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PHARMACOKINETIC AND PHARMCODYNAMIC STUDIES OF APOMORPHINE IN THE TREATMENT OF IDIOPATHIC PARKINSON'S DISEASE

INGRAM, WENDY MEREWYN

PHARMACOKINETIC AND PHARMACODYNAMIC STUDIES OF APOMORPHINE IN THE TREATMENT OF IDIOPATHIC PARKINSON'S DISEASE.

by

WENDY MEREWYN INGRAM

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Perspectives on Parkinson's disease....

It is now twenty-one years since my patients' awakenings...and yet, it seems to me, the subject is inexhaustible – medically, humanly, theoretically, dramatically. It is this which keeps the subject for me – and, I trust, my readers - evergreen and alive.
Oliver W. Sacks, 1990.

the real adventure is putting on your socks

the real adventure is putting on your socks,
it's difficult to do in the morning.
your eyes, they don't focus,
your fingers, they don't pinch,
your toes, they don't wiggle.
i sometimes sleep in my socks,
i'm not always in the mood for adventure.
Jeffery R. Romanyshyn, 1994.

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There were two aspects to the study of apomorphine in the treatment of Parkinson's disease: (i) a clinical pharmacokinetic-pharmacodynamic (PK-PD) study was designed and implemented in response to the challenges of apomorphine dose-titration in Parkinson's disease, and in view of the scarcity of available literature on the PK-PD relationships of apomorphine in Parkinson's disease, (ii) the PK (and tolerability) of apomorphine dosing using novel delivery/formulation combinations were explored in view of the inherent limitations associated with the conventional (i.e. subcutaneous) route of administration of apomorphine (e.g. cutaneous nodule formation, needle-phobia).
An HPLC assay was developed for the quantification of apomorphine in plasma, and stability issues relating to sample storage and assay were investigated.

With regards to the first aspect of the research, simultaneous PK-PD modelling was performed, using an effect compartment model to account for counterclockwise hysteresis in a sub-group of patients. According to the traditional two-stage approach to data analysis, mean (standard deviation) clearance following subcutaneous bolus was 2.2 (0.5) L/kg/h , (apparent) volume of distribution was 1.9 (0.8) L/kg, absorption half-life was 4.1 (2.1) minutes and elimination half-life was 69.5 (21.1) minutes (n=7). Equilibration half-life was estimated for two patients at 8.3 and 16.5 minutes.
Focus was given to investigating the relevance of a potential correlation (which had previously been identified using in-house pilot data) between post-distributional apomorphine PK and apomorphine-induced anti-parkinsonian response in patients with Parkinson's disease. It was hypothesised that this particular correlation may be of use in a dose-optimisation scheme. However it was demonstrated that, in the patients studied, the concept could not be applied to apomorphine dose-optimisation.

The novel delivery systems under scrutiny were: (i) Britaject® (Britannia Pharmaceuticals Ltd.) apomorphine formulation administered subcutaneously using a needle-free (jet) injector (J-TIP®, National Medical Products Inc.), (ii) an intranasal apomorphine powder formulation delivered using a turbospin insufflator (CDFS), and (iii) an apomorphine hydrogel co-polymer produced as a dosage-form for buccal delivery (Controlled Therapeutics (Scotland) Ltd.). As a result of this work, a rationale for subsequent development of the novel systems was provided. Indeed, the needle-free and buccal systems were, in their existing format, shown not to convey a net advantage over the existing system. However the intranasal formulation, with a mean (standard deviation) relative bioavailability of 41 (18) % (n=16) compared to subcutaneous bolus administration (and with a favourable outcome as regards to tolerability), was considered to be potentially suitable for further development.

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Contribution to publications:-

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- Pharmacokinetic Study of Single-Dose Intra-Nasal Apomorphine Powder (3 doses) in Healthy Volunteers 1999 (report held on file, Britannia Pharmaceuticals Ltd).

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External contacts:-
Mark Lovern (Pharsight Scientific Support), PharmPK Listserve (online discussion of pharmacokinetics, pharmacodynamics and related issues), Chris Ricketts and Hilary Sanders (statisticians, Plymouth University).

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SECTION 1:
LITERATURE REVIEW

1. Literature Review.

1.1. Pharmacokinetic-Pharmacodynamic Modelling Techniques.

Pharmacokinetics can be defined as the quantitative relationship between (observed) drug concentration (in plasma and/or tissue) and time. Pharmacodynamics describes the quantitative relationship between (observed) plasma and/or tissue concentration of the drug and (observed) pharmacological effect, the latter being defined as a drug induced change in a physiological parameter when compared to respective pre-dose (baseline) value[1] (Figure 1-1).

The combination of these two disciplines leads to a therapeutically relevant description of pharmacological effects and time. This description takes the form of a pharmacokinetic-pharmacodynamic model, whereby model parameter estimates provide information about intrinsic drug properties and hence enable predictions of concentration *versus* time and effect *versus* time profiles for different dosing schemes[1, 2].

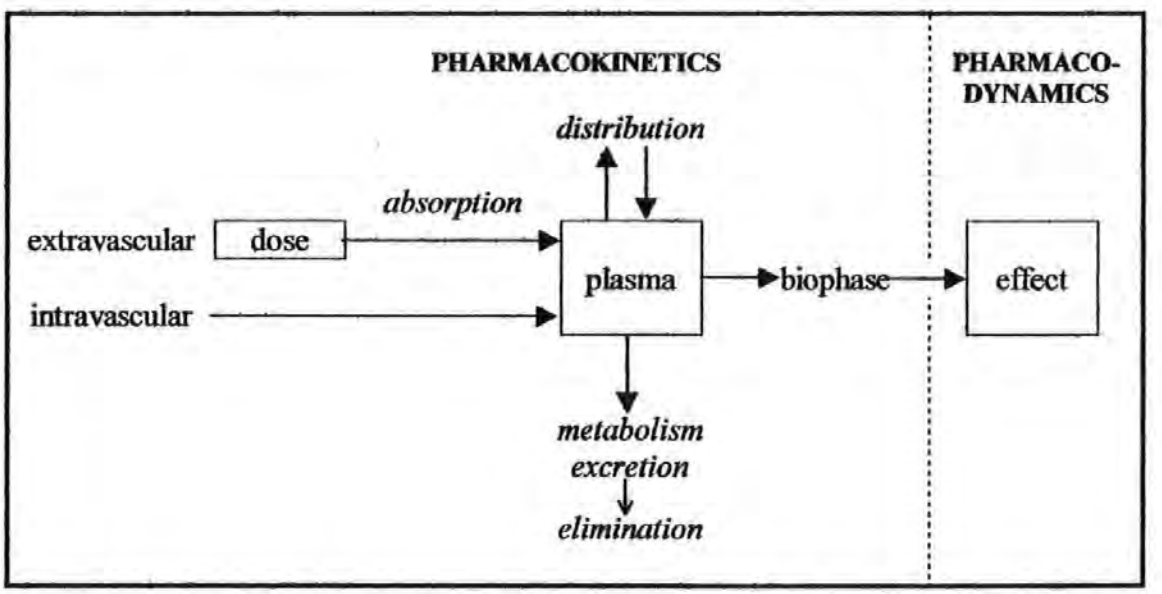

Figure 1-1 Schematic representation of the relationship between pharmacokinetics and pharmacokinetics (modified from [3-5]).

There are a number of distinct approaches to the estimation of pharmacokinetic and pharmacodynamic parameters for a population. The standard two-stage approach .[6-11]. is appropriate when rich datasets are available, i.e. when the number of drug concentration observations per patient far outnumbers the number of parameters being estimated, and when measurements are associated with only a small degree of error. Under these conditions, the standard two-stage approach is considered to be the "gold-standard" by which to compare other (more direct) approaches to population modelling, e.g. mixed effect modelling. Practically-speaking the standard two-stage approach involves the sampling of plasma (for plasma drug concentration) and response from each individual at a series of pre-specified times, and the subsequent generation of model parameter estimates for each individual within the study population (first stage). Thereafter the parameter mean and variance for the population are calculated from the individual estimates (second stage). Finally, the association between certain pharmacokinetic parameters and demographic characteristics are evaluated. A major disadvantage of this approach is that the residual inter-individual variability cannot be resolved from the other random effects, e.g. the (considerable) intra-individual error or measurement error

This constraint is avoided in the mixed effects modelling approach whereby both intra- and inter-individual variation are derived using built-in statistical protocols within the modelling process[6-11]. In contrast to the standard two-stage approach, the mixed effects modelling approach functions with sparse and/or observational data from individuals, and hence requires neither a stringently controlled study design nor intensive sampling from each individual. The observed data are pooled into a single dataset, although critically the individuals are still identifiable (allowing an uneven amount of data from different individuals to be input into the modelling process), and the population parameters are determined in a single stage of analysis.

1.1.1. Pharmacodynamic Models.

Pharmacodynamic models predict drug effect from (effect site) drug concentrations at steady state. There are four commonly encountered pharmacodynamic models.

The E_{max} model derives from drug-receptor interaction theory. Concentration (C) and effect (E) are related as follows: $E = E_0 + [(Emax \cdot C^{\gamma}) / (C^{\gamma} + EC_{50}^{\gamma})]$, where E_{max} denotes the maximum theoretical effect, EC_{50} is the concentration required to induce 50% of maximal effect (and hence represents drug potency), and γ is the Hill co-efficient. The Hill co-efficient accounts for the sigmoidicity of the relationship, allowing a better fit to observed data, and as such dose not have a direct biological interpretation.

In the simple (or hyperbolic) E_{max} model, $\gamma = 1$ (Figure 1-2).

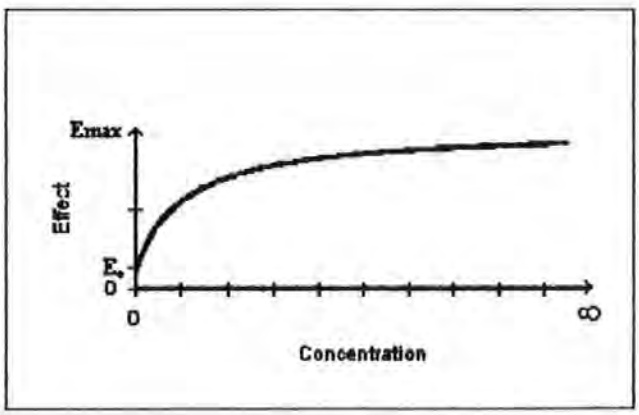

Figure 1-2 Simple E_{max} model with baseline effect parameter.

If $\gamma > 1$ then the concentration-effect relationship is sigmoid, and the larger the value of the exponent, the steeper the slope (Figure 1-3). The sigmoid E_{max} model is considered the most versatile of the basic pharmacodynamic models, however since the number of model parameters is relatively great (compared to other common pharmacodynamic models) the precision with which these parameters are estimated is decreased[2, 5, 11]. In order to obtain accurate and precise model parameter estimates, the observations throughout the entire range of the concentration-effect profile must be obtained. Generally this is achieved by the administration of ascending doses of drug to each individual[2].

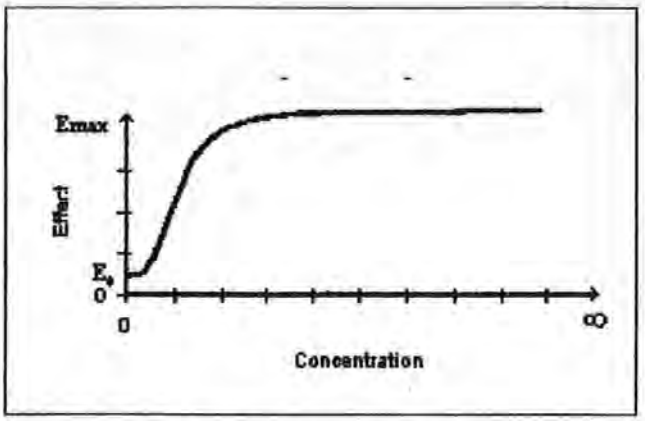

Figure 1-3 Sigmoid E_{max} model with baseline effect parameter.

The relationship between concentration (C) and effect (E) can be described more simply in terms of a linear function: $E = S.C + E_0$, where S denotes the proportionality factor (and hence is a measure of the sensitivity of the pharmacodynamic effect to changes in C) and E_0 is the intercept of the relationship (and the baseline value of E). The linear model parameters are easily obtained by linear regression (a feature which perhaps may be considered an advantage), however this model is limited in that maximum pharmacological effect cannot be described[5, 11].

Concentration-effect relationships may also be described using the log-linear function, i.e. $E = S. \log (C) + b$, where b is the *y*-axis intercept (an arbitrary constant). The derivation of the log-linear model came from the observation that for a hyperbolic concentration-effect relationship, log-transformed concentration *versus* effect is approximately linear in the range of 20 to 80% of maximum effect. In common with the limitation of the linear model, maximum effect cannot be predicted using the log-linear model. In addition, the latter model (always) predicts the existence of a threshold concentration below which there is no effect, indeed the effect at $C = 0$ cannot be estimated using this model [2, 5, 11].

1.1.2. Combined Pharmacokinetic-Pharmacodynamic Models.

Pharmacokinetic and pharmacodynamic data are linked directly or indirectly, depending on the nature of the relationship between concentration an effect. An exploration of a plot of observed drug concentration (usually the concentration in plasma) and observed effect, in which data points are connected in chronological order allows an insight into the relationship.

A direct link is implicated when the relationship between observed drug concentration and observed effect is independent of time, i.e. the times courses of observed plasma concentration and effect are in phase with each other. In this situation there is rapid equilibration of drug between the systemic and effect compartments. Under such conditions the observed drug concentration and (unobservable) effect site drug concentration are proportional, hence the observed plasma concentration is a good predictor of the concentration at the effect site and thus effect[1, 2, 12, 13].

In other circumstances the time courses of observed drug concentration and effect are *not* superimposed. Here the relationship between observed drug concentration and effect is time-dependent, and hysteresis is displayed in the plot of observed drug concentration *versus* observed effect. An indirect link between pharmacokinetics and pharmacodynamics is required in this situation.

Hysteresis in a clockwise direction (or proteresis) is indicative of : (i) the development of (pharmacodynamic) tolerance, e.g. by receptor desensitisation, (ii) the action of inhibitory metabolite(s), (iii) having sampled venous blood for pharmacokinetic-pharmacodynamic study when the equilibration of drug between the effect site and venous blood is relatively slow compared to that between effect site and arterial blood [11-15].

Counterclockwise hysteresis is displayed when the kinetics of effect are delayed with respect to the kinetics of observed drug concentrations. Mechanisms which explain this phenomenon include: (i) the time taken for distribution of drug from plasma to the effect

site, (ii) the action of active (synergistic) metabolite(s), (iii) delays due to post-receptor transduction processes, iv) receptor up-regulation[1, 2, 11-13].

Temporal disequilibrium between observed concentration and observed effect can be accounted for by the use of an effect compartment (or link) model (Figure 1-4) [2, 5, 11-14, 16]. This approach, which was first proposed by Segre[17] and Hull[18], was formalised by Sheiner *et al* in the context of the simultaneous characterisation of pharmacokinetics and pharmacodynamics of d-tubocurarine[19].

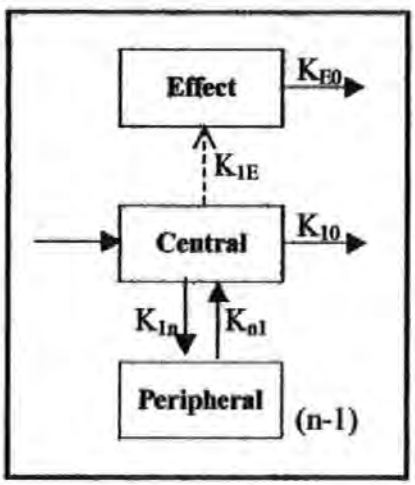

Figure 1-4 Schematic representation of effect compartment model described by Sheiner *et al*[19], whereby the hypothetical effect compartment receives input from the central compartment (modified from [12, 13, 16]).

The central compartment of the pharmacokinetic model (usually plasma) is linked to a hypothetical effect (or *biophase*) compartment by a first-order rate constant, k_{1E}; thus drug concentration in the effect compartment (i.e. Ce) depends on the kinetics of the central compartment. The observed effect is related to the drug concentration in the effect compartment by means of a pharmacodynamic model, e.g. E_{max}.

It is assumed that k_{1E} is very small relative to any other rate constant in the pharmacokinetic model, consequently the amount of drug which reaches the biophase is insignificant compared to the total amount of drug in other body compartments. In this respect the effect compartment cannot be distinguished pharmacokinetically from the plasma concentration-time profile as a peripheral compartment.

The first-order rate constant k_{E0} describes the dissipation of drug from the hypothetical effect site. This parameter is used to characterise the degree of hysteresis, in that the half-life of equilibration of drug between plasma and effect site (T_{eq}) is given by ln (2)/ k_{E0}. T_{eq} can also be interpreted as the half-life of the time required to "collapse" the two limbs of the hysteresis loop in the concentration-effect relationship.

Other approaches applicable to the indirect linking of pharmacokinetics and pharmacodynamics include linking the effect compartment to a peripheral compartment as opposed to the central compartment[16, 20, 21].

1.1.3. Assessing Goodness of Fit of Pharmacokinetic/Pharmacokinetic Models.

The characteristics of an well-defined model according to Gabrielsson and Weiner[5] are as follows:-

- the model has biological relevance,
- the fitted curve mimics trends in the observed data e.g. T_{max},
- the residuals display a random scatter and are free from systematic deviation,
- the parameter estimates have good precision,
- the parameters have low correlation,
- the condition number of the fit is (relatively) low and the rank is full.

These features, along with associated statistical tools, are used when comparing competing models for the best fit. No single statistic is more important than another, thus a set of diagnostic features (given below) should be considered when attempting to discriminate between models.

Weighted Sum of Squared Residuals (WSSR).

The process of producing a set of model parameter estimates (e.g. volume of distribution and elimination rate) is based on the minimisation of the difference between observed and predicted data, i.e. the minimisation of residuals. Thus nonlinear regression programs are

employed to determine parameter estimates by adjusting parameter values iteratively using WRSS as an objective function. Convergence is achieved when the relative change between $WRSS_{old}$ and $WSSR_{new}$ is less than the (user-defined) convergence criteria, the default (for WinNonlin®) being 0.0001. At convergence the iteration process ceases and the final parameter estimates are given.

Residual Analysis.

Residual plots should be inspected for evidence of systematic deviation, random scatter and the presence of outliers. Systematic deviation is evidenced by sequences of consecutive positive or negative residuals, or "runs". A model that possesses a lack of systematic deviation i.e. one that displays a relatively high number of changes in sign of consecutive residuals, is considered to be superior to one displaying systematic deviation. When plotted against the independent variable or the predicted dependent variable, residuals should be randomly distributed about the mean (i.e. zero) and fall within a narrow horizontal band (tramlines).

Visual inspection of residual plots also allows the detection of outliers i.e. isolated data values which were not fit well by the model. Such data values will possess unusually high residuals. An outlying data value may deviate in the concentration- or time-dimension, or both. Depending upon the point at which the deviation occurs within the data set, outliers may exert leverage on parameter estimation, compromising accuracy and/or affecting precision.

Parameter Correlation.

The parameter correlation matrix provides a means of assessing the co-dependence of parameters. Ideally model parameters should be totally uncorrelated with each other, i.e. $R = 0$. Furthermore where high correlations, say $R > |0.95|$, exist between a given pair of parameters, the associated parameter estimates should be interpreted with caution. This is because such correlations relate to there being insufficient information in the

concentration-time data to generate the parameter estimates with sufficient accuracy and precision.

Condition Number (of the matrix of partial derivatives).
The condition number is a marker of the stability of the model fitting process. The condition number is defined as the square root of the ratio of the largest to the smallest eigenvalue. A low condition number is desired (less than $10^{N_{par}}$, as a general rule, where N_{par} is the number of parameters); large condition numbers are indicative of instability in the minimisation process.

Rank of the matrix of partial derivatives of the model parameters.
If rank is less than N_{par} then the model is ill-conditioned i.e. there is not enough information contained in the data to precisely estimate all of the parameters in the model. When rank is equal to N_{par}, the matrix is said to possess full rank, this being an indication of a robust fit.

Akaike Information Criteria (AIC) and Schwarz Criteria (SC).
AIC and SC are measures of goodness of fit based on maximum likelihood. The use of AIC and SC is appropriate only when comparing competing models of the same weighting scheme. The model associated with the smallest AIC (or SC) is regarded as giving the best fit of those models under consideration. The distribution of these values is unknown, therefore no statistical significance can be associated with the AIC or SC values obtained for competing models. Computational formulae are given below:-

Akaike Information Criteria.

$AIC = N_{obs} * LOG(WSSR) + 2 * N_{par}$

Schwartz Criteria

$SC = N_{obs} * LOG(WSSR) + LOG(N_{obs}) * N_{par}$

where $WSSR = \sum wt\ (yi - \hat{yi})^2$

and $\hat{yi}$ denotes the predicted value of yi.
N_{obs} = number of observations
N_{par} = number of parameters

1.2. Parkinson's Disease.

1.2.1. Parkinsonism (The Parkinsonian Syndrome).

The Parkinsonian Syndrome was first described by James Parkinson in 1817 as paralysis agitans, or the "shaking palsy". He documented a state of:-

> "*...involuntary tremulous motion with lessened muscle power in patients not in action with a propensity to bend the trunk forward and to pass from a walking to a running pace*" [22].

Parkinson had described a clinical syndrome which was dominated by a disorder of muscle movement (dyskinesia) and muscle tone (dystonia). The characteristic clinical features of parkinsonism, each of which reflect the altered activity of the basal ganglia (see Section 1.1.1.1, page 1-13) are: akinesia, rigidity, and tremor.

Akinesia, i.e. loss of movement, is described as a symptom complex consisting of bradykinesia (slowness of movement) and hypokinesia (decreased amplitude of movement)[23]. Akinesia is considered to be the impairment which causes the most prominent disability to the parkinsonian individual[24, 25]. The broad consequence of akinesia is that difficulty in initiating movement and performing simultaneous, sequential and repetitive alternating motor tasks is experienced[24].

More specifically, fine motor tasks involving the hands and fingers, such as writing become inordinately difficult to accomplish. A slow, shuffling gait with impaired arm swing occurs. Difficulty may be experienced when the affected individual attempts to cease walking (festination). Facial amimia develops, which, coupled with reduced rate of blinking and swallowing, results in a "masked" facial appearance. The voice becomes quiet and monotonous[26].

The ultimate expression of akinesia is "freezing", where the individual suddenly becomes completely immobile. A parkinsonian individual will suddenly become "rooted to the spot" whilst walking, especially when attempting to change direction [27, 28].

Rigidity, that is, an increased resistance of muscle to passive movement, can occur throughout the full range of movement of the limbs, trunk and neck[26, 29]. Rigidity may be of a "lead pipe" or "cogwheel" quality, the former being likened to the sensation of bending a lead pipe, the latter, which is due to the combination of existing tremor and rigidity, being likened to turning a sticking cogwheel[23, 28]. The increase in muscle tone in flexor muscles is slightly more pronounced than in extensors. Thus when all four limbs are affected, a "stooped" or "simian" posture is produced[24].

Tremor has been described as the symptom which is publicly identified with parkinsonism. It is the most common symptom, although not universal, being absent in about 30% of individuals with parkinsonism due to idiopathic Parkinson's disease[24]. Where tremor *is* present it can affect the facial, jaw, tongue or leg muscles but it principally affects the hands at a frequency of 4-6Hz[23, 26, 28]. In the upper limb(s) tremor produces rhythmical pronation/supination and "pill-rolling" movement of the thumb and fingers[23]. Parkinsonian tremor is typically described as occurring at rest, being worsened by anxiety and being greatly reduced by voluntary movement, although it is documented that tremor may persist during activity in those individuals who normally experience a particularly well-developed tremor[23, 28].

1.2.2. Idiopathic Parkinson's Disease.

Idiopathic Parkinson's disease accounts for the majority of cases of true parkinsonism[24]. In this thesis, the term *Parkinson's disease* is used in reference to the idiopathic form. The "gold standard" for diagnosis of Parkinson's disease is the pathological finding of specific degeneration of nigrostriatal and other pigmented nuclei, with a characteristic inclusion, the Lewy body, in remaining nerve cells[30].

The following features are predictive of idiopathic Parkinson's disease : unilateral onset, classic rest tremor, and pronounced reduction of parkinsonian symptoms with L-dopa therapy[31]. Autonomic disturbance may occur as a late feature of the disease, as does

freezing and postural instability[24]. Parkinson's disease does not cause cerebellar or pyramidal signs[24].

Many individuals with Parkinson's disease exhibit a range of non-motor symptoms in addition to the characteristic motor deficits. It has been estimated that dementia occurs in over 15% of patients and that depression is common[32]. Depression is generally of a mild to moderate intensity and can be difficult to diagnose if hypomimia and hypokinesia are present. The presence of neuropsychiatric conditions can compromise the use of standard anti-parkinsonian pharmacotherapy.

Olfactory dysfunction has been demonstrated in Parkinson's disease. Typically, the olfactory deficit is bilateral, occurs for a wide range of odours and is not influenced by anti-parkinsonian drugs[32, 33].

Sensory symptoms such as pain and parasthesias have been reported[32]. In general the manifestations are mild, but distressing. Although these symptoms may be variable, they predominantly affect the side of the body with most severe motor symptoms.

Constipation is common in individuals with Parkinson's disease[32]. It is thought that physical inactivity, impaired forcefulness of abdominal musculature and dysfunction of the enteric nervous system and anal sphincter contribute to this condition[34].

Bladder dysfunction involving detrusor hyperactivity has been reported in some patients[32]. Abnormal heat regulation can be observed to different degrees. Sensations of cold and acute attacks nocturnal attacks of sweating have been documented[32]. Mild dysphagia and sialorrhoea is considered to be common in advanced Parkinson's disease [32]. Sleep disturbances, which include insomnia, parasonmias and excessive daytime somnolence have also been described[35].

1.2.3. Epidemiology of Idiopathic Parkinson's Disease.

The incidence of Parkinson's disease is rare before fifty years of age, but increases with age thereafter[36]. The prevalence of Parkinson's disease in North America and Europe is estimated to be between 100 and 200 cases per 100,000 population[36]. By the eighth decade of life, the estimated prevalence in North America and Europe rises to between 1,000 and 3,000 cases per 100,000 persons. In the UK, there is an annual incidence of 12 new cases per 100,000 of the population[37]. Parkinson's disease is known to be a world-wide disease, but is possibly less prevalent in China, Japan and Africa as compared to Western countries[38], and is slightly more common in men than in women[36, 38]

1.2.4. Pathogenesis of Idiopathic Parkinson's Disease.

1.1.1.1. The Nigrostriatal Pathway.

The nigrostriatal pathway is one of three dopaminergic systems in the human CNS. This pathway accounts for 75% of dopaminergic activity in the brain. Cell bodies lie in the substantia nigra. The axons project, via the medial forebrain bundle, to the corpus striatum and terminate at the neostriatum.

The corpus striatum is the principle input structure of the basal ganglia and receives excitatory glutaminergic input from many areas of the cortex. The majority of neurones within the striatum are projection neurones that innervate other basal ganglia structures. A small subgroup of striatal neurones are interneurones which do not project beyond the borders of the corpus striatum.

The outflow of the striatum proceeds along two routes, identified as the direct and indirect pathways. The direct pathway is formed by neurones in the striatum that project directly to the output stages of the basal ganglia, the substantia nigra pars compacta and the medial globus pallidus, using the inhibitory neurotransmitter GABA: these in turn relay to the thalamus, which provides excitatory input to the cortex. The striatal neurones giving rise to the direct pathway express primarily the excitatory D1 dopamine receptors.

The indirect pathway is composed of striatal neurones that project to the lateral globus. This structure in turn innervates the subthalamic nucleus, which provides outflow to the substantia nigra pars reticulata and medial globus pallidus output. This pathway involves two inhibitory GABA-mediated projections and one excitatory glutaminergic projection. The striatal neurones giving rise to the indirect pathway express primarily the inhibitory D2 dopamine receptors.

The substantia nigra pars compacta provides dopaminergic innervation to the striatal neurones giving rise to both the direct and indirect pathways, and regulates the relative activity of these two routes[23, 28, 39].

1.1.1.2. Dopamine Receptors

Dopamine exerts its physiological effects at the nigrostriatal pathway through receptors of the G-protein-coupled receptor superfamily. Two major classes of dopamine receptor, D1 and D2, are distinguishable by both pharmacological and biochemical criteria.

In terminals of dopamine neurones projecting from the midbrain to forebrain, levo-tyrosine is oxidised to levodopa by tyrosine hydroxylase. This is the rate-limiting step in catecholamine biosynthesis. Dopa is then decarboxylated to dopamine by aromatic L-amino acid decarboxylase and stored in vesicles. Following exocytotic release by depolarisation in the presence of Ca^{2+}, dopamine interacts with post-synaptic D1 and D2 receptors, as well as pre-synaptic D2 autoreceptors. Inactivation of trans-synaptic communication occurs primarily by active transport of dopamine into pre-synaptic terminals, with secondary deamination by mitochondrial monoamine oxidase-B to 3,4-dihydroxyphenylacetic acid and ultimately to noradrenaline. Postsynaptic D1 receptors, through G_s type G-proteins, activate adenylyl cyclase and the conversion of ATP to cAMP, while D2 receptors inhibit adenylyl cyclase through G_i proteins. D2 receptors also activate receptor-operated K^+ channels and stimulate phospholipase C, perhaps via the Bγ subunits liberated from activated G_i, to convert phosphatidylinositol to inositol triphosphate and diacylglycerol, with secondary modulation of Ca^{2+} and protein kinases[39].

D2 autoreceptors suppress the synthesis of dopamine by diminishing phosphorylation of rate-limiting tyrosine hydroxylase, as well as limiting dopamine release (possibly through modulation of Ca^{2+} or K^{+} currents[39]).

1.1.1.3. Consequences of Nigrostriatal Pathway Degeneration.

The primary deficit in Parkinson's disease is the degeneration of the dopaminergic neurones of the nigrostriatal pathway. Progressive loss of such neurones is a normal feature of ageing, however a loss of 80-90% of dopaminergic neurones occurs in symptomatic Parkinson's disease[39]. It has been elucidated, by post mortum and by positron emission tomography studies, that at this degree of neurone destruction up to 50% of brain dopamine may be lost[ ].

In Parkinson' disease the loss of the dopaminergic input to the corpus striatum has a differential effect on the two output pathways; the direct pathway to the substantia nigra pars reticulata and medial globus pallidus is less active, while the activity in the indirect pathway is increased. The net effect is that neurones in the substantia nigra pars reticulata and medial globus pallidus are more active. This leads to increased inhibition of the thalamus and reduced excitatory input to the motor cortex. This ultimately results in *akinesia, rigidity* and *tremor.*

1.2.5. Management of Idiopathic Parkinson's Disease.

1.1.1.4. Rationale for Anti-Parkinsonian Pharmacotherapy.

Currently pharmacotherapy is symptomatic only since there is insufficient evidence of the effect of any available drug on disease progression[37]. The preparations which are currently available aim to correct the neurohormonal imbalance at the basal ganglia. Levodopa is the treatment of choice for Parkinson's disease, being the most effective and reliable treatment available at the time of writing[37, 40, 41]. Levodopa is the natural intermediary in the enzymatic synthesis of dopamine from L-tyrosine, and has no pharmacological action of its own, but acts to replenish depleted striatal dopamine by crossing the blood-brain-barrier where it is decarboxylated to dopamine by L-amino acid decarboxylase. Levodopa is combined with a dopa-decarboxylase inhibitor (benserazide or carbidopa) which inhibits of breakdown of levodopa to dopamine in the periphery. Improvement in parkinsonian symptoms, especially bradykinesia and rigidity, occurs in approximately 80% of patients on levodopa pharmacotherapy[41], however treatment is limited by the development of neuro-psychiatric complications and the emergence of motor fluctuations. Such motor fluctuations take the form of end-of-dose deterioration (or wearing-off effect), inter-dose (or peak dose) abnormal involuntary movements, and unpredictable "on-off" fluctuations. In the latter case, periods of severe parkinsonian motor deficit alternate unpredictably with periods of relative mobility. It remains unclear whether duration of disease or duration of levodopa therapy causes such complications. It has been postulated that disease progression is accelerated as a consequence of potentially neurotoxic radicals produced by metabolism of levodopa, however this has not been confirmed in human studies[37]

The development of dopamine agonists has sought to produce a more predictable and sustained dopaminergic action than that of levodopa. Dopamine agonists currently available for use in the UK are: apomorphine, bromocriptine, cabergoline, lisuride, pergolide, pramipexole and ropinerole.

1.2.6. Apomorphine in the Treatment of Parkinson's Disease.

The discovery of apomorphine is credited to Matthiessen and Wright who, in 1869, published their work on the chemistry of opium bases[43]. Since then apomorphine has been used as emetic, expectorant and in various motor disturbances, however it was not until 1951 that Schwab showed that apomorphine could reduce rigidity in decerebrate animals[44]. Based on his pre-clinical work, Schwab attempted to alleviate parkinsonism in humans using subcutaneously administered apomorphine; an improvement in tremor and rigidity was seen in the patients. A decade would pass before it was realised that apomorphine acted at dopamine receptors, or that nigrostriatal dopamine deficiency occurred in Parkinson's disease.

Conformation of the therapeutic effects of apomorphine in Parkinson's disease followed, but introduction into clinical practice was limited by the need for parenteral administration, the occurrence of adverse reactions (such as vomiting, drowsiness, arterial hypotension, yawning), and also by the success of oral levodopa. An advance was made in 1979, when Agid found that domperidone (an extra-cerebral dopamine antagonist) could prevent the typical adverse effects of apomorphine (as given above), with the exception of yawning. Domperidone was subsequently recommended for the improvement of tolerance to orally active dopamine agonists. Key clinical studies were performed in patients in the 1980s [45-47]. Apomorphine was administered subcutaneously, either intermittently with the Penject® (Britannia Pharmaceuticals) to effect a "rescue" from "off" phases, or by continuous infusion using a Grasby MS 16A pump, the latter being applicable to patients who require multiple injections daily or who are unable to anticipate an "off" period with adequate time to self-administer a bolus. Administration of apomorphine by continuous infusion usually allows a reduction in oral dopaminergic medication.

Currently apomorphine is licensed in the UK[1] to manage motor fluctuations that are inadequately controlled by L-dopa and other agonists. Patients with Parkinson's disease who can expect to have a significant therapeutic benefit from apomorphine are those with severe off periods and with a good quality of on periods - particularly if not associated with troublesome interdose dyskinesias. Daily "off" time is reduced by more than 50% in patients with "on-off" motor fluctuations, with extended benefits seen up to eight years of follow-up[ ].

Adverse Effects.

The side effects are as those observed with other dopamine agonists, with the addition of yawning and drowsiness. The most frequent side effect is a local skin reaction at needle insertion points, consisting of itchy fibrotic nodules which may scab and occasionally become infected and bleed. It is possible to minimise these by diluting the apomorphine solution with saline and rotating injection sites[48-50].

Coombs-positive haemolytic anaemia which is drug-dependant and reversible has occurred in a few patients on apomorphine and L-dopa[46, 51]. This condition has been described in patients on L-dopa alone.

[1] Apomorphine was licenced as BRITAJECT® (Britannia Pharmaceuticals) for the duration of the clinical and analytical work contained in this thesis, however the formulation is now licensed as APO-GO® (Britannia Pharmaceuticals)

1.1.1.5. Chemistry of Apomorphine.

The molecular weight of apomorphine as a base is 267.3 and as a HCL-salt is 312.8 (Figure 1-5). Apomorphine readily undergoes oxidative changes, especially in solution under alkaline conditions, and in the presence of oxygen and light. On oxidation apomorphine turns green[52, 53].

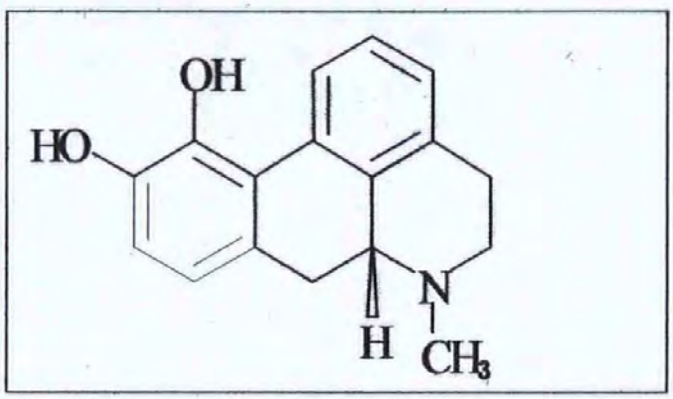

Figure 1-5 Structure of apomorphine.

The dopaminergic properties of aporphines results from the binding to both hydroxyl groups and the nitrogen atom. The hydroxyl group at the C11 position contributes most to the receptor binding and biological activity[ ].

The preferred conformation for central dopaminergic receptor agonist activity is the α-conformation, where both hydroxyl groups are located at the C10 and C11 atoms[52].

1.1.1.6. Metabolism of Apomorphine.

Data relating to apomorphine metabolism in humans is scarce. Bioavailability after an oral dose is low (17%), indicating an extensive first-pass effect[56]. Enantiomeric interconversion, methylation, sulphation and glucuronidation of R(-)-apomorphine have each been proposed as potential metabolic pathways[52] (Figure 1-). Apocodeine, isoapocodeine, and apomorphine-glucuronide conjugate(s) potentially exhibit dopaminergic stimulation, whereas S-apomorphine is a potential dopamine antagonist[54]. The contributions of the aforementioned pathways were examined in ten patients with Parkinson's disease by van der Geest *et al*[54]. The total excretion of unchanged apomorphine was only 0.3% of the administered subcutaneous dose, and as such accounted for only a minor proportion of the administered dose. The authors concluded that neither enantiomeric interconversion nor methylation occurred *in vivo* (based on the absence of detectable concentrations of S-apomorphine in the plasma, and apocodeine and isoapocodeine in the plasma or urine). The conjugation of apomorphine via glucuronidation and sulphation pathways was identified as having a minor role *in vivo*; the total excretion of sulphated and glucuronidated apomorphine was 3.8 and 6.0% of the administered dose, respectively. The authors suggested that *N*-demethylation of apomorphine to norapomorphine, by the cytochrome P-450 system, may contribute to apomorphine metabolism; norapomorphine possesses dopaminergic activity, but is significantly less potent than apomorphine in terms of stereotyped behaviour in animals[52]. Additionally (auto)oxidation to quinone derivatives may prove to be a quantitatively important process with regard to apomorphine metabolism[54] however the contribution of such derivatives to the pharmacological response to apomorphine in Parkinson's disease remains to be established.

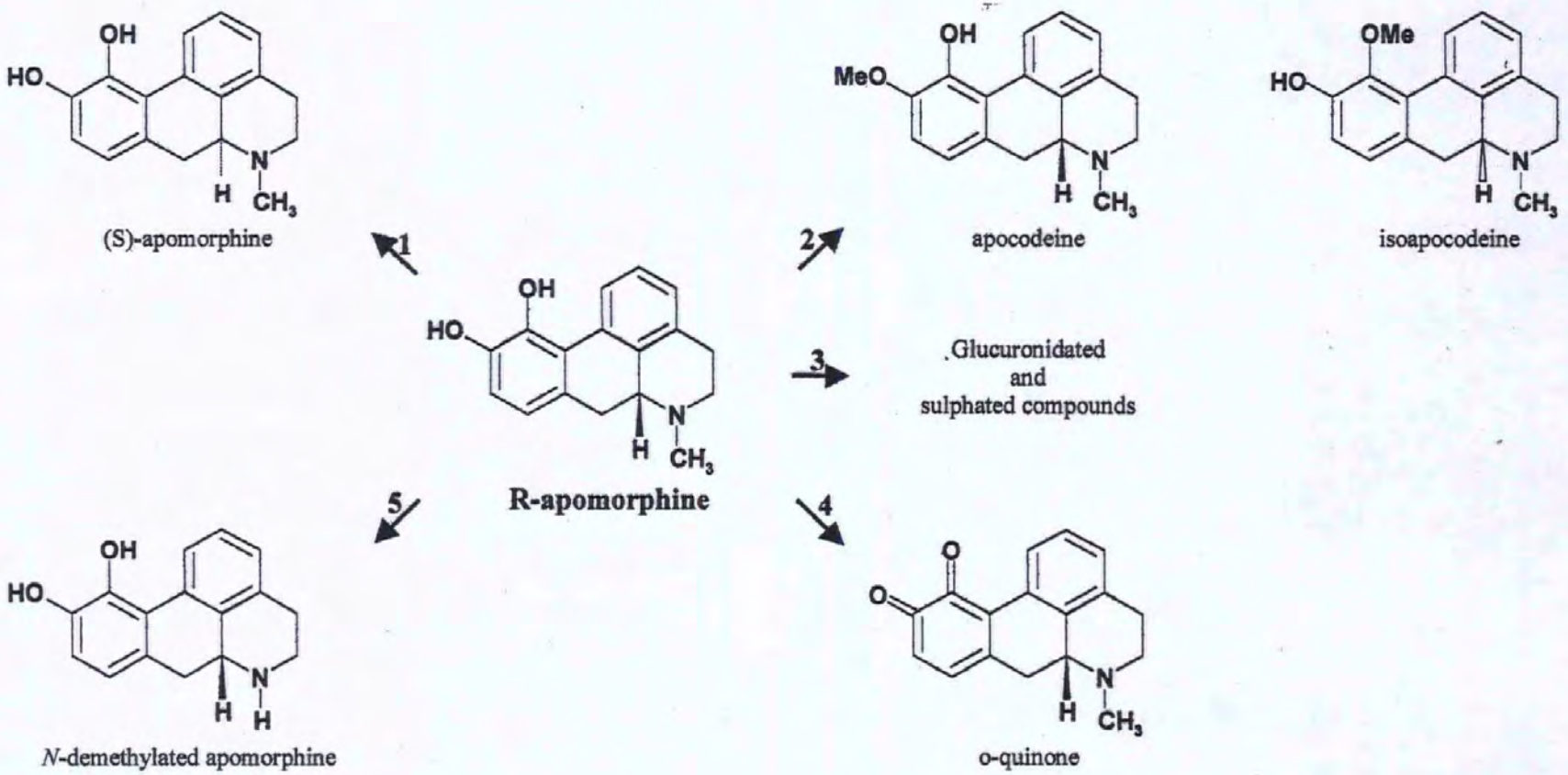

Figure 1- **Proposed metabolic pathways of R-apomorphine (modified from van der Geest[54])**

1 = racemisation, 2 = O-methylation, 3 = conjugation, 4 = (auto)oxidation, 5 = N-demethylation.

1.1.1.7. Pharmacokinetics-Pharmacodynamics of Subcutaneous Apomorphine.

The peripheral pharmacokinetics of apomorphine were first described, by Gancher *et al,* in 1989[56]. These data, along with those from more recent studies are summarised in Tables 6-2 and 6-3 (pages 6-4 to 6-6).

Apomorphine pharmacokinetics have been modelled using either a one- or two-compartment model. The bioavailability of the subcutaneous route (compared to intravenous administration) is 100% [56, 58]. Following subcutaneous injection, there is a short absorption half-live (approximately 6 minutes) and a brief T_{max} (approximately 14 minutes) which is dose-independent[56-58, 85]. Both C_{max} and AUC are linearly-related to dose, the latter ranging from approximately 10 to 90µg/kg [56-58, 61, 85]. A large inter-patient variation in absorption has been reported, but with lower intra-patient variation[56, 58]. Explanations for the variation in pharmacokinetic parameters have centred on differences in choice of injection site, local skin temperature, and local nodule formation. The distribution and elimination half-lives are short, i.e. approximately 10 and 50 minutes, respectively[54, 56, 58].

Clearance (at approximately 3L/h/kg) exceeds hepatic blood flow, indicating that metabolism must occur to a significant degree in extra-hepatic tissues[54]. The (apparent) volume of distribution is given as approximately 1.5L/kg, indicating extensive distribution outside the plasma compartment[54, 56-58, 61].

The onset of anti-parkinsonian effect following a subcutaneous bolus dose occurs shortly after dose administration (approximately 5 to 20 minutes), but the effect is short-lived (approximately 45 to 90 minutes).

The rapid distribution of apomorphine from the subcutaneous administration site into plasma, and the rapid clearance from plasma, are attributed to the high lipophilicity of apomorphine[54, 56-58, 61]. The short latency of onset to anti-parkinsonian effect, and the brief duration of effect, reflect a rapid passage across the blood-brain-barrier and a fast

equilibration with the site of action. These processes are similarly associated with the lipophilic properties of apomorphine.

Van Laar *et al* employed a multiple pseudo steady-state protocol in the characterisation of apomorphine concentration-effect relationship in ten patients with Parkinson's disease[62]. The dose ranged from 10 to 100 μg/kg/h, comprising of a 10μg /kg/h increase every 20 minutes. It was thus demonstrated that a narrow therapeutic window exists, and furthermore that there is a high inter-patient variation in the minimum plasma concentration for onset of beneficial effect (1.4 to 10.7 ng/mL), onset of dyskinesia, (2.7 to 20.0 ng/mL) and for onset of adverse effects, e.g. nausea, hypotension, (8.5 to 24.5 ng/mL).

Harder *et al* performed a pharmacokinetic-pharmacodynamic study in which four ascending bolus doses of apomorphine (0.5, 1, 2, 4mg) were administered subcutaneously to ten patients with Parkinson's disease[60]. The authors demonstrated the existence of a steep sigmoidal relationship between dose and response, with a minimal effective threshold dose (the percentage of patients who responded at 0.5, 1, 2 and 4mg was 0, 20, 60 and 75, respectively), and whereby a dose increase above the threshold resulted in an extension to the duration of effect, but not to an augmentation of the response.

A direct relationship between concentration and effect was evident in five (out of forty) series. Conversely in other cases proteresis was displayed (n=6), whilst in seven series counterclockwise hysteresis was apparent. In three series, no clear relationship between concentration and effect could be detected, and in the remaining series modelling was precluded due to the absence of detectable plasma apomorphine concentrations and/or a lack of demonstrable effect.

A hypothetical effect compartment was incorporated to account for the counterclockwise hysteresis observed in a sub-set of the study population. The effect site equilibration half-life was short (approximately 6 minutes) thus accounting for the rapid onset of clinical effects.

1.3. Pharmacodynamic Assessments in Parkinson's Disease.

A tool employed for pharmacodynamic assessment should be objective, reliable, and valid[63]. The method must be standardised, with attention paid to all the steps of the test, e.g. executive instructions and conditions[63]. In terms of the study of apomorphine pharmacodynamics, it must be feasible to complete the assessment within a very short time frame (approximately one minute) due to rapid changes in motor performance.

The methods used to quantify symptoms in movement disorders such as Parkinson's disease include rating scales and instrumented measurements. The Unified Parkinson's Disease Rating Scale[64] (UPDRS) (see Appendix 8.1) was designed to provide a measure of signs and symptoms of Parkinson's disease in clinical practice and research. The UPDRS Part III sub-score is intended to assess parkinsonian severity, but not necessarily disability. The UPDRS is considered to be the "gold standard" for assessing therapeutic efficacy in clinical trials in Parkinson's disease and is the most widely used standardised quantitative measure in Parkinson's disease[63]. However, the time taken to complete the assessment is too long to obtain replicate measurements in a study of apomorphine (bolus injection) in Parkinson's disease.

Rating scales which have been used previously in pharmacokinetic-pharmacodynamic studies of apomorphine in Parkinson's disease include a four-point tremor score[4, 54, 62], a four-point dyskinesia score[4, 54, 62] and the Columbia University Rating Scale (CURS)[60]. The major disadvantage of the latter rating scale is that the task takes approximately five minutes to complete[60].

The tapping test is commonly used as an assessment of parkinsonian bradykinesia because it is simple, rapid and objective[4, 54, 57, 65, 66]. The subject is required to alternately depress two counters which are (usually) placed 30cm apart, using one hand only, for a period of (usually) 30 seconds. (Figure 1-6). The task must be performed at the maximal

rate of movement using the hand of the side of the body most affected with parkinsonism. Tapping speed has been shown to correlate with other measures of parkinsonism, such as the bradykinesia and rigidity assessments of the CURS[67-69]. Disadvantages of the tapping test are that the task is subject to learning effects[68, 70, 71] and, in common with many other assessments of symptoms in movement disorders, relies on the active co-operation and motivation of the subject.

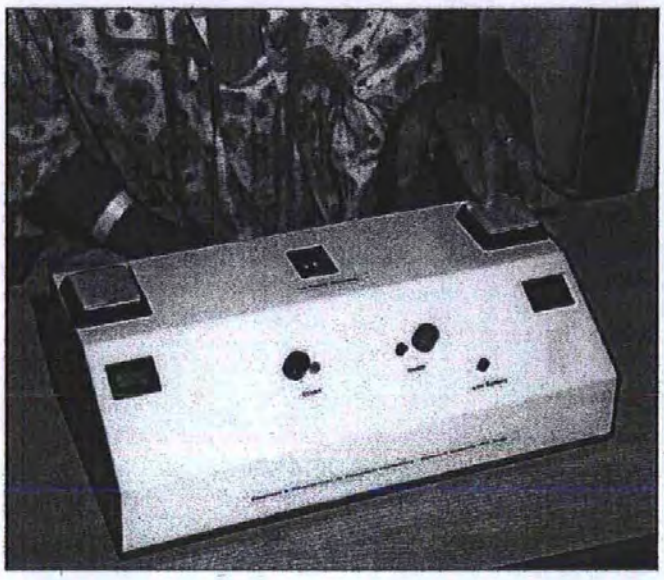

Figure 1-6 The tapping tester used in the study presented in this thesis.

The Bradykinesia Akinesia Inco-ordination Test (BRAIN TEST©) is a computerised keyboard tool, which has been used to quantify upper limb motor function in movement disorders[72]. This test is based on the tapping test but generates a dysmetria score (i.e. the number of incorrectly hit keys corrected for speed) and an inco-ordination score (i.e. the variance of the time between keystrokes) in addition to a kinesia score (or tap rate). Both the kinesia score and inco-ordination score correlate with the UPDRS Part III sub-section.

Ambulation tasks have been used to monitor anti-parkinsonian response, e.g. in the form of a simple walking test in which the subject is required to walk at maximal speed over a distance of 12m[65, 66], and also in the form of an analysis of gait parameters such as stride length, which correlated with the CURS bradykinesia score[70], and velocity, which correlated with UPDRS III Axial motor score[73]

There are other techniques which have not been widely used, probably because these involve the use of specialist equipment and are therefore less accessible. Examples include the use of activity monitors and accelerometers[74], computer-derived indices of handwriting kinematics[75], and computer-assisted analysis of whole body (posturo-locomotion-manual) movement patterns and motor performance[76].

1.4. The Intranasal Route for Drug Delivery.

Nasal respiratory epithelium consists of ciliated pseudostratified columnar epithelial cells, many of which are covered with microvilli[77, 78]. Hence the nasal cavity possesses a large and permeable surface area for the absorption of drugs. The sub-epithelium layer is highly vascularised, and since the venous blood passes directly into the systemic circulation, first pass hepatic metabolism is avoided[78].

As a result of experiments in rodents, it has been proposed that intranasally administered drugs may be transferred (more) directly to the central nervous system[79], i.e. directly along the olfactory pathway to the brain following nasal administration[80] and by diffusion through the perineural space, a compartment which is continuous with the sub-arachnoid space[78]. Transneuronal absorption (in humans) is considered to be slow in relation to absorption by the supporting mucosal cells and capillary bed[81].

A two layered mucus covers the respiratory epithelium. The mucus consists of a low viscosity fluid (sol layer) which surrounds the cilia and a more viscous gel layer which is situated on the surface of the sol layer[77, 78]. The typical pH of nasal secretions is 5.5 to 6.5[77, 78].

Inhaled particles are cleared from the nasal cavity by mucociliary clearance, i.e. mucus (and the particles that are trapped in or on the mucus layer) is transported posteriorly in the nose and down the throat by the movement of the cilia. Mucus flow rate in humans is approximately 5mm per minute[77, 78] and theoretically the mucus layer is renewed every 15 to 20 minutes[78]. The volume of mucus in the nasal cavity is typically 0.5 to 1mL[82].

1.4.1. Application of Intranasal Delivery of Apomorphine to Parkinson's Disease.

The nasal mucosa represents a route for apomorphine administration for which first pass metabolism is avoided. Being highly lipophilic, apomorphine has the potential for extensive absorption via the nasal mucosa. However with a pK_b of 7.2[83], apomorphine is predominantly (approximately 95 to 100%) charged at the local environment pH (pH 5.5 to 6.5[77, 78]) hence the likelihood of absorption is very much reduced.

The nasal route has been investigated for efficacy, tolerability and bioavailability in patients with Parkinson's disease[84-90]. These were all relatively small scale studies, with patient numbers ranging from 5 to 10.

The intranasal formulation used was, in the majority of studies, an aqueous solution of apomorphine which was delivered by means of a metered dose nebuliser[84, 87, 89]or nasal spray[85, 86, 88]. A single actuation from the inhaler delivered 1 mg of apomorphine in 0.1 mL of solution[84, 86-89] or 1.3mg apomorphine in 0.065mL of solution[85]. The intranasal doses used in these studies ranged from 1 to 10 mg apomorphine[84-90].

Patients were titrated to doses of intranasal apomorphine that elicited an optimum clinical response[84-89], optimum clinical response being defined as a satisfactory "on"-phase. The intranasal dose required for an optimum response was reported to be the same as[87], similar to[84] or higher than (up to five times higher[85], but with a mean of approximately two times higher[85, 89]) that required for an equivalent motor improvement using the subcutaneous route. A comparison of the peripheral pharmacokinetics of apomorphine administration via the intranasal and subcutaneous routes was undertaken by Sam[85], and a partial comparison of the two routes has been reported by van Laar *et al*[84].

Apomorphine was rapidly absorbed after intranasal administration, as evidenced by the rapid absorption half life (mean was 9 minutes, range was 2 to 24 minutes[85]), short T_{max}

(means were 23[85] and 7[84] minutes, with ranges of 11 to 37 and 4 to 9 minutes, respectively) and brief lag-time (mean of 3 minutes, range of 0 to 9 minutes[85]). Variation in the estimates of T_{max} following intranasal administration between the two studies may be due to differences in the performance of the devices used, e.g. deposition of drug, or the number of puffs required to deliver total dose.

Both the mean absorption half life and T_{max} for intranasal apomorphine administration were longer than those given for subcutaneous administration, whereas the lag time was shorter than that for subcutaneous administration[85]. However, there were no *significant* differences between the two routes with regard to these parameters which describe absorption kinetics[85]. There was a positive correlation between intranasal dose and C_{max}[84].

The bioavailability of intranasal apomorphine administration, in terms of the area under the plasma concentration-time curve for an intranasal dose compared to that estimated for a subcutaneous dose, was highly variable. Sam *et al* reported that the mean relative bioavailability for the whole group (n=6 patients) was 45%. In fact for five of the six patients, the relative bioavailability was in the range of 13 to 46%, but was 117 % for the remaining patient[85]. Van Laar *et al* reported the relative bioavailability of the intranasal route for one of the seven patients in their study; this was estimated to be 90 to 100%[84].

The mean latency to onset of effect following intranasal dosing ranged from 9 to 18 minutes[84-88, 90]. In the two studies which made a direct comparison of the pharmacodynamic effects of apomorphine administration using the intranasal and subcutaneous routes, the mean delay to onset of effect was longer following intranasal administration, however the difference in latency did not reach statistical significance (where $p<0.05$ was considered statistically significant)[85, 87]. Nor was there a significant difference in the duration of the apomorphine-induced response between the two routes (where $p<0.05$ was considered statistically significant)[85, 87]. The mean duration of effect following intranasal dosing ranged from 44 to 61 minutes[84-88, 90].

Dyskinesia was a common adverse effect of intranasal apomorphine administration, but was considered to be comparable in severity to that which occurred following levodopa[86, 88] or subcutaneous apomorphine[84] administration. Yawning was reported in a minority of patients as a result of intranasal apomorphine administration[84, 86, 88], as was paresthesia[84] and nausea (despite anti-emetic therapy)[86, 88]. The aforementioned signs are commonly experienced adverse effects of (subcutaneous) apomorphine therapy. The adverse effects specific to intranasal administration of apomorphine were mild nasal stinging and the occurrence of a bitter taste following the intranasal dose[86, 89].

After approximately 4-6 weeks of usage, up to half of the patients in the long-term studies had been affected with mild to moderate local adverse reactions to intranasal apomorphine, i.e. slight vestibulitis with nasal blockage and nasal crusting (which was black in colour in one case[84]), and did not limit the use of intranasal apomorphine[84, 86, 87]. Whilst this was the case, the local adverse effects of intranasal use were considered to be more disabling than those resulting from subcutaneous use[87].

Adverse events at the nasal mucosa, including erythema and crusting, also occurred in (unknown number of) asymptomatic patients receiving placebo nasal spray [86].

However, up to one third of patients receiving intranasal apomorphine developed severe and disabling (reversible) local tissue reactions (e.g. severe nasal vestibulitis, with secondary infection, crusting with pain and bleeding on intranasal administration, superficial mucosal ulceration) leading to the discontinuation of treatment in some cases[84, 86, 87, 89]. Severe adverse reaction of this nature was reported to substantially lower absorption at the nasal mucosa[84].

Patient acceptability was good; in the absence of severe local adverse reaction the intranasal route was preferred over subcutaneous administration. Patients who were unable to administer subcutaneous injections were able to self-administer intranasal apomorphine[89].

It has been demonstrated that preferential delivery to the CNS occurred following nasal administration of water soluble prodrugs of L-dopa in rats (evidenced by the finding that olfactory bulb and CSF L-dopa concentration were higher compared to an equivalent intravenous dose)[91] and that dopamine was transferred into the olfactory bulb following nasal administration in mice[80]. It is reasonable to suggest that the same outcome would apply to intranasal administration of apomorphine, especially given the high lipophilicity of apomorphine.

Although the delivery of apomorphine via the olfactory pathway and/or via CSF represents the opportunity for enhanced CNS delivery, there is no evidence for an augmented apomorphine-induced response in the literature, i.e. the intranasal dose required for pharmacodynamic equivalence with subcutaneous injection, in terms of the magnitude and duration of the response, is greater than that for subcutaneous injection, with a similar latency to onset reported for each route. This suggests that the contribution of the olfactory transneuronal and/or direct CSF routes in the delivery of apomorphine to the CNS is inferior to absorption at the respiratory mucosa into the capillary bed, however, the relative contribution of the delivery mechanisms following intranasal administration of apomorphine remain to be established.

It is well established that patients with Parkinson's commonly exhibit olfactory dysfunction [33, 92, 93] and that the olfactory deficit is severe and present in early stage Parkinson's disease[94, 95]. Whilst the underlying mechanisms remain contested, it has been proposed that olfactory dysfunction in Parkinson's disease is a consequence of a lesion in the olfactory neural pathway[96], including the olfactory neuroepithelium[97]. In this case, the absorption of drugs across the olfactory epithelium and subsequent delivery to the brain via the olfactory pathway may be precluded. The olfactory function of the patients with Parkinson's disease in the published studies of intranasal administration of apomorphine was not commented upon by the authors.

It has also been reported that patients with Parkinson's disease display reduced sniffing capability compared to healthy controls (and that this impairment contributes to olfactory dysfunction in Parkinson's disease)[98]. One patient in the study by Sam *et al* exhibited a poor sniff effort[85]. This finding has implications for the intranasal delivery of apomorphine using self-powered delivery devices.

1.5. The Buccal Route for Drug Delivery.

Oral epithelium consists of a mitotically active basal cell layer, progressing through a number of differentiating intermediate layers to the superficial layers, where cells are shed from the epithilial surface. Oral membranes are covered in mucous and are continually provided with fresh serous and mucous saliva. The pH of human saliva varies from 6.0 to 7.4. [99].

Administration of drug via the buccal mucosa results in rapid absorption into the systemic circulation due to the rich local network of systemic veins and lymphatics. Buccal tissue is better perfused than gingival, sublingual and palatal tissue (in that order)[99].

The buccal mucosa is considerably more permeable than gingival and palatal mucosae, but is less permeable than that at the sublingual region, and as such the buccal mucosa is generally not considered to be able to provide the rapid absorption and high bioavailability possible with sublingual administration[99] .

A limitation of the oral mucosa as a site for drug delivery is the challenge of retaining the dosage form in place, despite the presence of food and beverages, and without interfering with mastication and speech. Drug delivery via the buccal mucosa is also limited by the possibility that the recipient may chew or swallow the dosage form.

1.5.1. Application of Buccal Delivery of Apomorphine to Parkinson's Disease.

The buccal mucosa represents a route for apomorphine administration for which first pass metabolism is avoided. Being highly lipid soluble, apomorphine has the potential for efficient absorption via the oral mucosa. However with a pK_b of 7.2, apomorphine is predominantly charged at salivary pH (pH 6.0-7.4), which indicates that absorption is limited.

The sublingual route has been investigated for efficacy, tolerability and bioavailability in patients with Parkinson's disease[65, 66, 100-105]. These were all relatively small scale studies, with patient numbers ranging from 5 to 10. The sublingual dose of apomorphine used in the above studies varied from 3 to 57 mg, and comprised of either single or multiple tablets, which were allowed to dissolve under the tongue. Dose titration was performed as a prelude to acute[100, 103] and chronic[102] pharmacokinetic studies by certain groups. The efficacy of sublingually administered apomorphine was stated as being similar to that achieved using levodopa, with the exception of two (out of five) patients in a single study[66] who did not exhibit improvement in motor function following a sublingual dose of 18mg.

Generally it was reported that C_{max} and AUC were correlated to the administered sublingual dose. The mean T_{max} observed in the studies of sublingual apomorphine administration ranged from 35 to 62 minutes. The variation in T_{max} (and in onset of effect) was related by some authors to the dissolution time of the tablet(s)[65, 101, 103], a process which took an average of 10 to 30 minutes [66, 101-104]. Notably in one study was it reported that the dissolution time of the sublingual formulation used was not related to T_{max}[105]. The dissolution time quoted in the latter study was, at 10 to 20 seconds, considerably faster than that reported by the other investigators.

The mean bioavailability, in terms of the AUC following sublingual administration relative to that for subcutaneous administration in the same patients, ranged from

10 to 18%[65, 66, 104, 105]. The relatively poor bioavailability was attributed to a proportion of the administered dose being swallowed by the patients[101, 105].

The mean latency to onset of effect was, at 15 to 40 mins, relatively long compared to that reported for subcutaneous administration of apomorphine[100, 103, 105]. The extended delay to onset of effect was identified as a disadvantage in the application of the sublingual route to apomorphine administration, especially in the cases of severely akinetic patients with Parkinson's disease.

The mean duration of response varied from 55 to 137 mins, and as such was considered to be equivalent or longer than that which was achieved following subcutaneous administration[100, 103, 105].

Acute apomorphine administration via the sublingual route resulted in adverse (systemic) effects which were typical of the adverse events commonly reported following subcutaneous administration, i.e. dyskinesia[100, 103, 105] in many patients, yawning and dizziness in a minority[103]) and were generally mild to moderate in nature. In addition, an unpleasant (bitter or acidic) taste was reported in some studies[101, 102, 104]. Chronic sublingual administration of apomorphine resulted in the development of stomatitis[65, 101] and ulceration[65] of the oral mucosa which was severe enough to lead to the discontinuation of treatment in some cases[65].

Based on the latency to onset of effect which has been reported for sublingual delivery of apomorphine, it was envisaged that the buccal mucosa represented a site which may be useful for sustained release of apomorphine, rather than rescue action.

Salivary secretion rate decreases with increasing age[106] and furthermore "dry mouth" can result as a consequence of the use of anti-cholinergic drugs in the management of Parkinson's disease. However in contrast to this, patients with Parkinson's disease often exhibit hypersialorrhea and/or drooling, a characteristic which has been attributed to diminished motor function relating to swallowing, and also to changes in autonomic function[107, 108]. Such extremes of salivary flow that may occur in Parkinson's disease have the potential to affect the administration of apomorphine via the buccal mucosa.

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SECTION 2:
INTRODUCTION

2. Introduction.

2.1. Pharmacokinetic-Pharmacodynamic Study of Subcutaneous Apomorphine Administration in Patients with Parkinson's Disease.

Previous studies on apomorphine in Parkinson's disease have revealed the large inter-patient variation in pharmacokinetics and pharmacodynamics, and also the existence of a narrow therapeutic window[1-3]. These factors clearly demonstrate the need for individualised dose optimisation of apomorphine in the management of Parkinson's disease.

Currently apomorphine dose optimisation consists of a basic threshold dose-finding protocol (Section 8.2). Given that apomorphine has a narrow therapeutic window, a threshold dose-finding protocol might not be the most appropriate procedure for dose optimisation. Certain clinical issues are important here: are all patients who do not respond to the higher (7mg or 10mg) doses non-responders? Can the transition from multiple bolus dosing to continuous infusion be facilitated?

With these issues in mind, a pharmacokinetic study involving two patients on established clinically effective subcutaneous infusions of apomorphine was undertaken in-house[4], and a potential correlation between (peripheral) pharmacokinetics and general therapeutic outcome in the two patients was identified, i.e. *despite* a difference in dosing requirements and in pharmacokinetic parameter estimates between the two patients, values calculated for the beta-phase intercept (Box 2-1) were consistent (Figure 2-1 and Table 2-1). The beta-phase intercept was also estimated from published apomorphine concentration-time profiles; the values were similar to those obtained in the in-house study, *irrespective* of dose and route and *despite* large variation in other pharmacokinetic parameters (Table 2-2).

Thus, under the assumption that the peripheral compartment contains the effect site (i.e. the CNS), it was proposed that the beta-phase intercept might function as a marker of drug concentration at the effect site, and furthermore that the consistent value observed in optimised patients, i.e. approximately 10ng/mL, might therefore represent a threshold concentration for effect. In this context the beta-phase intercept would be predictive of anti-parkinsonian response and could therefore be exploited in a dose-optimisation scheme whereby patients could be dosed to a therapeutic apomorphine concentration at the effect site.

In this thesis the significance of this relationship between the beta-phase intercept and anti-parkinsonian effect of apomorphine in patients with Parkinson's disease was examined.

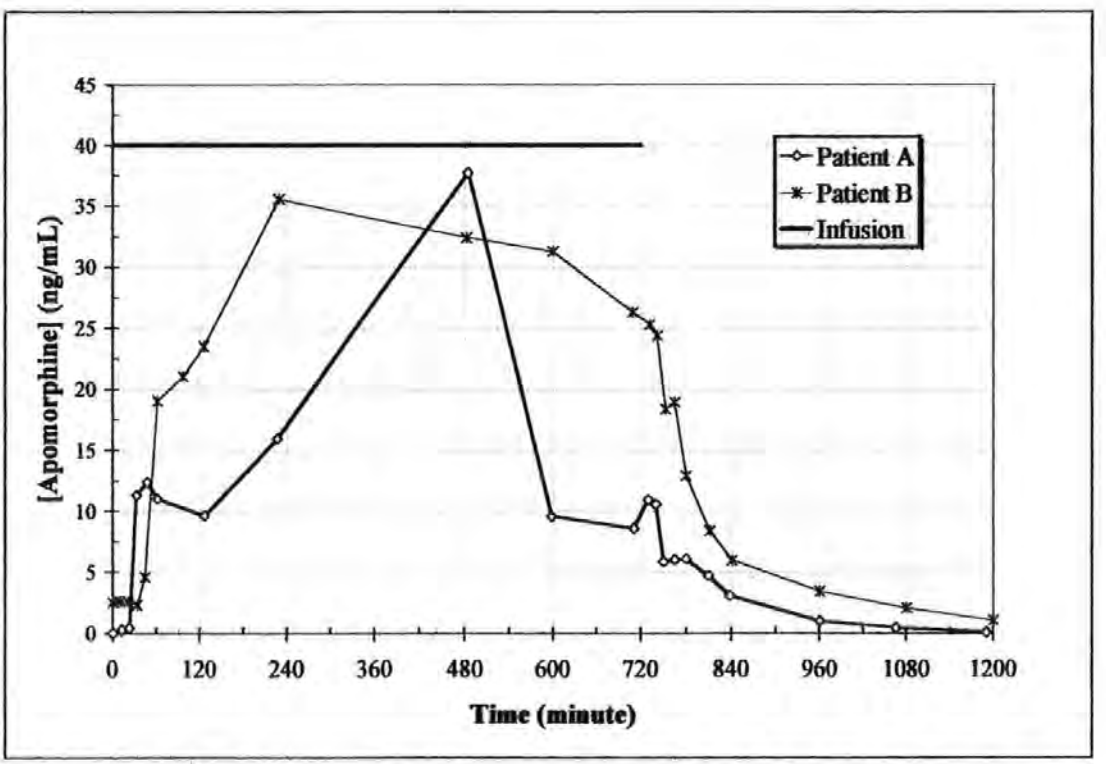

Figure 2-1 Plasma apomorphine concentration following 12h subcutaneous infusion of apomorphine in two patients with Parkinson's disease. Reproduced from Priston[4], with permission.

Patient	Dose (mg)	Cmax[a] (ng/mL)	$AUC_{0\text{-infinity}}$[a] ($ng.mL^{-1}.h$)	t½ α (min)	t½ β (min)	B (ng/mL)
A	35 (2.9mg/h x 12h)	1.1	6.7	8.2	76.4	10.9
B	141 (11.8mg/h x 12h)	0.3	2.9	46.6	166.5	10.8

Table 2-1 **Selected pharmacokinetic parameter estimates from a study of subcutaneous apomorphine infusion to dose-optimised patients with Parkinson's disease.**

Reproduced from Priston[4] with permission

Abbreviations: t½ α = distribution half-live, t½ β = (apparent) elimination half-live.

Route	Dose	B (ng/mL)	Publication
subcutaneous bolus	2.6mg	13.2	Nicolle[3]
	3.0mg	8.0 *	Hofstee[5]
subcutaneous infusion	4.9mg/h x 24h	11.3	Nicolle[3]
sublingual	57mg	14.4 *	Hughes[6]
	57mg	14.8 *	Hughes[6]
	0.6mg/kg	9.7 *	Durif[7]
intravenous bolus	2.6mg	10.5	Nicolle[3]
intravenous infusion	0.6mg/h x 12h	11.3	Gancher[2]
	30mg/kg x 15min	13.8	van der Geest[8]
intranasal	1mg	5.5	van Laar[9]
	4mg	16.2	van Laar[9]
rectal	10mg	18.2 *	van Laar[10]

Table 2-2 **Estimates of the beta phase intercept (B) from published articles.**

Asterisked values indicate that it was stated in the publication that the administered dose had elicited a therapeutic effect. In the remaining publications, the nature of the effect was not stated.

[a] Normalised to 1mg dose.

The two-compartment pharmacokinetic model resolves the body into two distinct mathematical compartments: (i) a central compartment which consists of blood and rapidly equilibrated organs (e.g. liver), and (ii) a peripheral (or "deep") compartment which consists of more slowly equilibrating tissues (e.g. tissues which are surrounded by protective membranes), and which is usually inaccessible to direct measurement[11, 12].

The assumptions of this model are that (i) irreversible drug elimination (e.g. by hepatic biotransformation) takes place only from the central compartment, and (ii) reversible distribution occurs between the central and peripheral compartments[11, 13].

For a drug which exhibits two-compartment pharmacokinetics, drug disappearance from the sampling window (plasma) following extravascular dosing is described by the linear sum of exponential terms:-

$$C(t) = A.e^{(-\alpha.t)} + B.e^{(-\beta.t)} + D.e^{(-K01.t)}$$

Alpha and beta correspond to the initial and terminal slope factors, respectively, and A and B to the *y*-axis intercepts of the initial and terminal slopes, respectively. D is given by -(A+B), and K01 is the absorption rate[12, 13] (Figure 2-2).

The beta-phase intercept represents the concentration of drug in the peripheral compartment had the total dose been instantaneously distributed to this compartment[14].

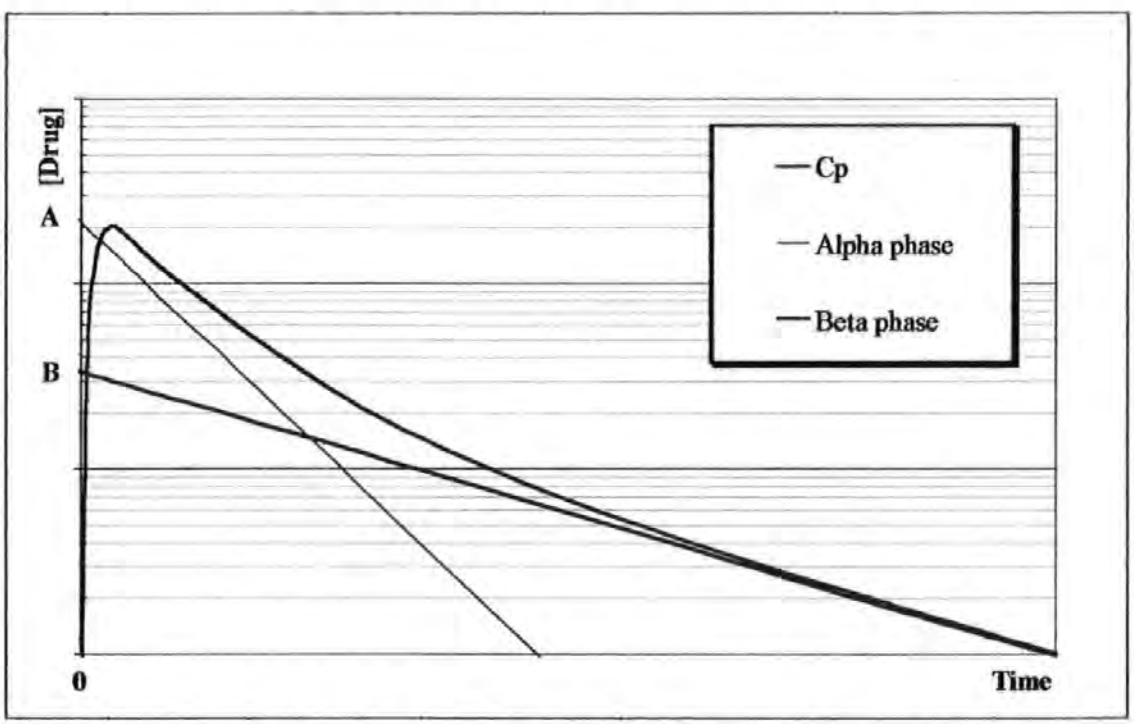

Figure 2-2 Drug disposition (according to a two compartment model) following extravascular (1st order input) dosing.

Box 2-1 The beta-phase intercept.

2.2. Studies on Novel Apomorphine Delivery Systems with Application to Parkinson's Disease.

There are inherent limitations associated with subcutaneous injection or infusion of apomorphine to patients with Parkinson's disease. The high risk of developing cutaneous nodules, first described by Stibe *et al*[15], can be treatment-limiting. The technique itself can be problematic for some patients[16-18], indeed "off" period disability adversely affects the capability of the patient to self-inject using a syringe or "Penject®" (Britannia Pharmaceuticals Ltd)[6, 19, 20]. Manual difficulties such as these have led to dependence on others to administer the drug on behalf of the patient[7, 19, 20].

Furthermore there are issues surrounding the use of needles for drug delivery, including fear or anxiety associated with (self) injection[21], and the range of health and safety imperatives and social issues involved[22]. Additionally, the requirement for parenteral delivery may result in a reluctance to prescribe by some neurologists[23]. Such limitations have prompted research of alternative modes of delivery of apomorphine, e.g. intranasal spray[24], rectal suppositories[25] and transdermal iontophoresis[26].

As a contribution to the research of alternative delivery systems for apomorphine, studies were undertaken on the properties of three novel systems, i.e. intranasal administration of apomorphine powder to healthy volunteers, buccal administration of an apomorphine hydrogel formulation to healthy volunteers, and subcutaneous administration of apomorphine (Britaject®) by needle-free injector to patients with Parkinson's disease. Where applicable the beta-phase intercept was included as an outcome measure in these studies as part of the research outlined in Section 2.1.

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SECTION 3:
MATERIALS

3. Materials.

3.1. Analytical Reagents.

Product.	Source.
alumina for column chromatography, type WA-A, activity grade: super I	Sigma-Aldrich Company Ltd, Poole, Dorset, UK.
ammonium chloride	BDH Merck, Leicester, Leics, UK.
ammonium hydroxide	Sigma-Aldrich Company Ltd, Poole, Dorset, UK.
L- ascorbic acid ACS reagent	Sigma-Aldrich Company Ltd, Poole, Dorset, UK.
citric acid	BDH Merck, Leicester, Leics, UK.
diaminoethanetetra-acetic acid sodium salt (EDTA)	Fisher Scientific, Loughborough, Leics, UK.
diethyl ether	Fisher Scientific, Loughborough, Leics, UK.
diphenylboric acid 2-aminoethyl ester	Sigma-Aldrich Company Ltd, Poole, Dorset, UK.
ethyl acetate	Fisher Scientific, Loughborough, Leics, UK.
heptane	Fisher Scientific, Loughborough, Leics, UK.
1- heptanesulphonic acid sodium salt "*HiPerSolv for HPLC*"	BDH Merck, Leicester, Leics, UK.
hydrochloric acid	Fisher Scientific, Loughborough, Leics, UK.
hydrogen peroxide (6%) 20vol	BDH Merck, Leicester, Leics, UK.
2- mercaptoethanol (electrophoresis reagent)	Sigma-Aldrich Company Ltd, Poole, Dorset, UK.
methanol, HPLC Grade	Fisher Scientific, Loughborough, Leics, UK.
octanol	Sigma-Aldrich Company Ltd, Poole, Dorset, UK.
orthophosphoric acid	Fisher Scientific, Loughborough, Leics, UK.
perchloric acid	BDH Merck, Leicester, Leics, UK.
sodium dihydrogen orthophosphate	Fisher Scientific, Loughborough, Leics, UK.
sodium dodecyl sulphate	BDH Merck, Leicester, Leics, UK.
sodium hydroxide	BDH Merck, Leicester, Leics, UK.
sodium metabisulphite	Fisher Scientific, Loughborough, Leics, UK.
tetraoctylammonium bromide	Sigma-Aldrich Company Ltd, Poole, Dorset, UK.
TRIZMA pre-set crystals pH 8.4 (tris(hydroxymethyl)aminomethane and tris hydrochloride, reagent grade)	Sigma-Aldrich Company Ltd, Poole, Dorset, UK.

3.2. Clinical Equipment.

Product.	Source.
blood collection tubes: Vacutainer Hemogard tubes (EDTA K3, 7mL)	Southern Syringe Services, Southgate, London, UK.
venflon flush solutions: • *hepsol* • 0.9% w/v NaCl i.v. infusion BP	Braun Medical Ltd, Sheffield, S. Yorks, UK.

3.3. Laboratory Equipment.

Product.	Source.
autosampler: • *AS-950* • *AS3000*	Jasco, Gt Dunmow, Essex, UK. Thermo Separations Products, Manchester, UK.
autosampler vials: 2mL, Gold grade glass	Chromacol Ltd, Welwyn Garden City, Herts, UK
bulk column packing: • *Columbus* (C18, 5um) • *Prodigy* (C18, 10um)	Phenomenex, Macclesfield, Cheshire, UK.
data acquisition software: • *Borwin* v1.22 Build 03 • *Chromquest*	MBS Developments, Grenoble, France. Thermo Separations Products, Manchester, UK.
HPLC analytical columns: • *Columbus* (C18, 5µm, 150 x 4.6mm) • *Techopak 10* (C18, 10µm, 250 x 4.0mm)	Phenomenex, Macclesfield, Cheshire, UK. HPLC Technology, Macclesfield, Cheshire, UK.
HPLC pre-columns: *Security Guard*, C18	Phenomenex, Macclesfield, Cheshire, UK.
pharmacodynamic tool: tapping tester	Dept of Biomedical Engineering, Derriford Hospital, Plymouth, Devon, UK.
photodiode array detector: *UV6000P*	Thermo Separations Products, Manchester, UK.
solid phase extraction columns: • *Bond Elut* C8 columns (1mL, 100mg) • *Bond Elut* C18 columns (1mL, 100mg)	Varian Sample Preparation Ltd, Walton-on-Thames, Surrey, UK.
solid phase extraction vacuum manifold: *Techelut* (12 position)	HPLC Technology Co. Ltd, Macclesfield, Cheshire, UK.
solvent delivery system: *constaMetric 3200*	LDC Analytical/Thermoquest, Manchester, UK.
spectrofluorometer: • *FP-821* • *FP-920*	Jasco, Gt Dunmow, Essex, UK.
test tubes: polypropylene (7 and 15 mL)	Sarstedt, Leicester, Leics, UK.

3.4. Reference Compounds.

Product.	Source.
R(-)- apocodeine hydrochloride	Sigma-Aldrich Company Ltd, Poole, Dorset, UK.
R(-)- apomorphine hydrochloride	Sigma-Aldrich Company Ltd, Poole, Dorset, UK.
R(-)- apomorphine hydrochloride (Britaject®)	Britannia Pharmaceuticals Ltd, Redhill, Surrey, UK.
apomorphine orthoquinone	SPA Contract Synthesis, Coventry, UK.
benserazide hydrochloride (DL-serine 2-(2,3,4-trihydroxybenzyl)-hydrazide)	Sigma-Aldrich Company Ltd, Poole, Dorset, UK.
boldine (1,10-dimethoxy-2,9-dihydroxyaporphine)	Sigma-Aldrich Company Ltd, Poole, Dorset, UK.
bromocriptine mesylate (2-bromo-α-ergocryptine methanesulphonate salt)	Sigma-Aldrich Company Ltd, Poole, Dorset, UK.
bromocriptine mesylate capsules (Parlodel®)	Novartis Pharmaceuticals UK, Camberley, Surrey, UK.
cabergoline (Cabaser®)	Pharmacia & Upjohn Ltd, Milton Keynes, UK
carbidopa (S(-)-α-hydrazino-3,4-dihydoxy-2-methylbenzenepropanoic acid)	Sigma-Aldrich Company Ltd, Poole, Dorset, UK.
co-beneldopa capsule (Madopar®): benserazide HCl + levodopa	Roche Products Ltd, Welwyn Garden City, Herts, UK.
co-careldopa tablets (Sinemet®): cabidopa monohydrate + levodopa	Du Pont Pharmaceuticals Ltd, Stevenage, Herts, UK.
domperidone maleate tablets (Motilum®)	Sanofi Winthrop Ltd, Guildford, Surrey, UK.
entacapone tablets (Comtess®)	Orion Pharma (UK) Ltd, Newbury, Berks, UK.
levodopa (L-3,4-dihydroxyphenylalanine)	Sigma-Aldrich Company Ltd, Poole, Dorset, UK.
lisuride maleate tablets (Revanil®)	Cambridge Laboratories, Newcastle-upon-Tyne, UK.
paracetamol	Sterling Health, Brentford, Middx, UK.
pergolide mesylate (Celance®)	Eli Lilly & Co. Ltd, Basingstoke, Hants, UK.
pramipexole HCl (Mirapexin®)	Pharmacia & Upjohn Ltd, Milton Keynes, UK
R(-)- propylnorapomorphine hydrochloride (NPA)	Sigma-Aldrich Company Ltd, Poole, Dorset, UK.
ropinerole HCl tablets (Requip®)	SmithKline Beecham Healthcare, Brentford, Middx, UK.
selegiline HCl oral liquid (Eldepryl®)	Orion Pharma (UK) Ltd, Newbury, Berks, UK.
selegiline HCl tablets (Eldepryl®)	Orion Pharma (UK) Ltd, Newbury, Berks, UK.

3.5. Stock Solutions.

Name.	Composition, conditions of use.
apomorphine stock solution(s)	1mg/mL R(-)-apomorphine hydrochloride powder in diluent A (prepared in silanised, amber glassware, stored at 4-8°C for 28 days). Intermediate stock solutions of 10µg/mL, 1µg/mL and 75ng/mL were prepared in diluent A, as required.
2-mercaptoethanol	1% (v/v) 2-mercaptoethanol in water, stored at room temperature.
propylnorapomorphine stock solution(s) (internal standard)	0.1mg/mL R(-)-propylnorapomorphine powder in diluent A (prepared in silanised, amber glassware, stored at 4-8°C for 28 days).

3.6. Working Solutions.

Name.	Composition, conditions of use.
diluent A	0.10% (w/v) EDTA, 0.15% (w/v) ascorbic acid, stored at 4-8°C.
HCl in methanol wash solution	0.01M HCl in 50:50 methanol:water
propylnorapomorphine	aliquots of 5µg/mL in diluent A, stored at 4-8°C.
sodium metabisulphate solution	2% (w/v) sodium metabisulphate in diluent A, stored at 4-8°C.
solid phase extraction eluting solution	0.25M NaH_2PO_4 to pH 3.30 with orthophosphoric acid containing 40% (v/v) methanol, stored at 4-8°C.

SECTION 4:
METHODS

4. Methods.

4.1. Pharmacokinetic-Pharmacodynamic Study of Subcutaneous Apomorphine Administration in Patients With Parkinson's Disease: Development of Clinical Protocol.

4.1.1. Investigators.

The protocol was devised in collaboration with Drs V. Pearce and D. MacMahon. The clinical study was undertaken under the supervision of Dr D. MacMahon at The Camborne and Redruth Community Hospital, Drs V. Pearce and T. Malone at The Royal Devon and Exeter Hospital, and Dr J. O'Sullivan at The National Hospital for Neurology and Neurosurgery.

4.1.2. Objectives.

The aim of this work was to examine the pharmacokinetic-pharmacodynamic relationships of apomorphine in Parkinson's disease. The primary objective was to determine the significance of the proposed relationship between the beta-phase intercept and clinical response. (Details on the proposed correlation between beta-phase intercept and clinical response are given in Section 2.1, pages 2-1 and 2-2).

4.1.3. Treatment Administered.

Apomorphine HCl (Britaject® 10mg/mL, Britannia Pharmaceuticals Ltd.).

4.1.4. Study Design.

Open, controlled.

4.1.5. The Study Population.

It was proposed that apomorphine pharmacokinetic-pharmacodynamic relationships were initially investigated in those patients who had previously been individually optimised on subcutaneous apomorphine therapy, as a means of piloting the study in the group known to respond to apomorphine.

The recruitment criteria were as follows:-

Inclusion Criteria.

Include subject if *all* the following apply:-

i) an established diagnosis of idiopathic Parkinson's disease according to the United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria[1] (Appendix 8.3),

ii) currently receiving apomorphine therapy for Parkinson's disease,

iii) exhibits a clear and predictable response to subcutaneous apomorphine,

iv) experiences well-defined "on" and "off" phases,

v) given informed consent to participate in the study.

Exclusion Criteria.

Exclude if *any* of the following apply:-

i) significant concomitant medical condition,

ii) significant and current psychiatric morbidity,

iii) physical disability which precludes the use of the primary efficacy variable (the tapping tester),

iv) pregnancy,

v) current participation in other clinical study.

Twelve patients were recruited in total; however two patients were withdrawn from the study at their own request, patient 03 at approximately sixty minutes post-apomorphine dose and patient 06 at approximately ninety minutes prior to dosing with apomorphine. Both patients cited the severity of parkinsonian symptoms that they each experienced as a consequence of the provoked "off"-state as the reason for withdrawal (see page 4-4 Induction of "Of" Period). Whilst no pharmacokinetic or pharmacodynamic sampling was performed for patient 06, a limited number of observations were made for patient 03.

Quantification of plasma apomorphine was not possible in the case of one patient (patient 11) due to the presence of a compound in the plasma samples which co-eluted with apomorphine[a].

Thus the study group was comprised of ten individuals: six males and four females. Of the ten patients recruited, seven were currently being treated with intermittent subcutaneous bolus apomorphine, whilst the remaining three patients were currently receiving apomorphine by 24-hour subcutaneous infusion. The mean (range) age of the patients was 63 (49 to 77) years. Patients had been diagnosed with Parkinson's disease for a mean (range) of 13 (10 to 17) years. The mean (range) duration of levodopa and apomorphine therapy was 12 (5 to 20) years and 3 (1 to 9) years, respectively. The mean (range) UPDRS score[b] when "on" was 50 (35 to 74), n=9. The median (range) Hoehn and Yahr score[c] when "on" was 4 (2 to 4), n=9. Detailed patient demographic data are given in Appendix 8.4.

4.1.6. Investigational Plan.

Patients were admitted to either the Camborne and Redruth Community Hospital, Royal Devon and Exeter Hospital, or National Hospital for Neurology and Neurosurgery for the duration of the study.

The study protocols differed depending on the mode of apomorphine administration used, i.e. subcutaneous bolus or subcutaneous infusion, therefore each is presented separately.

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[a] The contaminant was subsequently shown (using UV–diode array detection) to be dantron, which was present as a consequence of the concomitant administration of dantron-based laxatives (see Appendix 8.5).

[b] UPDRS score range: 0 to 206, where 0 represents normal and 206 represents maximal parkinsonian disability (see Appendix 8.1).

[c] Hoehn and Yahr score range: 0 to 5, where 0 represents normal and 5 represents maximal parkinsonian disability (See Appendix 8.1).

4.1.6.1. Protocol for Subcutaneous Bolus Apomorphine Administration.

Induction of "Off" Period.

All anti-parkinsonian medication was withdrawn from midnight preceding the study day in order to avoid potential pharmacokinetic and pharmacodynamic interferences, with the effect that the patient was "off" at the start of the study day. This element of the protocol is common practice in published pharmacokinetic studies of apomorphine in Parkinson's disease [2-8], efficacy studies [3, 9-16] and in the current dose titration protocol[17].

Administration of Apomorphine.

Following insertion of a peripheral intravenous cannula and baseline measurements (blood sample, UPDRS assessment, tapping test and walking test), a single bolus of apomorphine was administered at the dose routinely used by individual patients for therapeutic effect. The mean (range) dose was 5.3mg (2 to 10), i.e. 77μg/kg (35 to 167), n=8 (Table 4-2, page 4-7).

In order to avoid potential diurnal variation in apomorphine pharmacokinetics, the apomorphine dosing time was standardised; a window between 8am and 10am was deemed appropriate (to accommodate patient travel to hospital and to limit length of provoked parkinsonian "off" period).

The use of further anti-parkinsonian medication was disallowed until the last blood sample was collected so that pharmacodynamic interferences were avoided. All other necessary medication required by the patient was made available.

Blood Sampling Scheme.

The development of the blood sampling schedule was based on an examination of published concentration-time courses[4, 7, 13, 18]. These data were used to construct semi-log plots which were then used as a guide to the time course of sampling points. Emphasis was placed on obtaining sufficient samples to characterise the beta-phase. It was deemed appropriate to aim to sample on at least two occasions for each phase of the plasma concentration-time curve. Thus samples (of 7mL) were taken pre- apomorphine dose and at 5, 15, 30, 45, 60, 90, 120, 180, 240 and 360 minutes post-dose. A summary of simultaneous blood and pharmacodynamic sampling is given in Table 4-1 (page 4-6).

Pharmacodynamic Sampling Scheme (relevant to bolus *and* infusion treatments).

Two timed tests of motor function were employed in the quantitative assessment of apomorphine-induced effects: the tapping test and the walking test. Both are commonly used tools in Parkinson's disease [3-6, 12]. The tapping test was designated as the primary pharmacodynamic test on the basis that the test was objective, self-evident and could be completed in a short time period (thus allowing multiple measurements to be made during the relatively short duration of drug effect), and also because the tapping tester itself was portable (Figure 1-6, page 1-24).

The tapping test was standardised in terms of the hand used, i.e. the hand of the side of the body most affected with parkinsonian symptoms was selected, and in terms of the conditions of the test and executive instructions (Appendix 8.6).

Similarly, the conditions of the walking test were standardised (Appendix 8.7) in that the 6m course was consistently kept clear of obstacles that might induce freezing, and the flooring was non-patterned to avoid the use of a visual cue[19, 20].

No encouragement was given during the testing period of each of the two tests, and patients were blinded to results, although it could not be ruled out that patients kept a mental record of their own tapping test scores.

The apomorphine-induced response was described qualitatively by observation of parkinsonian symptoms and apomorphine-related effects, and by patients' self-reporting of symptom changes.

A summary of simultaneous blood and pharmacodynamic sampling is given in Table 4-1.

Time (mins post bolus)	Blood sample volume (7mL)	Observation of parkinsonian symptoms	Tapping test	Walking test	Blood pressure and pulse
pre-	✓	✓	✓	✓	
Administer bolus apomorphine *or* stop infusion.					
5	✓		✓		To be monitored as appropriate
15	✓		✓	✓	
30	✓		✓		
45	✓		✓	✓	
60	✓		✓	✓	
90	✓	✓	✓	✓	
120	✓				
180	✓				
240	✓				
360	✓				

Table 4-1 Simultaneous blood/pharmacodynamic sampling scheme.

Where multiple tasks exist at a single time point, the priority was as follows: (1) blood sampling, (2) tapping test, (3) walking test.

Shaded region indicates predicted apomorphine-induced "on" phase.

Patient ID	Apomorphine dose			Time of administration	Site of administration	Position of patient on administration	Washout period (h)
	mode	(mg)	(μg/kg)				
01	bolus	2	41.7	09.56	thigh	sitting	8.0
02	bolus	5	47.6	09.41	abdomen	sitting	NR
03	bolus	10	142.8	09.54	abdomen	lying	11.9
04	bolus	10	166.7	10.02	abdomen	sitting	11.0
05	bolus	5	77.5	09.22	abdomen	sitting	12.4
07	infusion	180 mg/24h	(87.5 μg/kg/h x 24h)	stopped at 10.30	upper back	NA	NA
08	infusion	45 mg/24h	(25.8 μg/kg/h x 24h)	stopped at 10.52	upper back	NA	NA
09	bolus	2	35.1	10.09	thigh	standing	8.2
10	bolus	3.5	47.3	09.50	thigh	sitting	4.6
12	bolus	5	55.6	11.04	thigh	sitting	7.6

Table 4-2 **Administration of apomorphine (by subcutaneous route) to patients with Parkinson's disease.**

Abbreviation: NA = not applicable, NR = not recorded.

4.1.6.2. Protocol for Subcutaneous Apomorphine Infusion Administration.

Administration of Apomorphine.

Apomorphine was administrated at the individual patients' typical dose and schedule (Table 4-2, page 4-7). Following insertion of a peripheral intravenous cannula and baseline measurements (blood sample and pharmacodynamic measurements), the apomorphine infusion was stopped (at approximately 10am).

The use of further anti-parkinsonian medication was disallowed until the last blood sample was collected so that pharmacodynamic interferences were avoided. All other necessary medication required by the patient was made available.

Blood Sampling Scheme.

Blood samples (of 7mL) were taken prior to stopping the infusion, i.e. 15 minutes prior to stopping the infusion and also immediately prior to stopping the infusion. The time that the infusion was stopped was designated as time = 0. Blood sampling was performed at 5, 15, 30, 45, 60, 90, 120, 180, 240 and 360 minutes from time = 0. A summary of simultaneous blood and pharmacodynamic sampling is given in Table 4-1 (page 4-6).

Pharmacodynamic Sampling Scheme (relevant to bolus *and* infusion treatments).

The rationale for the pharmacodynamic sampling scheme is detailed previously (page 4-6). A summary of simultaneous blood and pharmacodynamic sampling is given in Table 4-1 (page 4-6).

4.1.7. Pre-Treatment of Blood Samples.

Treatment of blood samples was carried out according to Priston[18], i.e. each blood sample was collected into a pre-cooled (4-8°C) EDTA vacutainer tube, immediately transferred into a polypropylene tube containing 6mg ascorbic acid and mixed. If required the sample was stored at this stage at 4-8°C for up to three hours. The sample was then centrifuged at 4°C (1250g for 5 minutes) and the plasma immediately aspirated into a polypropylene tube. The plasma was stored at -20°C until required for analysis.

4.1.8. Plasma Apomorphine Quantification.

Plasma samples were prepared for assay according to the protocol given in Section 4.3.3.3 (page 4-35) and assayed using the methods given in Section 4.4 (pages 4-50 and 4-51).

4.1.9. Criteria for Evaluation.

Plasma apomorphine concentration and apomorphine-induced anti-parkinsonian response over six hours post-dose. Pharmacokinetic parameters: C_{max}, T_{max}, $AUC_{0\text{-infinity}}$, and beta-phase intercept. Response variables: onset and duration of response, extent of improvement in tapping test score and ambulatory time from baseline.

4.1.10. Data Handling.

Pharmacokinetic and pharmacodynamic parameters were estimated using WinNonlin modelling program (Standard version 2.0, Pharsight, CA, USA) according to the method given in Section 4.6 (page 4-103). Bivariate correlations were performed for salient pharmacokinetic parameters using SPSS (version 9.0.0, SPSS Inc.).

4.1.11. Study Ethics.

Ethical approval was sought and obtained from the Local Research Ethics Committees of and Cornwall and Isles of Scilly (September 1997) and Exeter and North Devon (October 1997). Written informed consent from all volunteers was obtained using the Patient Information Leaflet (Appendix 8.8) and Consent Form given in the protocol (Appendix 8.9).

4.1.12. Protocol Review.

A review of the study protocol was undertaken following the first five patients in the light of patient recruitment difficulties.

Withdrawal of Anti-Parkinsonian Medication.

The withdrawal of anti-parkinsonian medication was identified as a major barrier to recruitment. Experience with the first five patients had shown that prolonged wash-out exerted a detrimental effect on therapeutic response, i.e. doses of apomorphine that had previously evoked a therapeutic response produced a sub-optimal effect following withdrawal of anti-parkinsonian medication from midnight. This effect has been documented in the literature[21]. In view of this the wash-out period was reduced, i.e. patients were instructed to maintain their anti-parkinsonian regimens until the early morning (approximately 6am) dose on the day of the study. This allowed a baseline "off" period to be established prior to the apomorphine dose, but avoided a protracted "off" state. Additionally the withdrawal of anti-parkinsonian medication *during* the sampling time course was rescinded. Consequently the anti-parkinsonian regimen, with the exception of additional apomorphine, was re-established following the cessation of the apomorphine-induced "on" period.

Blood Sampling Scheme.

The blood sampling scheme was evaluated after the study of five patients. Using parameter estimates generated for these patients, the schedule was restructured in order to include:-

i) time points at which the model was most sensitive to changes in the model parameters. These points were identified by inspection of the relationship between the partial derivatives of the predicted function with respect to each of its parameters and time. The time at which a maximum or minimum occurs indicates the point at which the

model is most sensitive to changes in the model parameters. This is illustrated in Figure 4-1 where a turning point in the temporal relationship of the partial derivative of predicted concentration with respect to alpha occurred at 30 minutes (Patient 01).

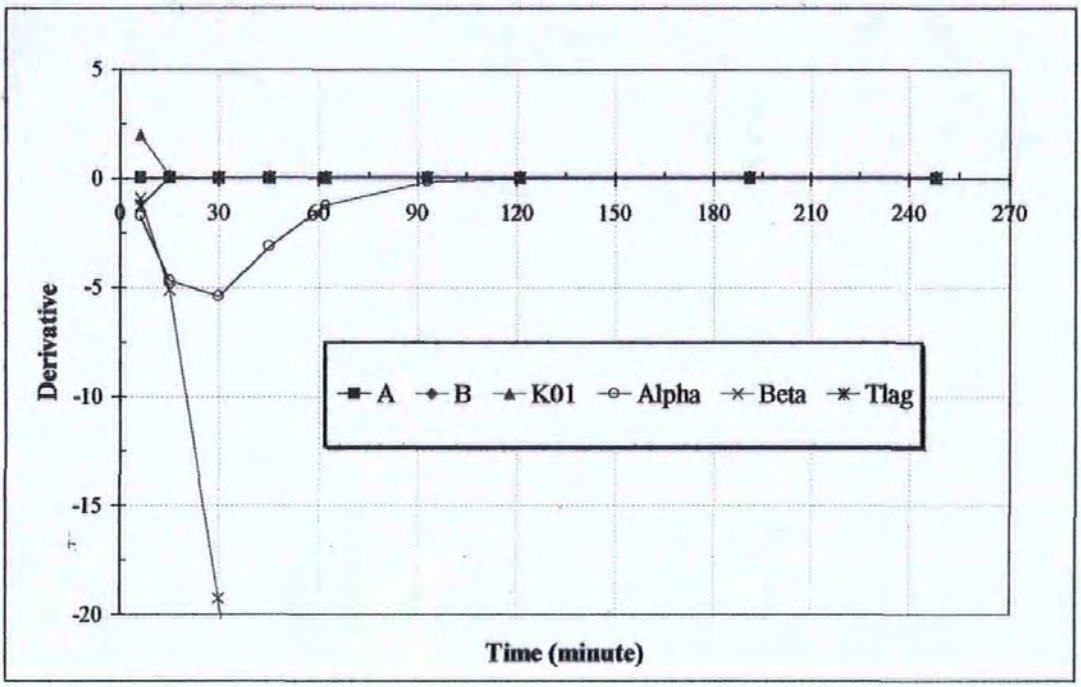

Figure 4-1 Sensitivity analysis (Patient 01).

ii) time points given by the reciprocal of the macro constants alpha and beta[22].

iii) a sufficient distribution of samples in order to define multiple compartments of an (individual) pharmacokinetic model. It was found that at least three samples per disposition phase were required in the modelling process. Thus additional sampling times were interspersed between the key sampling points derived from (i) and (ii) according to the traditional approach of constructing blood sampling schemes, i.e. with increasing time intervals at later times[22].

The restructured sampling schedule was evaluated using variance inflation factor (VIF) analysis. The variance of a model parameter can be expressed as the product of the underlying variance of the residuals and a multiplier which is known as the VIF (variance = σ^2.VIF). VIF does not depend on the actual data values; it is dependent only on the

sampling schedule. In comparing experimental designs, the design for which VIF values are the lowest yields more precise estimates of the model parameters.

Competing designs were compared by running simulations of each using WinNonlin. VIF were computed for the model parameters and predicted plasma apomorphine concentrations of each design. It could be demonstrated that the VIF associated with the updated schedule had the impact of considerably improving the precision with which model parameters were estimated (Appendix 8.10).

In addition to the timing of samples, the issue of sample volume was addressed. Experience with the first five patients demonstrated that the sample volume could be reduced to 4mL over the first 30 minutes, and to 6mL up to 180 minutes post-dose, whilst allowing a sufficient quantity of apomorphine for analysis (see Section 4.5.2, pages 4-88 and 4-89). A summary of the restructured blood sampling scheme is given in Table 4-3 (page 4-13).

Pharmacodynamic Sampling Scheme.

Following a review of the protocol, the UPDRS Part III was included as a further measurement of apomorphine-induced changes in parkinsonian motor symptoms. The assessment was made immediately prior to apomorphine bolus administration and also during the period of effect.

A summary of the restructured pharmacodynamic sampling scheme is given in Table 4-3.

Time (mins post bolus)	Blood sample volume	Obs of Parkinsonian symptoms	UPDRS	Tapping Test	Walking Test	Blood Pressure and Pulse
pre-	4mL	✓	Part III	✓	✓	
Administer apomorphine bolus *or* stop infusion.						
5 (5)	4mL	✓	Parts I - VI	✓		To be monitored as appropriate
10	4mL			✓	✓	
15 (15)	4mL			✓		
22	4mL			✓	✓	
30 (30)	4mL			✓	✓	
50 (45,60)	6mL			✓	✓	
80 (90)	6mL			✓	✓	
125 (120)	6mL			Until other anti-parkinsonian medication is taken		
180 (180)	6mL					
240 (240)	7mL					
310	7mL					
360 (360)	7mL					

Table 4-3 Restructured simultaneous blood/pharmacodynamic sampling scheme.

The original sampling times are given in parentheses.

Where multiple tasks exist at a single time point, the priority was as follows: (1) blood sampling, (2) tapping test, (3) walking test.

4.2. Clinical Protocols for Studies on Novel Delivery Systems of Apomorphine in Humans.

Studies were undertaken on the properties of three novel modes of administration of apomorphine, namely, intranasal administration in healthy volunteers, buccal administration in healthy volunteers, and subcutaneous administration by needle-free injector to patients with Parkinson's disease.

4.2.1. Preliminary Study of Needle-Free Subcutaneous Injections of Apomorphine in Parkinson's Disease.

To date there are no references concerning the use of needle-free technology with application to apomorphine administration. It was surmised that the needle-free technique might provide a treatment option for those patients with Parkinson's disease for whom the conventional system presents a barrier to therapy, i.e. those who experience a fear of needle injection or who are unable to self-inject due to parkinsonian disability. It was also speculated that the mode of action of the needle-free delivery system may confer an advantage over the standard method to a wider population of patients with Parkinson's disease should there be an (even) faster onset of action as a consequence of more rapid absorption, or should the risk of nodule formation be reduced.

4.2.1.1. Investigators.

The protocol was developed by John O'Sullivan[1], Steve Barker[2], Kirsten Turner[1] and Hasmet Hanagasi[1] (Departments of Neurology[1] and Vascular Surgery[2], Middlesex Hospital, London, UK).

4.2.1.2. Objectives.

The aim of this work was to examine the pharmacokinetics, pharmacodynamics and tolerability of apomorphine following subcutaneous needle-free delivery in comparison with subcutaneous delivery using the conventional apparatus, i.e. needle and syringe or Penject® (Britannia Pharmaceuticals Ltd).

4.2.1.3. Needle-Free Delivery Device.

The needle-free injection system used was the J-TIP® (National Medical Products, Inc, California, USA), see Figure 4-2. The device is a single use, disposable syringe which delivers drug solution by jet (pressurised) injection. Injection is powered by compressed

carbon dioxide contained in a cartridge within the device, and is activated manually by depressing the trigger. This causes the release of compressed carbon dioxide into the compartment behind the plunger which is then driven forward. The contents of the syringe are expelled through the micro-orifice, thus producing a pressurised stream of liquid. The drug solution punctures the epidermis and dermis and is delivered into the subcutaneous space, reaching a depth of approximately 3-8mm in 0.2s[23]. A spray-like dispersion pattern is achieved, in contrast to the depot of drug which is produced by injection through a needle[24]. It is claimed that the injection is almost pain free as a consequence of the transfer of solution through a micro-orifice at high velocity[23, 25].

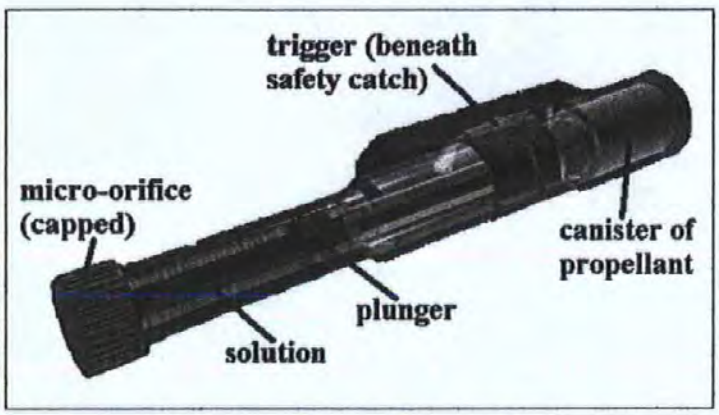

Figure 4-2 Needle-free delivery device: J-TIP® (National Medical Products, Inc.). Reproduced from website[25].

4.2.1.4. Study Design.

Open, controlled, within-subject comparison.

4.2.1.5. The Study Population.

The utility of the J-TIP® needle-free system was studied in three patients with Parkinson's disease. Recruitment criteria are given in Section 4.1.5 (page 4-1), but included the additional criteria that patients who were unable to attend the Hospital for the study days, or who refused to try the novel system were excluded.

Two males and one female were recruited. The mean (range) age of the patients was 61 (55 to 70) years. Patients had been diagnosed with Parkinson's disease for a mean (range) of 14 (10 to 16) years. The mean (range) duration of levodopa and apomorphine therapy was 12 (5 to 20) years and 5 (3 to 9) years, respectively. The mean (range) UPDRS score[a] when "on" was 42 (36 to 48). All patients had a Hoehn and Yahr score[b] of 2 when "on". In the case of one patient (patient 12), parkinsonian motor fluctuations were currently being managed with pallidal stimulation.

4.2.1.6. Treatment Administered.

Apomorphine HCl (Britaject® 10mg/mL, Britannia Pharmaceuticals Ltd.).

4.2.1.7. Investigational Plan.

Patients were admitted to The National Hospital for Neurology and Neurosurgery, London, for the duration of the study. Each patient received two doses of apomorphine: one dose was delivered using the conventional device and, on a separate day, one dose using the needle-free device (Table 4-5, page 4-20). An exception was made to this schedule in the case of one patient (patient 10) whereby, due to an unexpected adverse local tissue reaction following needle-free delivery, a second trial of apomorphine administration using the needle-free device was performed. The additional trial of needle-free apomorphine in patient 10 was performed on a separate, i.e. third, day.

Within each patient the doses used were identical, both in terms of the amount of apomorphine and the volume of drug solution (Britaject®, Britannia Pharmaceuticals Ltd). The dose used was that which the individual patients has previously been titrated to in the

[a] UPDRS score range: 0 to 206, where 0 represents normal and 206 represents maximal parkinsonian disability (see Appendix 8.1).
[b] Hoehn and Yahr score range: 0 to 5, where 0 represents normal and 5 represents maximal parkinsonian disability (see Appendix 8.1).

management of their Parkinson's disease symptoms. All apomorphine doses were administered to the patients by the same clinician.

An attempt was made to randomise the order of the treatments, thus two patients received apomorphine by conventional delivery first, whereas one patient received needle-free apomorphine first (Table 4-5). However, neither the patients nor the investigators were blinded to the treatments. Given that the release of solution from the needle-free device was accompanied with a loud "pop" and a "hiss" as the propellant was discharged, blinding of the study was not practical.

There was a difference in the design of the needle-free device used for patient 12 compared to those used for the first two subjects, i.e. patients 09 and 10, in that the capacity of the drug reservoir used for patient 12 was greater. All other aspects of the needle-free injection system were identical.

Methods were common to those given in Section 4.1.6.1 (see Table 4-4 for a summary, page 4-19) with the notable inclusion of (i) a pain rating of the injection event using a visual analogue scale, where zero represented absence of pain and ten represented the maximum pain rating, and (ii) the inspection of the injection site during the course of the study day and reporting on the condition of the injection site to the clinician following the study day.

Pre-treatment of blood samples was performed according to the protocol given in Section 4.1.7 (page 4-9). Plasma samples were prepared for assay according to the protocol given in Section 4.3.3.3 (page 4-35) and assayed using the methods given in Section 4.4 (page 4-50).

Time (mins post-bolus)	Blood sample volume	Clinical observations (including inspection of injection site)	Pain rating	UPDRS	Tapping test	Walking test
pre-bolus	4mL	✓		Part III	✓	✓
Administer apomorphine ✓						
5	4mL				✓	
10	4mL				✓	✓
15	4mL			Parts I - VI	✓	
22	4mL				✓	✓
30	4mL				✓	✓
50	6mL	✓			✓	✓
80	6mL				✓	✓
125	6mL				Until other anti-parkinsonian medication is taken	
180	6mL					
240	7mL					
310	7mL					
360	7mL					

Table 4-4 **Simultaneous blood/pharmacodynamic sampling scheme used in the study of needle-free subcutaneous injections of apomorphine in Parkinson's disease.**

Shaded region indicates predicted apomorphine-induced "on" phase.

Where multiple tasks exist at a single time point, the priority is as follows: (1) blood sampling, (2) tapping test, (3) walking test.

Patient ID		Order of Treatment	Apomorphine bolus dose		Time of administration	Site of administration	Patient's posture on administration	Washout period[a]	Stimulator downtime[b]
No.	Treatment		(mg)	(µg/kg)				(h)	(h)
09	CON	1st	2	35.1	10.09	Right thigh	Standing	8.2	NA
	NF	2nd			09.57			8.0	
10	CON	1st	3.5	47.3	09.50	Right thigh	Sitting	4.6	NA
	NF 1	2nd			10.20	Left thigh		5.3	
	NF 2	3rd			10.29	Right thigh		5.5	
12	CON	2nd	5	55.6	11.04	Right thigh	Sitting	7.6	0.8
	NF	1st			12.16			8.8	1.5

Table 4-5 Subcutaneous administration of apomorphine to patients with Parkinson's disease by conventional (needle) and novel (needle-free) delivery devices. Abbreviations: CON = conventional, NA = not applicable, NF = needle-free.

[a] Defined as the time period between the last anti-parkinsonian medication that was taken and administration of the study dose of apomorphine.
[b] Defined as the time period between stopping the pallidal stimulation and administration of the study dose of apomorphine.

4.2.1.8. Criteria for Evaluation.

Plasma apomorphine concentration and apomorphine-induced anti-parkinsonian response over six hours post-dose.

Pharmacokinetic parameters: C_{max}, T_{max}, $AUC_{0\text{-infinity}}$, and beta-phase intercept.

Response variables: onset and duration of response; extent of improvement in tapping test score, ambulatory time and UPDRS rating from baseline.

Tolerability indices: condition of the injection site, pain rating of the injection event.

4.2.1.9. Data Handling.

Pharmacokinetic parameters were estimated using WinNonlin modelling software (Standard version 2.0, Pharsight, CA, USA) using the method given in Section 4.6 (page 4-103).

4.2.1.10. Ethics.

Ethical approval was obtained from the University College London Hospitals Committee on the Ethics of Human Research by the investigative team given in Section 4.2.1.1 (page 4-15). Written informed consent from all volunteers was obtained by the investigative team given in Section 4.2.1.1.

4.2.2. Pharmacokinetic Study of Single-Dose Intranasal Apomorphine Powder (Three Doses) in Healthy Volunteers.

Much of the information given in this section has been summarised from the study protocol[26].

4.2.2.1. Investigators.

The protocol was developed by Dr. J. Whittington (Mediscience Services Ltd), Dr. M. Buraglio (LCG Bioscience, Bourn Hall Clinic, Cambridge), Dr. D. Anderson Davies (Britannia Pharmaceuticals Ltd), Mr. K. Davies (Britannia Pharmaceuticals Ltd) and Ms. S. Mercer (Britannia Pharmaceuticals Ltd).

4.2.2.2. Objectives.

The aim of this work was to examine the pharmacokinetics and bioavailability of single, ascending doses of apomorphine powder given by the intranasal route to healthy volunteers, in comparison with subcutaneous delivery using the conventional apomorphine formulation and apparatus.

(The secondary objective was to determine the safety and tolerability of single, ascending doses of intranasal apomorphine powder in healthy volunteers; however this was not within the remit of this thesis).

4.2.2.3. Intranasal Delivery Device.

Apomorphine is predominantly formulated for intra-nasal use as a liquid nasal spray[27-32]. In contrast to this, a dry powder formulation was the focus of this study. Hence, this was an exploratory study of the viability of the intranasal delivery of apomorphine powder using the "Turbospin" insufflator (CDFS, UK, Figure 4-3). Potential benefits to be gained by the departure from intranasal solutions are the avoidance of significant drainage from the

application site and, in the specific case of apomorphine, an improvement over the limited stability of apomorphine in solution[33].

Under the conditions of use, the chamber of the insufflator is loaded with a capsule containing the pharmaceutical preparation. The capsule is then pierced using the retractable prongs attached to the base section of the device. The nozzle is inserted into the selected nostril, the other nostril having been occluded. The intranasal formulation is delivered into the nasal cavity when the recipient makes a deliberate inhalation through the device. As air is inspired through the vents a simple spin mechanism is activated which, in turn, drives the expulsion of the powder from the chamber into the nasal cavity.

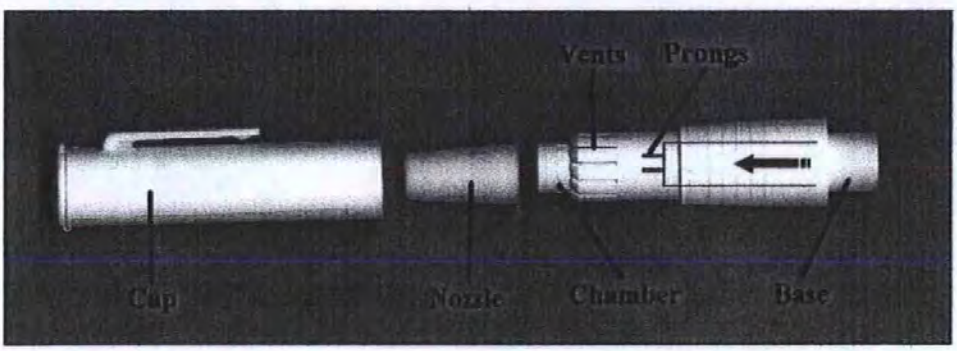

Figure 4-3 Turbospin nasal insufflator, disassembled (CDFS, UK).

4.2.2.4. Study Design.

Open, controlled, non-randomised, ascending dose, within-subject comparison.

4.2.2.5. The Study Population.

Six volunteers were recruited from a volunteer panel by LCG Bioscience. The main recruitment criteria were:-

i) able to provide written informed consent,

ii) male,

iii) in good health,

iv) a body mass index between 18 and 28 kg/m^2,

v) nasal inspiratory flow of at least 30 L/min,

vi) no history of nasal disorders or abnormalities including any current medical condition that affects the nose or throat.

4.2.2.6. Treatments Administered.

Apomorphine HCl (Britaject® 10mg/mL, Britannia Pharmaceuticals Ltd.).

Apomorphine intranasal powder capsules: clear size 2 gelatine capsules containing 1, 3 or 5 mg apomorphine HCl, 1% w/w ascorbic acid, made up to 10 mg total powder weight with dextrose monohydrate (Unival Clinical Trials Management, Bolton, UK).

4.2.2.7. Investigational Plan.

Volunteers were resident at Bourn Hall Clinic, Cambridge, during the clinical phase.

The use of prescription or over-the-counter medication was disallowed for 28 days prior to the onset of apomorphine dosing, with the exception of domperidone which the volunteers were required to take for three days prior to the trial (20 mg three times daily) as a prophylactic anti-emetic. The use of prescription or over-the-counter medication was also disallowed for the duration of the trial, with the exception of paracetamol.

Each volunteer received a single dose of each of the four treatments on consecutive days and in the following order: i) subcutaneous injection 3 mg (to the anterior abdominal wall), ii) intranasal powder 1 mg, iii) intranasal powder 3 mg, iv) intranasal powder 5 mg. Capsules were recovered from the delivery device after use and assayed for residual drug content (performed by Penn Pharmaceuticals Ltd, Gwent, UK.)

For each of the treatments administered blood samples were collected for apomorphine assay, via a peripheral intravenous cannula, at baseline, i.e. within the 60 minute period prior to administration, and at 5, 10, 15, 20, 25, 30, 60, 90, 120, 240 and 360 minutes post-dose.

Pre-treatment of blood samples was performed according to the protocol given in Section 4.1.7 (page 4-9) by LCG Bioscience staff. Plasma samples were prepared for assay according to the protocol given in Section 4.3.3.3 (page 4-35) and assayed using the methods given in Section 4.4 (page 4-50).

4.2.2.8. Criteria for Evaluation.

Plasma apomorphine concentration over six hours post-dose.

Pharmacokinetic parameters: C_{max}, T_{max}, $AUC_{0\text{-infinity}}$.

4.2.2.9. Data Handling.

Pharmacokinetic parameters were estimated using WinNonlin modelling software (Standard version 2.0, Pharsight, CA, USA). Bivariate correlations and ANOVA with repeated measures (significance level (α) = 0.05) were performed for salient pharmacokinetic parameters using SPSS (version 9.0.0, SPSS Inc.).

4.2.2.10. Ethics.

Ethical approval was obtained from Local Research Ethics Committee of Addenbrook's Hospital, Cambridge, by the investigative team given in Section 4.2.2.1 (page 4-22).

Informed consent from all volunteers was obtained by the investigative team given in Section 4.2.2.1.

4.2.3. Pharmacokinetic Study of Single-Dose Buccal Apomorphine Powder (Three Doses) in Healthy Volunteers.

Much of the information given in this Section has been summarised from the study protocol[34].

4.2.3.1. Investigators.

The protocol was developed by Dr. J. Whittington (Mediscience Services Ltd), Dr. M. Buraglio (LCG Bioscience, Bourn Hall Clinic, Cambridge), Dr. D. Anderson Davies (Britannia Pharmaceuticals Ltd), Mr. K. Davies (Britannia Pharmaceuticals Ltd) and Ms. S. Mercer (Britannia Pharmaceuticals Ltd).

4.2.3.2. Objectives.

The aim of this work was to examine the pharmacokinetics and bioavailability of apomorphine administered to healthy volunteers by the buccal route in comparison with subcutaneous delivery using the conventional apomorphine formulation and apparatus. (The secondary objective was to determine the safety and tolerability of single, ascending doses of buccal apomorphine in healthy volunteers; however this was not within the remit of this thesis).

4.2.3.3. Treatments Administered.

Apomorphine HCl (Britaject® 10mg/mL, Britannia Pharmaceuticals Ltd.).

Apomorphine buccal formulation: hydrogel containing 5, 10 or 20 mg apomorphine HCl powder (Controlled Therapeutics (Scotland) Ltd, East Kilbride, UK).

4.2.3.4. Buccal Delivery Device.

Hydrogels are cross-linked polymers which have the ability to absorb and retain (aqueous) solvent(s), forming a swollen gel-phase in the process, and remaining un-dissolved[35]. When loaded with drug, hydrogels can function as drug delivery systems. The release of drug from a hydrogel involves the absorption of water into the polymer and simultaneous desorption of drug via diffusion[35].

The hydrogel used was a poly(ethylene glycol) matrix which has approval from the MCA for vaginal delivery of prostaglandin E2 in the induction of labour (Propess®, Ferring Pharmaceuticals)[36]. The use of this particular hydrogel as a buccal delivery system for apomorphine administration constituted a novel application, both in terms of the drug used *and* the site of administration. In fact, the use of *any* hydrogel, regardless of composition, has not been previously documented for apomorphine administration. Furthermore, the buccal mucosa itself is a novel route for delivery of apomorphine (regardless of delivery device).

The hydrogel polymer was formulated as an "insert" which was 5mm wide x 17mm long x 1.1 mm thick (Controlled Therapeutics (Scotland) Ltd, East Kilbride, UK). The *in vitro* swelling profile of the inserts used in this study was commensurate with *in vitro* apomorphine release (Figure 4-4). *In vitro* swelling was determined using the USP paddle method[ ], and apomorphine was quantified using a spectroscopic method (Controlled Therapeutics (Scotland) Ltd, East Kilbride, UK).

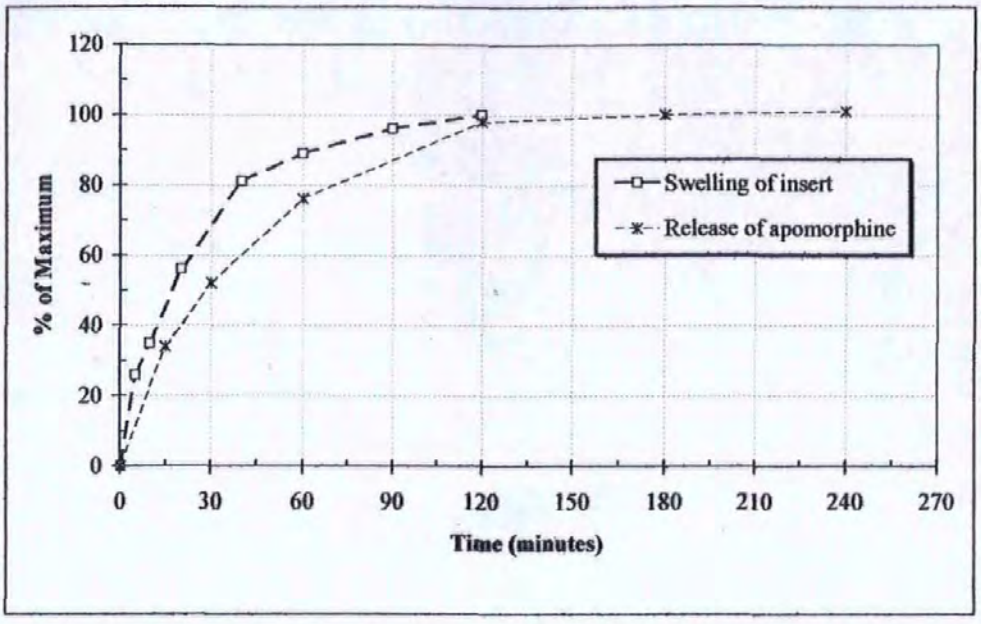

Figure 4-4 ***In vitro*** **buccal insert swelling and apomorphine release tests. (Controlled Therapeutics (Scotland), East Kilbride, UK). Reproduced with permission.**

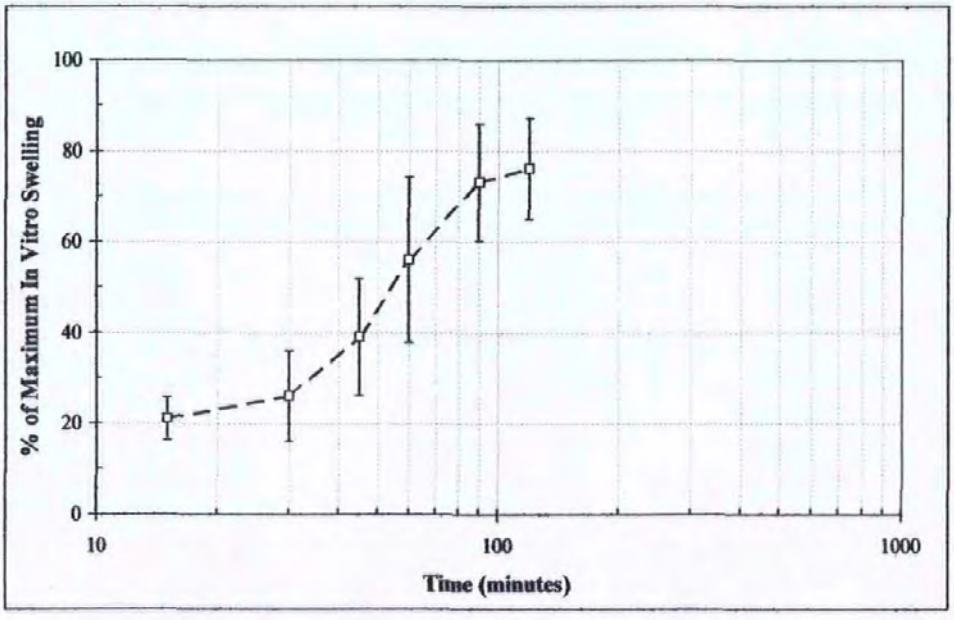

Figure 4-5 ***In vivo*** **buccal insert swelling test.**

The mean percentage swelling of placebo buccal inserts is presented, *n*= five individuals (Controlled Therapeutics (Scotland), East Kilbride, UK). Reproduced with permission.

The swelling-time profile of the buccal insert *in vivo* was sigmoid in nature, i.e. there was a lag in the swelling process over the first 30 minutes *in situ*, followed by a period of relatively rapid swelling which continued until a plateau was reached at about 100 minutes. The plateau lasted until (at least) 120 minutes, this being the final sample point in the experiment (Figure 4-5, page 4-29).

The plateau achieved *in vivo* in the latter stage of the time course corresponded to a degree of swelling that was approximately 75% of the maximum achievable *in vitro* for the insert. In this condition the hydrogel tended to detach from the mucosa.

Under the conditions of use the dry buccal insert is placed between the upper gingiva and buccal mucosa, and is held in place by the upper lip. The insert is inconspicuous *in situ* and does not interfere with drinking or speaking, however eating usually causes the insert to detach from the mucosa. Hydration of the insert by saliva at the site of administration confers muco-adhesive properties to the surface of the insert, resulting in rapid attachment of the insert to the gingival mucosa. As water is absorbed the hydrogel swells up progressively, becoming gel-like in consistency. Consequently the apomorphine contained in the polymer is dissolved and diffuses (via multidirectional release) from the hydrogel matrix to the absorption site(s) in the oral cavity.

4.2.3.5. Study Design.

Open, controlled, non-randomised, ascending dose, within-subject comparison.

4.2.3.6. The Study Population.

Six volunteers were recruited from a volunteer panel by LCG Bioscience. The main recruitment criteria were:-

i) able to provide written informed consent,

ii) male,

iii) in good health,

iv) a body mass index between 18 and 28 kg/m^2,

v) no history of oral disorders or abnormalities or any current medical condition that affects the mouth or gums.

4.2.3.7. Investigational Plan.

Volunteers were resident at Bourn Hall Clinic, Cambridge during the clinical phase.
The use of prescription or over-the-counter medication was disallowed for 28 days prior to the onset of apomorphine dosing, with the exception of domperidone which the volunteers were required to take for three days prior to the trial (20 mg three times daily) as a prophylactic anti-emetic. The use of prescription or over-the-counter medication was also disallowed for the duration of the trial, with the exception of paracetamol.
Each volunteer received a single dose of each of the four treatments on consecutive days and in the following order: i) subcutaneous infusion 2 mg/h x 2 h, ii) buccal insert 5 mg, iii) buccal insert 10 mg, iv) buccal insert 20 mg. The buccal inserts were positioned on the upper gum and held in place by the upper lip. The buccal inserts were left *in situ* for 120 minutes post-commencement of administration; specifically the insert was removed from the mouth directly after the blood sample designated at 120 minutes was collected.
Subsequently the used inserts were analysed for residual apomorphine content (Controlled Therapeutics (Scotland) Ltd, East Kilbride, UK).

For each of the treatments administered blood samples were collected for apomorphine assay, via a peripheral intravenous cannula, at baseline, i.e. within the 60 minute period prior to administration, and at 5, 15, 30, 60, 90, 120, 240 and 360 minutes after the insertion of the buccal device.

Pre-treatment of blood samples was performed according to the protocol given in Section 4.1.7 (page 4-9) by LCG Bioscience staff. Plasma samples were prepared for assay according to the protocol given in Section 4.3.3.3 (page 4-35) and assayed using the methods given in Section 4.4 (page 4-50).

4.2.3.8. Criteria for Evaluation.

Plasma apomorphine concentration over six hours post-dose.

Pharmacokinetic parameters: C_{max}, T_{max}, and $AUC_{0\text{-infinity}}$.

4.2.3.9. Data Handling.

Pharmacokinetic parameters were estimated using WinNonlin modelling software (Standard version 2.0, Pharsight, CA, USA). Bivariate correlations and ANOVA with repeated measures (significance level (α) = 0.05) were performed for salient pharmacokinetic parameters using SPSS (version 9.0.0, SPSS Inc.).

4.2.3.10. Study Ethics.

Ethical approval was obtained from Local Research Ethics Committee of Addenbrook's Hospital, Cambridge, by the investigative team given in Section 4.2.3.1 (page 4-27). Informed consent from all volunteers was obtained by the investigative team given in Section 4.2.3.1.

4.3. Development of Analytical Methods

Given that plasma apomorphine concentrations in the post-distribution phase were likely to be low, i.e. in the region of 1ng/mL at three hours post-bolus[7], an assay that was capable of determining drug concentration pertaining to this period was developed. The assay was based on the published method of Priston and Sewell[18], details of which are given below (Sections 4.3.1 and 4.3.2).

4.3.1. Solid Phase Extraction of Apomorphine According to Priston.

A Bond-Elut C_{18} 1mL/100mg solid phase extraction column (HPLC Technology, Macclesfield, UK) was attached to a Techelut vacuum manifold (HPLC Technology, Macclesfield, UK). The column was conditioned with 2mL methanol followed by 2mL water. A 1mL sample was then aspirated, followed sequentially by 2mL water, 1mL 10% (v/v) methanol in water, 1mL 20% (v/v) methanol in water and 1mL 50% (v/v) methanol in water. The column was not allowed to dry out at any of the conditioning and washing stages.

Apomorphine (and boldine, the internal standard) were eluted with 2 x 200µL 0.1M hydrochloric acid in methanol into a silanised 1mL volumetric flask containing 400µL 1% (w/v) sodium metabisulphite and made up to volume with diluent A (see Section 3.6).

4.3.2. HPLC Method for Apomorphine Determination According to Priston.

The HPLC system consisted of an LDC Analytical ConstaMetric 3200 solvent delivery system (Thermoquest, Manchester, UK) and a Jasco FP-821 spectrofluorometer (Jasco, Great Dunmow, UK) set at λex 270nm, λem 450nm, attenuation 1, gain 1000.

Data acquisition was performed by a Milton-Roy Computing Integrator 4000 (Stone, Staffs, UK) and, later, Borwin Chromatography software (version 1.0, JMBS Developpements, France).

The HPLC column was a Techopak C18 10μm 250 x 4mm I.D. column used in conjunction with a 2cm pre-column containing the same stationary phase as the main column (HPLC Technology, Macclesfield, UK).

The mobile phase contained 70% (v/v) aqueous and 30 % (v/v) methanol portions. The constituents of the aqueous portion, expressed as final concentrations in the mobile phase, were: 0.25 M sodium dihydrogen orthophosphate and 0.25 % (w/v) heptane sulphonic acid which were adjusted to pH 3.30 with orthophosphoric acid, and 0.003 % (w/v) EDTA.

The flow rate was 1.5mL/min. Injection of sample was made using a Rheodyne injection system and was 50μL in volume.

4.3.3. Preparation of Matrix for Use in Analytical Method Development.

Blood collection and preparation was based on methods described by Priston[18].

4.3.3.1. Control Blood Collection and Pre-Treatment.

Venous blood was collected from volunteers (number of individuals ≥ 3) into 7mL EDTA k3 vacutainer tubes. Blood was transferred into polypropylene tubes containing ascorbic acid to give a final concentration of 5mM ascorbic acid, and centrifuged (4°C, 1250g x 5 minutes). The resulting plasma was pooled and stored in polypropylene tubes at 4-8°C until required.

4.3.3.2. Preparation of Plasma Samples (1)

Plasma was spiked to 1 and 20ng/ml R(-)-apomorphine HCl using stock apomorphine solutions in diluent A (see Section 3.6). These concentrations were selected because they represented the range of apomorphine concentrations reported in published pharmacokinetic studies[4, 7]. Aliquots of appropriate volumes were stored in polypropylene tubes at –20°C.

4.3.3.3. Preparation of Plasma Samples (2)

When required, aliquots of plasma were thawed at 4-8°C. On thawing, 1% (v/v) 2-mercaptoethanol solution was added to give a final concentration of 0.01% (v/v) 2-mercaptoethanol. Plasma was then centrifuged (4°C, 1250g x 5minutes) in order to pellet precipitated protein and the resultant supernatant was transferred to a polypropylene tube.

Plasma was then spiked to give a final concentration of 100ng/mL internal standard using a stock solution of internal standard in diluent A (see Section 3.5), mixed gently and left to equilibrate at 4-8°C for 5 minutes prior to solid phase extraction.

4.3.4. Development of Apomorphine Assay.

4.3.4.1. Choice of HPLC Column.

A recently marketed HPLC column, the Columbus[a] (Phenomenex, UK), was selected for evaluation against the existing column, i.e. the Techopak[b] (HPLC Technology, UK). Whilst the functional group was the same for each column, the Columbus bonded phase consisted of extremely high purity silica onto which the octadecylsilane had been bonded. Furthermore residual silanol groups had been extensively deactivated, using a process known as "double endcapping", resulting in an inert bonded surface. Such properties potentially improve chromatography by limiting non-specific interactions between the analyte and stationary phase.

4.3.4.1.1. Development of Mobile Phase.

Experimental.

A solution of 50ng/mL R(-)-apomorphine HCl and 5ng/mL R(-)-boldine HCl in diluent A were assayed according to the method given in Section 4.3.2 (page 4-34), with the exception that the alternative HPLC column (the Columbus) was employed.

The retention times of boldine and apomorphine were approximately 13 and 30 minutes, respectively, at 1mL/minute flow rate using the alternative analytical column. In order to allow rapid assay of apomorphine (at 1mL/min), the percentage of methanol in the mobile phase was increased so as to shorten the assay run time. A range of 30 to 45 % (v/v) methanol concentration in the mobile phase, run at a flow rate of 1mL/minute, was investigated.

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[a] C18, carbon loading: 19%, spherical 5 μm particles, 150 mm x 4.6 mm I.D.
[b] C18, carbon loading: 14%, irregular 10μm particles, 250 mm x 4.0 mm I.D.

Results.

Given that (i) the greater the value for resolution factor[a], the greater the resolution between two compounds, and (ii) a resolution factor of 1.5 is considered to be desirable for the rapid assay of given analyte(s)[37], it was evident that a mobile phase methanol content of 45% or greater would be required for optimal assay performance in terms of resolution of apomorphine and boldine (Figure 4-6). However, at a mobile phase methanol content of 45%, the resolution between the unretained solute (ascorbic acid) and boldine was compromised, i.e. the resolution factor was 0.9. Therefore a mobile phase composition of 40% (v/v) methanol was deemed satisfactory for use in the assay of boldine and apomorphine. At a methanol content of 40% (v/v), the retention times of unretained solute, boldine and apomorphine were 1.5, 3.9 and 6.9 minutes, respectively.

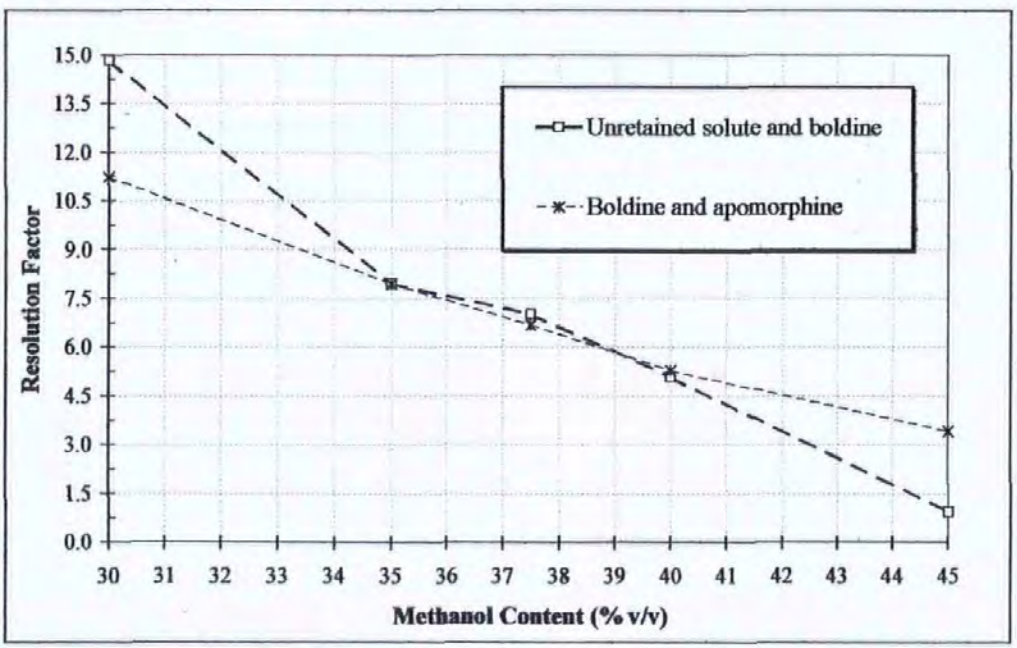


Figure 4-6 Effect of mobile phase methanol content on retention behaviour of apomorphine and boldine.

[a] Computational formula for resolution factor (R_s) of two peaks A and B: $R_s = 2.(t_B - t_A)/(W_A + W_B)$, where t is retention time and W is peak width[37].

4.3.4.1.2. Comparative Performance of HPLC Columns.

Experimental.

Apomorphine (50ng/mL) was extracted from diluent A according to the method given in Section 4.3.1 (page 4-33). A direct comparison of the Techopak and Columbus columns was made whereby both non-extracted and extracted solutions were assayed under the conditions given in Section 4.3.2 (page 4-34) using each of the two columns. N.B. the mobile phase developed in Section 4.3.4.1.1 (page 4-37) was employed.

Results.

Assay of the extract using the Columbus column demonstrated that apomorphine had degraded as a result of the solid phase extraction process and furthermore that the constituent peaks were not resolved using the Techopak column (Figure 4-7).
This indicated that a modified solid phase extraction procedure was necessary, and that the Columbus analytical column should be used in favour of the Techopak column in the assay of apomorphine. The development of a modified solid phase extraction procedure is addressed in Section 4.3.4.3 (page 4-41).

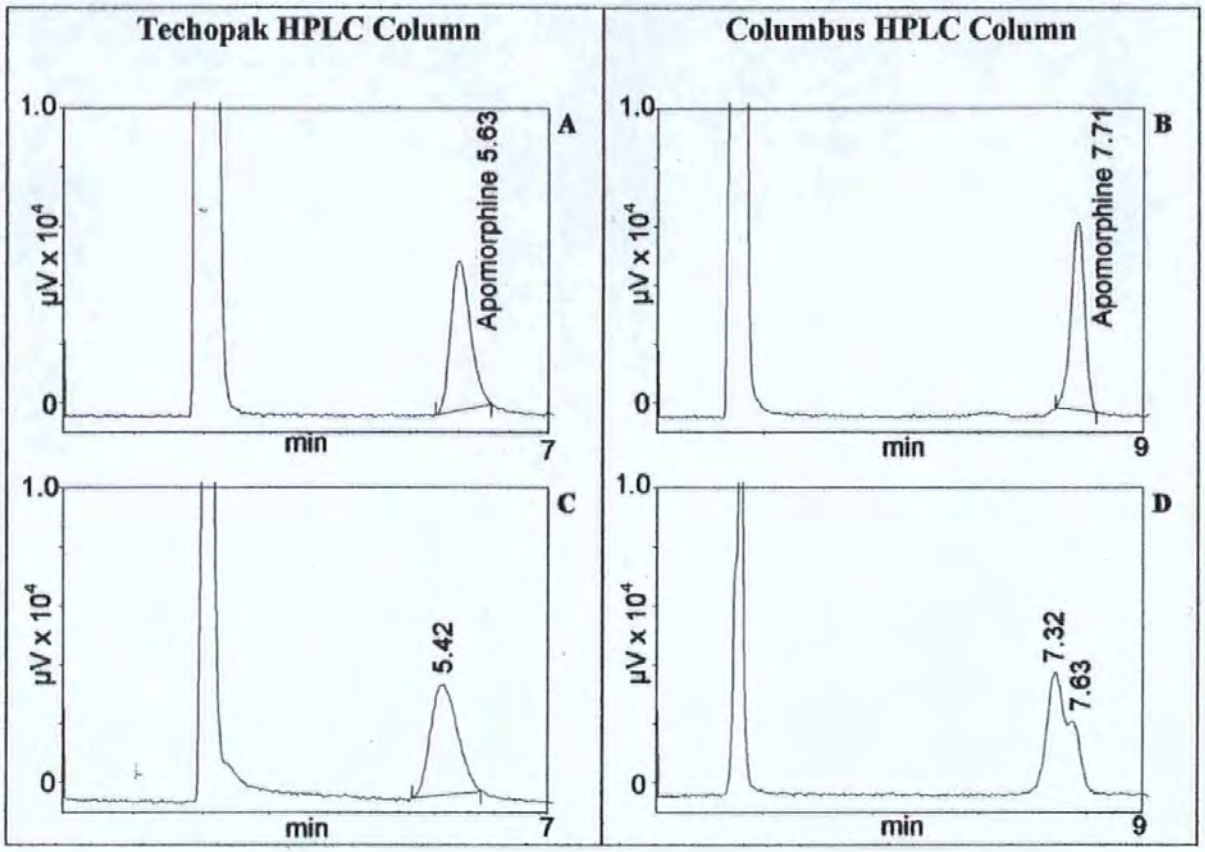


Figure 4-7 **Comparison of Techopak and Columbus HPLC columns: 50 ng/mL R(-)-apomorphine HCl in diluent A.**

Panels A and B: standard (non-extracted) apomorphine solution.

Panels C and D: extracted apomorphine.

Retention times are given as peak labels.

4.3.4.2. Choice of Internal Standard.

During the initial stages of method development there was, intermittently, a peak observed at the retention time of boldine, i.e. 4 minutes. It was therefore decided to evaluate an alternative internal standard, i.e. R(-)-propylnorapomorphine (NPA) (Figure 4-8). This compound was utilised as an internal standard in the HPLC apomorphine assays developed by Smith *et al* [38], Gancher *et al* [4], Essink *et al* [39], Sam *et al* [40] and Ameyibor *et al* [41], and in subsequent publications[7, 12, 42-44] which employed modified versions of these assays for apomorphine determination.

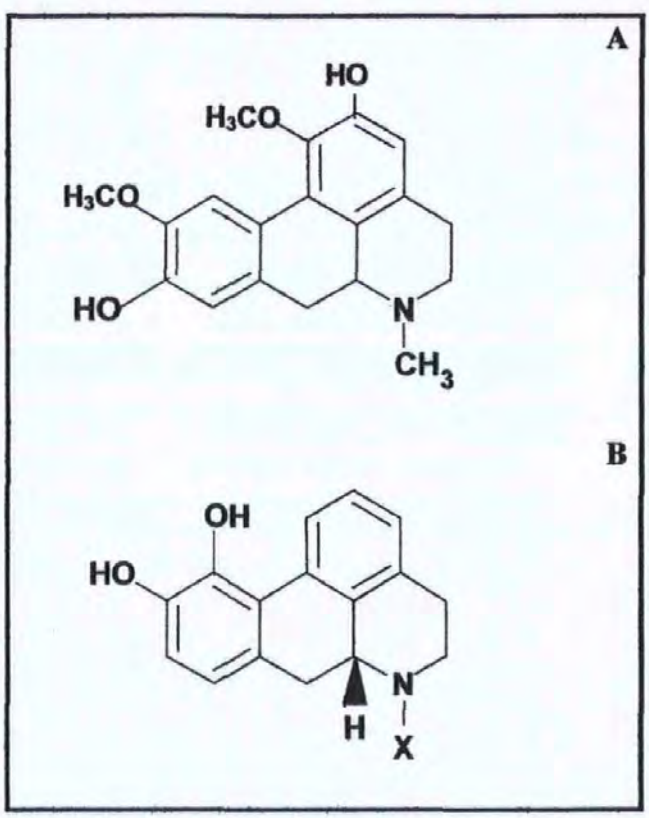


Figure 4-8 Structures of internal standards used.

Panel A: Boldine.

Panel B: R(-)-propylnorapomorphine: X = $CH_2CH_2CH_3$.
NB. Structure of R(-)-apomorphine: X = CH_3.

Using the mobile phase composition developed in Section 4.3.4.1.1, NPA eluted at approximately 13 minutes. NB. The retention time of apomorphine under these conditions was approximately 8 minutes (see Figure 4-13, page 4-52).

4.3.4.3. Modification to Solid Phase Extraction Eluting Solution.

The role of the eluting solution (0.1M hydrochloric acid in methanol) in the degradation of apomorphine as a result of solid phase extraction was investigated.

Experimental 1.

R(-)-Apomorphine HCl (50ng/mL) in diluent A was assayed using the method developed thus far, i.e. under the conditions given in Sections 4.3.1 and 4.3.2 (pages 4-33 and 4-34) but using the Columbus column in place of the Techopak column, and a mobile phase which contained 40% (v/v) methanol (see Section 4.3.4.1, page 4-36). Alternative eluting solutions were employed in the solid phase extraction procedure. These are given in Table 4-6 with the associated outcome.

Results 1.

Eluting Solution.			Outcome.
Solutions based on acidified methanol.			
0.01M HCl in methanol			Degradation of apomorphine.
0.001M – 1.0M CH_3CO_2H in methanol			Degradation of apomorphine.
Solutions based on mobile phase.			
$[Na_2H_2PO_4]$	pH	methanol (%)	
0.25M	3.30	10	No degradation of apomorphine, but poor recovery[a] (~20%).
0.25M	3.30	35	No degradation of apomorphine, recovery[a]: ~50%.
0.25M	3.30	40	No degradation of apomorphine, recovery[a]: ~70%.
0.25M	4.20	40	No degradation of apomorphine, recovery[a]: ~60%.
0.25M	3.30	50	Deterioration in peak shape.
0.25M	3.30	60	Degradation of apomorphine.

Table 4-6 Evaluation of alternative eluting solutions for solid phase extraction of apomorphine.

[a] Recovery of apomorphine in terms of peak area in comparison to that of (non-extracted) *standard.*

A suitable eluting solution was identified, i.e. 0.25M sodium dihydrogen orthophosphate, adjusted to pH 3.30 with orthophosphoric acid, in 40% (v/v) methanol. However it became apparent that, whilst a satisfactory outcome was achieved in the extraction of apomorphine from diluent A, degradation of the same nature as shown in Figure 4-7D (page 4-39) occurred in the extraction of apomorphine from plasma when this eluting solution was used (see Figure 4-9C).

The retention behaviour of the additional peak present in the plasma extract was concordant with the peak produced by forced degradation of apomorphine under oxidative conditions (see Figure 4-20F, page 4-71). Thus it was proposed that apomorphine oxidation had occurred as a consequence of the solid phase extraction procedure. Based on this an anti-oxidant was introduced into the solid phase extraction procedure. This development is described in *Experimental 2* (below).

Experimental 2.

R(-)-Apomorphine HCl (50ng/mL) in diluent A was assayed using the method developed thus far, i.e. under the conditions given in Sections 4.3.1 and 4.3.2 but with the following modifications:-

- the Techopak column was superseded by the Columbus column (see Section 4.3.4.1.2, page 4-38),
- the mobile phase contained 40% (v/v) methanol (see Section 4.3.4.1.1, page 4-36),
- the eluting solution consisted of 0.25M sodium dihydrogen orthophosphate, adjusted to pH 3.30 with orthophosphoric acid, in 40% (v/v) methanol (Section 4.3.4.3 *Experiment 1*).

Sodium metabisulphite in diluent A, in the form of either 200µL x 2% (w/v) or 100µL x 4% (w/v), was aspirated through the solid phase extraction column after the 50% (v/v) methanol in water wash step and, therefore, prior to application of eluting solution.

Results 2.

Degradation of apomorphine following extraction from plasma persisted when 100µL x 4% (w/v) solution was used, but was prevented by the inclusion of 200µL x 2% (w/v) sodium metabisulphite in diluent A (Figure 4-9).

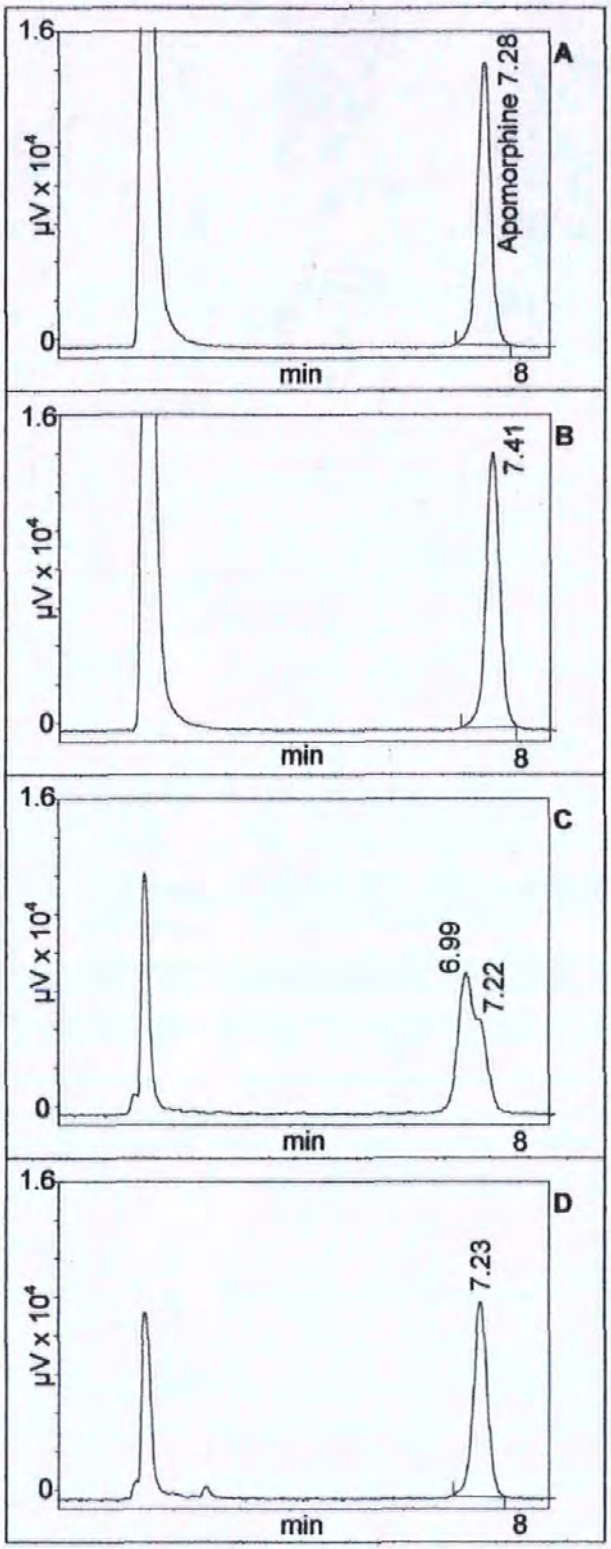


Figure 4-9 Modification of solid phase extraction procedure.

Panel A: standard (non-extracted) solution: 50 ng/mL R(-)-apomorphine HCl in diluent A.

Panel B: 50 ng/mL R(-)-apomorphine HCl in diluent A, extracted in absence of 2% sodium metabisulphite in diluent A wash step.

Panel C: 50 ng/mL R(-)-apomorphine HCl in plasma, extracted in absence of 2% sodium metabisulphite in diluent A wash step.

Panel D: 50 ng/mL R(-)-apomorphine HCl in plasma, extraction procedure included 2% sodium metabisulphite in diluent A wash step.

4.3.4.4. Modification to Solid Phase Extraction Eluting Solution Volume.

An examination of the elution profiles of apomorphine and NPA was performed in order to optimise recovery following solid phase extraction.

Experimental.

The elution profile of apomorphine at two concentrations (1 and 20 ng/mL in plasma) in the presence and absence of NPA (100 ng/mL in plasma) was investigated (in duplicate) for potential concentration or competition effects.

Apomorphine and NPA were extracted according to method given in Section 4.4.1 (page 4-33), with the exception that eluting solution was applied to the solid phase extraction column in six successive 200 µL portions. The extracts that were yielded from each discrete (200µL) portion of eluting solution were individually collected in succession into vials containing 200µL of 2 % sodium metabisulphite in diluent A for assay using the method described in Section 4.4.2 (page 4-51).

The solid phase extraction column was not allowed to dry out, except after the final 200µL portion of eluting solution had been applied. Consequently the volume of eluting solution collected at this stage included the volume contained in the sorbent bed and port manifold, measured at 180µL, in addition to the 200µL directly applied.

Recovery was estimated by comparing analyte peak areas in the portions of eluting solution to that of a non-extracted standard in diluent A.

Results.

There was no evidence of concentration effects in the recovery of apomorphine, nor was there an indication that the presence of NPA in plasma affected the extraction of apomorphine from that plasma (Figure 4-10). However it could be shown that there was a difference in the elution characteristics of apomorphine compared to NPA, with the greatest recovery of apomorphine occurring in the *second* portion of eluting solution whereas the *third* portion contained the highest concentration of NPA (Figure 4-11). The prolonged retention of NPA (relative to apomorphine) was to be expected due to the greater degree of non-polar interactions between the sorbent functional groups and analyte molecule that is afforded by the additional alkyl group of NPA (see Figure 4-8, page 4-40).

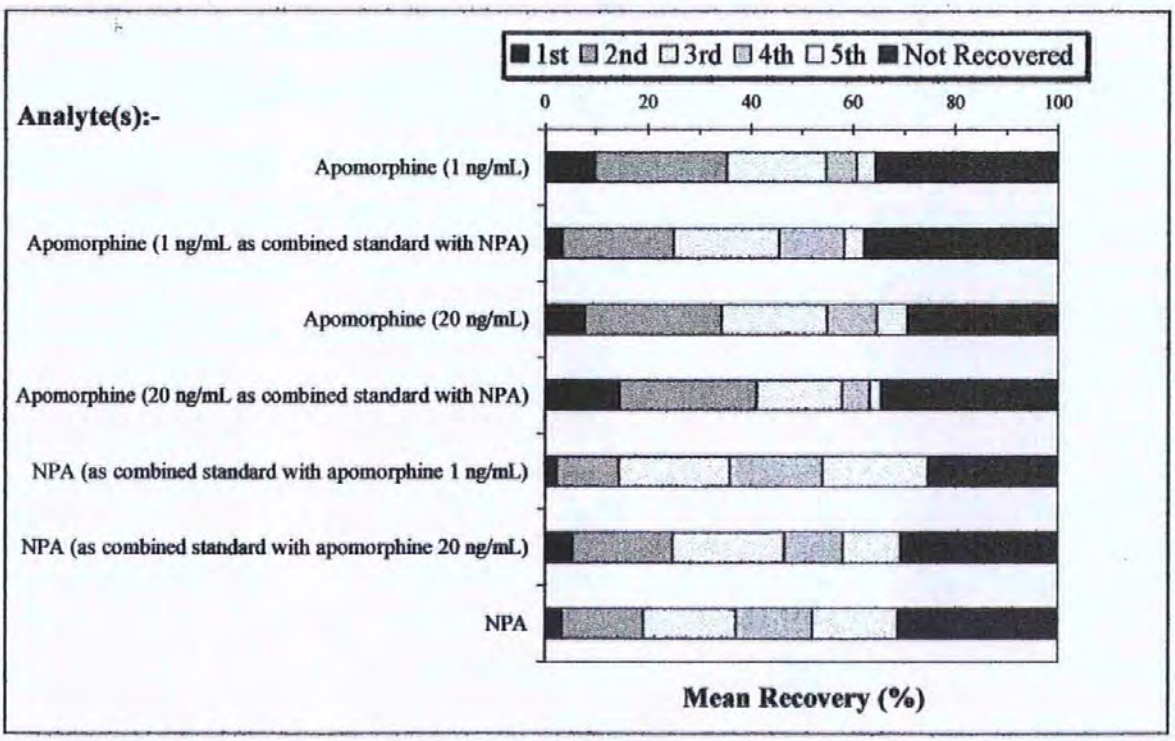


Figure 4-10 Recovery of analyte from plasma in successive portions of eluting solution.

A volume of 800 µL eluting solution was considered to be optimal, since this volume was the maximum that could be used to elute analytes without having the effect of diluting apomorphine extracted from samples of 1mL or less.

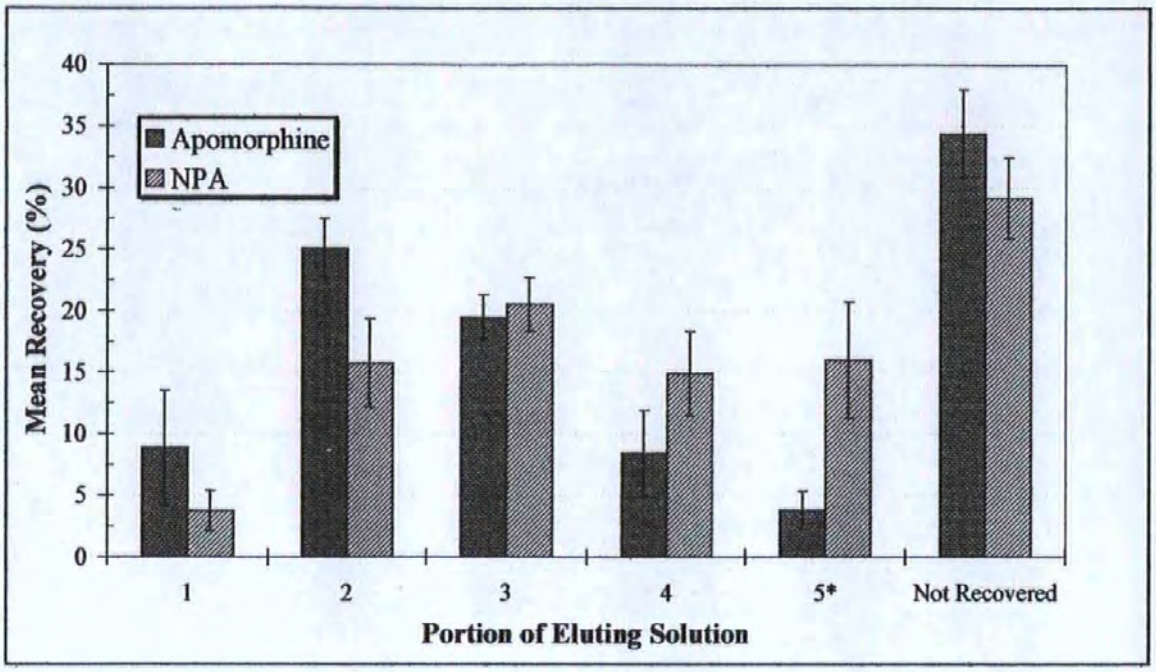


Figure 4-11 Elution profiles of apomorphine and NPA extracted from plasma.

4.3.4.5. Capacity of Sorbent Bonded Phase for Apomorphine and NPA.

The capacity of the bonded phase, i.e. the mass of analyte that a specific sorbent mass can maximally retain in a given solvent environment, was investigated. It was anticipated that clinical samples would yield approximately 2.5mL of plasma per sample point. Thus the linearity of response over a volume range of 0.25 to 2.5mL was studied.

Experimental.

Plasma was prepared according to Section 4.3.3 (page 4-35) such that 6.5mL aliquots of 3 and 20 ng/mL R(-)-apomorphine HCl in plasma were produced. Volumes of 0.25, 0.5, 1.0, 2.0 and 2.5mL of plasma at each apomorphine concentration were extracted using the method given in Section 4.4.1 and assayed according to Section 4.4.2 (pages 4-50 and 4-51).

Results.

The mean concentrations of apomorphine for the extractions of 3 and 20 ng/mL were calculated to be 3.0 ng/mL (S.D. = 0.2ng/mL, C.V. = 5.3%) and 19.0 ng/mL (S.D. = 0.5 ng/mL, C.V. = 2.4%), respectively (n=6).

There was no indication that the capacity of the solid phase extraction bonded phase had been reached. This was evidenced by the linearity of the response with increasing mass of analyte ($y = 1.3\text{x}10^5x - 1.9\text{x}10^5$, $R^2 = 0.9936$, $p<0.001$, see Figure 4-12).

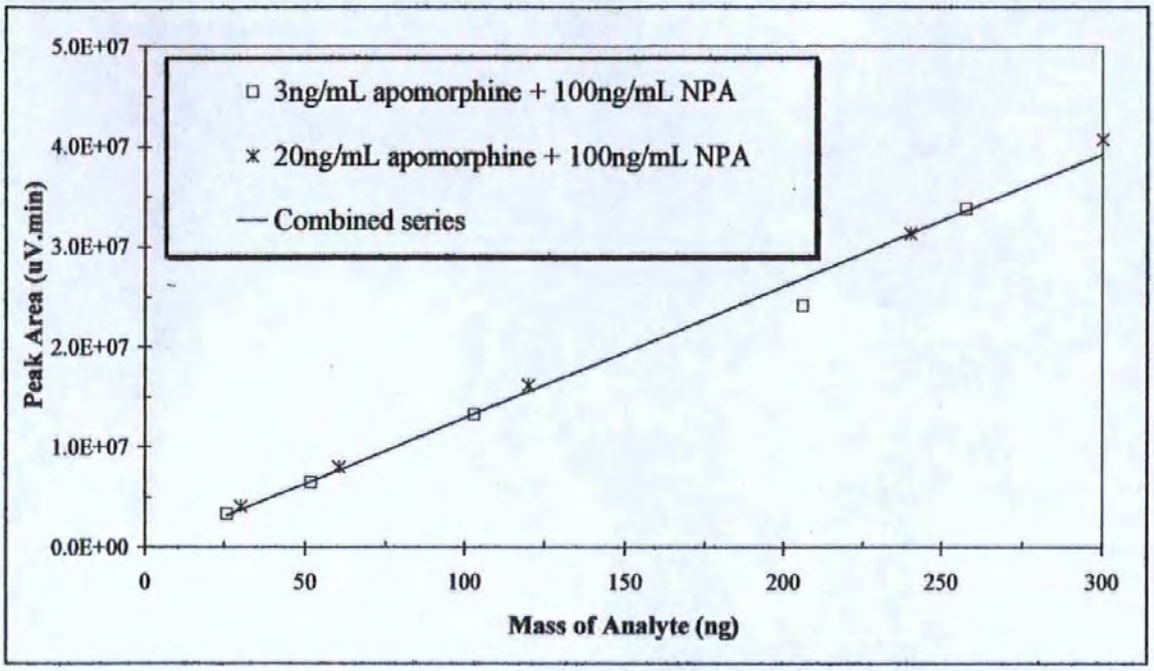


Figure 4-12 Evaluation of the capacity of sorbent (1mg x C18, Bond Elut, Varian, UK) in the extraction of apomorphine and NPA from plasma using method given in Section 4.4.1.

4.4. Final Analytical Method

4.4.1. Solid Phase Extraction

Apomorphine was extracted under vacuum using Bond-Elut C_{18} 1mL, 100mg SPE columns. With the column attached to a port in the extraction row[a] of the manifold, conditioning washes were applied, i.e. 2 x 1mL methanol followed by 2 x 1mL water. The sample was then passed through the column under negative pressure (approximately 9in Hg) and washed with 2 x 1mL distilled water followed sequentially by 1mL 10% (v/v) methanol in distilled water, 1mL 20% (v/v) methanol in distilled water, 1mL 50% (v/v) methanol in distilled water and finally 200µL of 2 % (w/v) sodium metabisulphite in diluent A. The column matrix was not allowed to dry out at any of the conditioning and washing stages. The column was transferred to a port in the elution row[b] and analytes were eluted with 800µL SPE eluting solution (0.25M sodium dihydrogen orthophosphate (pH 3.30), 40 % (v/v) methanol) into an autosampler vial containing 200µL 2 % (w/v) sodium metabisulphite in diluent A. The vial was sealed and placed at 4°C prior to assay by L.C.

In order to avoid contamination of subsequent extractions with residual analyte, the elution port manifold was washed with 5mL x 0.01M HCl in 50 % (v/v) methanol , followed by 10mL distilled water and air-dried using suction generated by the vacuum pump.

[a] The front row of the solid phase extraction manifold was designated as the extraction row.
[b] The back row of the solid phase extraction manifold was designated as the elution row.

4.4.2. HPLC System

The solvent delivery system (LDC Analytical constaMetric 3200) supplied mobile phase at a flow rate of 1 mL/min. The mobile phase contained 60% (v/v) aqueous and 40 % (v/v) methanol portions. The constituents of the aqueous portion, expressed as final concentrations in the mobile phase, were: 0.25 M sodium dihydrogen orthophosphate and 0.25 % (w/v) heptane sulphonic acid which were adjusted to pH 3.30 with orthophosphoric acid, and 0.003 % (w/v) EDTA.

The L.C. column employed was a C18, 5 μm, 150 mm x 4.6 mm I.D. (Columbus, Phenomenex, UK) used in conjunction with a 2 cm pre-column containing the same stationary phase as the main column.

The spectrofluorometer (FP-821 and later the FP-920, both Jasco, UK) configurations were: λex 270nm, λem 450nm, attenuation 1, gain 1000.

Samples were injected onto the column using the autosampler (AS-950, Jasco, UK), whereby an injection of 100 μL was made and the injection manifold was flushed (x3) with diluent A.

Data acquisition was performed using Borwin Chromatography software (v. 1.13, JMBS Developpements, France), according to parameters given in Table 4-7. Example chromatograms are presented in Figure 4-13.

Peak Parameters	Value
minimum slope (μV/minute)	0.500
minimum area (μV.minute)	4000
smoothing factor	13
minimum height (μV)	10
peak width calculated at:	50% height
number of theoretical plates calculated at:	50% height
peak asymmetry calculated at:	10% height
run time (minute)	18

Table 4-7 User-defined peak parameter settings.

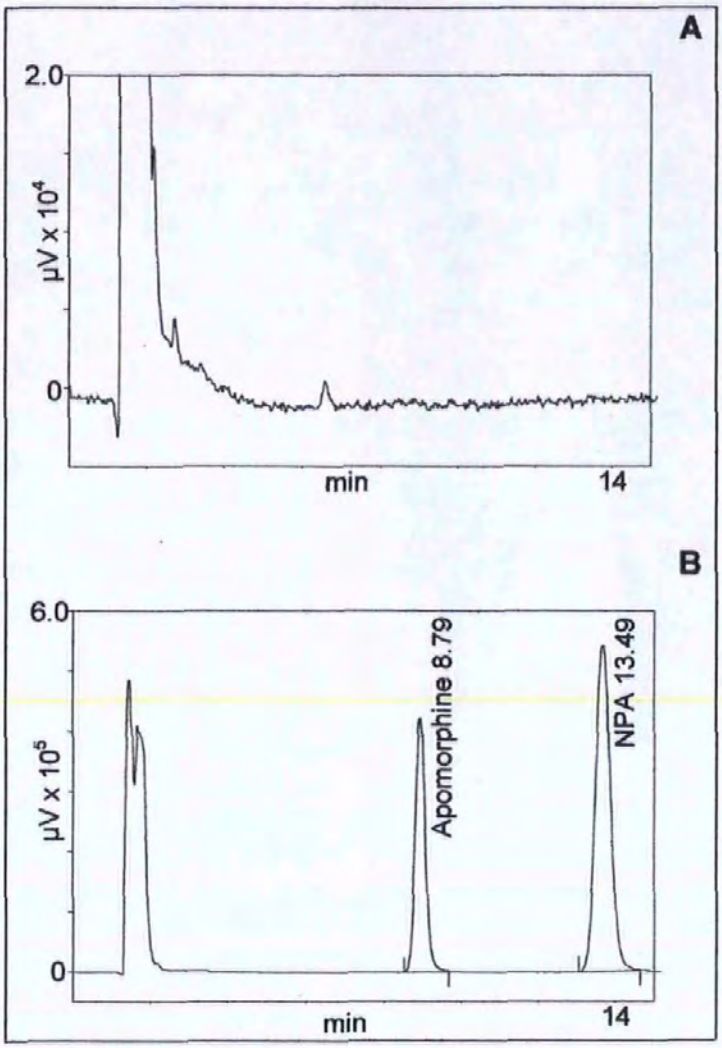


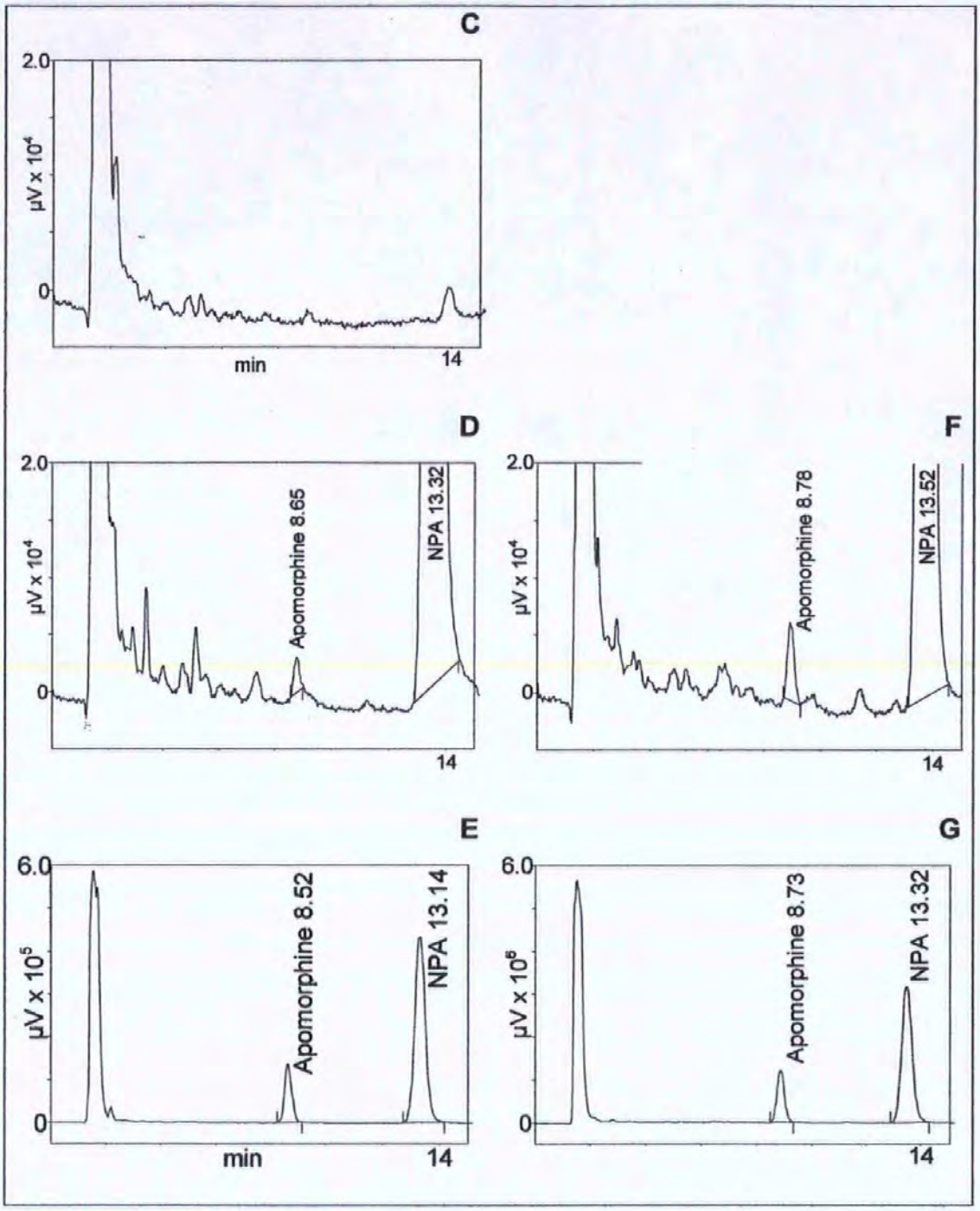
Figure 4-13 **Assay of R(-)-apomorphine HCl and R(-)-NPA HCl using the final analytical method.**

Panel A: diluent A.

Panel B: 50ng/mL R(-)-apomorphine HCl and 100ng/mL R(-)-NPA HCl in diluent A.

Retention times are given as peak labels.

continued ...



Panel C: blank plasma extract (patient 09[a], t=10 minutes prior to apomorphine bolus dose, 1.2mL of plasma extracted)..

Panel D: patient plasma extract (patient 09[a], t=240 minutes post-bolus, 2.0mL of plasma extracted): apomorphine concentration = 0.2 ng/mL.

Panel E: patient plasma extract (patient 09[a], t=6 minutes post-bolus, 1.4mL of plasma extracted): apomorphine concentration = 16.6 ng/mL.

Panel F: control plasma extract (1.0mL of plasma extracted): expected apomorphine concentration = 1.0ng/mL, observed apomorphine concentration = 1.1ng/mL.

Panel G: control plasma extract (1mL of plasma extracted): expected apomorphine concentration = 20ng/mL, observed apomorphine concentration = 19.7ng/mL

[a] (Needle-free) apomorphine bolus dose of 2 mg.

4.4.3. Evaluation of Published Extraction Methods.

As part of the apomorphine assay development, the extraction methods described by Durif *et al* [42], Sam *et al*[43], van der Geest *et al* [44], Bolner *et al* [45] and Ameyibor *et al* [41] were evaluated using the HPLC assay given in Section 4.4.2 (page 4-51). These particular methods were selected for evaluation since they were representative of the range of published techniques for apomorphine assay (see Appendix 8.11).

Experimental.

The performance of the extraction procedures was assessed using solutions of R(-)-apomorphine HCl in diluent A and in pooled plasma (e.g. 1 and 20 ng/mL), and also using blank matrices. Chromatographic peaks were identified on the basis of retention time; no further analysis leading to the definitive identification of peaks was performed. The recovery of apomorphine was determined by comparing the peak area of the extracted standard in plasma to that of a known standard in diluent A which had not been extracted (having taken account of the dilution or concentration of analyte in a given extraction method). Where satisfactory extraction was achieved, a precision of replicate extractions of apomorphine (and internal standard, i.e. NPA) was performed. The performance of the published methods was compared to the extraction method developed in-house (Section 4.4.1, page 4-50) using performance characteristics such as recovery and intra-batch precision as indicators of merit.

Durif *et al*[42].
Durif *et al* describe a solvent (ethyl acetate) extraction method which involves back-extraction of apomorphine (and internal standard) into an acidic aqueous phase for subsequent quantification by HLPC.

Results.
An unsatisfactory outcome was observed following extraction of apomorphine from both diluent A and pooled plasma (Figure 4-14), i.e. there was an absence of a peak at the retention window of apomorphine, coupled with the presence of additional peaks that were not evident in the blank matrices. The cause of the degradation was investigated; it was found that apomorphine was unstable in the presence of the ethyl acetate and that, from a practical point of view, it was difficult to entirely eliminate the organic solvent from the aqueous portion used for HPLC analysis.

Sam *et al*[43].
This method is the same as that given by Durif *et al* (above) with the exception that the solvent used is diethylether, and 2-mercaptoethanol is included in the acidic aqueous phase.

Results.
The outcome was similar to that described for Durif *et al*. Similar practical difficulties were experienced.

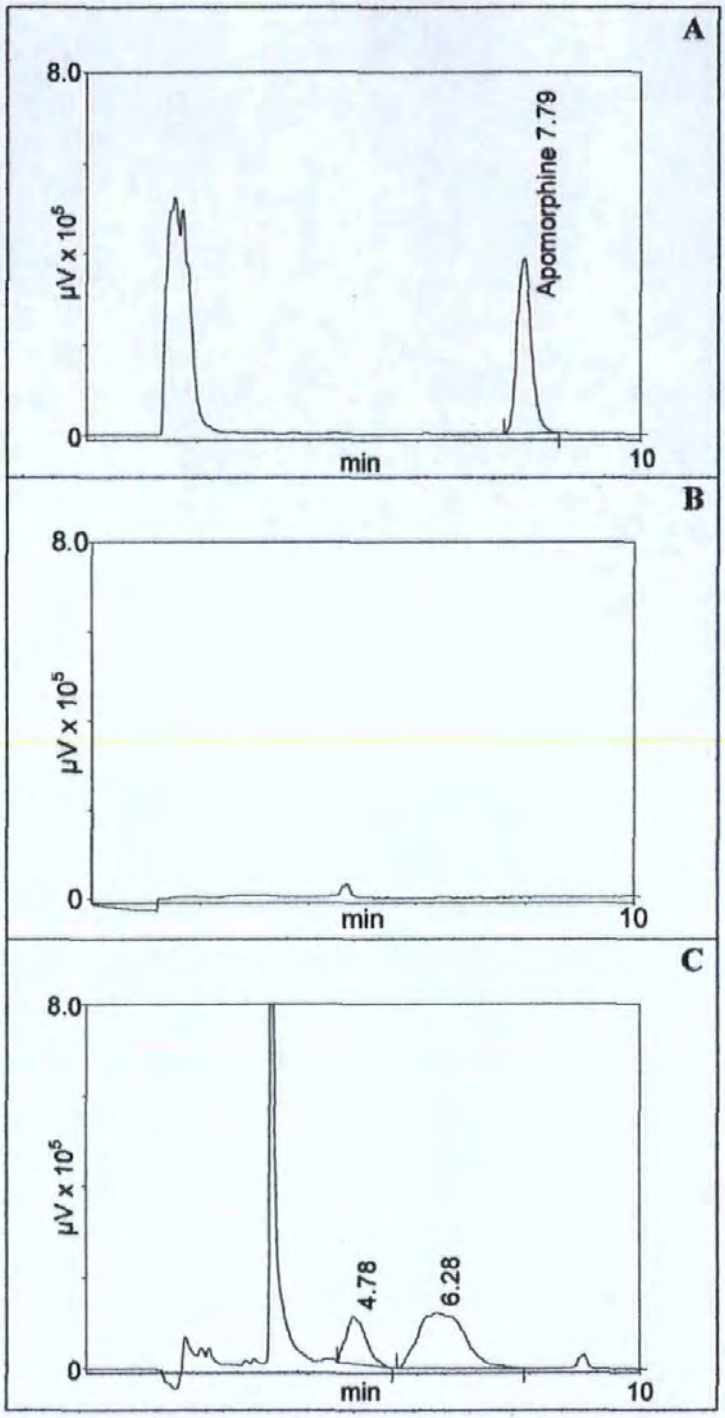


Figure 4-14 Extraction of apomorphine according to Durif[42].

Panel A: R(-)-apomorphine HCl standard (not extracted): 20 ng/mL in diluent A.

Extraction of blank plasma (Panel B) and 20 ng/mL R(-)-apomorphine HCl in plasma (Panel C).

Van der Geest *et al.*

Van der Geest *et al* describe a solvent extraction method in which plasma samples containing apomorphine are reacted with diphenylborinic acid ethanolamine ester (DPBEA) in an alkaline medium. Under these conditions the borate group of DPBEA specifically binds to the diol group of apomorphine, resulting in a negatively charged complex which is reported to confer stability to apomorphine during the extraction[39]. Organic solvent (octanol:hexane, 1:10) containing tetraoctyl ammonium bromide (TOABr) (as a cation) is mixed with the aqueous portion, with the result that an ion pair is formed with the apomorphine-DPBEA complex and TOABr. Consequently the ion pair is extracted into the organic phase, followed by back-extraction into an aqueous acidic phase, where, at low pH, apomorphine dissociates from the borate group of DPBEA.

The mean recovery of apomorphine from plasma (20ng/mL) compared to a standard in diluent A was 47% (S.D = 5%, n=5). (The percentage recovery given in the published article was, at 79% for a 25ng/mL standard of apomorphine in plasma, substantially higher than that achieved in this evaluation). In this the extraction method given by Van der Geest compared unfavourably to the in-house method (page 4-50), for which a mean recovery of 74% (S.D = 5, n=7) was demonstrated for the extraction of 20ng/mL apomorphine in plasma (see Section 4.5.2.3, page 4-89).

The performance of the two methods were similar with regards to intra-batch precision of extractions of apomorphine in plasma (20ng/mL), 1.9% (n=5) for the method described by van der Geest, and 2.1% (n=7) for the in-house method (see Section 4.5.3.1, page 4-90).

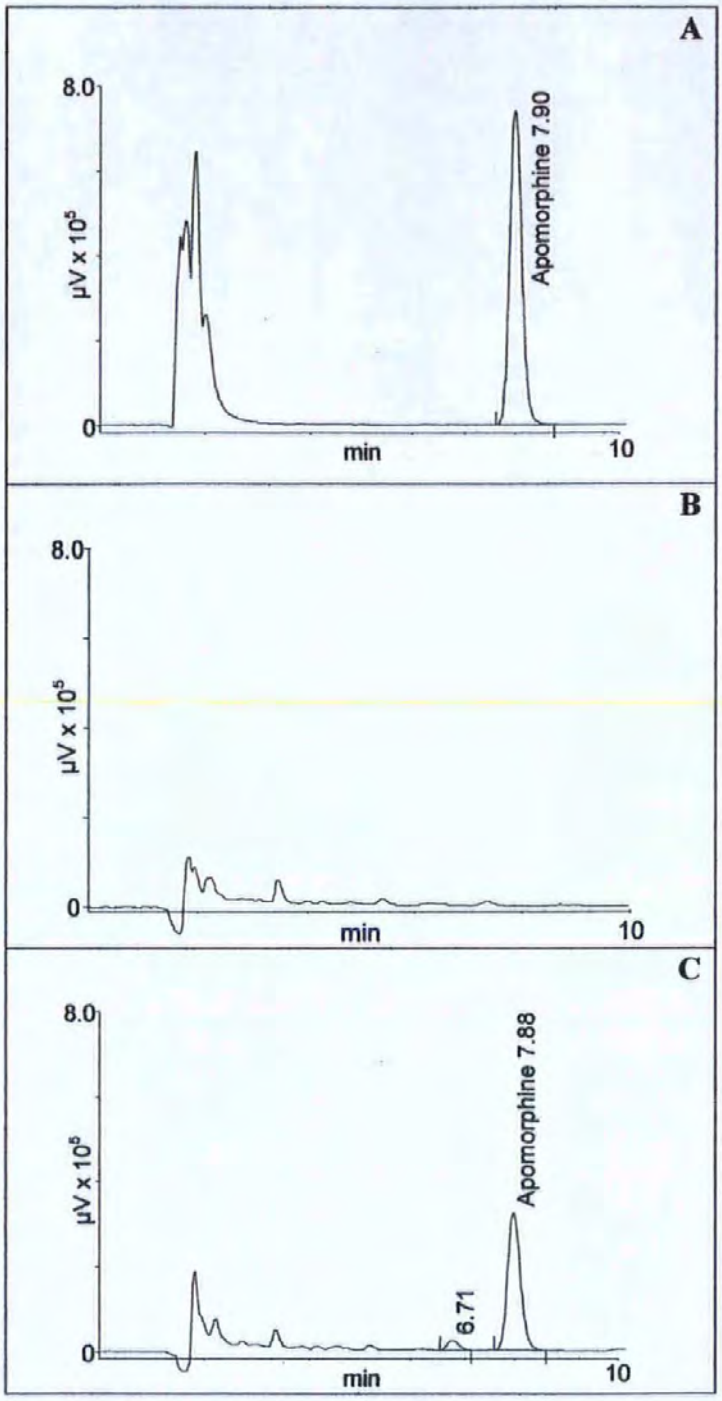


Figure 4-15 Extraction of apomorphine according to van der Geest[44].

Panel A: R(-)-apomorphine HCl standard (not extracted): 40 ng/mL in diluent A, represents 100% recovery of sample in Panel C

Extraction of blank plasma (Panel B) and 20 ng/mL R(-)-apomorphine HCl in plasma (Panel C).

Bolner *et al.*

Bolner *et al* describe an extraction method whereby apomorphine is extracted from plasma using alumina. The extraction of analytes from plasma is performed in the presence of tris buffer. The alumina pellet is repeatedly washed with distilled water, and apomorphine is eluted into perchloric acid in acetonitrile. One volume of the acid supernatant is then mixed with two volumes of phosphate buffer, and a sample of this mixture is injected onto the HPLC column.

Results.

An unsatisfactory outcome was observed following extraction of apomorphine from both diluent A and pooled plasma (Figure 4-16), i.e. recovery was only 3 % and 4%, respectively, and there were additional peaks present that were not evident in the blank matrices.

The cause of the degradation was investigated; it was found that apomorphine was not stable in the extraction nor the injection mixture. This is illustrated in Figure 4-16F.

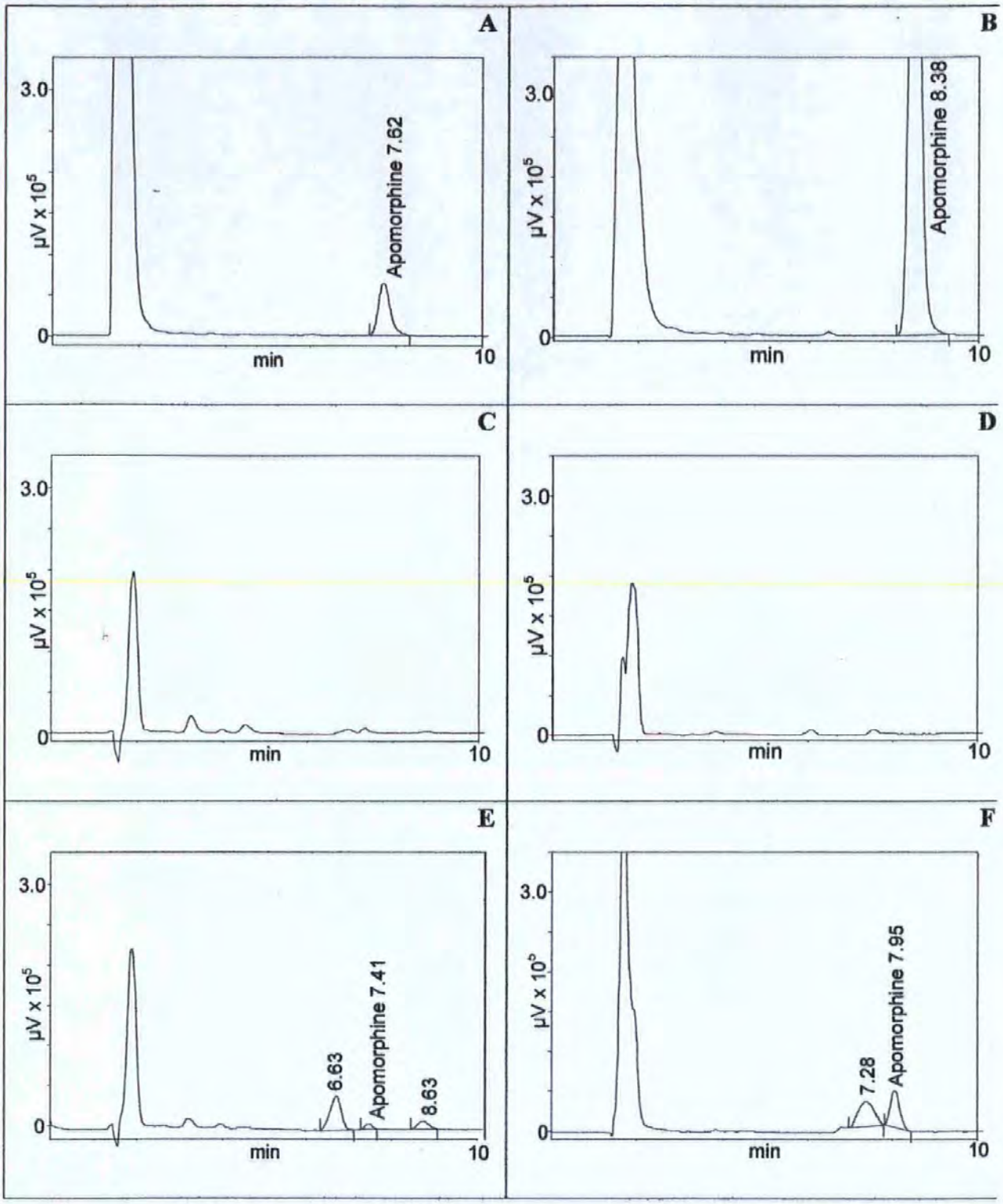


Figure 4-16 Extraction of apomorphine according to Bolner[45].

Panel A: R(-)-apomorphine HCl standard (not extracted): 6.7ng/mL in diluent A, represents 100% recovery of sample in Panel E.
Panel B: R(-)-apomorphine HCl standard (not extracted): 50ng/mL in diluent A.
Panel C: Extraction of: blank plasma
Panel D: Extraction of blank diluent A.
Panel E: Extraction of 20ng/mL R(-)-apomorphine HCl in plasma
Panel F: R(-)-apomorphine HCl standard (not extracted): 50ng/mL in injection mixture, i.e. 0.2M perchloric acid in acetonitrile and 0.6M phosphate buffer pH 3.6 (1:2, v/v)

Ameyibor *et al*[41].

This method involves solid phase extraction of apomorphine, whereby, having applied a sample of apomorphine solution to the conditioned solid phase extraction column, the column is washed with a solution of potassium dihydrogen phosphate (pH 7.4) and methanol (7:3, v/v), and apomorphine (plus internal standard) is eluted with a solution of potassium dihydrogen phosphate (pH 3.0) and methanol (3:7, v/v). The eluant is evaporated under nitrogen prior to reconstitution of extracted analytes with mobile phase (the volume of which did not constitute concentration-step), and subsequent injection onto the HPLC column.

Results.

There was evidence that apomorphine had degraded as a result of the extraction procedure, i.e. peaks with equivalent retention times to those resulting from the forced degradation of apomorphine were present (Figure 4-17, also see Section 4.5.1.3, page 4-73). The mean recovery of apomorphine from plasma (20ng/mL) compared to a standard in diluent A was 58% (S.D = 9%, n=5). (The percentage recovery given in the published article was, at 98% (n=3), substantially higher than that achieved in this evaluation). The extraction method given by Ameyibor compared unfavourably to the in-house method (page 4-50), for which a mean recovery of 74% (S.D = 5%, n=7) was demonstrated for the extraction of 20ng/mL apomorphine in plasma (see Section 4.5.2.3, page 4-89).

An assessment of intra-batch precision of extractions of apomorphine in plasma (20ng/mL) was made using the method given by Ameyibor and compared to in-house data. In this the two methods were similar; precision being 2.2% (n=5) for the method described by Ameyibor, and 2.1% (n=7) for the in-house method (see Section 4.5.3.1, page 4-90).

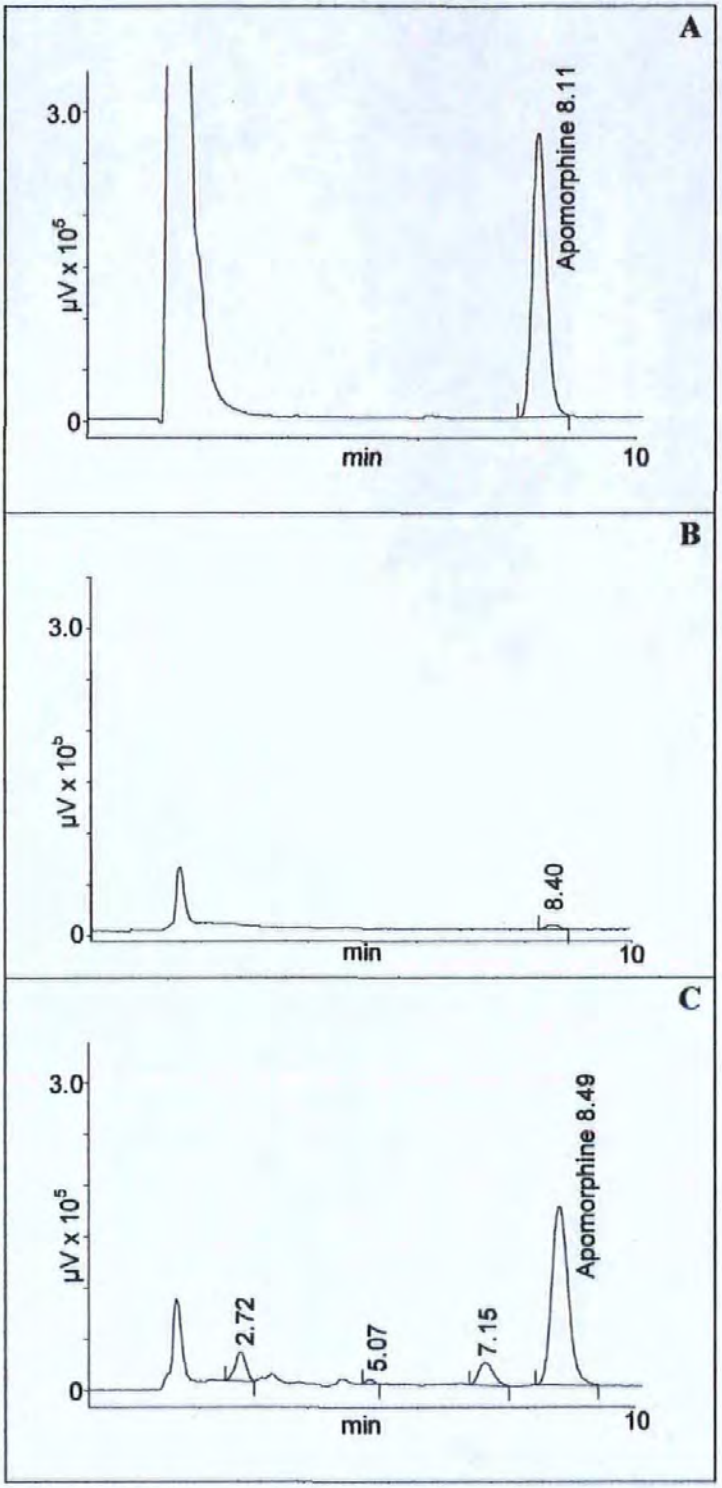


Figure 4-17 Extraction of apomorphine according to Ameyibor[41].

Panel A: R(-)-apomorphine HCl standard (not extracted): 20 ng/mL in diluent A. (represents 100% recovery of sample in Panel C)

Extraction of blank plasma (Panel B[a]) and 20 ng/mL R(-)-apomorphine HCl in plasma (Panel C).

[a] Samples were contaminated by carry-over of apomorphine. Carry-over was estimated to be at a level of 1.4% of the peak area of the standard (20ng/mL in diluent A).

General Outcome of the Evaluation of Published Methods.

Theoretically the published extraction methods offered certain potential advantages over the in-house method, e.g. a concentration-step[42, 44] or smaller volume of plasma[42, 43], however, the (brief) evaluation revealed that in general there were no major benefits afforded by the published methods in comparison with the in-house method. The performance of the extraction methods given in the published articles could not be not reproduced in the evaluation performed. Factors which may have contributed to this include deficiencies in the robustness or in the stability indicating properties, of the published methods. Also, certain practical difficulties were experienced, e.g. in the isolation of the aqueous (analyte-containing) portion in the solvent extraction methods of Durif *et al* and Sam *et al*, which greatly contributed to the poor performance of the methods in comparison to published data.

4.5. Analytical Method Validation.

4.5.1. Assay Selectivity.

The ability of the assay to detect the analyte in the presence of endogenous compounds and to separate the analyte from degradation products, co-administered drugs and metabolites, was investigated.

4.5.1.1. Assay Specificity.

Experimental.

Blood was collected from six individual normal controls using the method given in Section 4.3.3 (page 4-35) and assayed according to methods given in Section 4.4 (pages 4-50 to 4-51).

Results.

A lack of response across the retention time windows of apomorphine and NPA was demonstrated in six independent sources of plasma. There was no evidence of interference from endogenous compounds in the sample matrices that were analysed (Figure 4-18).

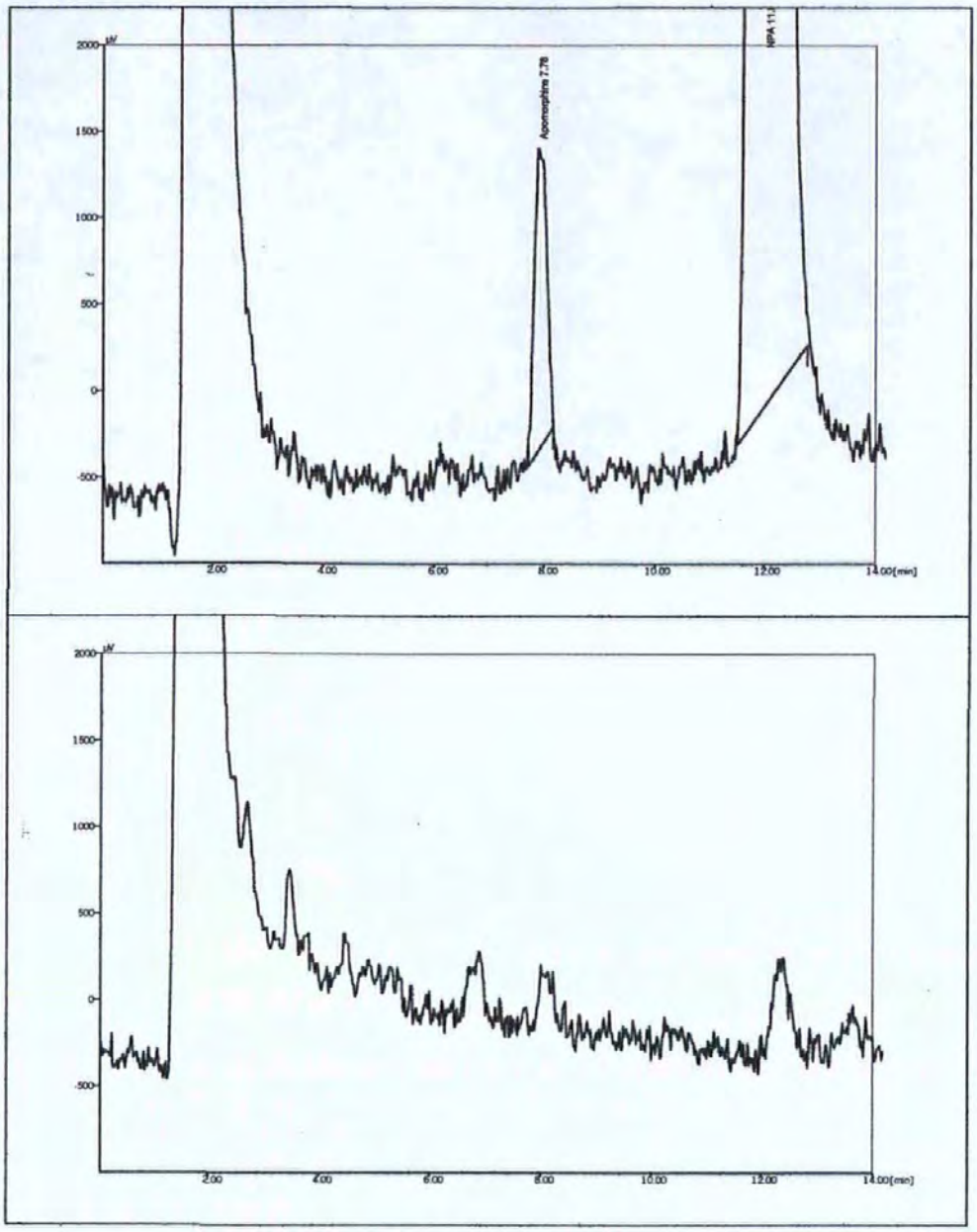


Figure 4-18 Assay specificity. Retention times are given as peak labels

Panel A: R(-)-apomorphine HCl (1ng/mL) and R(-)-NPA HCl (100ng/mL) in diluent A.

Panel B: Typical chromatogram of blank plasma collected from normal control.

4.5.1.2. Peak Purity.

Conventional spectroscopic detectors monitor at a discrete wavelength only. Photodiode array detection (DAD) allows the continuous monitoring of column effluent over a wavelength range of 190 to 800nm, resulting in an absorption spectrum for any point of interest on the chromatogram.

Thus the use of DAD permits the purity of a peak to be calculated. Spectra from within a peak are compared against other spectra in that same peak, to give a spectral similarity index (S.S.I.)[a], where S.S.I. = 1 represents a pure peak.

Having obtained the UV spectrum for a given peak, this can be stored electronically in a spectral library and compared against other spectra of interest. In the comparison of spectra for identification purposes, a S.S.I. of greater than or equal to 0.98 is indicative that a match has been obtained[46]. However, since UV spectra are not as distinctive as mass spectra, or indeed infrared spectra, further analysis is desirable for unequivocal identification.

The peak purity for apomorphine and NPA, both in diluent A and extracted from plasma, were obtained.

Experimental.

The assay was identical to the method given in Section 4.4.2 (page 4-51), with the exception of the detector. A detector with DAD capabilities (UV6000LP, Thermo Separations Products, UK) was employed specifically for the determination of peak purity. The detector operated over a wavelength range of 198 to 798nm. The data acquisition

[a] Computational formula for spectral similarity index: S.S.I. = A*B / (N-1), where A is the first normalised spectra (a vector of normalised absorbance versus time), B is the second normalised spectra, and N is the number of points in the vector.

software used was Chromquest (Thermo Separations Products, UK). Peak purities were calculated using a scan threshold of 5mAU and a peak coverage requirement of 95%. Solutions of R(-)-apomorphine HCl and R(-)-NPA HCl (both 1μg/mL in diluent A) were assayed under the conditions given above. R(-)-Apomorphine HCl and R(-)-NPA HCl (both 1μg/mL) were extracted from plasma according to the method given in Section 4.4.1 and assayed under the conditions given above.

Results.

S.S.I. of 0.995 and 0.990 were obtained for apomorphine and NPA in diluent A, respectively, and 0.995 and 0.992 for apomorphine and NPA extracted from plasma, respectively. These results indicate that there was no evidence of co-elution of an additional product with apomorphine or NPA in the standard solutions used or following extraction of each from plasma.

Furthermore, having stored the spectra of the non-extracted and extracted analytes in a spectral library (Appendix 8.12), the identity of the peaks present in the chromatogram (at the retention times of apomorphine and NPA) following extraction from plasma were verified in that (i) the spectra of extracted apomorphine matched that of non-extracted apomorphine in diluent A with an S.S.I. of 0.995, and (ii) the spectra of extracted NPA matched that of non-extracted NPA in diluent A with an S.S.I. of 0.993 (Figure 4-19).

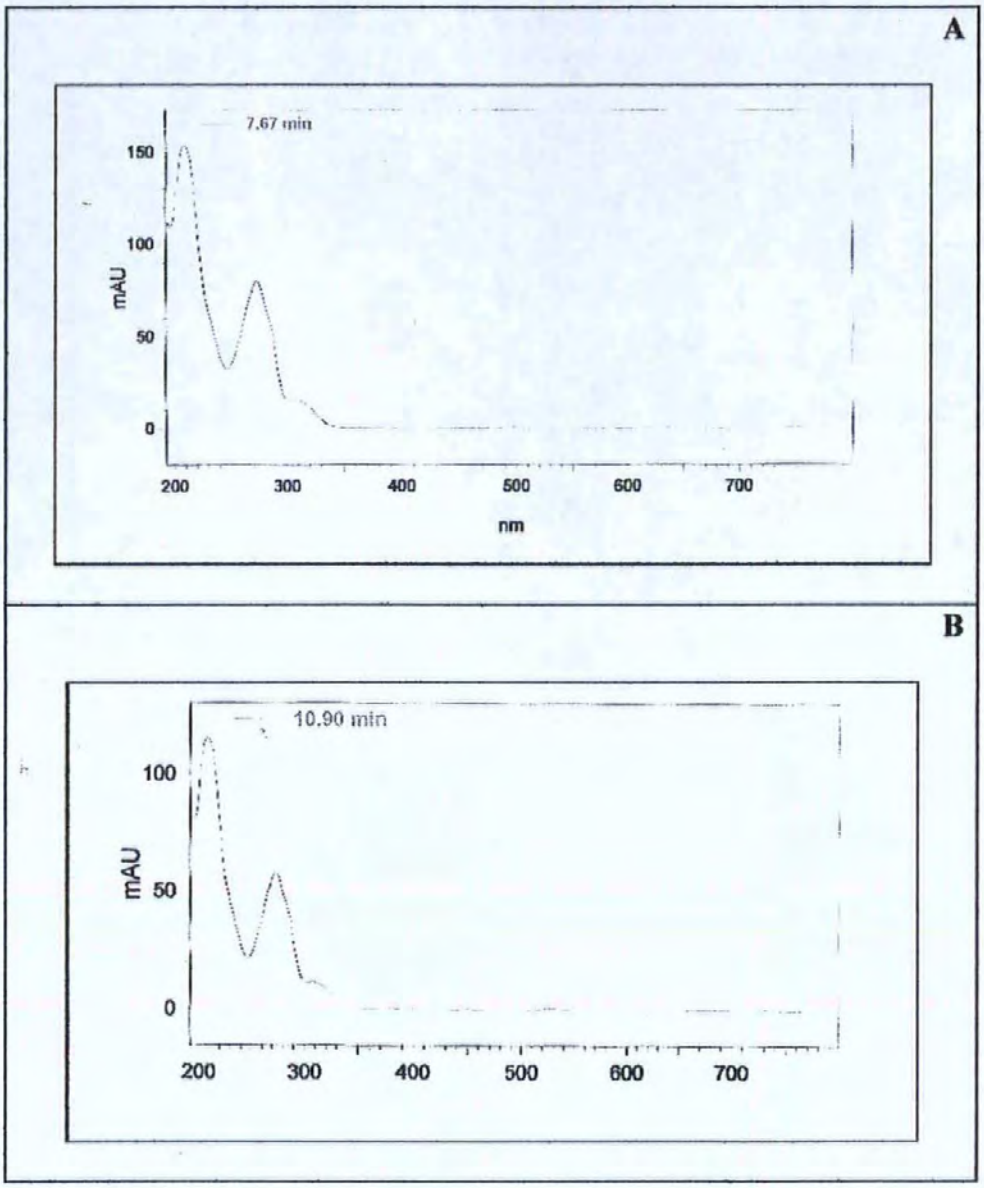


Figure 4-19 Peak spectra.

Panel A: R(-)-apomorphine HCl (1μg/mL) in diluent A.

Panel B: R(-)-NPA HCl (1μg /mL) in diluent A.

4.5.1.3. Stability Indication.

A stability indication study was performed in order to establish whether apomorphine and NPA could be distinguished, in terms of retention time, from their degradation products. Thus apomorphine and NPA in solution were forcibly degraded under acidic, alkaline and oxidative conditions, and by heating.

Experimental 1.

Aliquots of R(-)-apomorphine HCl (1 mL x 0.5µg/mL in distilled water) and R(-)-NPA HCl (1mL x 1.0µg/mL in distilled water) were reacted separately with the following:-

- 1mL x 0.1M hydrochloric acid for:
  - 30 minutes at 63⁺/-1°C[a]
  - 10 minutes at 63⁺/-1°C[a]
  - 1 minute at room temperature (approximately 25°C)[a]
  - 3 seconds at room temperature (approximately 25°C),
- 1mL x 0.1M sodium hydroxide for
  - 30 minutes at 63⁺/-1°C[a]
  - 10 minutes at 63⁺/-1°C[a]
  - 1 minute at room temperature (approximately 25°C)[a]
  - 3 seconds at room temperature (approximately 25°C),
- 1mL x 6 vol. hydrogen peroxide at 63⁺/-1°C for 30 minutes,
- 1mL x distilled water at 63⁺/-1°C for 30 minutes,
- 1mL x distilled water at 4-8°C for 30 minutes as a control for the effect of heating.

Solutions of R(-)-apomorphine HCl (50 ng/mL) and R(-)-NPA HCl in diluent A (100 ng/mL) stored at 4-8°C acted as controls for the use of distilled water as a diluent.

The acidic and alkaline solutions were neutralised after the reaction period by addition of 1mL 0.1M sodium hydroxide and 0.1M hydrochloric acid, respectively.

[a] Performed for apomorphine only.

Subsequently, each reaction mixture and control solution was made up to volume with distilled water to give a final concentration of 50 ng/mL for apomorphine and 100 ng/mL for NPA. All solutions were protected from light throughout. Final solutions were then assayed using the method given in Section 4.4.2 (page 4-51).

Results 1.

The solutions of apomorphine and NPA remained colourless during the reaction and/or incubation period.

Chromatograms which illustrate the following results are given in Figures 4-20 and 4-21 (pages 4-71 and 4-72). A summary of results is given in Table 4-8 (page 4-73). The effect of using water as a diluent was to reduce the peak area of apomorphine by 19 % and NPA by 2 % as compared to control solutions in diluent A. Incubation of the analyte solutions in distilled water at approximately 60°C for 30 minutes caused a reduction in peak area of 21 and 75 % for apomorphine and NPA, respectively, as compared to peak areas of the control solutions stored at 4-8°C.

There was a large instantaneous reduction (90 – 96 %) in peak area of both analytes when exposed to an acidic or alkaline environment.

Compounds were detected in the test solutions that were absent from the control solutions of analyte in diluent A. The reactions of apomorphine with hydrochloric acid, sodium hydroxide and distilled water generated a peak which eluted at 2.7 minutes. The reactions of NPA with hydrochloric acid, sodium hydroxide and distilled water resulted in a peak at a retention time of 3.2 minutes.

The reaction of both apomorphine and NPA with hydrogen peroxide resulted in a peak at 7.2 minutes retention time. This peak was consistent, in terms of retention behaviour, with the peak produced as a result of solid phase extraction of apomorphine using the method of Priston[18] (see Figure 4-7, page 4-51).

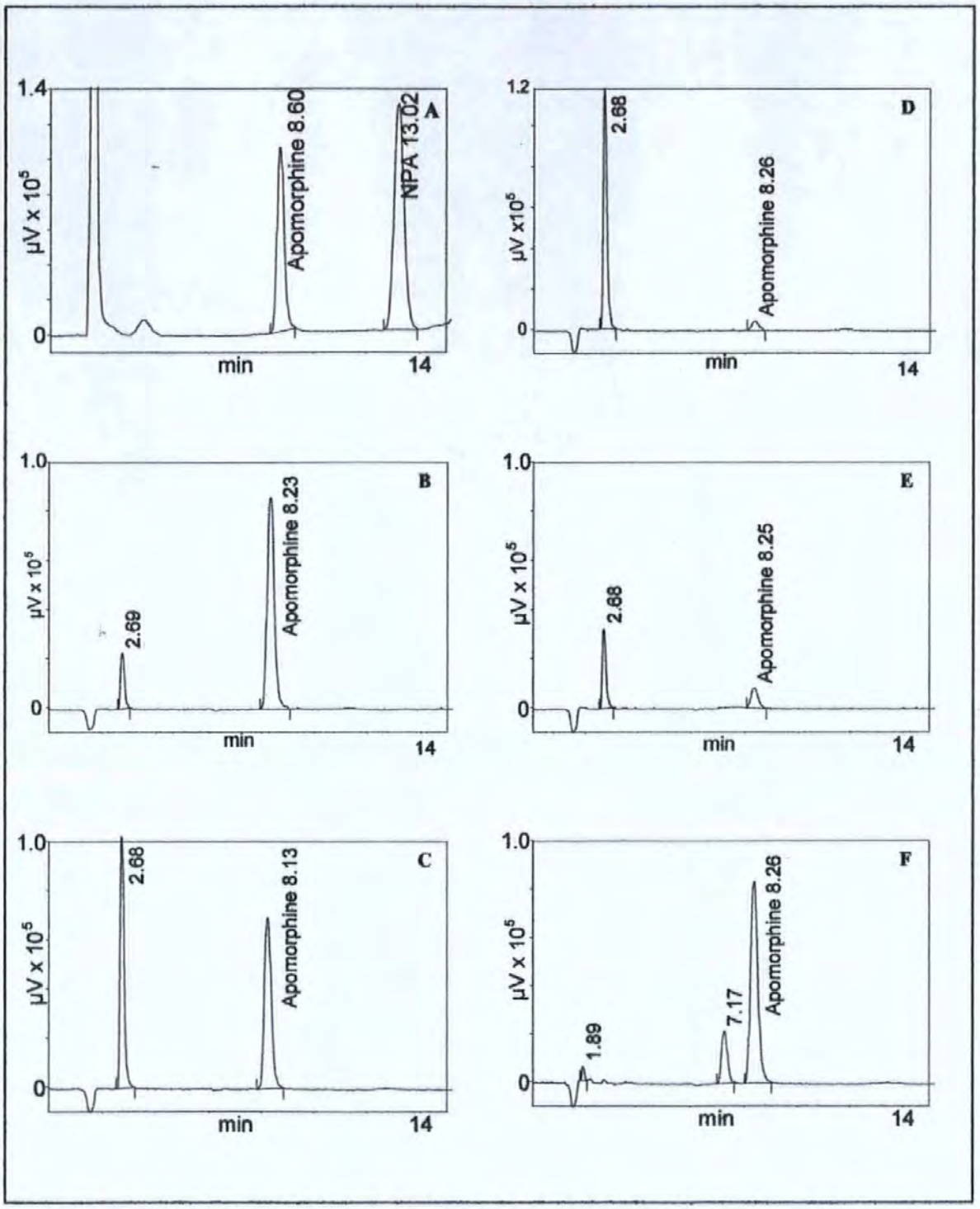


Figure 4-20 Forced degradation of R(-)-apomorphine HCl.

Panel A: R(-)-apomorphine HCl (50ng/mL) and R(-)-NPA HCl (100ng/mL) in diluent A (4-8°C, standard).

Panel B: Control in distilled water (4-8°C). Panel C: Control in distilled water (60°C). Panel D: Reaction with HCl (3s at 25°C). Panel E: Reaction with NaOH (3s at 25°C). Panel F: Reaction with H_2O_2.

Retention times are given as peak labels.

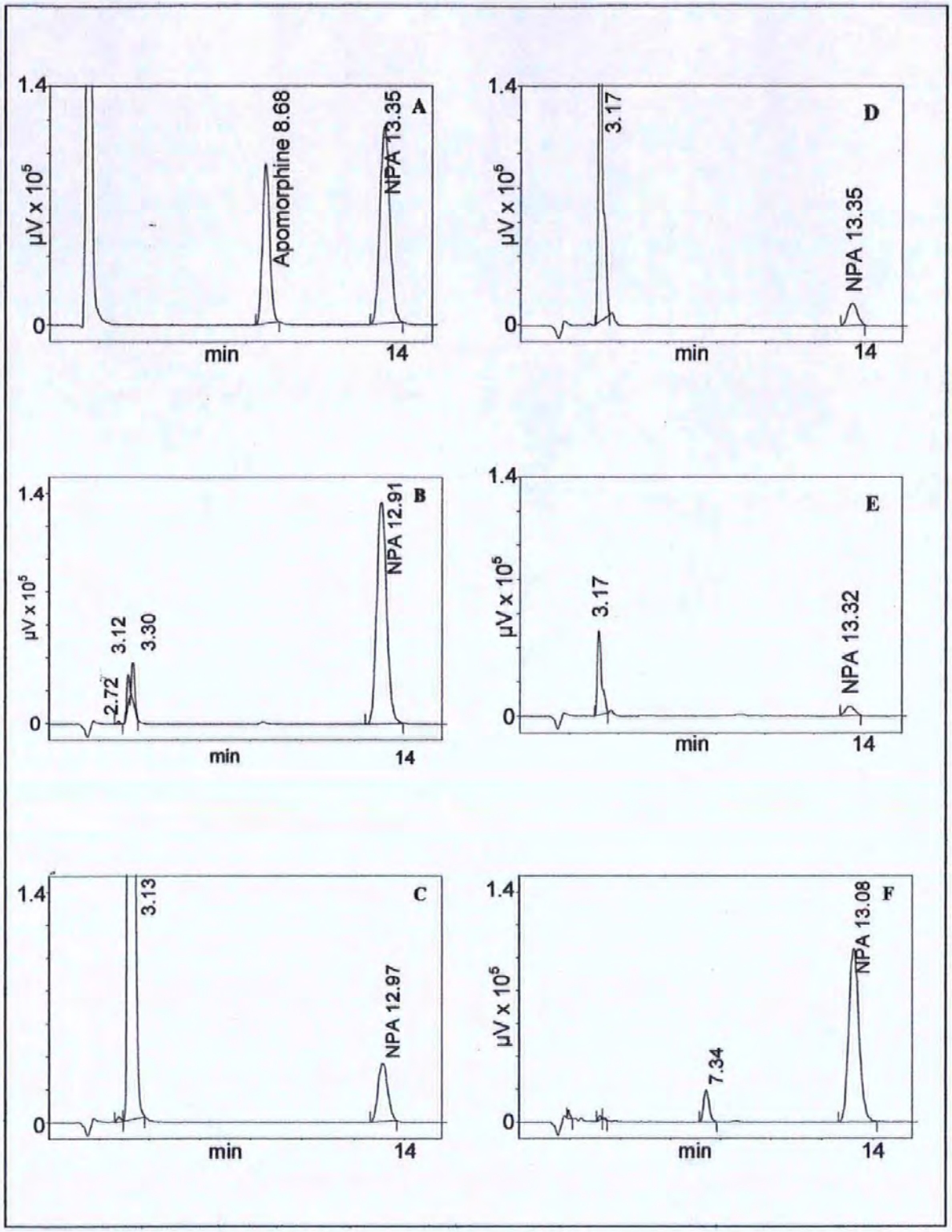


Figure 4-21 Forced degradation of R(-)-NPA HCl.

A: R(-)-apomorphine HCl (50ng/mL) and R(-)-NPA HCl (100ng/mL) in diluent A (standard, 4-8°C).

B: Control in distilled water (4-8°C), C: Control in distilled water (60°C), D: Reaction with HCl (3s at 25°C), E: Reaction with NaOH (3s at 25°C), F: Reaction with H_2O_2.

Retention times are given as peak labels.

Reaction Conditions.			% Apomorphine remaining:		Additional peaks t_R.(mins)	% NPA remaining:		Additional peaks t_R.(mins)
			cf H_2O control	*cf* diluent A control		*cf* H_2O control	*cf* diluent A control	
Control in diluent A	30 minutes	4-8°C.	NA	NA	None	NA	NA	None
Control in H_2O	30 minutes	4-8°C.	NA	81	2.6	NA	98	3.2, 3.4
	30 minutes	60°C.	79	65	2.6	25	28	3.1, 3.3
+Hydrochloric acid	30 minutes	60°C.	0	0	2.6	NA	NA	NA
	10 minutes	60°C.	NA	4	2.7	NA	NA	NA
	1 minute	25°C.	NA	8	2.7	NA	NA	NA
	3 seconds	25°C.	NA	4	2.7	10	11	3.2
+Sodium hydroxide	30 minutes	60°C.	0	0	None	NA	NA	NA
	10 minutes	60°C.	NA	2	2.2	NA	NA	NA
	1 minute	25°C.	NA	1	2.7	NA	NA	NA
	3 seconds	25°C.	NA	8	2.7	4	5	3.2
+Hydrogen peroxide	30 minutes	60°C.	122	79	7.2	312	77	7.3

Table 4-8 Stability indication of apomorphine and NPA (final concentrations of 50 and 100 ng/mL, respectively) using fluorescence detection.

Experimental 2.

On a separate occasion the forced degradation of apomorphine was repeated and the resultant solutions were assayed using DAD (see Section 4.5.1.2, page 4-66). The degradative conditions used were as those given previously (in *Experimental 1*, page 4-69), however a more concentrated solution of apomorphine in distilled water was used, i.e. 1µg/mL final concentration, due to the comparatively poor absorbance of apomorphine at UV wavelengths as opposed to the response obtained using fluorescence detection.

Peak spectra were obtained for the control solution of R(-)-apomorphine HCl, i.e. 1µg/mL in diluent A stored at 4-8°C, and for all peaks present in the test solutions as a result of forced degradation. Spectra were stored for reference in a spectral library (Appendix 8.12).

Results 2.

A summary of results is given in Table 4-9 (page 4-77). The outcome regarding percentage of parent peak remaining following the degradation process was comparable to that reported previously in *Results 1* (page 4-73).

Also in common with the initial findings was the presence of a peak at a retention time of 2.6 minutes when apomorphine was in solution with distilled water, and when subjected to acidic and alkaline environments. A comparison of the spectra of such peaks was performed. With an S.S.I. of 0.998, the spectra of the peaks produced on contact with acid and alkali exhibited high similarity, the peak produced on incubation with distilled water being below the scan threshold (see Section 4.5.1.2, page 4-61). On incubation of apomorphine with hydrogen peroxide, two peaks which were not present in the control solutions were detected (Figure 4-22), one of which (with a retention time of 9.3 minutes) was not detected using fluorescence detection (see Figure 4-20F, page 4-71).

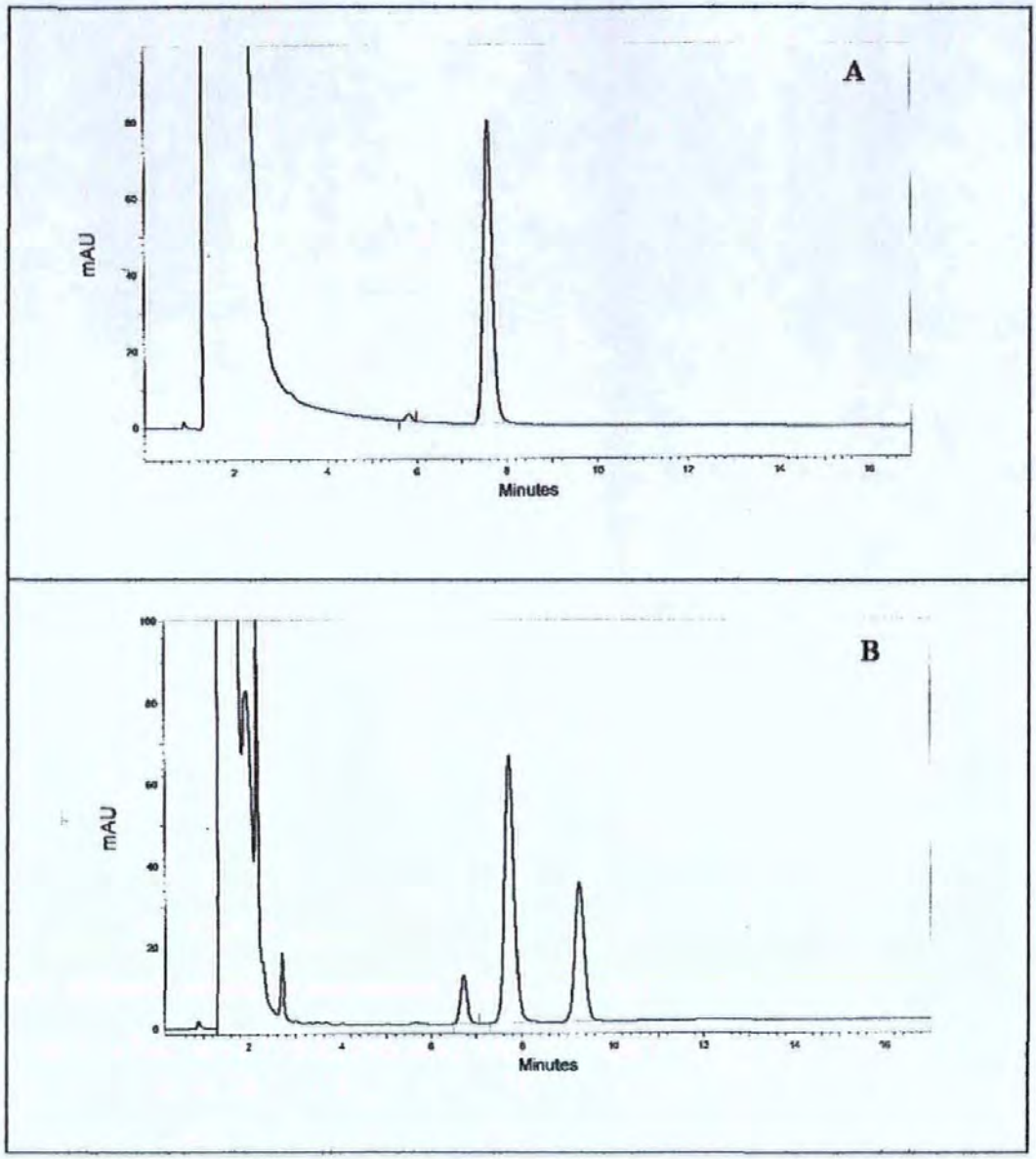


Figure 4-22 Forced degradation of R(-)-apomorphine HCl under oxidative conditions (detection wavelength of 270nm).

Panel A: Standard (1µg/mL) in diluent A (4-8°C).

Panel B: Reaction with H_2O_2.

NB. Scale: 0 to 17 minutes, 0 to 100 mAU.

It was demonstrated that the assay was stability indicating for degradation products forcibly produced under acidic, alkaline, and oxidative conditions.

Reaction Conditions	% Apomorphine Remaining:		Spectra Library Match	S.S.I.	Apomorphine Peak Purity	Additional Peaks			
	cf H_2O control	*cf* diluent A control				t_R. (mins)	Peak Purity	Spectra Library Match	S.S.I.
Control in diluent A (30 minutes, 4-8°C).	NA	NA	NA	NA	0.996	None	NA	NA	NA
Control in H_2O (30 minutes, 4-8°C).	NA	94	Apomorphine in dil. A Apomorphine in H_2O	0.998 0.998	0.999	2.7	*	*	NA
(30 minutes, 60°C).	47	42	Apomorphine in dil. A Apomorphine in H_2O	0.999 0.999	0.920	2.7	*	*	NA
+Hydrochloric acid (3 seconds, 25°C).	6	6	*	NA	*	2.7	*	Apomorphine degradation by NaOH	0.998
+Sodium hydroxide (3 seconds, 25°C).	3	3	*	NA	*	2.7	*	Apomorphine degradation by HCl	0.998
+Hydrogen peroxide (30 minutes, 60°C).	219	96	Apomorphine in dil. A Apomorphine in H_2O	1.000 1.000	0.987	6.5 9.5	0.977 0.987	None	NA

Table 4-9 Stability indication of apomorphine (final apomorphine concentration of 1μg/mL) using UV-DAD system.

Abbreviations: dil = diluent, * = peak below scanning threshold of 5 mAU.

4.5.1.4. Co-Administered Drugs.

Drugs which are commonly encountered in anti-parkinsonian drug regimens were assayed using the method given in Section 4.4.2 (page 4-51). Paracetamol was also investigated since this drug was made available to volunteers in the studies of intranasal and buccal apomorphine formulations in healthy subjects (see Sections 4.2.2 and 4.2.3, pages 4-24 and 4-31).

Experimental.

The following preparations were assayed according to the method given in Section 4.4.2:-

- bromocriptine (approximately 10 mg/mL in distilled water)
- cabergoline (approximately 40 µg/mL in water)
- co-beneldopa (approximately 33 mg/mL levodopa in distilled water, approximately 8 mg/mL benserazide in distilled water)
- co-careldopa (approximately 10 mg/mL levodopa in distilled water, approximately 1 mg/mL carbidopa in distilled water)
- domperidone (approximately 10 mg/mL in distilled water)
- entacapone (approximately 67 mg/mL in distilled water)
- lisuride (approximately 20 µg/mL in distilled water)
- paracetamol (approximately 100 µg/mL in distilled water)
- pergoline (approximately 1 mg/mL in distilled water)
- pramipexole (approximately 180 µg/mL in distilled water)
- selegiline (500 µg/mL in distilled water).

Results.

Whilst compounds were detected in each of the solutions given above, generally these were not retained on the analytical column to any significant extent and thus eluted mainly at the solvent front. This is illustrated using co-beneldopa in Figure 4-23.

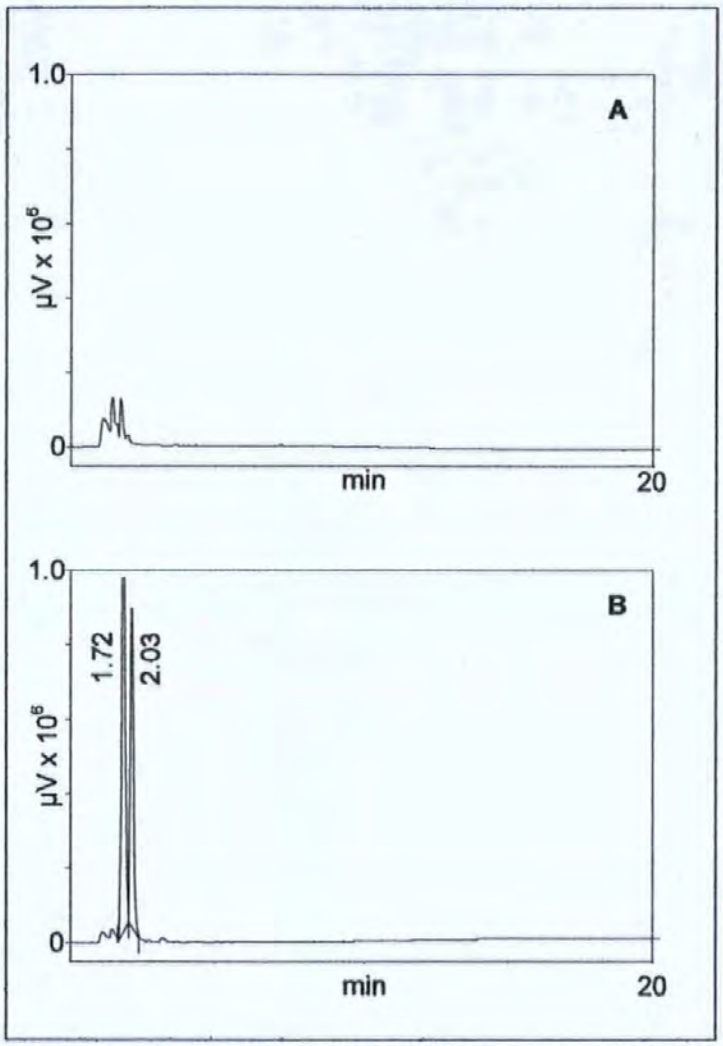


Figure 4-23 Assay of co-beneldopa.

Panel A: distilled water.

Panel B: co-beneldopa (approximately 33 mg/mL levodopa in distilled water, approximately 8 mg/mL benserazide in distilled water).

Retention times are given as peak labels.

There were no cases of interference with apomorphine or NPA determination, with the exception of the selegiline solution, in which a peak at the retention time of apomorphine was detected. It was demonstrated that, following solid phase extraction according to the method given in Section 4.4.1 (page 4-50), the interference was entirely removed from the extract, with approximately 93 % (in terms of peak area compared to the non-extracted solution) of the interfering compound eluted in the 50 % methanol wash and approximately 6% in the sodium metabisulphite in diluent A wash (Figure 4-24).

Thus it was proposed that co-administration of any of the drugs tested would not adversely affect the assay of apomorphine, at least in terms of co-elution of the constituents (parent drug and excipients) of the aforementioned drugs with apomorphine or NPA. However, co-elution of the metabolites of the drugs tested with either apomorphine or NPA was not examined and therefore remained a possibility.

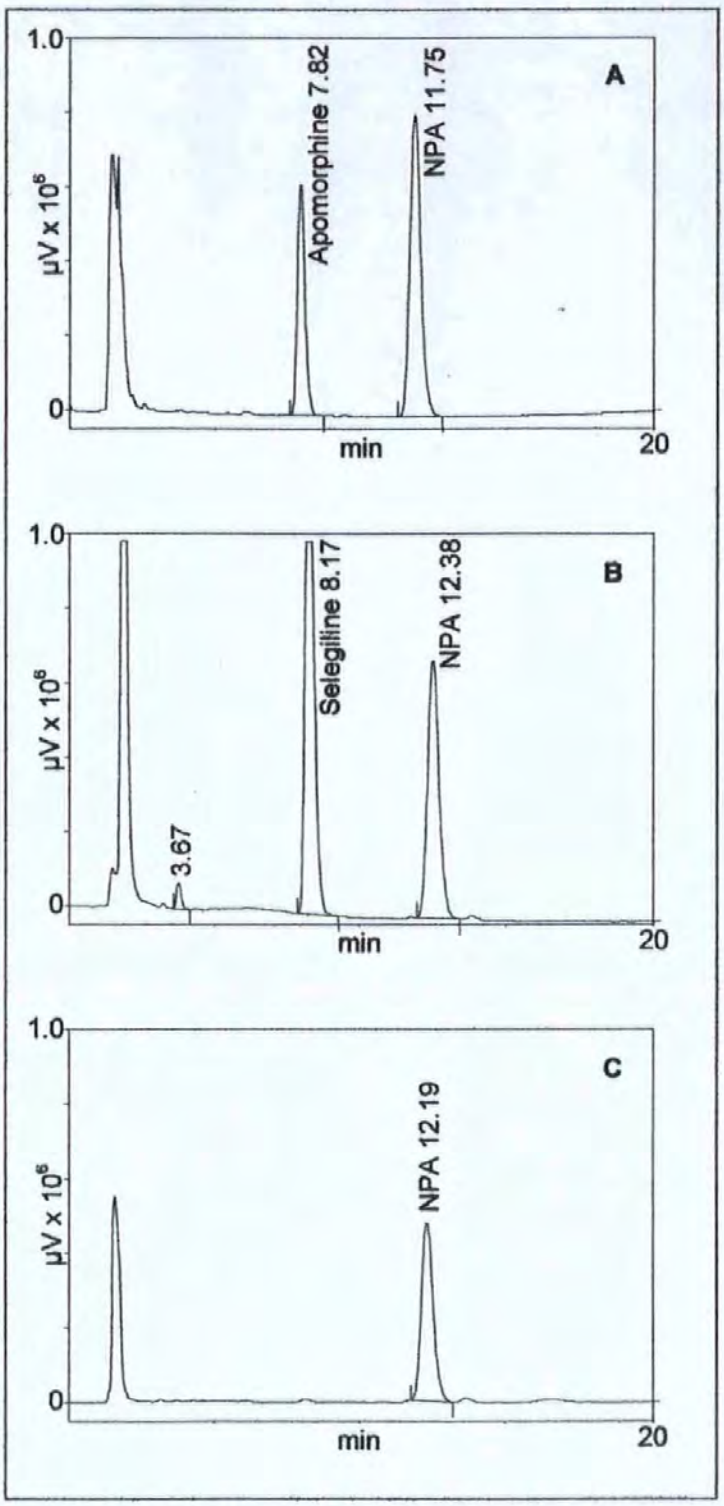


Figure 4-24 Assay of selegiline. Retention times are given as peak labels.

Panel A: R(-)-apomorphine HCl (50ng/mL) and R(-)-NPA HCl (100ng/mL) in diluent A.

Panel B: Selegiline (0.25mg/mL) and R(-)-NPA HCl (100ng/mL) in distilled water (prior to solid phase extraction).

Panel C: Result of extraction of solution given in Panel B.

4.5.1.5. Metabolites.

Experimental.

The proposed apomorphine metabolites, R(-)-apocodeine HCl (Sigma-Aldrich Company Ltd, UK) and R(-)-apomorphine orthoquinone (SPA Contract Synthesis, UK) were assayed according to the method given in Section 4.4.2.

Results.

Apocodeine typically eluted at approximately 17 minutes (Figure 4-25B). There were two peaks present due to apomorphine orthoquinone, the retention times of which were approximately 9 and 10 minutes (Figure 4-25C).

It was demonstrated that apomorphine and NPA could be distinguished in terms of retention time from both apocodeine and apomorphine orthoquinone.

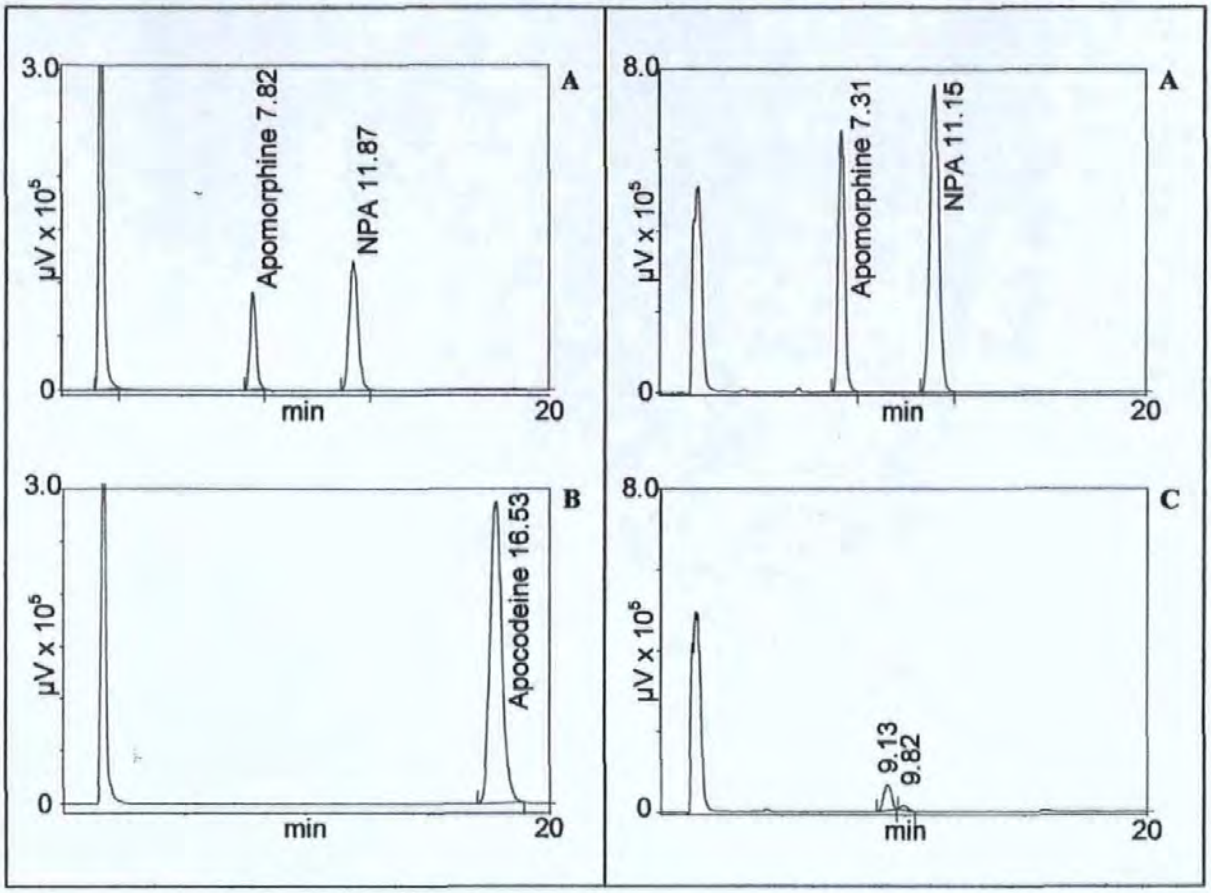


Figure 4-25 Assay selectivity with respect to proposed apomorphine metabolites.

Panel A: R(-)-apomorphine HCl (50ng/mL) and R(-)-NPA HCl (100ng/mL) in diluent A.

Panel B[a]: R(-)-Apocodeine (50ng/mL) in diluent A.

Panel C[b]: R(-)-Apomorphine orthoquinone (0.14mg/mL) in diluent A.

Retention times are given as peak labels.

[a] The detector used in the assay of apocodeine, apomorphine and NPA, i.e. left-hand panel, was the FP-821 (Jasco, UK).

[b] The detector used in the assay of apomorphine orthoquinone, apomorphine and NPA, i.e. right-hand panel, was the FP-920 (Jasco, UK).

4.5.2. Assay Calibration.

4.5.2.1. Apomorphine Calibration Curve in Plasma.

Experimental.

Calibration standards were prepared using plasma which was obtained according to the method given in Section 4.3.3.1 (page 4-35). The calibration curve was constructed using fifteen standards which represented the expected range of apomorphine concentrations, i.e. up to 75.0ng/mL. The standards were prepared by adding the appropriate volume of stock R(-)-apomorphine HCl solution (10μg/mL, 1μg/mL, 75ng/mL or 10ng/mL in diluent A) to plasma, whereby the volume used was between 0.30 and 2.50% of the total volume of the calibration standards. Sufficient volumes of calibration standards were prepared to allow for extraction of 1mL of plasma for the 1 to 75ng/mL standards, and 2.5mL of plasma for 0.03 to 0.5 ng/mL standards. Calibration standards in plasma were then stored in polypropylene tubes at –20°C. Preparation of the standards was completed using the method given in 4.3.3.3 (page 4-35) and assayed according to methods given in Section 4.4 (page 4-50). Linear regression analysis was performed using MS Excel.

Results.

It was found that the response was not linear over the entire range of concentration standards, as evidenced by systematic deviation in the relative error[a] of predicted apomorphine concentration with respect to expected apomorphine concentration (Table 4-10). Residual plots were used as a guide to the identification of linear relationships within the concentration range used. The calibration curve was defined by three such partial concentration ranges, i.e. 0.05 to 0.75ng/mL (n=5), 0.75 to 10ng/mL (n=5) and 5 to 70ng/mL (n=6), see Figure 4-26 and Table 4-10.

[a] Relative error (%) = ((expected [apomorphine] – observed [apomorphine])/(expected [apomorphine])).100

The point of intersection of the linear regression equations were used to define the useful range of the curves, i.e. 0.80 ng/mL for the low and mid range plots, and 12.7ng/mL for the mid and high range plots. The range of the lower concentration calibration curve was defined as the limit of quantitation (0.05 ng/mL, see Section 4.5.2.2, page 4-88) up to the point of intersection of the curves

The mean (S.D.) relative error of the observed versus calculated data points was –2.6 (11.2) %, n=14. The mean absolute value of the relative error of the observed versus calculated data points was 8.3%.

Expected [apo] (ng/mL) x_i	Observed [apomorphine] (ng/mL) y=0.0158x+0.0068,	Relative error (%) R^2=0.9960	Observed [apomorphine] (ng/mL) y=0.0339x+0.0020,	Relative error (%) R^2=9709	Observed [apomorphine] (ng/mL) y=0.0142x+0.0181,	Relative error (%) R^2=0.9995	Observed [apomorphine] (ng/mL) y=0.0160x-0.0047..	Relative error (%) R^2=0.9930
0.030	<3N	NA						
0.050	-0.21	521.4	0.06	9.8				
0.075	-0.11	245.7	0.09	-23.0				
0.100	-0.05	145.8	0.12	-21.7				
0.500	0.63	-25.3	0.41	17.9				
0.750	1.43	-90.0	0.81	-7.4	0.78	-4.6		
1.00	2.19	-119.3			2.90	3.2		
3.00	3.34	-11.3			5.02	-0.4		
5.00	5.25	-5.0			7.60	-1.4	5.89	-17.9
7.50	7.58	-1.1			9.94	0.6		
10.00	9.69	3.1					10.28	-2.8
20.00	17.99	10.1					18.45	7.8
35.00	36.84	-5.3					37.03	-5.8
50.00	46.57	6.9					46.62	6.8
70.00	72.08	-3.0					71.76	-2.5

Table 4-10 Calibration curve standards: relative error analysis.

Abbreviations: <3N = apomorphine peak area was less than three times the baseline (blank mobile phase) noise, NA = not applicable.

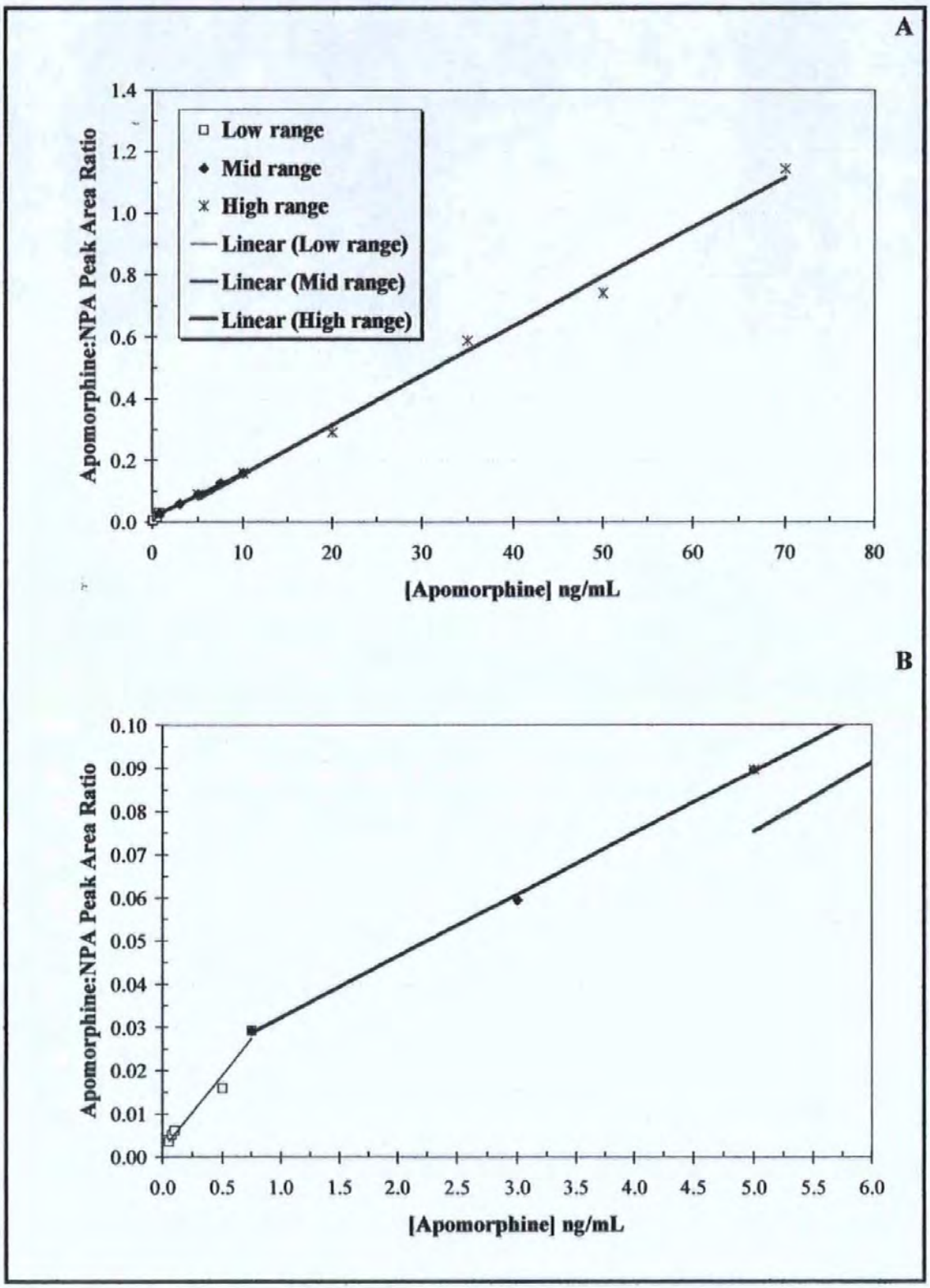


Figure 4-26 Calibration curve of R(-)-apomorphine HCl in plasma.

4.5.2.2. Assay Detection Limits.

The limit of quantitation (LOQ), i.e. the lowest concentration of apomorphine that could be distinguished from the noise level at a signal:noise ratio of 10:1[47] was 0.05ng/mL (extracted from 2.5mL of plasma). This is illustrated in Figure 4-27, where the baseline noise in blank mobile phase across the retention window of apomorphine was visually estimated to be approximately 700μV, and the peak height of 0.05ng/mL apomorphine extracted from plasma was measured as 6880μV. The limit of detection (LOD) is defined as the lowest concentration of apomorphine that could be distinguished from the noise level at a signal:noise ratio of 3:1[47]. The LOD was therefore defined as the concentration of apomorphine which corresponded to an apomorphine peak height 2100μV; this was 0.03ng/mL (extracted from 2.5mL of plasma), with a peak height of 2416μV.

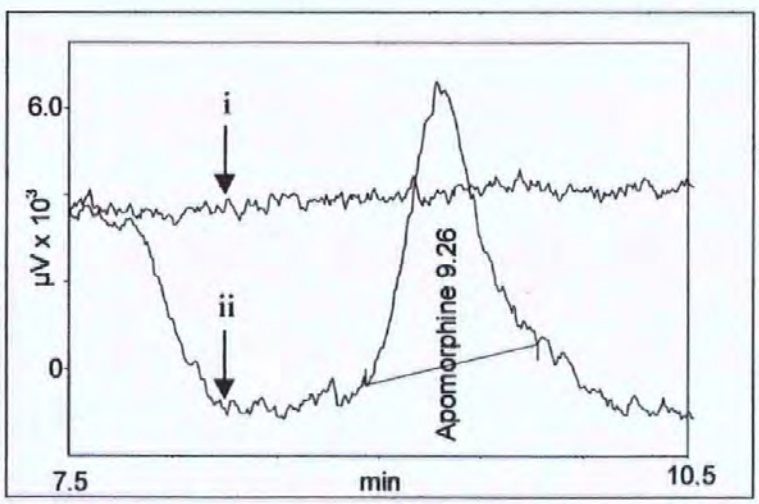


Figure 4-27 Definition of assay lower detection limits.

(i) Baseline noise in blank mobile phase across the retention window of apomorphine.

(ii) Extraction of 0.05ng/mL apomorphine in control plasma (2.5mL of plasma extracted).

4.5.2.3. Recovery of Analyte.

The recovery of analyte, i.e. the peak area of the standard in plasma expressed as a percentage of the peak area of a standard which had not been subjected to pre-treatment, was calculated for all 1mL extractions of apomorphine and NPA over a calibration curve range (n=8, range 1 to 50 ng/mL).

Experimental.

The calibration standards in plasma which are described in Section 4.5.2.1 (page 4-84) were used. The control solutions of apomorphine were prepared in diluent A by adding the appropriate volume of stock R(-)-apomorphine HCl solution (10µg/mL, 1µg/mL or 75ng/mL in diluent A), whereby the volume used was between 0.35 and 2.00% of the final volume of the control. These were then spiked to a final concentration of 100ng/mL R(-)-NPA HCl using a stock solution of 5µg/mL in diluent A, gently mixed, and stored at 4-8°C prior to assay using the method given in Section 4.4.2 (page 4-51).

Results.

Data are presented in Table 4-11. The mean recovery of apomorphine was 72% with a S.D. of 5% and C.V. of 7% (n=8). The mean recovery of NPA was 67% with a S.D. of 2% and a C.V. of 3% (n=8). The recovery of each analyte, whilst being less than maximal, was considered to be acceptable given the consistency in recovery throughout the calibration standard range[48].

Apomorphine standard (ng/mL) + NPA (100 ng/mL)	Recovery of apomorphine (%)	Recovery of NPA (%)
1.0	79.9	66.8
3.0	79.0	65.0
5.0	70.2	68.7
7.5	66.9	68.3
10.0	74.1	68.2
20.0	68.3	64.5
35.0	68.8	68.8
50.0	69.7	67.5

Table 4-11 Recovery of analyte following solid phase extraction from plasma.

4.5.3. Assay Precision.

4.5.3.1. Intra-Batch Precision: Quantification of Apomorphine in Plasma.

Experimental.

Apomorphine standards were prepared according to Section 4.3.3 (page 4-35), with the exception that the following concentrations of apomorphine were prepared: 0.5, 20 and 50ng/mL. Solid phase extraction (Section 4.4.1, page 4-50) was performed with seven individual aliquots of each apomorphine standard in a single session. The resultant extracts were assayed using the method given in Section 4.4.2 (page 4-51).

Results.

Data are presented in Table 4-12. Using the criteria that the precision around the mean should not exceed 15% of the C.V., and the mean value should be within +/-15% of the deviation of the nominal value for accuracy[49], it was concluded that the assay operated within acceptable limits for precision and accuracy.

Apomorphine standard (ng/mL)	Mean [apomorphine] (ng/mL)	S.D (ng/mL)	C.V. (%)	Mean relative error (%)	Mean Recovery (%)	
					Apomorphine	NPA
0.5	0.54	0.09	8.13	7.14	73.4	58.6
20	18.26	0.76	2.08	-8.70	74.4	70.9
50	48.23	0.79	1.63	-3.54	67.6	55.9

Table 4-12 Intra-batch precision of R(-)-apomorphine HCl extracted from plasma (*n*=7).

4.5.3.2. Inter-Batch Precision: Quantification of Apomorphine in Plasma.

Experimental.

Apomorphine standards were prepared according to Section 4.3.3 (page 4-35) with the exception that the following concentrations of apomorphine were additionally prepared: 0.5, and 50ng/mL. Solid phase extraction (Section 4.4.1, page 4-50) was performed on single aliquots of each apomorphine standard once a day for seven days and the resultant extract was assayed according to method given in Section 4.4.2 (page 4-51).

Results.

Data are presented in Table 4-13. Using the criteria that the precision around the mean should not exceed 15% of the C.V., and the mean value should be within +/-15% of the deviation of the nominal value for accuracy[49], it was concluded that the assay operated within acceptable limits for precision and accuracy.

Apomorphine Standard (ng/mL)	Mean [Apomorphine] (ng/mL)	S.D. (ng/mL)	C.V. (%)	Mean relative error (%)	Mean Recovery (n=3) (%)	
					Apomorphine	NPA
0.5	0.49	2.5×10^{-2}	1.48	-1.14	75.6	65.9
1	0.87	3.4×10^{-2}	3.94	-1.14	78.3	55.9
20	19.69	1.1	5.66	-1.59	66.1	60.9
50	50.46	2.1	4.14	0.91	73.1	65.0

Table 4-13 Inter-day precision of R(-)-apomorphine HCl extracted from plasma (n=7).

4.5.3.3. Intra-Batch Precision: Quantification of Apomorphine in Extract.

Experimental.

Apomorphine standards were prepared according to Section 4.3.3 (page 4-35). Solid phase extraction (Section 4.4.1, page 4-50) was performed on 2 x 1mL aliquots of each apomorphine standard in plasma. For each standard concentration, the resulting extracts were pooled in an autosampler vial and five injections were made from each using the assay method given in Section 4.4.2 (page 4-51).

Results.

The C.V. of the apomorphine:NPA peak area ratio was 0.39 and 0.75% for 1 and 20 ng/mL apomorphine, respectively (n=5). This compared favourably to the intra-batch precision of apomorphine:NPA peak area ratios demonstrated for apomorphine standards of 1 and 20 ng/mL in diluent A, i.e. C.V.s of 0.66 and 0.52%, respectively, n=5 (Section 4.5.3.4, page 4-93).

Using the criteria that the precision around the mean should not exceed 15% of the C.V.[49], it was concluded that the assay operated within acceptable limits for precision and accuracy.

4.5.3.4. Intra-Batch Precision: Quantification of Apomorphine in Diluent A.

Experimental.

Solutions of 1 and 20ng/mL R(-)-apomorphine HCl in diluent A were prepared according to Section 3.5 (page 3-4) and each was assayed in replicate (n=5 injections) according to Section 4.4.2 (page 4-51).

Results.

The C.V. of the apomorphine:NPA peak area ratio was 0.66% for 1ng/mL and 0.52% for 20ng/mL (n=5). It was demonstrated that the assay operated within the acceptable limits given for precision of analyte in simple diluent, i.e. a C.V. of <1%.

4.5.3.5. Intra-Batch Precision: NPA in Diluent A.

A solution of 100ng/mL R(-)-NPA HCl in diluent A was prepared according to Section 3.5 (page 3-4) and assayed in replicate (n=5 injections) according to Section 4.4.2 (page 4-51).

Results.

The C.V. of the apomorphine:NPA peak area ratio was 0.18%; thus it was demonstrated that the assay operated within the acceptable limits given for precision of analyte in simple diluent, i.e. a C.V. of <1%.

4.5.4. Stability Studies.

The stability of apomorphine and internal standard under working assay conditions and at pertinent stages of the plasma preparation procedures was investigated.

All apomorphine stability studies were performed at two concentrations, i.e. 1 and 20ng/mL.

The assessment of stability was based on the following criteria:-

- potency of analyte; the acceptable limits for analyte stability were defined as $^{+}$/-2 x S.D. from the analyte concentration at time = 0, whereby the C.V. used was that obtained at time = 0 under directly comparable experimental conditions.
- appearance of degradation products, e.g. those identified in Section 4.5.1.3 (page 4-73).

4.5.4.1. Stability of Apomorphine in Plasma at -20°C Containing Ascorbic Acid.

The objective was to assess the long term stability of apomorphine in plasma stored at -20°C .

The limits for analyte stability were defined as $^{+}$/-2 S.D. from the analyte concentration at time = 0, whereby the C.V. used were those obtained for the intra- (and inter-day) precision of apomorphine in plasma (Sections 4.5.3.1 and 4.5.3.2, pages 4-90 and 4-91). Thus the acceptable variation from time zero was $^{+}$/-7.88 % for 1ng/mL apomorphine and $^{+}$/-11.32 % for 20ng/mL apomorphine.

Experimental.

On three separate occasions pooled plasma was prepared and spiked with apomorphine and internal standard as detailed in Section 4.3.3 (page 4-35). An aliquot from one of the three batches was assayed as given in Section 4.4 (page 4-50) on days 0, 1, 2, 4, 7, 10, 14 and weekly thereafter until day 161.

Results.

Chromatograms were scrutinised for the presence of peaks with equivalent retention times to those resulting from the forced degradation of apomorphine (see Section 4.5.1.3, page 4-73); no such peaks were found.

Although individual calculated concentrations went beyond pre-defined limits of variation for apomorphine in plasma, there was an absence of consecutive observations beyond the acceptable limits until day 126 and day 98 for 1 and 20 ng/mL apomorphine concentrations, respectively (Figure 4-28 and Figure 4-29), therefore the time period of refrigerated storage for apomorphine in plasma was defined as 98 days.

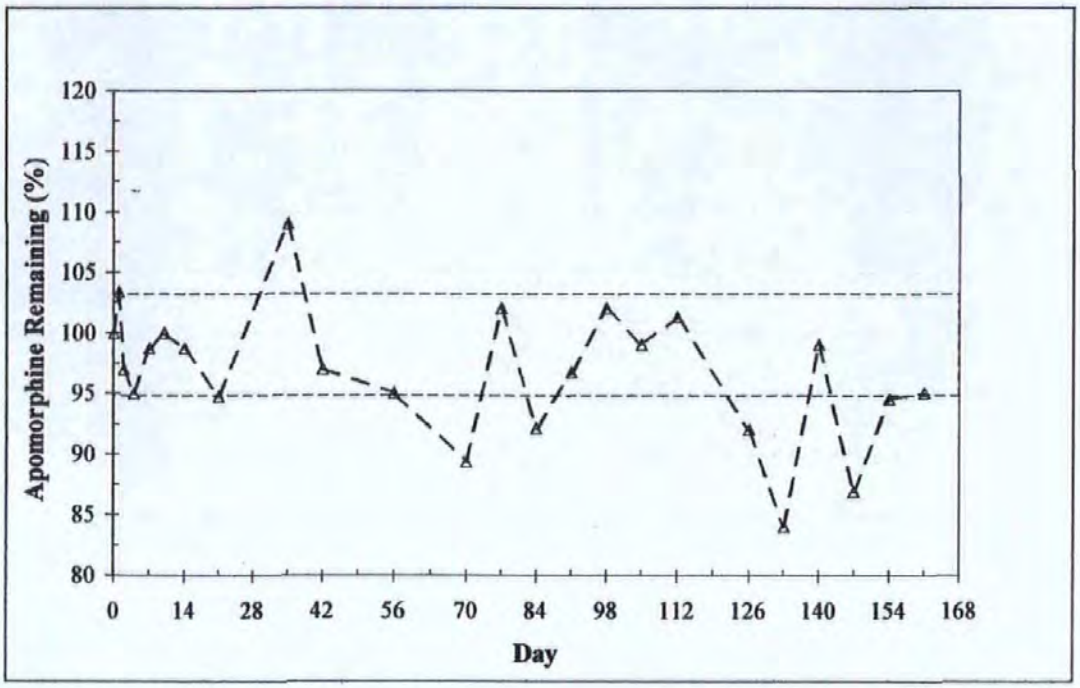


Figure 4-28 Stability of R(-)-apomorphine HCl (1ng/mL) in plasma at –20°C.

Limits (----) are defined as $^{+}$/- 2 x S.D. from the concentration at t=0, whereby the S.D. used was that demonstrated for the inter-batch precision of apomorphine in plasma (1ng/mL).

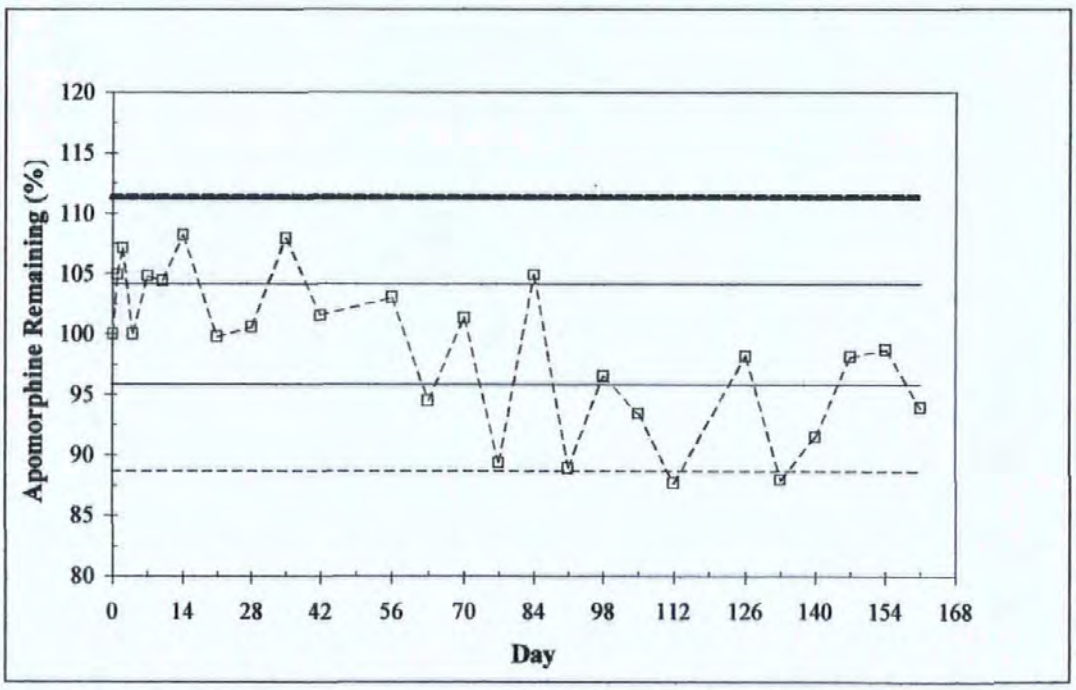


Figure 4-29 Stability of R(-)-apomorphine HCl (20ng/mL) in plasma at –20°C.

Limits are defined as $^{+}$/- 2 x S.D. from the concentration at t=0, whereby the S.D. used was that demonstrated for the intra-batch (——) and inter-batch (----) precision of apomorphine in plasma (20ng/mL).

4.5.4.2. Freeze-Thaw Cycles: Apomorphine in Plasma.

The objective was to investigate the stability of apomorphine in plasma samples when subjected to repeated freeze-thaw cycles.

The limits for analyte stability were defined as $^{+}$/-2 S.D. from the analyte concentration at time = 0, whereby the C.V. used were those given for the precision of the experimental controls (see below). Thus the acceptable variation from time zero was $^{+}$/-4.38 % for 1ng/mL apomorphine and $^{+}$/-4.72 % for 20ng/mL apomorphine.

Experimental.

Duplicate aliquots of stock plasma (6.0mL) were prepared according to Sections 4.3.3.1 and 4.3.3.2 (page 4-35), with the exception that, prior to placing the stock plasma at –20°C, 1.2mL of each stock was removed, spiked with 2-ME to a final concentration of 0.01 % (v/v), centrifuged (2000RCF, 5mins, 4°C) and assayed according to Section 4.4 (day 0). On days 1, 2, 3 and 4 the stock plasma was thawed at 4-8°C, one aliquot (1.2mL) was removed and processed as described in Section 4.4 (page 4-50), and the remaining stock plasma was replaced in the –20°C freezer.

On each sampling day, individual aliquots (1.2mL) of plasma, prepared according to Section 4.3.3.1, were assayed as described in Section 4.4. These acted as controls, not having been subjected to multiple freeze-thaw cycles.

Results.

It was demonstrated that during the course of four freeze-thaw cycles, apomorphine concentration did not go beyond the defined acceptable limits (Figure 4-30). Furthermore there was an absence of chromatographic peaks at the retention time windows of apomorphine degradation products (the latter having been identified as such by the forced degradation of apomorphine, see Section 4.5.1.3, page 4-73).

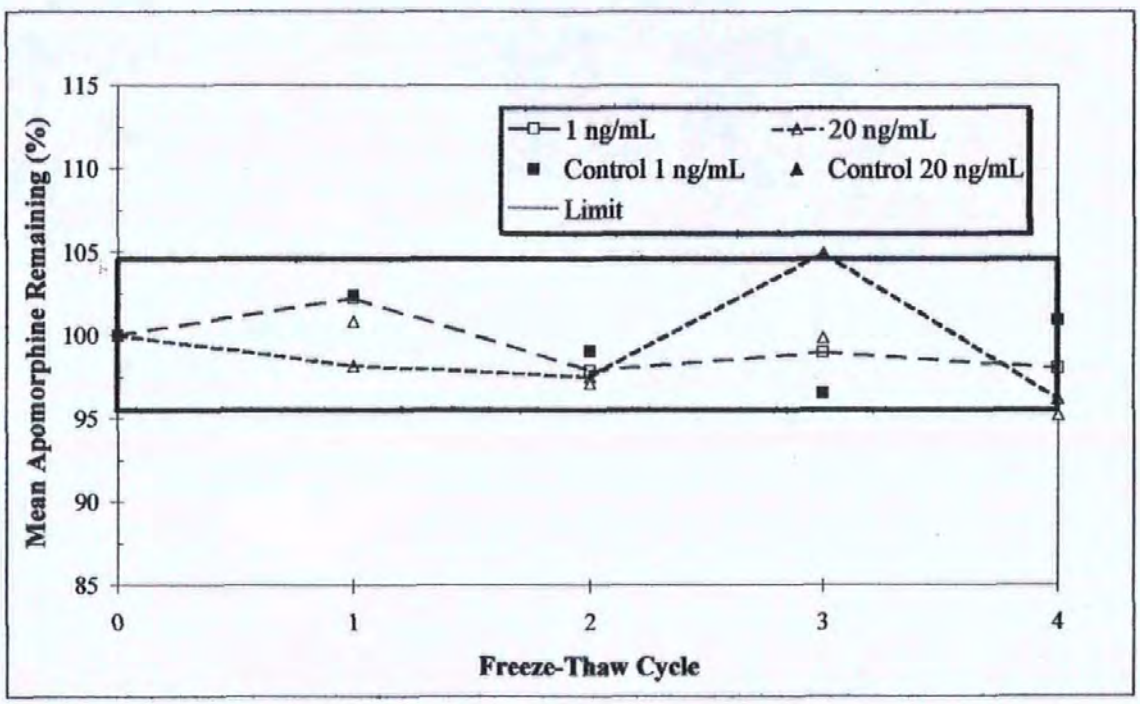


Figure 4-30 Stability of R(-)-apomorphine HCl in plasma following multiple freeze-thaw cycles.

Limits (——)are defined as $^{+}$/- 2 x S.D. from the concentration at t = 0, whereby the S.D. used was (the mean of) that given for the precision of the experimental controls (at 1 and 20 ng/mL).

4.5.4.3. NPA in Diluent A at 4-8°C.

The objective was to assess the long term stability of NPA, at a concentration of 5µg/mL in diluent A, at fridge temperature. This concentration was chosen as it represented a suitable working concentration of NPA, i.e. the working solution contributes only 2% of the final volume of sample to be assayed. This experiment was performed on two separate occasions.

The limits for analyte stability were defined as $^{+}$/-2 S.D. from the analyte concentration at time = 0, whereby the C.V. used was that demonstrated for the intra-day NPA in diluent A (100ng/mL). Thus the acceptable variation from time zero was $^{+}$/-1.44 %.

Experimental.

On 12 occasions over a period of 24 weeks, aliquots (1mL) of 5µg/mL were prepared from a stock solution of NPA in diluent A (0.1mg/mL, stored at fridge temperature, see Section 3.5) and stored in polypropylene microtubes in a light-proof container at fridge temperature. At 24 weeks each 5µg/mL aliquot was used to spike 1mL of diluent A to 100ng/mL. The 100ng/mL solutions was assayed according to the HPLC method given in Section 4.4.2 (page 4-51), bracketed with an external standard in the form of a solution of 100ng/mL NPA which had been made that day from a freshly prepared stock solution of NPA in diluent A. The peak area of a test solution was compared to that of the external standard in order to calculate the concentration of the test.

Results.

It was demonstrated that for a period of 20 weeks, (mean) NPA concentration did not go beyond the pre-defined acceptable limits, see Figure 4-31. There was an absence of chromatographic peaks at the retention time windows of NPA degradation products for all samples assayed. (NPA degradation products were identified as such by the forced degradation of NPA, see Section 4.5.1.3, page 4-73). It was considered that NPA, at a concentration of 5µg/mL in diluent A, was stable at fridge temperature for a period of 20 weeks. This defined the conditions of use for the working concentration of internal standard in the assay of apomorphine.

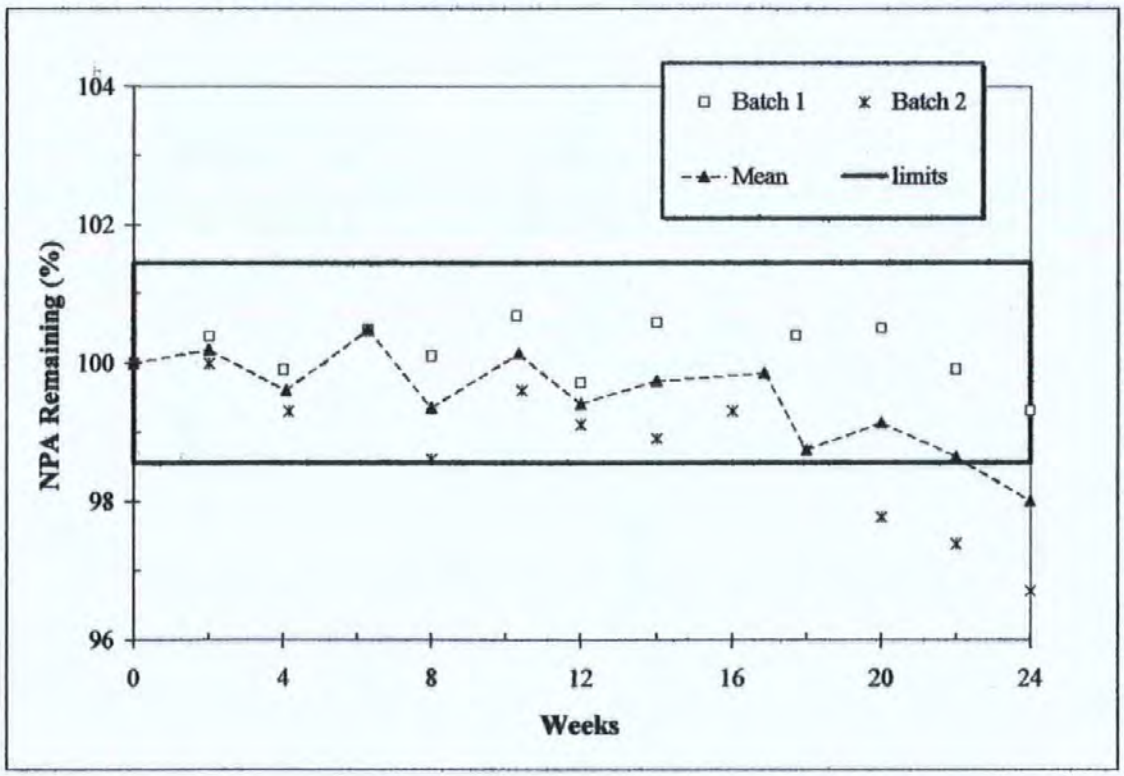


Figure 4-31 Stability of R(-)-NPA HCl (100ng/mL) in Diluent A at 4-8°C.

Limits are defined as $^{+}$/- 2 x S.D. from the concentration at t = 0, whereby the S.D. used was that demonstrated for the intra-day precision of NPA in diluent A (100ng/mL).

4.5.4.4. Plasma Extract at 4-8°C.

The objective was to examine the stability of apomorphine (and internal standard) following extraction from plasma, i.e. as analytes in the eluting solution. The extract was stored at 4-8°C.

The acceptable limits for analyte stability were defined as $^{+}$/-2 S.D. from the apomorphine:NPA peak area ratio at time = 0, whereby the C.V. used was that obtained for the intra-batch precision of the apomorphine:NPA peak area ratio of the extract (Section 4.5.3.3, page 4-92). Thus the acceptable variation from time zero was $^{+}$/- 2.98% for 1ng/mL apomorphine and $^{+}$/- 1.66% for 20ng/mL apomorphine.

Experimental.
Blood collection and preparation of plasma was performed according to Section 4.3.3 (page 4-35). The eluate which was produced on extraction (Section 4.4.1, page 4-50) of four individual aliquots of 1mL was pooled in a polypropylene tube and then distributed between two autosampler vials, which were stored at fridge temperature. A sample from alternate vials was assayed (Section 4.4.2, page 4-51) on days 0, 1, 4, 7, 10, 14, 21, 28 and 31. The apomorphine:NPA peak area ratio, rather than the absolute potency of the analytes, was used as a functional marker of stability of the extract.

Results.
There was an absence of chromatographic peaks at the retention time windows of apomorphine and NPA degradation products for all samples assayed. (Degradation products were identified as such by forced degradation of both analytes, see Section 4.5.1.3, page 4-73). Although individual observations went beyond pre-defined limits, there was an absence of a consecutive observations beyond the acceptable limits (see Figure 4-32 and Figure 4-33), therefore the time period for storage at fridge temperature of extracts of 1 and 20 ng/mL apomorphine in plasma was defined as 31 days.

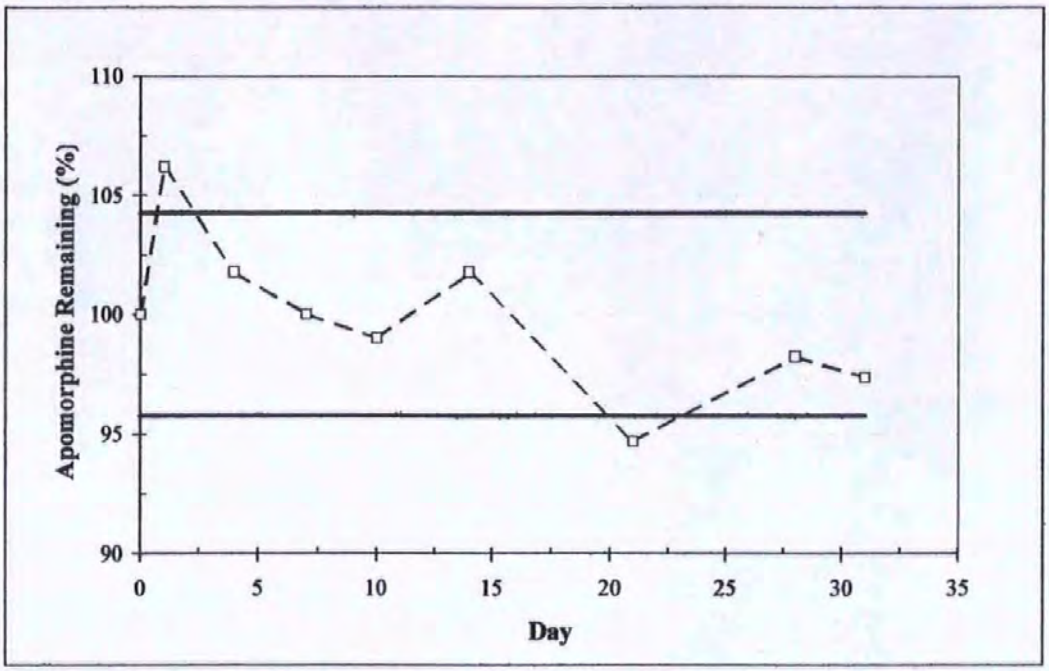


Figure 4-32 Stability at 4-8°C of apomorphine (1ng/mL) and NPA following extraction from plasma.

Limits are defined as +/- 2 x S.D. from the concentration at t = 0, whereby the S.D. used was that demonstrated for the intra-day precision of plasma extract (1ng/mL apomorphine).

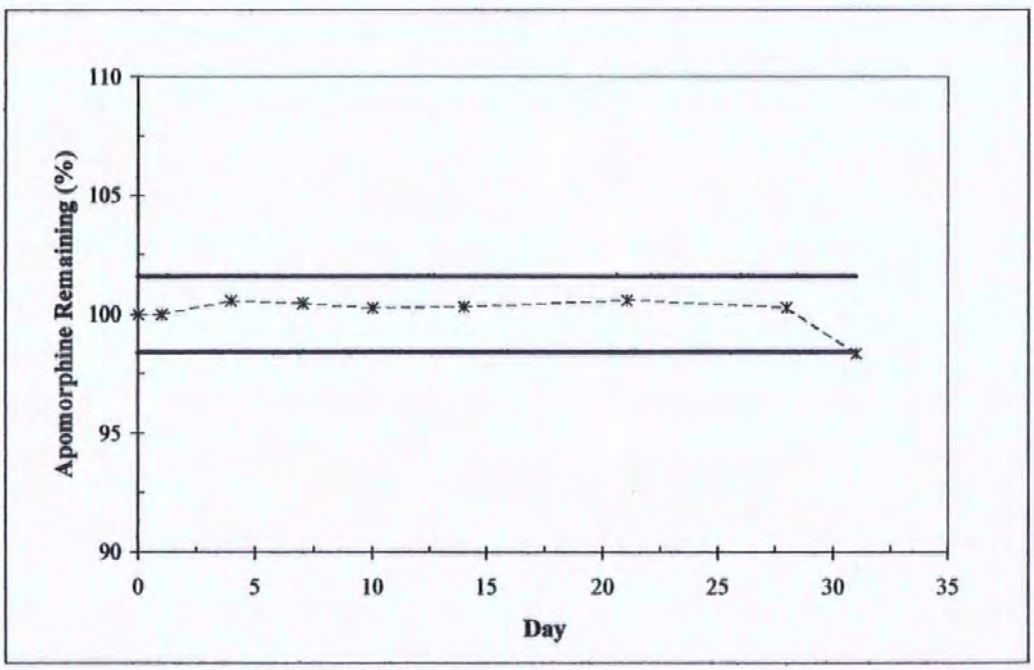


Figure 4-33 Stability at 4-8°C of apomorphine (20ng/mL) and NPA following extraction from plasma.

Limits are defined as +/- 2 x S.D. from the concentration at t = 0, whereby the S.D. used was that demonstrated for the intra-day precision of plasma extract (20ng/mL apomorphine).

4.6. Pharmacokinetic and Pharmacodynamic Modelling Strategy.

The standard two-stage approach was used in the analysis of pharmacokinetic and pharmacodynamic data. Modelling was performed using WinNonlin Standard version 2.0. (Pharsight Corporation, Palo Alto, CA , USA).

Inspection of the (log-linear) relationship between observed plasma apomorphine concentration *versus* time, or observed plasma apomorphine concentration *versus* observed improvement in tapping test score, was performed in the first instance in order to determine (i) the fundamental structure of the relationship, e.g. the number of compartments in the case of pharmacokinetic models, or linear *versus* hyperbolic relationship in the case of pharmacodynamic models, and (ii) initial estimates for model parameters, e.g. A, alpha, or E_{max}, EC_{50}.

Having identified the likely structural model(s) for each dataset in this way, modelling was performed using, in the main, the Guass-Newton algorithm with Hartley and Levenberg-Marquardt modifications. This was used since it is a powerful algorithm that performs well on most data sets[50]. Upper and lower constraints (boundaries) on initial parameter estimates were routinely applied, since the use of boundaries can prevent the situation where unrealistic parameter estimates are generated, or where the model actually fails to converge.

Weighting was applied to data in order to account for heterogeneity in the variance of the data. In most cases the protocol used was that of iteratively reweighted least squares (IRLS). The weighting scheme often employed is one where an observation is weighted by the reciprocal of the variance of that observation (1 / variance of Y), however Gabrielsson and Weiner advise that IRLS, i.e. weighting by the reciprocal of the *predicted* value (1 / variance of $\hat{Y}$) , is a superior approach[50]. The authors give the following rationale: the

true variance of the observed value is generally unknown, but is often taken to be the square of the observed concentration (Y^2). However, assuming the model is correct, the predicted concentration is actually a better estimate of the true concentration than is the observed concentration. It is therefore more appropriate to use the variance of the predicted value ($\hat{Y}^2$) in the weighting scheme.

The advantage conveyed by this approach can be demonstrated by the effect on outlying data; the IRLS process does not incorporate the observed outlying data value, rather, it is the predicted value derived from the model that is used.

With each iteration of the minimisation process the predicted value changes, and the associated weight is updated for use in the subsequent iteration. This process is continued until convergence is achieved.

The goodness of fit was assessed using diagnostic features such as residual analysis (see Section 1.1.3, page 1-7). In the situation where competing models existed, model discrimination was performed according to the following criteria: WSSR, S, residual analysis, parameter correlation, the condition number, rank, Akaike Information Criteria and the C.V. of final parameter estimates (see Section 1.1.3, pages 1-7 to 1-9).

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SECTION 5:
RESULTS

5. Results.

5.1. *Pharmacokinetic-Pharmacodynamic Study of Subcutaneous Apomorphine Administration in Patients With Parkinson's Disease.*

The time courses of apomorphine-induced effect are described for each patient as follows (see Table 5-1 on page 5-23 for a summary and Appendix 8.13 on page 8-26 for raw data):-

Patient 01.

A change in facial expression was identified by the patient as a qualitative marker of parkinsonian status, i.e. when "off" the face was often masked or fixed, and when "on" the face was often more expressive and relaxed.

Following a washout period of eight hours the patient displayed mild to marked rigidity of major joints, moderate action tremor of the hands, mild to moderate resting tremor of the hands, and moderate to severely impaired performance in tests of rapid, alternating hand movement. The patient also exhibited a moderate hypomimia ("masked face") and slight impairment in speech (in terms of volume and expression).

The walking test was attempted at baseline however, due to impaired postural stability and severe disturbance of gait, the patient was unable to walk unaided. Therefore the walking test was aborted at this time and was not attempted during the remainder of the sampling period.

The time course of plasma apomorphine concentration and clinical status following apomorphine bolus administration is given in Figure 5-1A. The onset of effect was defined by the patient's comment that the drug was "just beginning to have effect". This event coincided with urinary urgency (which might have reflected a resolution of possible "off" phase urinary retention[1]) and the patient walked with assistance, to the bathroom. On the patient's return (three minutes later) there had been an improvement in mobility which

allowed the patient to walk unaided back from the bathroom. Additionally there was a substantial change in facial expression and quality of speech, and an improvement in tapping score was observed.

The patient became somewhat anxious immediately prior to venous blood sampling, on which occasions tremor (e.g. affecting the lower extremities) was observed to increase in severity.

There were no observations of adverse effects resulting from apomorphine administration. In fact there was a notable absence of yawning, this symptom being a common apomorphine-related (adverse) effect.

At 38 minutes post-dose, the patient experienced a worsening in parkinsonian symptoms, culminating in a return to an "off" state which, in terms of the tapping test, was more pronounced than that at baseline. A second period of improvement in parkinsonian state subsequently ensued whereby an improvement in symptoms, e.g. facial expression, was evident and was reflected in the improved performance in the tapping test. This "second wind" was potentially facilitated by the patient stretching and standing (with assistance). Cessation of effect was defined by the patient stating that she was "just fully turning "off"". The patient rated the response following the apomorphine bolus as sub-optimal in terms of quality and duration in comparison to her typical experience.

Visual inspection of the (observed) plasma apomorphine concentration verses (observed) effect plot (Figure 5-1B) revealed that no clear relationship existed between effect and plasma concentration.

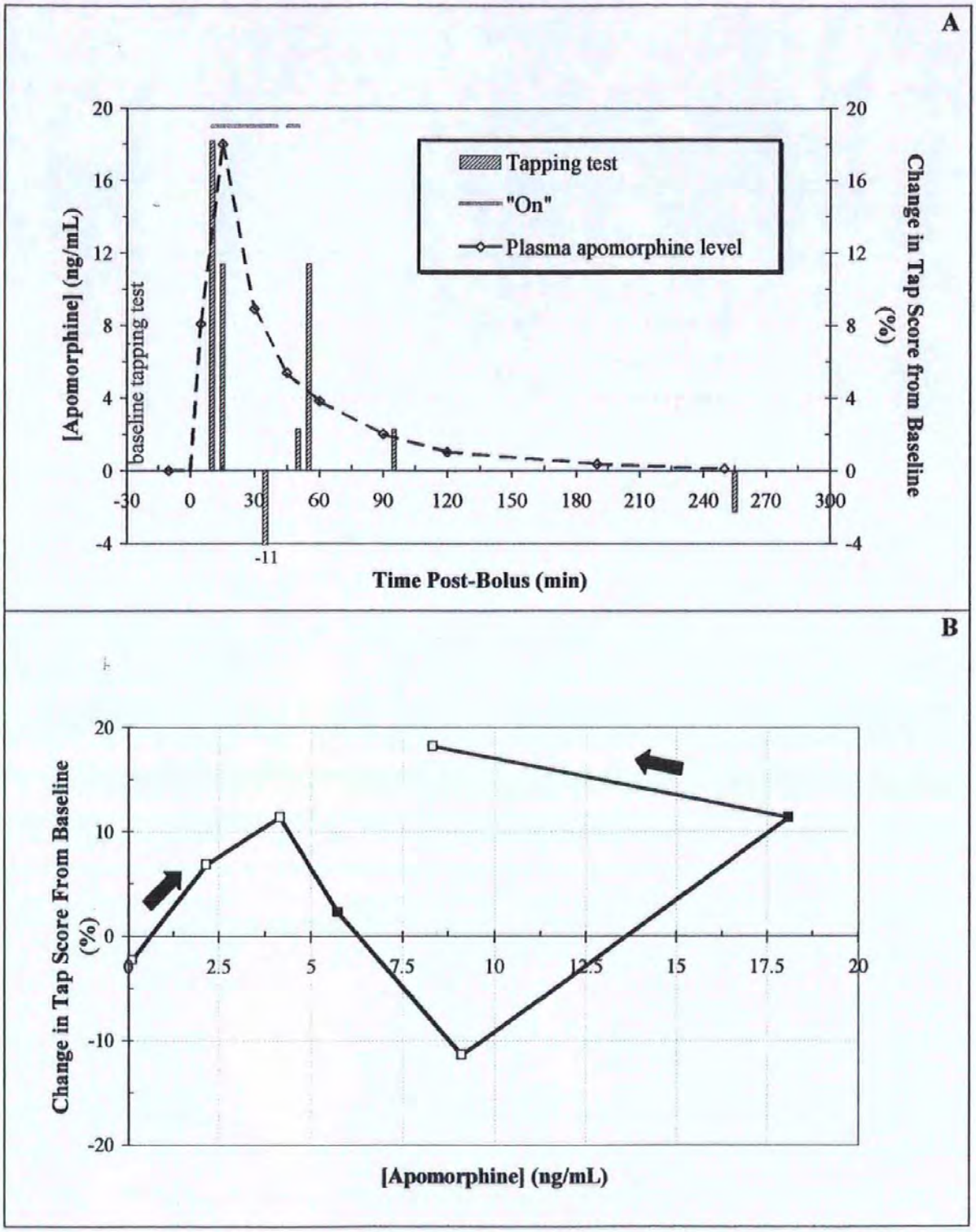

Figure 5-1 **Panel A: Plasma apomorphine concentration/clinical status profile following apomorphine bolus administration (2mg) to Patient 01.**

Panel B: Relationship between plasma apomorphine concentration and anti-parkinsonian effect.

Arrows indicate chronological order of occurrence.

Filled squares indicate that, at the time of sampling, the patient was in an apomorphine-induced "on" phase. Open squares indicate that, at the time of sampling, the patient was in a parkinsonian "off" phase.

Patient 02.

At the culmination of the wash-out period the patient admitted to feeling "very lousy". Due to the extent of parkinsonian disability, walking unaided was not possible at this time. The time course of plasma apomorphine concentration and clinical status following apomorphine bolus administration is illustrated in Figure 5-2A.

In the twenty-five minutes following apomorphine administration, there was mild improvement in some parkinsonian symptoms, e.g. speech quality, and by twenty-nine minutes post-dose the patient stated that he felt "a sensation of wanting to move". Yawning occurred at this time (thirty and thirty-three minutes post-dose). Although the aforementioned signs and symptoms were indicative of a response to apomorphine, the response was considered to be sub-optimal as compared to the patient's typical experience. Indeed, the patient felt a sensation of being "underwater" at a time during the period of improvement in parkinsonian symptoms. Given the limited improvement in parkinsonian state, the walking test was not instigated during the time course, rather, priority was focussed on the tapping test in order to obtain a more comprehensive record of drug response. From eighty-six minutes post-dose onwards the patient became progressively tired and slept periodically for the remaining sampling period. There were no observations of adverse effects resulting from apomorphine administration. The patient admitted to disliking the tapping test throughout the entire pharmacodynamic monitoring period.

Visual inspection of the (observed) plasma apomorphine concentration verses (observed) anti-parkinsonian effect plot (Figure 5-2B) suggested that there was a direct relationship between plasma concentration and effect.

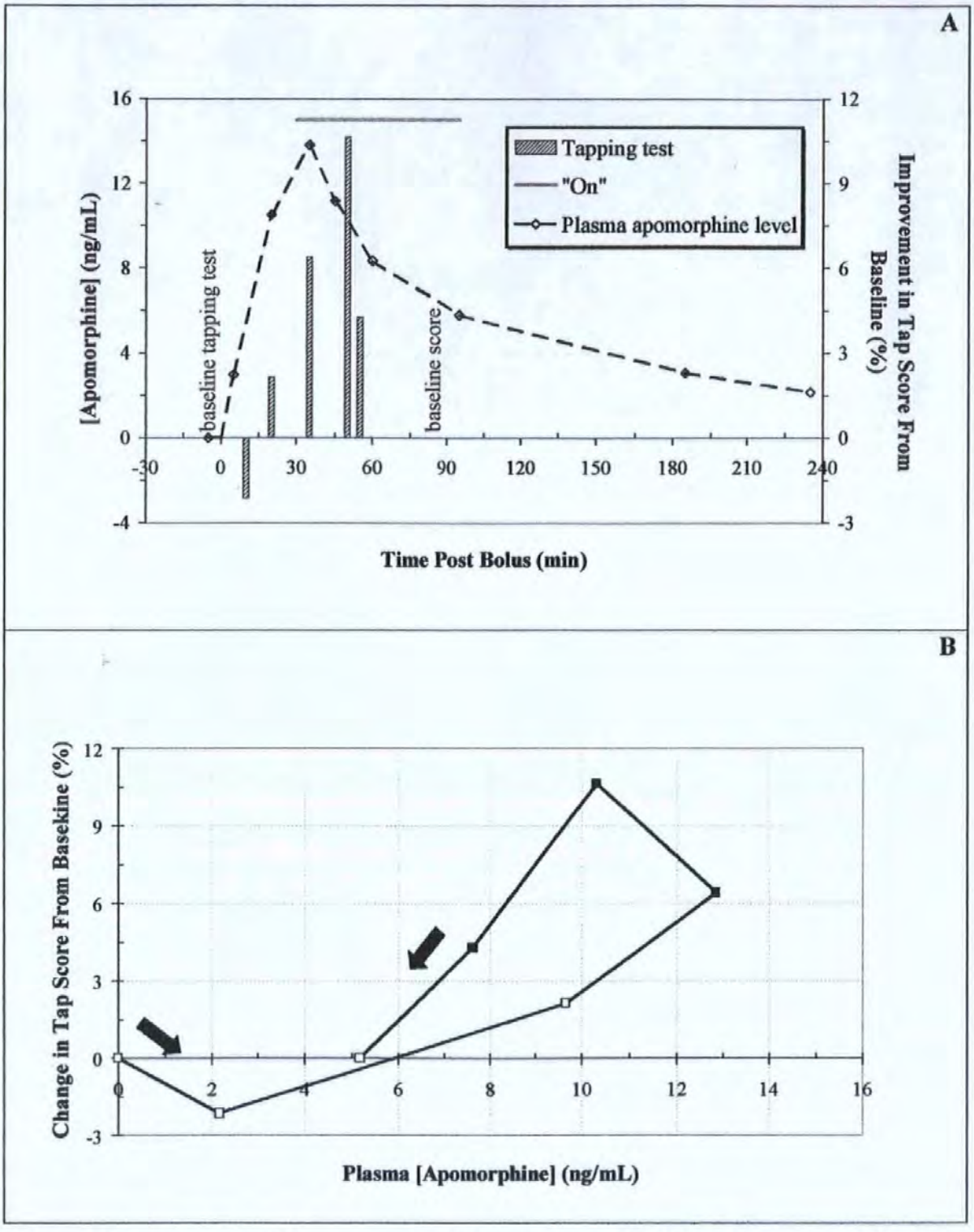

Figure 5-2 **Panel A: Plasma apomorphine concentration/clinical status profile following apomorphine bolus administration (5mg) to Patient 02.**

Panel B: Relationship between plasma apomorphine concentration and anti-parkinsonian effect.

Arrows indicate chronological order of occurrence.

Filled squares indicate that, at the time of sampling, the patient was in an apomorphine-induced "on" phase. Open squares indicate that, at the time of sampling, the patient was in a parkinsonian "off" phase.

Patient 03.

Whilst patient 03 displayed an absence of action and resting tremor at baseline, other parkinsonian symptoms were present in a severe form, i.e. marked impairment of speech, hypomimia, moderate rigidity of major joints, extreme abnormality of posture, and an inability to stand or walk even with assistance. There was also severe impairment in the performance of the tests of rapid, alternating hand movements, and the patient was unable to perform the tapping test at baseline.

At twelve minutes post-dose the patient remained unable to depress the counter on the tapping tester. The anti-parkinsonian response amounted to a minimal improvement in leg mobility (which was noted by the clinician at twenty-two minutes post-dose) and a tapping test score of four taps per 30s at thirty-seven minutes post-dose (compared to a mean baseline score in the other patients of 55 taps per 30s, range = 36 to 96 taps per 30s, n=7). Yawning occurred at forty-four minutes post-dose. Due to the degree of discomfort and anxiety expressed by the patient, the study was terminated at sixty-six minutes post-dose. See Figure 5-3 for plasma concentration-time profile.

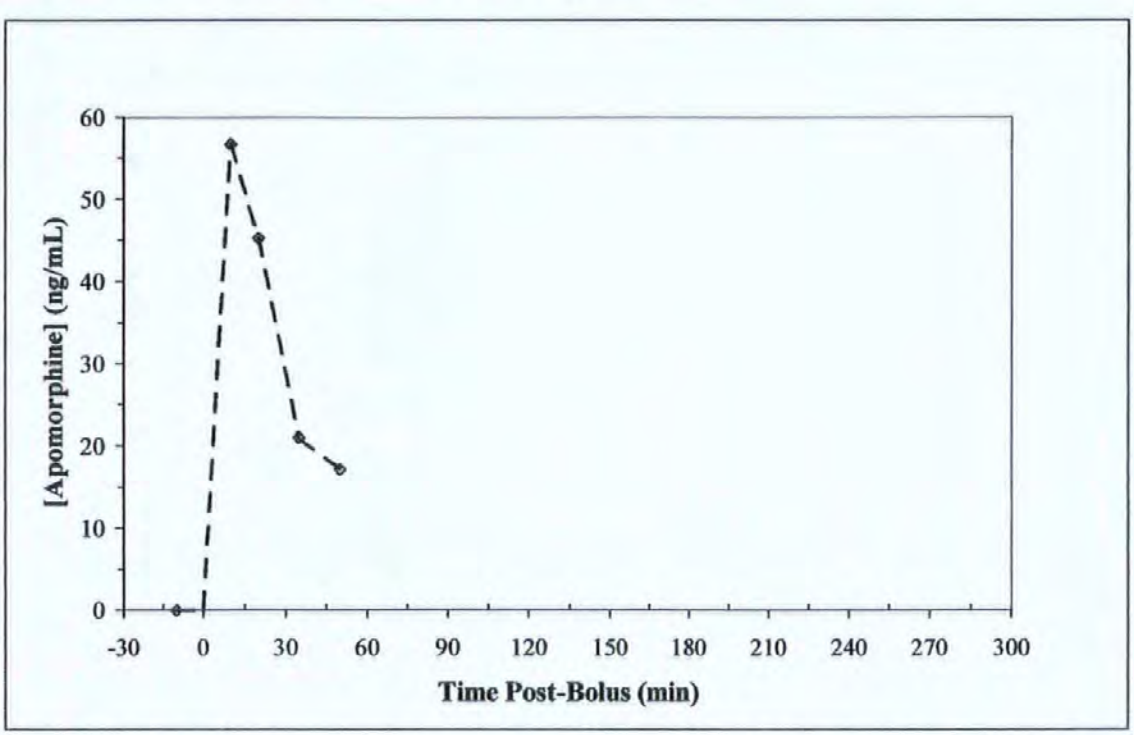

Figure 5-3 Plasma apomorphine concentration following apomorphine bolus administration (10mg) to Patient 03.

Patient 04.

At baseline patient 04 exhibited moderately impaired speech and moderate hypomimia. Tremor was present at rest and on action (to a slight to moderate degree). Rigidity was present in the major joints, ranging from a slight affliction in the lower extremities to a severe form at the neck. Moderate to severe impairment in ability to perform tests of rapid, alternating hand movements was demonstrated. Moderately abnormal posture, impaired postural stability and a severe disturbance of gait were additionally observed.

The time course of apomorphine administration is illustrated in Figure 5-4A. Within two minutes of subcutaneous apomorphine injection the patient stated that he was "confused". Diaphoresis, yawning, micturition, dyskinesia, a feeling of "giddiness" and drowsiness occurred variously in the twenty-three minutes that followed apomorphine administration, during which time the tapping test scores were sub-baseline level. Whilst there was a general amelioration of parkinsonian symptoms, e.g. tremor, the episodes of drowsiness and sleeping continued up to and beyond the point at which the patient stated that he was "just going off" (at 129 minutes post-dose).

This was not a typical response to apomorphine compared to the patient's usual experience with sweating and loss of bladder control in particular being novel occurrences. Clearly the patient exhibited a number of the common adverse effects attributed to apomorphine and for this reason the response was deemed a "toxic" response.

Visual inspection of the (observed) plasma apomorphine concentration verses (observed) effect plot (Figure 5-4B) suggested that there was no clear relationship existed between effect and plasma concentration.

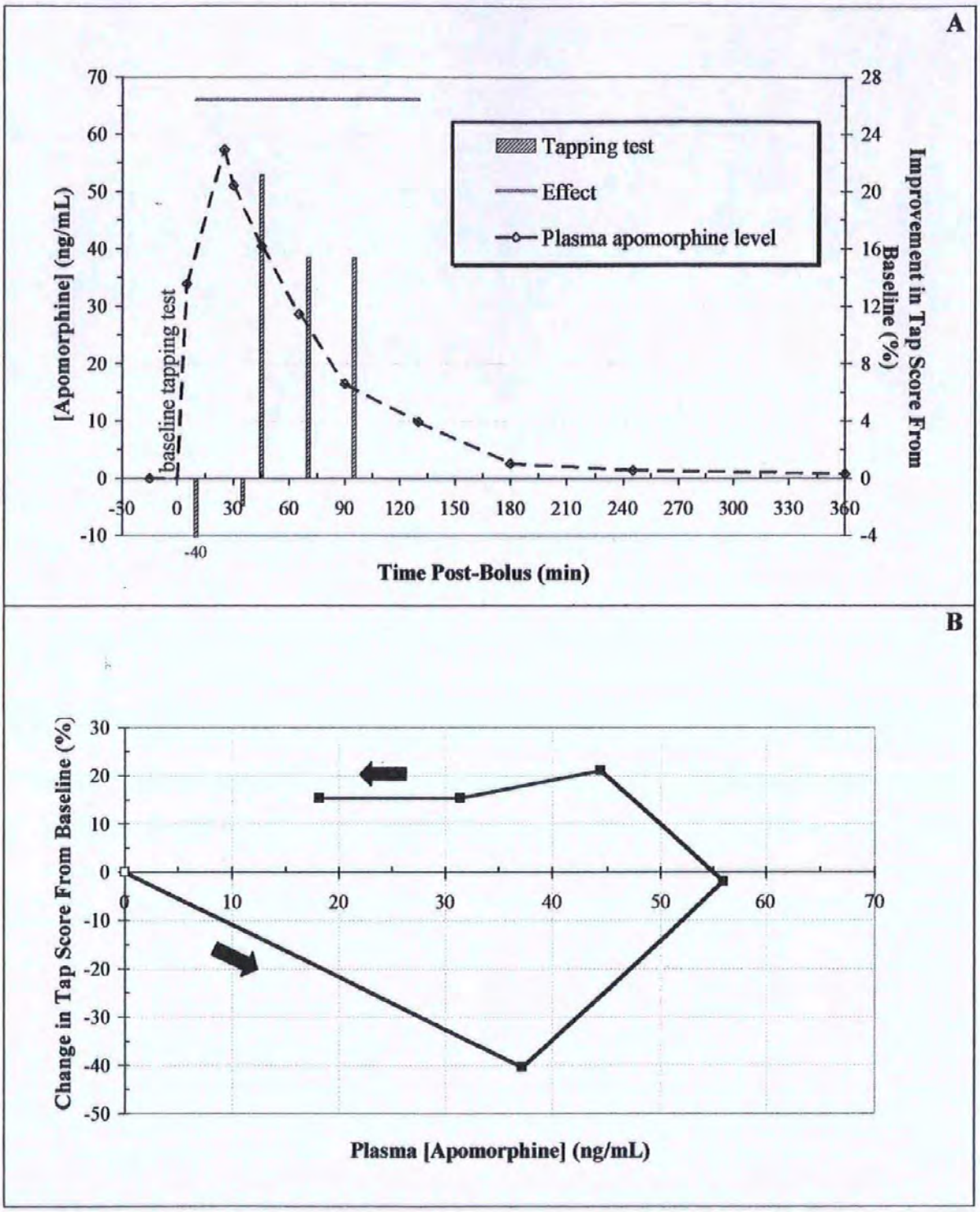

Figure 5-4 **Panel A: Plasma apomorphine concentration/clinical status profile following apomorphine bolus administration (10mg) to Patient 04.**

Panel B: Relationship between plasma apomorphine concentration and anti-parkinsonian effect.

Arrows indicate chronological order of occurrence.

Filled squares indicate that, at the time of sampling, the patient was in an apomorphine-induced "on" phase. Open squares indicate that, at the time of sampling, the patient was in a parkinsonian "off" phase.

Patient 05.

At baseline this patient exhibited moderately impaired speech and hypomimia. Resting tremor, action tremor, and rigidity of the major joints were considered to be mild to moderate in nature. Posture and gait were more severely affected and consequently the patient was not able to rise from a chair or stand without assistance, and could not walk even with assistance. Repetitive, alternating tasks involving the hands were moderately to extremely severely impaired, to the degree at which the task could barely be performed. The time course of plasma apomorphine concentration and clinical status following apomorphine bolus administration is illustrated in Figure 5-5A.

Within ten minutes of apomorphine bolus administration, an intense period of yawning occurred, and the patient stated that she felt "droopy" during this time. There began a period of improvement in parkinsonian bradykinesia, as evidenced by the improvement in tapping test performance. At thirty-one minutes post-dose muscle cramp developed in the lower left extremity. Muscle cramp returned intermittently over the following eleven minutes, and was more severe than typically experienced by this patient. Shortly after this period the patient complained of feeling "weak" and at sixty-two minutes post-dose, she requested the administration of further anti-parkinsonian medication, since she felt that the effects of apomorphine were beginning to wane. The tapping test score post-sixty minutes was at sub-baseline levels, but improved dramatically when assessed two hours later as a result of the administration of the further anti-parkinsonian medication. The apomorphine-induced response was deemed to be sub-optimal compared to the typical response in terms of magnitude and quality of effect.

Visual inspection of the (observed) plasma apomorphine concentration verses (observed) anti-parkinsonian effect plot (Figure 5-5B) suggested that there was a direct relationship between plasma concentration and effect.

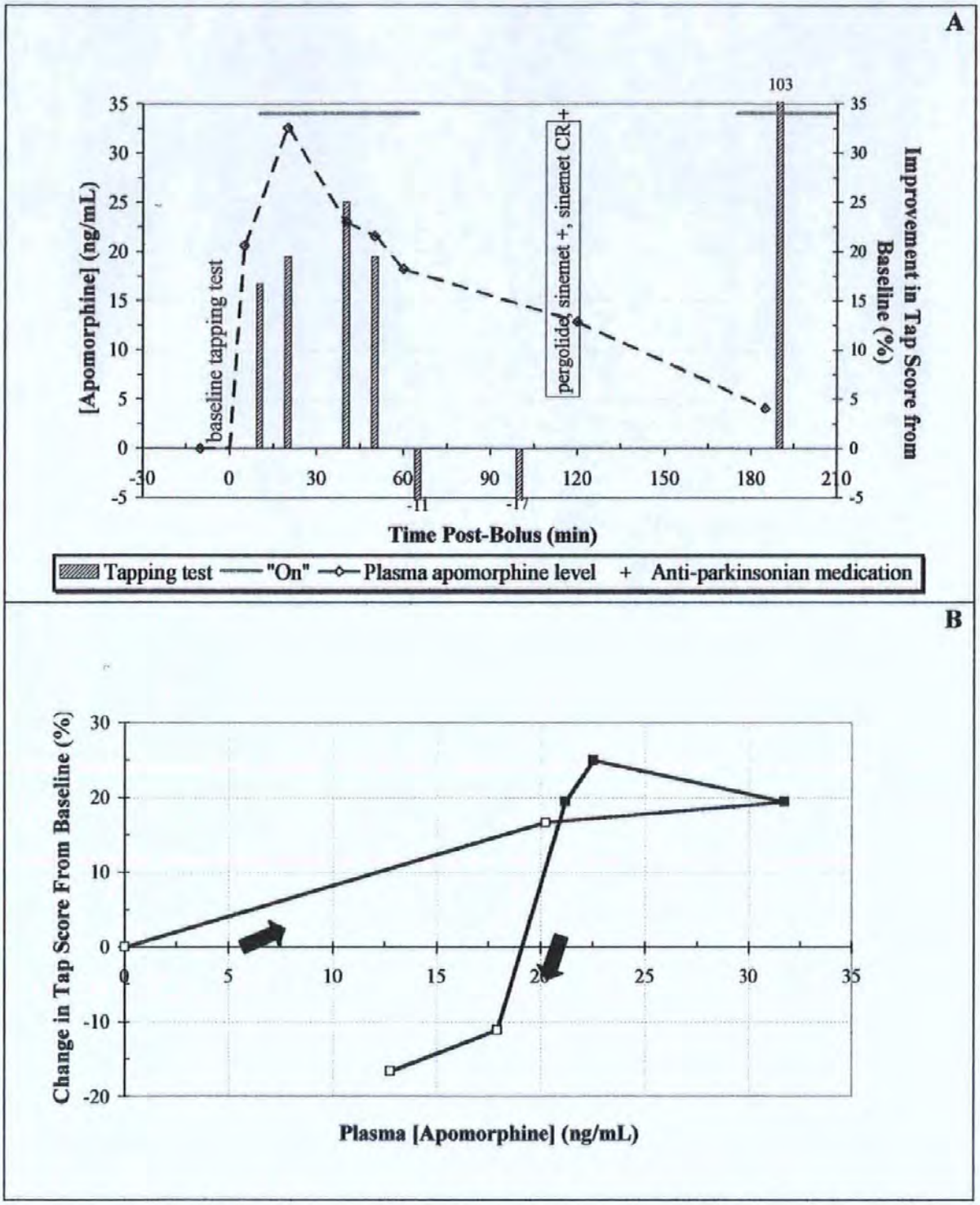

Figure 5-5 **Panel A: Plasma apomorphine concentration/clinical status profile following apomorphine bolus administration (5mg) to Patient 05.**

Panel B: Relationship between plasma apomorphine concentration and anti-parkinsonian effect.

Arrows indicate chronological order of occurrence.

Filled squares indicate that, at the time of sampling, the patient was in an apomorphine-induced "on" phase. Open squares indicate that, at the time of sampling, the patient was in a parkinsonian "off" phase.

Patient 07

The time course of plasma apomorphine concentration and clinical status following cessation of (24h) apomorphine infusion is illustrated in Figure 5-6. The last anti-parkinsonian medication taken prior stopping the infusion was Sinemet CR; this was administered 3.5 hours prior stopping the infusion. Nine minutes after the infusion was stopped, the patient commented on "feeling..high". At this time abnormal involuntary movements were present; a symptom that was absent at baseline. At twenty-nine minutes after the infusion was stopped, the patient commented that he was "definitely going "off"". At forty-five minutes after stopping the infusion, the patient experienced dystonia in the left foot (which persisted for approximately thirty minutes), and was unable to walk even with assistance, whereas at baseline unassisted walking was demonstrated. Despite resuming other anti-parkinsonian medication, the tapping test scores did not return to baseline level. At 247 minutes after stopping the infusion, dystonia had returned and the patient commented that he felt "off".

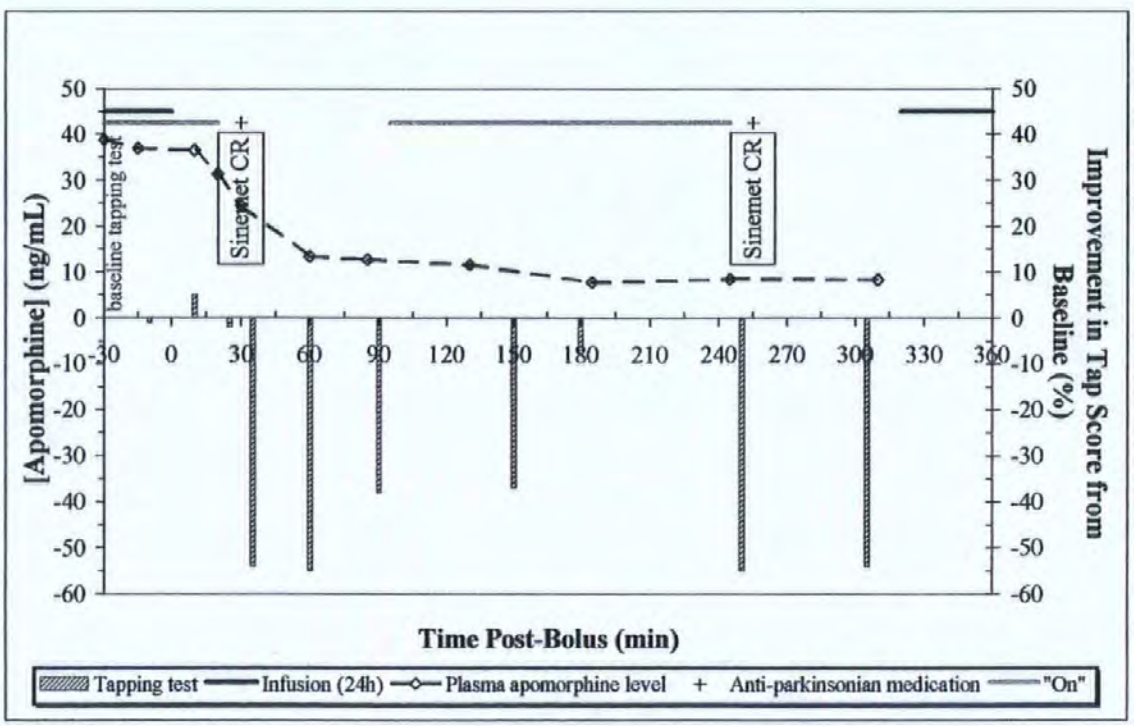

Figure 5-6 Plasma apomorphine level and clinical status profile: Patient 07.

Patient 08.

The time course of plasma apomorphine concentration and clinical status following cessation of (24h) apomorphine infusion is illustrated in Figure 5-7. The last anti-parkinsonian medication taken prior stopping the infusion was Sinemet CR; this was administered approximately three hours prior stopping the infusion.

Postural stability, gait, body bradykinesia and the ability to rise from a chair deteriorated from being minimally to mildly affected during the apomorphine infusion to being markedly or severely affected in the absence of apomorphine. There was only minimal deterioration in rigidity of the neck and of the lower extremities on cessation of the apomorphine infusion, and also in tasks involving rapid, repetitive hand movements such as pronation-supination motion, but not in tapping test performance, which actually improved on cessation of the apomorphine infusion. Potential factors which may have contributed to this unusual outcome include: inaccuracy in the baseline tapping test score, practice (or learning) effects, changes in the motivation of the individual, or the continued benefit from controlled release levodopa medication (see page 6-31 for a discussion on the limitations of the tapping test).

At 138 minutes after the infusion was stopped, the patient commented that she was "a bit dopey...feel like I want a bit more strength". Approximately 60 minutes after this, anti-parkinsonian medication was re-established.

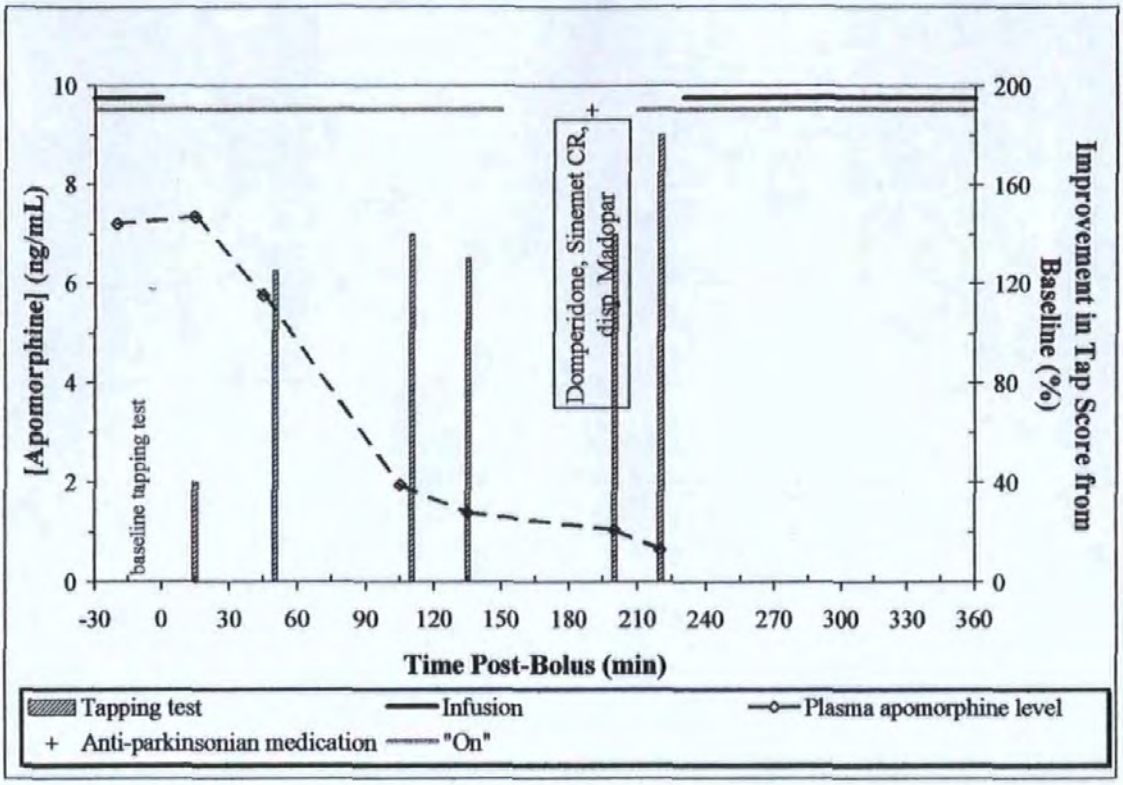

Figure 5-7 Plasma apomorphine level and clinical status profile: Patient 08.

Patient 09.

The time course of plasma apomorphine concentration and clinical status following apomorphine bolus administration is illustrated in Figure 5-8 and a detailed patient commentary given by patient 09 is represented in Figure 5-14.

The onset of apomorphine-induced effect, at five minutes post-dose, was defined by the patient's comment that she was "loosening up". Cessation of effect was defined subjectively by the clinician, who noted the recurrence of tremor. Some mild adverse effects were observed, e.g. yawning, which occurred intermittently during the "on" phase.

The effect of apomorphine on individual parkinsonian symptoms is documented below, whereby the assessment of effect was made forty-one minutes post-dose.

Speech

Speech was impaired to a minimal degree both prior to and following apomorphine dosing.

Facial expression.

At baseline moderate hypomimia was observed; this was reduced to a minimal impairment following apomorphine administration.

Tremor.

Resting tremor, whilst mild to moderate in the "off" state, was absent following the apomorphine-induced "on" state. Similarly, action tremor was improved from a slight impairment to being absent.

Rapid, repetitive hand movements.

Performance was rated as being moderately to severely impaired at baseline. Improvement was observed in certain aspects of the set of tests, whereas in other aspects, apomorphine administration did not effect a change in performance.

Posture

The effect of apomorphine was to improve the mild postural instability observed at baseline to normal postural status.

Gait

Patient 09 displayed some difficulty in walking, however did not require assistance in doing so. Following apomorphine administration walking ability was improved, but not to the point at which gait could be considered normal.

Body bradykinesia

The patient exhibited mild body bradykinesia and hypokinesia at baseline; symptoms which were improved to following apomorphine administration, although a minimal impairment was considered to persist in the "on" state.

The patient rated the response to the apomorphine bolus as typical of that experienced under normal circumstances.

Visual inspection of the (observed) plasma apomorphine concentration verses (observed) anti-parkinsonian effect plot (Figure 5-8B) revealed the existence of counter-clockwise hysteresis. This was indicative of an indirect, i.e. time-dependant, relationship between plasma concentration and effect.

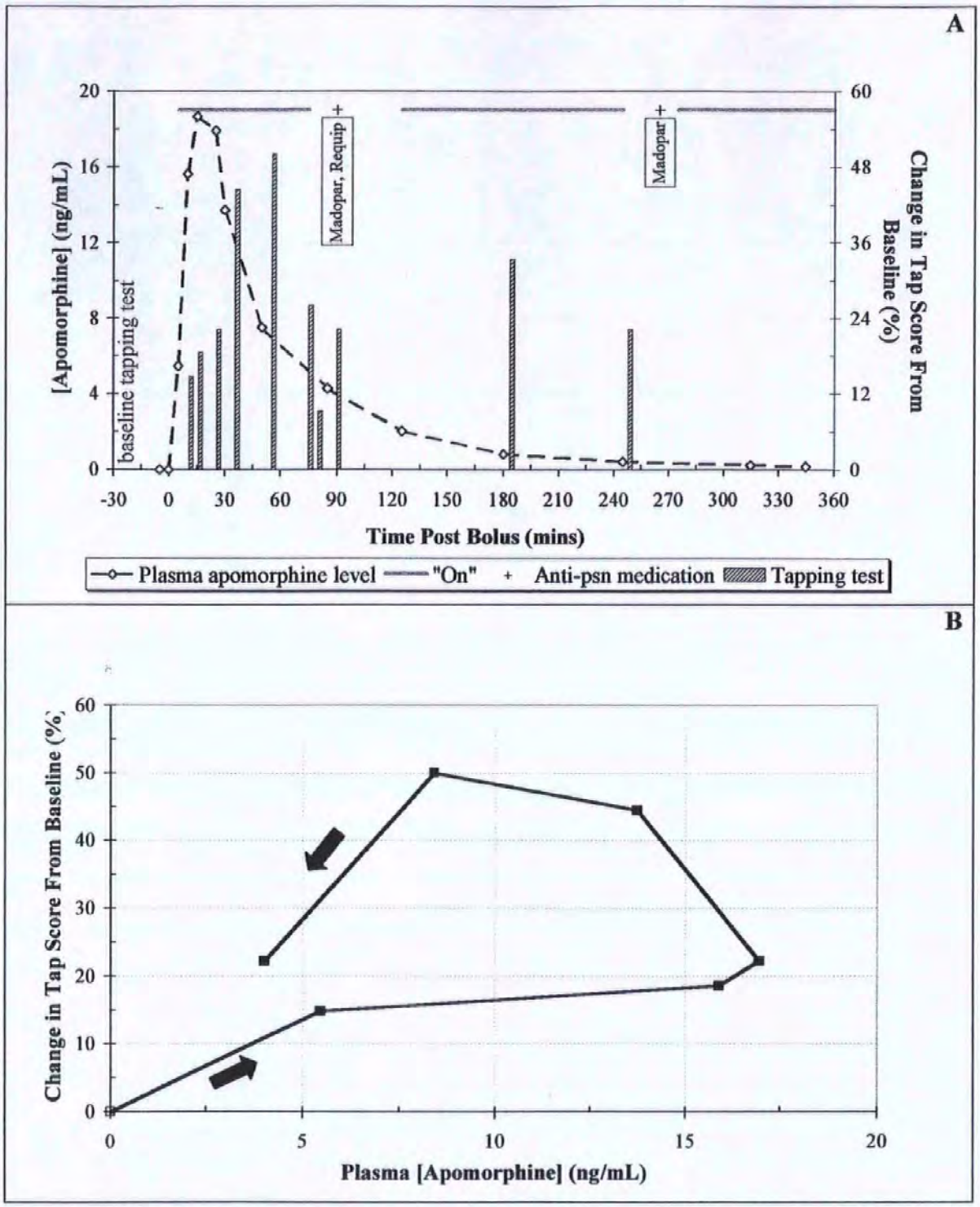

Figure 5-8 **Panel A: Plasma apomorphine concentration/clinical status profile following apomorphine bolus administration (2mg) to Patient 09.**

Panel B: Relationship between plasma apomorphine concentration and anti-parkinsonian effect.

Arrows indicate chronological order of occurrence.

Filled squares indicate that, at the time of sampling, the patient was in an apomorphine-induced "on" phase. Open squares indicate that, at the time of sampling, the patient was in a parkinsonian "off" phase.

Patient 10.

At baseline the patient was affected by sciatica independently of parkinsonian symptoms. The patient identified leg stretching as a characteristic sign that he was switching "on". This sign occurred at four minutes post-apomorphine. A period of improvement in parkinsonian symptoms then ensued until 23 minutes post dose, when parkinsonian symptoms, e.g. bradykinesia, returned. The return to a parkinsonian "off" state was short-lived, lasting only 3 minutes. The second period of improvement in parkinsonian symptoms ended at 82 minutes post dose; cessation of effect was defined by the by patient's increasing difficulty moving, as evidenced by the tapping test score. The time course of plasma apomorphine concentration and clinical status following apomorphine bolus administration is illustrated in Figure 5-9.

The effect of apomorphine on individual parkinsonian symptoms is documented below, whereby the assessment of effect was made in the second period of improvement in parkinsonian symptoms.

Speech and facial expression.

Both were considered to be moderately impaired at baseline, and were improved following apomorphine administration, although minimal impairments in both persisted.

Tremor.

Resting and postural tremor was absent both prior to and following apomorphine administration.

Rigidity.

At baseline rigidity was judged to be slightly affecting the right lower extremity, was mild to moderate in the neck and the left extremities, and was marked in the right upper extremity. Apomorphine did not effect a change in these parameters.

Rapid, repetitive hand movements.

Performance at baseline ranged from being mild to severely impaired, depending on the exact test and hand used. Following apomorphine administration, performance was predominantly only mildly impaired.

Posture

Slight improvements in posture were observed following apomorphine administration, however there was no improvement in postural stability; this remained slightly abnormal.

Gait

Patient 10 displayed some difficulty in walking at baseline, but did not require assistance in doing so. Following apomorphine administration walking ability was improved, but not to the point at which gait could be considered normal.

Body bradykinesia

The effect of apomorphine was to improve the moderate bradykinesia and hypokinesia that was evident at baseline to a mild, but definitely abnormal, state.

The patient rated the response to the apomorphine bolus as sub-optimal compared to typical experience.

Visual inspection of the (observed) plasma apomorphine concentration verses (observed) effect plot (Figure 5-9B) revealed that no clear relationship existed between effect and plasma concentration.

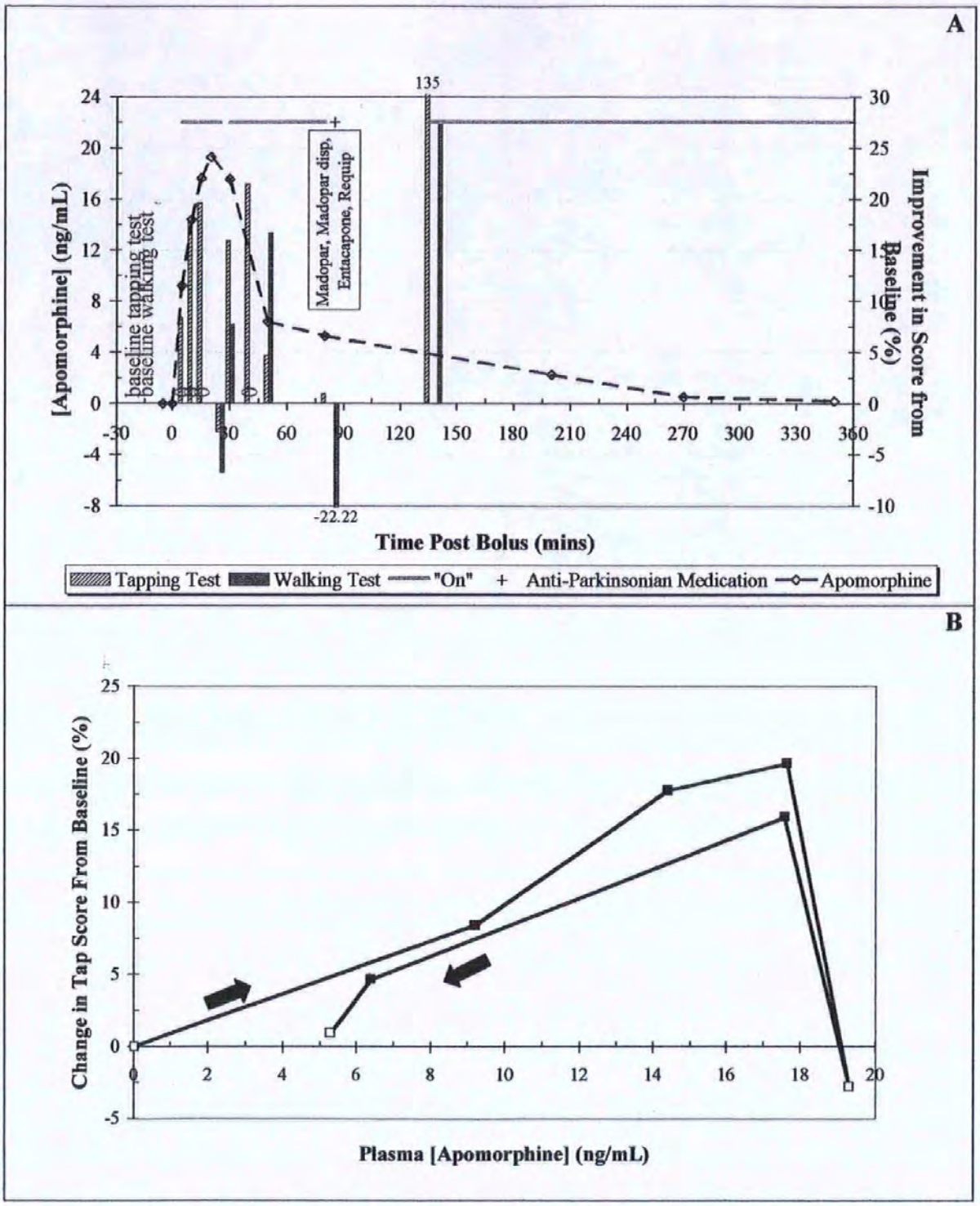

Figure 5-9 **Panel A: Plasma apomorphine concentration/clinical status profile following apomorphine bolus administration (5mg) to Patient 12.**

Panel B: Relationship between plasma apomorphine concentration and anti-parkinsonian effect.

Arrows indicate chronological order of occurrence.

Filled squares indicate that, at the time of sampling, the patient was in an apomorphine-induced "on" phase. Open squares indicate that, at the time of sampling, the patient was in a parkinsonian "off" phase.

Patient 12.

The time course of plasma apomorphine concentration and clinical status following apomorphine bolus administration is illustrated in Figure 5-10. Onset of effect occurred at nine minutes post dose according to the patient's commentary. The period of improvement in parkinsonian symptoms lasted for sixty minutes, the cessation of effect being defined by a reduction in tapping test scores to below baseline level.

The effect of apomorphine on individual parkinsonian symptoms is documented below, whereby the assessment of effect was made thirty-three minutes post-dose.

Speech.

Apomorphine had no effect on speech, which remained moderately impaired.

Facial expression.

There was moderate hypomimia at baseline, which was improved by apomorphine to what was considered a slight diminution of facial expression.

Tremor.

At baseline a slight resting tremor affected the face, lips, chin, hands and right foot. Additionally a slight postural tremor affected the right hand. The effect of apomorphine was to abolish tremor from the aforementioned regions.

Rigidity.

Apomorphine had no effect on rigidity of the neck (which was markedly affected) or the lower left extremity (which was slightly affected). However, the mild to moderate rigidity in the upper extremities was reduced to a very slight level following apomorphine administration.

Rapid, repetitive hand movements.

Performance at baseline in tests of repetitive hand movements ranged from being mild to severely impaired, depending on the exact test and hand used. Following apomorphine administration, improvement was seen in the majority of parameters, such that the performance was mild to moderately impaired.

Posture

It was demonstrated that postural reflexes were normal at baseline and that posture was only slightly impaired. These features did not change upon apomorphine administration.

Gait

Patient 10 displayed a normal gait at both baseline and post apomorphine dosing.

Body bradykinesia

The effect of apomorphine was to improve the mild bradykinesia that was evident at baseline to a minimal movement impairment that could be considered normal for some persons.

The patient rated the response to the apomorphine bolus as typical of that experienced under normal circumstances.

Visual inspection of the (observed) plasma apomorphine concentration verses (observed) anti-parkinsonian effect plot (Figure 5-10B) revealed the existence of counter-clockwise hysteresis. This was indicative of an indirect, i.e. time-dependent, relationship between plasma concentration and effect.

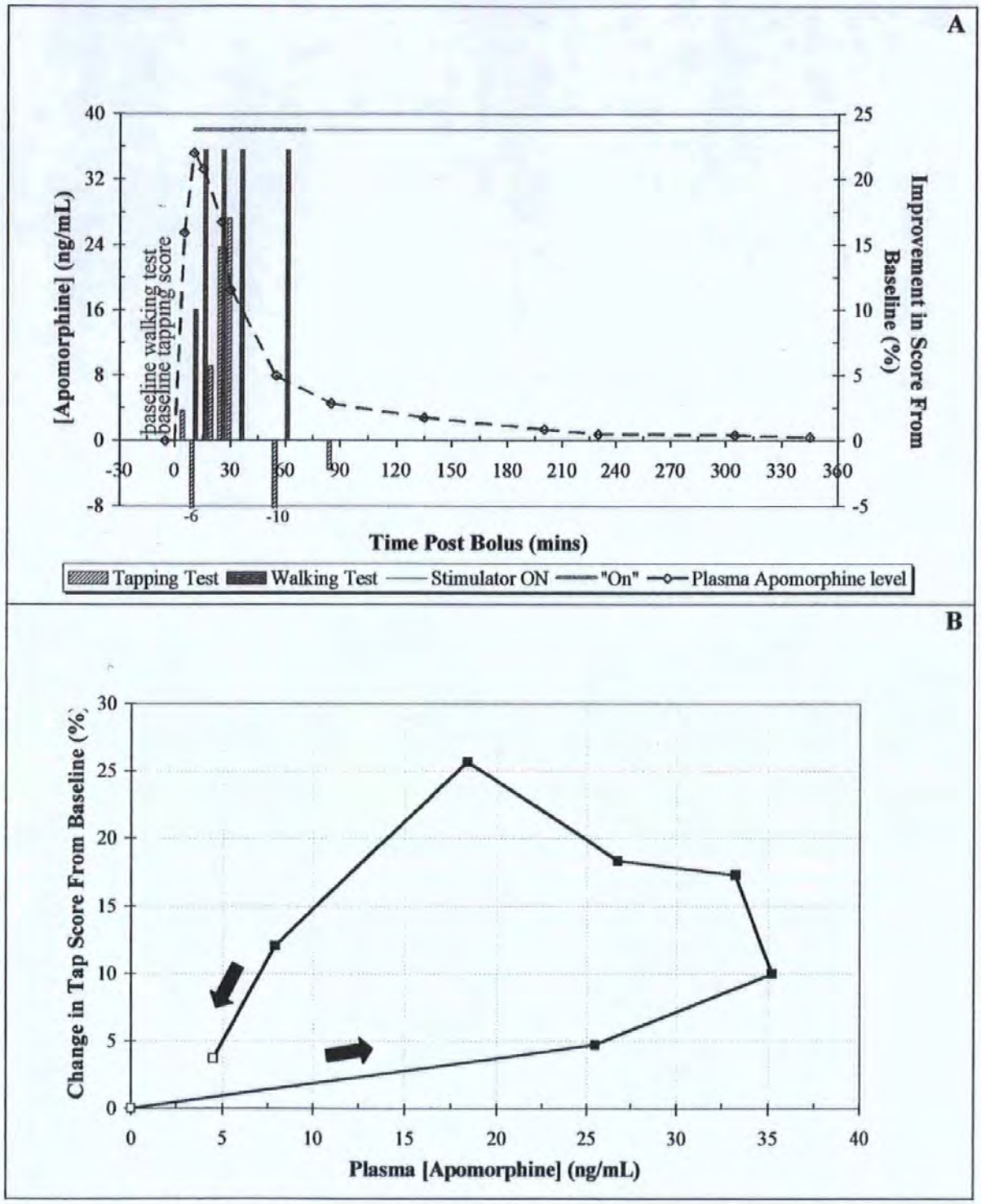

Figure 5-10 **Panel A: Plasma apomorphine concentration/clinical status profile following apomorphine bolus administration (5mg) to Patient 12.**

Panel B: Relationship between plasma apomorphine concentration and anti-parkinsonian effect.

Arrows indicate chronological order of occurrence.

Filled squares indicate that, at the time of sampling, the patient was in an apomorphine-induced "on" phase. Open squares indicate that, at the time of sampling, the patient was in a parkinsonian "off" phase.

Patient ID	UPDRS Part III		Tapping Test		Onset of effect	Duration of effect	Quality of "on"[a]
	Change in score: "off" to "on"[b] (% improvement)	Time of "on" phase rating (mins post-dose)	Change in score: baseline to peak "on" score[c] (% improvement)	Time of maximum score (mins post-dose)	(mins post-dose)	(mins)	
01	NA	NA	11	17 and 57	12	44	Sub-optimal
02	NA	NA	11	49	29	96	Sub-optimal
04	NA	NA	21	47	8	121	Toxic
05	NA	NA	25	42	10	54	Sub-optimal
09	31	41	50	51	5	70	Optimal
10	10	43	22	39	4	78	Sub-optimal
12	18	33	26	32	9	60	Optimal

Table 5-1 Anti-parkinsonian effect of subcutaneous bolus administration of apomorphine. Abbreviation: NA = not applicable

[a] Relative to patients' usual experience.
[b] Calculated using (("off" score – "on" score) / 108) x 100.
[c] Calculated as ((peak "on" score – baseline score) / baseline score) x 100.

5.2. Preliminary Study Of Needle-Free Subcutaneous Injections Of Apomorphine In Parkinson's Disease.

The time courses of apomorphine concentration and pharmacodynamic response following needle-free administration are given in Figure 5-11 to Figure 5-13.

The improvements in tap score and in UPDRS score in each case reflected the patients' rating of the "on" phase (Table 5-2, raw data is given in Appendix 8.14).

Some mild adverse effects were observed in or reported by patient 09, i.e. the patient reported being "hot" and "light-headed", and was observed to be restless and flushed in the face. These occurred following both apomorphine doses, but to a slightly greater extent following needle-free administration, see Figure 5-14. Yawning was observed in two patients following each treatment, and in the third patient as a result of needle-free apomorphine only (patient 12).

Needle-free delivery was rated as the same or more painful than conventional delivery (Table 5-3). Administration of apomorphine resulted in slight bleeding at the site on two occasions, each following needle-free delivery (patient 10 trial 2 and patient 12 - see Table 5-3).

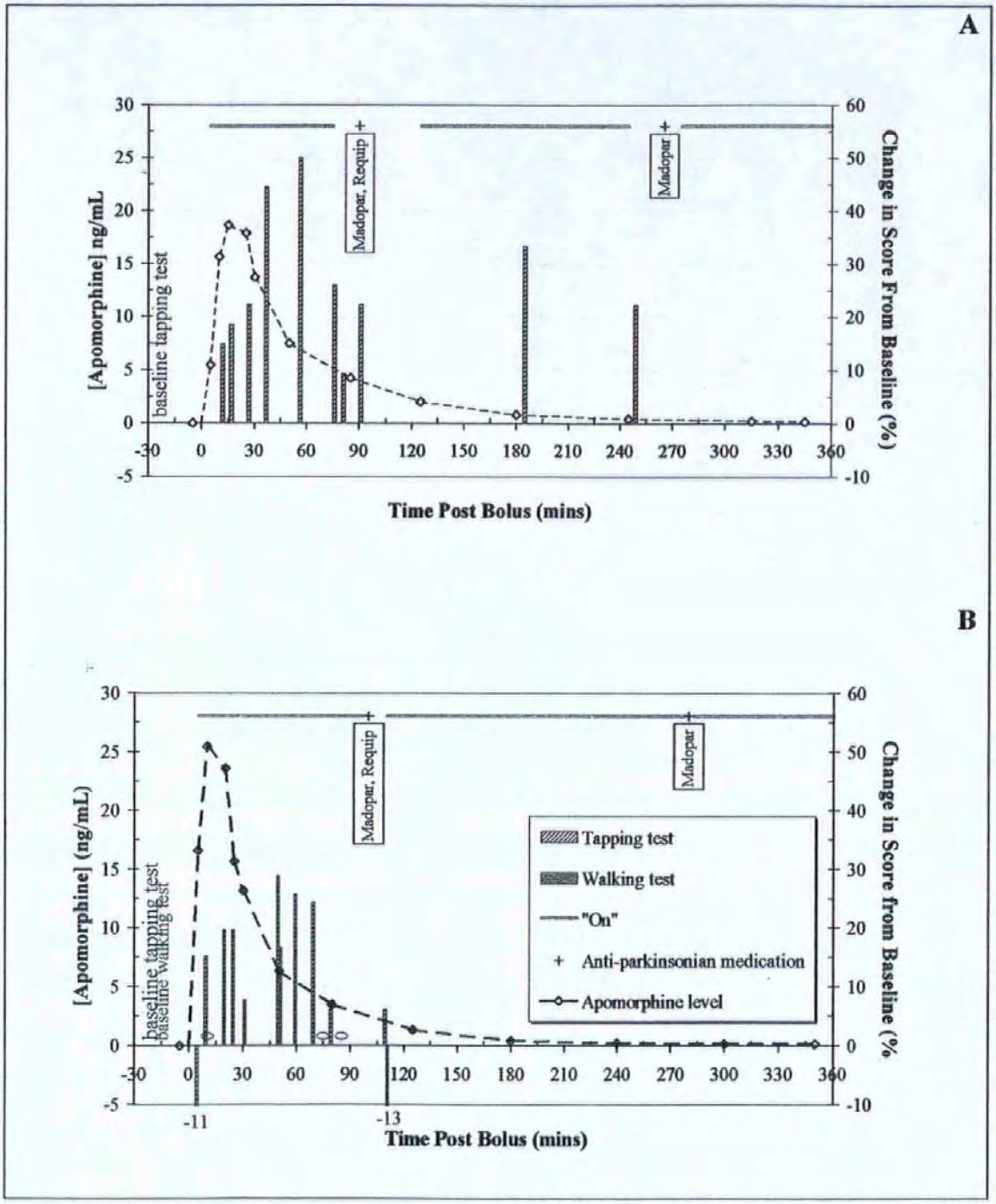

Figure 5-11 Plasma apomorphine level and clinical status profile following conventional (panel A) and needle-free (panel B) delivery: patient 09.

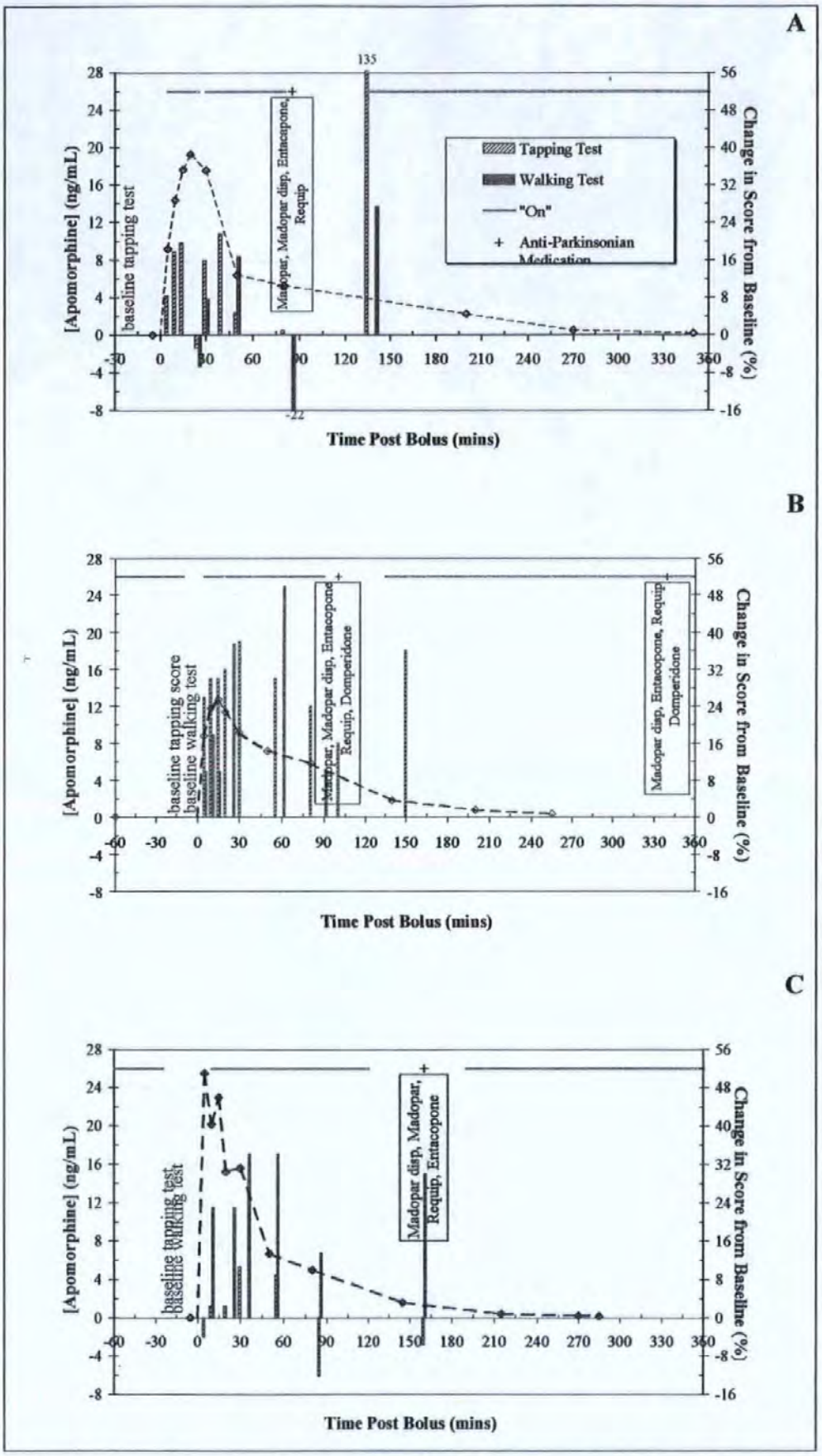

Figure 5-12 Plasma apomorphine level and clinical status profile following conventional (panel A) and needle-free delivery trials 1 and 2 (panels B and C respectively): patient 10.

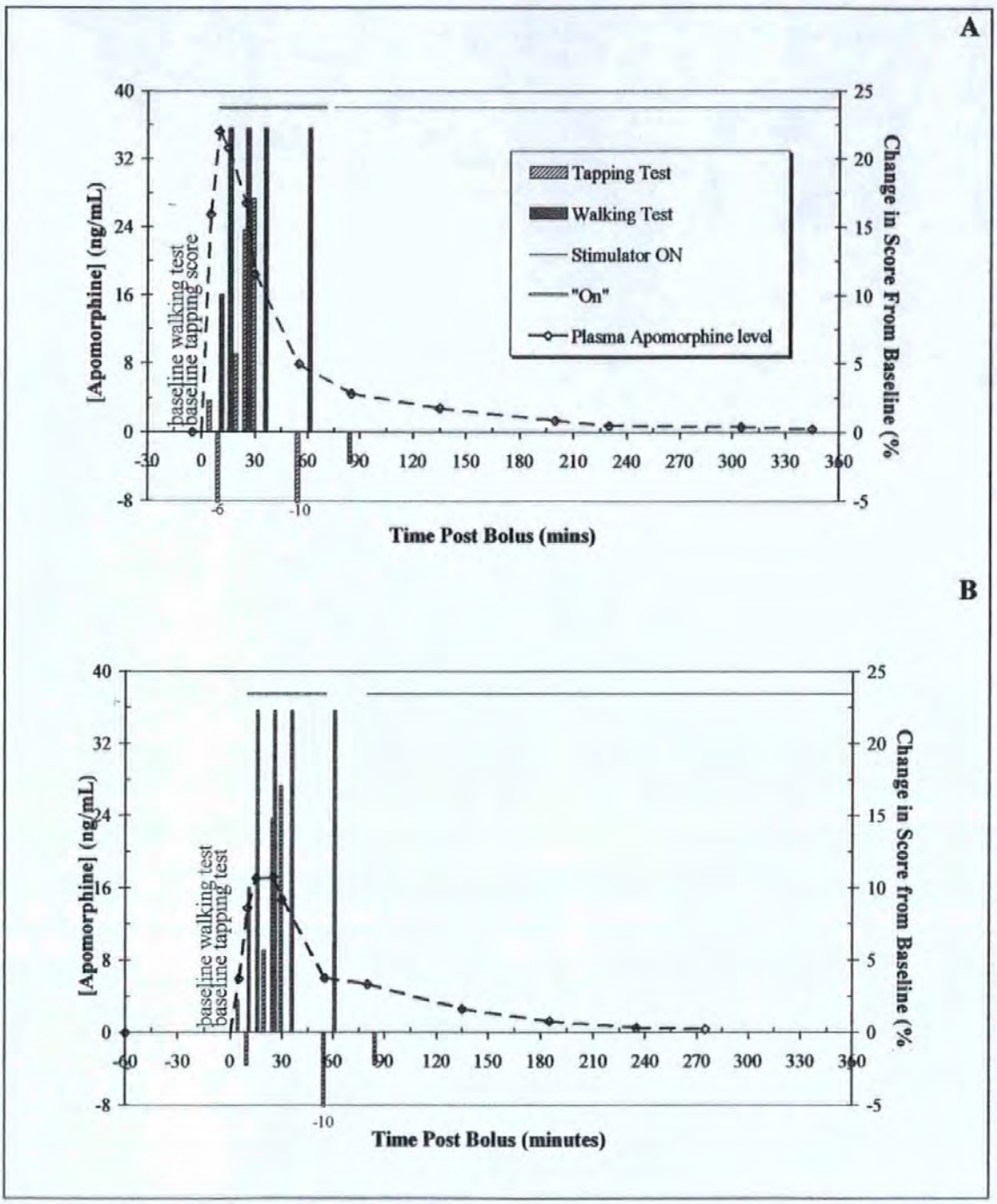

Figure 5-13 Plasma apomorphine level and clinical status profile following conventional (panel A) and needle-free (panel B) delivery: patient 12.

Patient ID		UPDRS Part III		Tapping Test			Onset of effect	Duration of effect	Quality of "on"[a]
		Change in score: "off" to "on" (% improvement)	Time of "on" phase rating (mins post-dose)	Hand used	Change in score: baseline to peak "on" score (%)	Time of maximum score (mins post-dose)	(mins post-dose)	(mins)	
09	CON	31	41	Right	50	51	5[b]	70[c]	Optimal
	NF	29	26		29	48	3[b]	74[c]	Optimal
10	CON	10	43	Right	11	39	4[d]	78[e]	Sub-optimal
	NF 1	14	33		33	30	4[d]	85[f]	Optimal
	NF 2	20	36		38	31	15[d]	105[g]	Optimal
12	CON	18	33	Left	26	32	9[h]	60[i]	Optimal
	NF	9	38		17	31	13[j]	45[i]	Sub-optimal

Table 5-2 Anti-parkinsonian effect of subcutaneous apomorphine administered by conventional (needle) and novel (needle-free) devices.

[a] Relative to patients' typical experience.
[b] Onset of effect was defined by patient's comment: "loosening up now".
[c] Cessation of effect was defined subjectively by clinician: "slowing down now", tremor recurring.
[d] Onset of effect was defined by leg stretching – identified as a qualitative marker of apomorphine effect in this patient.
[e] Cessation of effect was defined by patient's increasing difficulty moving, supporting evidence from tapping test score.
[f] Cessation of effect was defined by patient's comment: "going "off"".
[g] cessation of effect was defined by patient's comment: "gone "off"".
[h] Onset of effect was defined by patient's comment: "taken away dead feeling".
[i] Cessation of effect was defined subjectively by clinician, supporting evidence from tapping test score.
[j] Onset of effect was defined by observation that body movement became more fluid, especially evident in right upper limb.

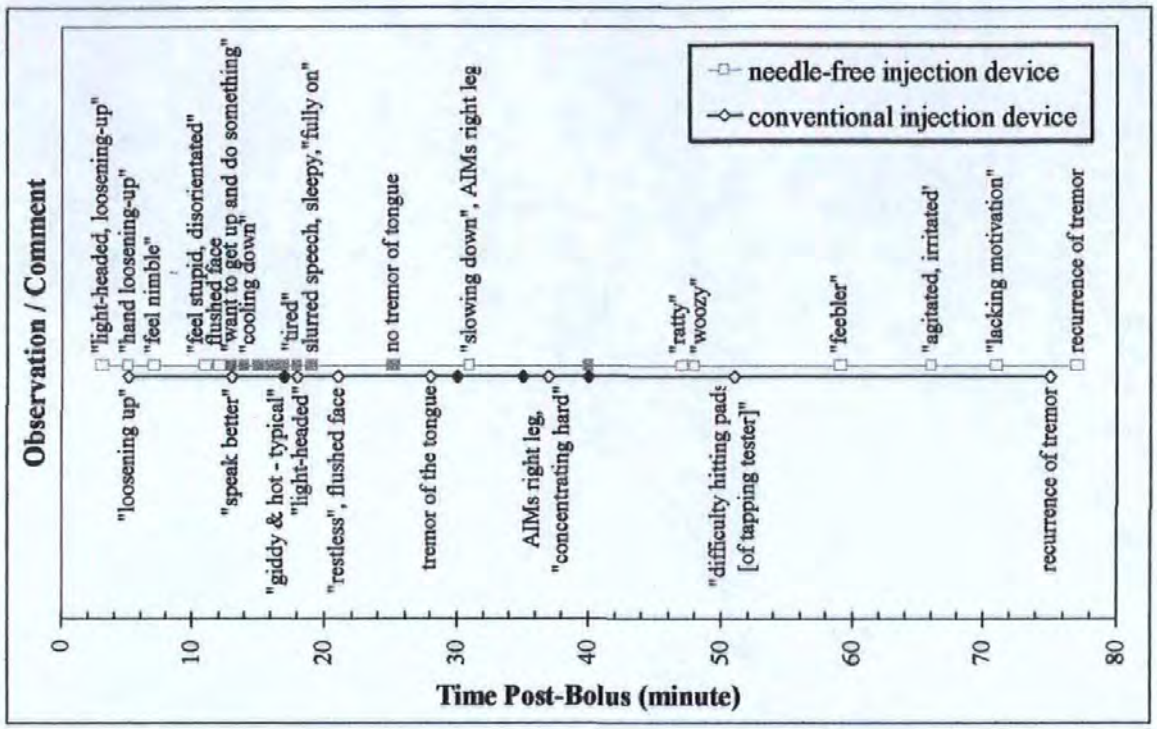

Figure 5-14 Symptom observation and patient commentary following apomorphine dosing: patient 09. Filled symbols indicate yawning.

Abbreviation: AIM = abnormal involuntary movement.

Patient ID No.	Treatment	Pain score (0=min, 10=max)	Local tissue reaction: Bleeding at puncture site?	Local tissue reaction: Significant adverse event?
9	CON	1	No	No
	NF	3	No	No
10	CON	1	No	No
	NF 1	1	No	Yes
	NF 2	3	Yes	No
12	CON	1	No	No
	NF	1	Yes	Yes

Table 5-3 Tolerability of apomorphine administration.

Abbreviations: CON = conventional, NF = needle-free.

On each occasion following conventional delivery of apomorphine, there was no evidence of abnormal local tissue reaction on the study day. Follow-up on the injection site was possible for patients 09 and 10 on day seven and day four, respectively. At this time, there had been no abnormal reaction at the site of injection. There was no follow-up on patient 12.

Administration via the needle-free jet injection system produced a "bull's-eye" marking on the epidermis, the outer ring having been imprinted by the syringe and the inner dot, a pinprick-sized spot of blood, at the actual puncture site (as described by Florentine *et al*[2]).

On two occasions (patient 09 and patient 10 trial 2) there was no abnormal local reaction following needle-free delivery of apomorphine during the study day. Confirmation was received from patient 10 four weeks after the study day (needle-free trial 2) that there had been no subsequent adverse reaction at the injection region. There was no follow-up on patient 09.

However an adverse local tissue reaction *did* occur as a result of needle-free delivery on the other two occasions (patient 10 trial 1 and patient 12). In fact it was due to this development that patient 10 volunteered to return for a second trial of needle-free delivery of apomorphine.

In the case of patient 10 (trial 1) it was noted that at approximately four hours post-dose a region of approximately 15mm in diameter around the injection site had become hardened. The indurated tissue was surrounded by a bruise which extended to approximately 25mm from the injection point. The hardened area persisted for five weeks post-dose and the bruise, which was described as being vivid in colour (purple and pink), remained present for a further week. This local tissue reaction was described as being distinct from the

bruising which occasionally had been experienced as a result of apomorphine administration via a needle. The latter was reported to be very dark blue in colour, and present only for ten to fourteen days post dose.

In the case of patient 12, a very slightly raised and indurated region of approximately 15mm in diameter around the injection point was noted at 183 minutes post-dose and, whilst no further changes were recorded on the study day, bruising was present at follow-up (ten days post-dose). The bruising, which was purple/brown in colour at this time, affected an area of approximately 70mm in diameter around the injection site. A region of approximately 10mm in diameter directly surrounding the injection site, and corresponding to the cross sectional area of the J-TIP® syringe, appeared entirely unaffected.

5.3. *Pharmacokinetic Study of Single-Dose Intra-Nasal Apomorphine Powder (Three Doses) in Healthy Volunteers.*

Analysis of the residual apomorphine contained in the used capsules (and insufflators) revealed that the mean amount of apomorphine released was 77.8% of the initial amount, with a range of 8.0 to 97.0% (Penn Pharmaceuticals, UK, reproduced with permission), see Figure 5-15.

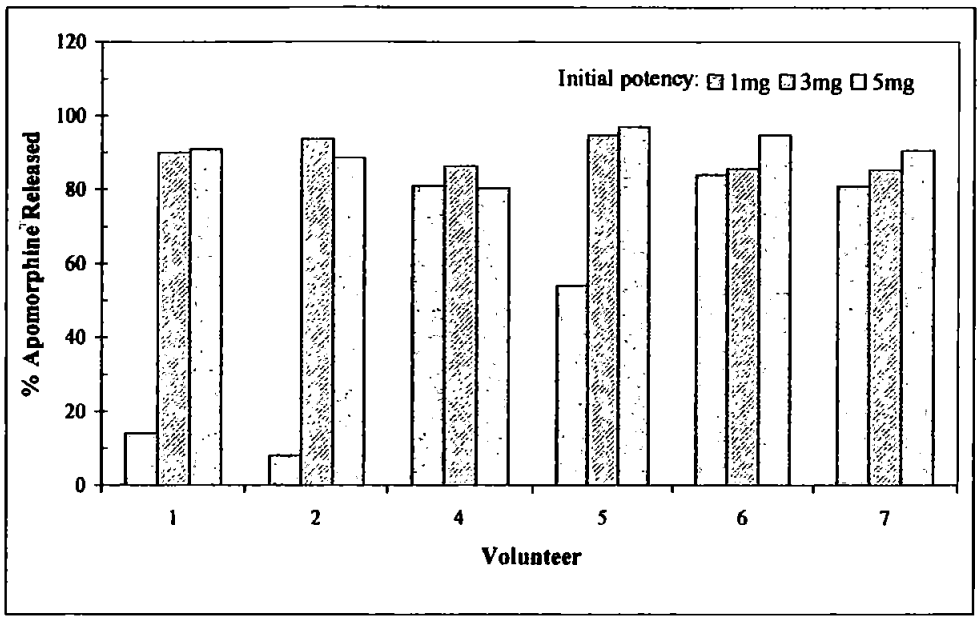

Figure 5-15 Release of apomorphine from intranasal delivery system. Reproduced with permission (Britannia Pharmaceuticals Ltd, Redhill, UK).

The apomorphine concentration in two series of plasma samples, i.e. the low dose intranasal dose for volunteers 1 and 2, was found to be below the assay detection limits. Plasma apomorphine concentration-time profiles for the remaining series are given in Figure 5-16 to Figure 5-21.

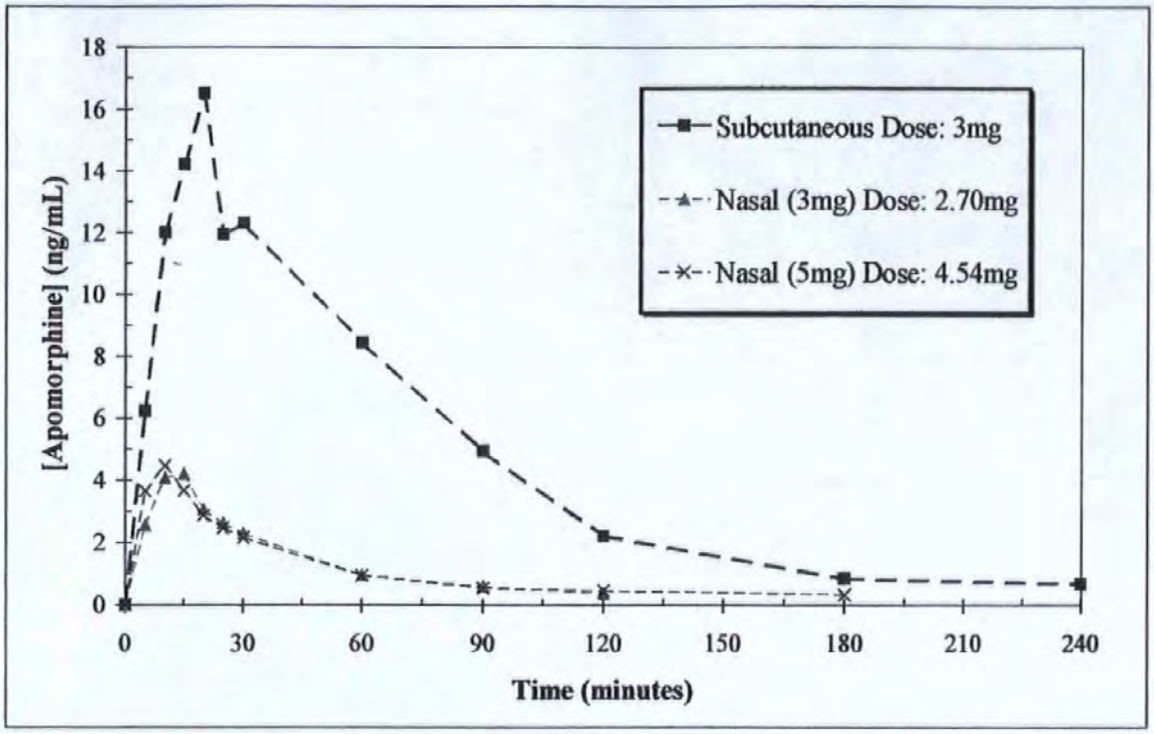

Figure 5-16 Plasma apomorphine concentration following subcutaneous and intranasal administration to volunteer 1. Initial potency of intranasal capsule is given in parentheses.

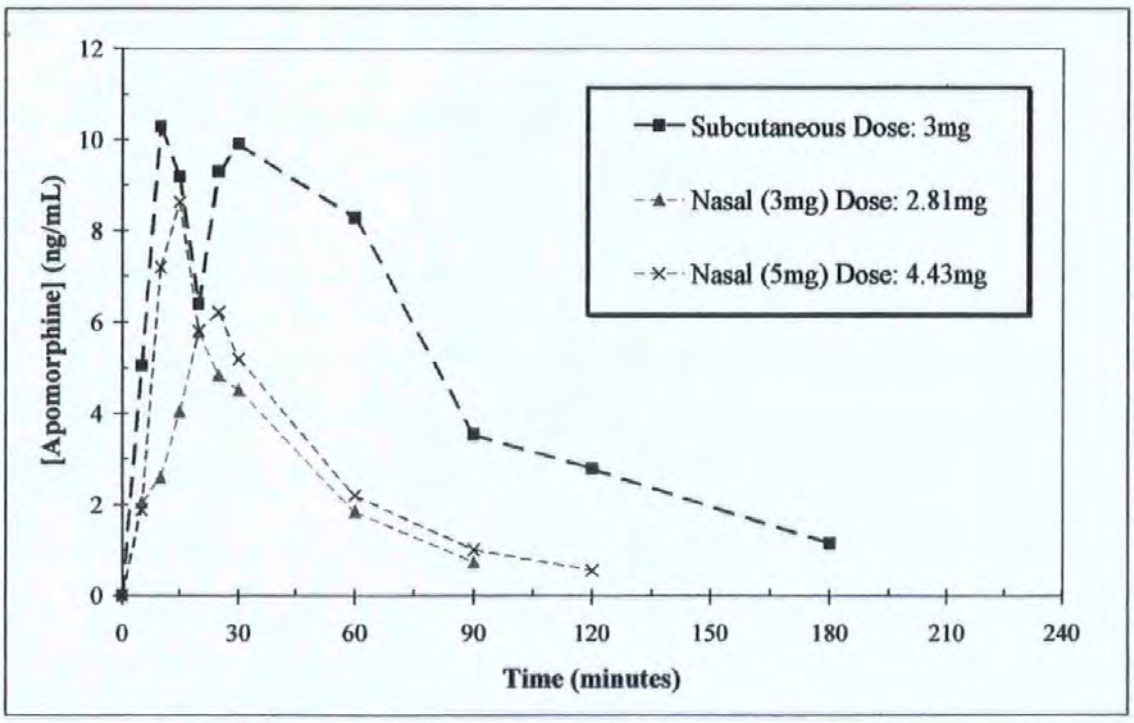

Figure 5-17 Plasma apomorphine concentration following subcutaneous and intranasal administration to volunteer 2. Initial potency of intranasal capsule is given in parentheses.

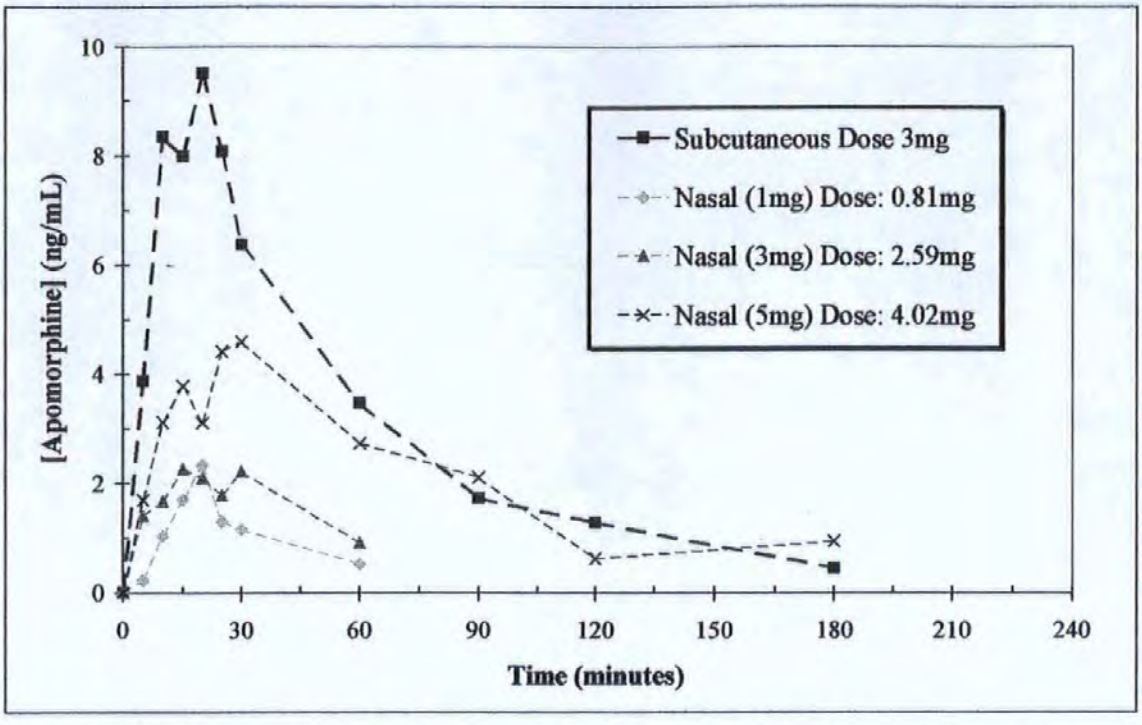

Figure 5-18 Plasma apomorphine concentration following subcutaneous and intranasal administration to volunteer 4. Initial potency of intranasal capsule is given in parentheses.

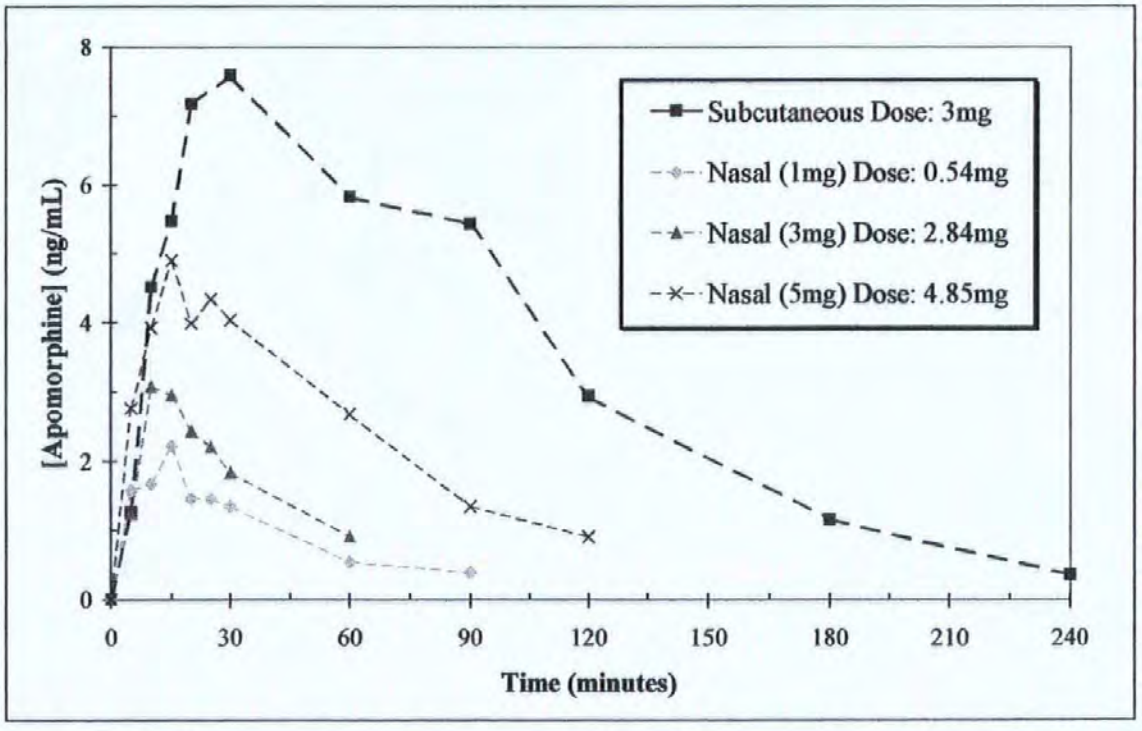

Figure 5-19 Plasma apomorphine concentration following subcutaneous and intranasal administration to volunteer 5. Initial potency of intranasal capsule is given in parentheses.

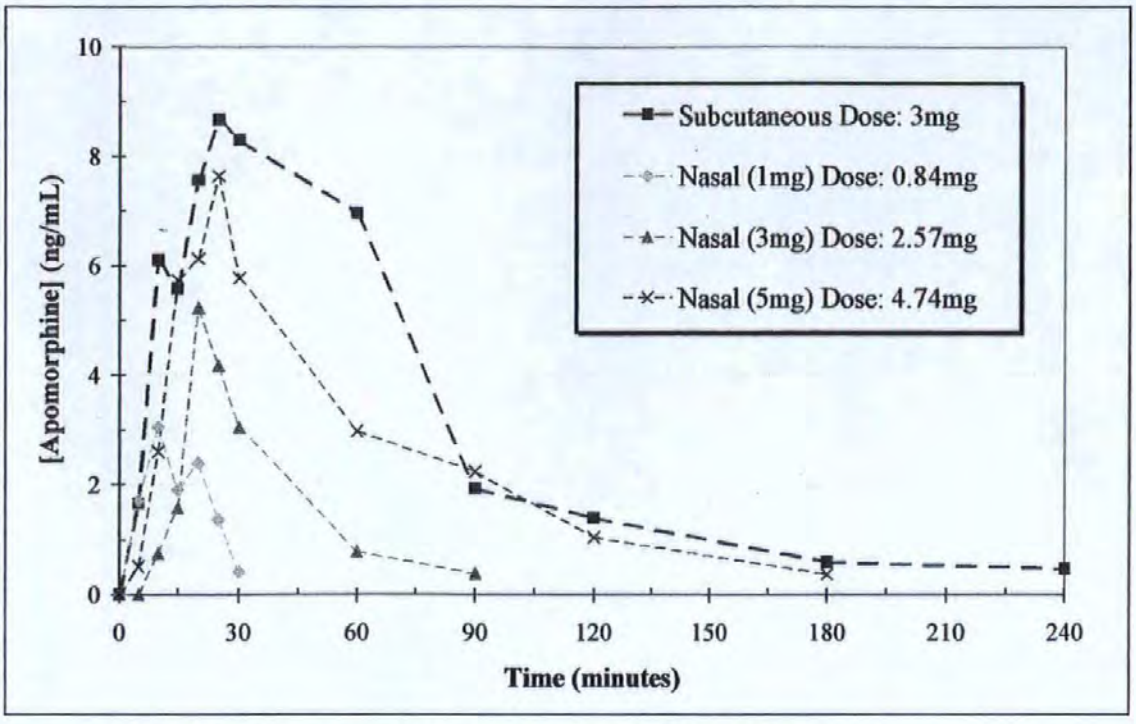

Figure 5-20 Plasma apomorphine concentration following subcutaneous and intranasal administration to volunteer 6. Initial potency of intranasal capsule is given in parentheses.

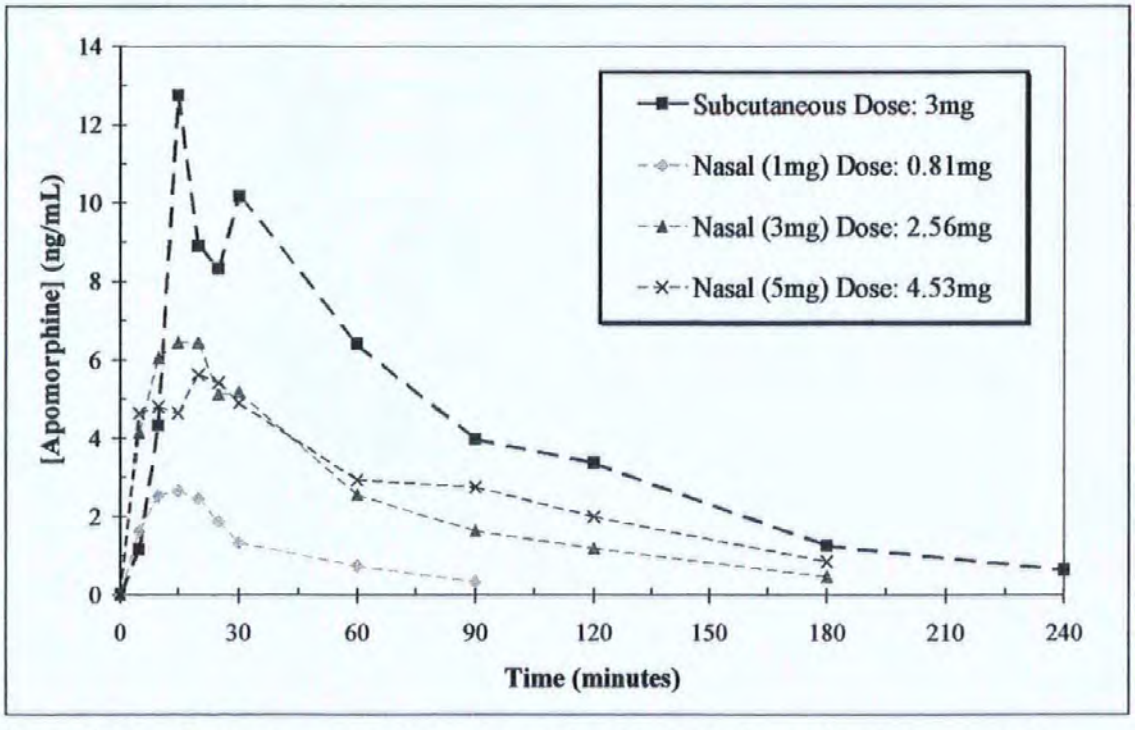

Figure 5-21 Plasma apomorphine concentration following subcutaneous and intranasal administration to volunteer 7. Initial potency of intranasal capsule is given in parentheses.

There were no serious adverse effects observed as a result of apomorphine administration. The adverse effects that did occur were those which are commonly associated with apomorphine therapy in Parkinson's disease. The highest incidence of adverse effects occurred after the high (5mg) intranasal dose (unpleasant or bitter taste, tingling in nostril, lethargy, difficulty in maintaining concentration), although the number of reported adverse events was not dissimilar to that reported for the mid (3mg) intranasal dose or the subcutaneous dose. The incidence of adverse effects following the low (1mg) intranasal dose was extremely small, probably as a result of the very low administered doses of apomorphine. Nasal examination revealed that there were limited cases of mild, transient inflammation and crusting[3].

5.4. Pharmacokinetic Study of Single-Dose Buccal Apomorphine (Three Doses) in Healthy Volunteers.

Analysis of the residual apomorphine contained in the used inserts revealed that the release of apomorphine from the inserts was low and variable: the mean amount of apomorphine released was 9.6% of the initial potency, with a range of 0.1 to 35.1% (Controlled Therapeutics (Scotland) Ltd, reproduced with permission, see Figure 5-22). This corresponded to a range of apomorphine doses of 0.01 to 7.37mg over 120 minutes. It was evident that at each dosage level the greatest release of apomorphine occurred in the *same* individual, i.e. volunteer 1.

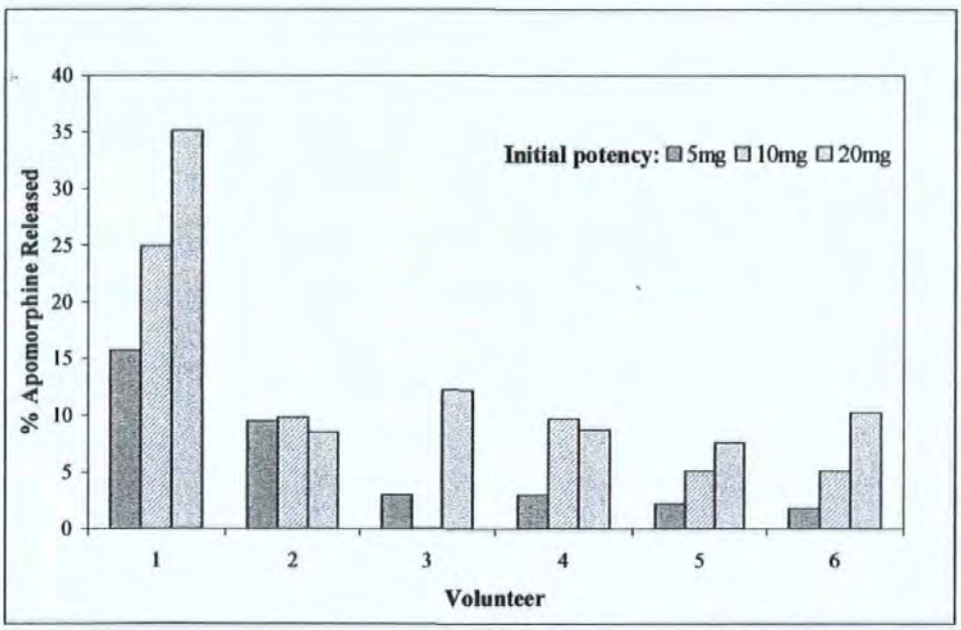

Figure 5-22 Release of apomorphine from buccal hydrogel inserts (Controlled Therapeutics (Scotland) Ltd, reproduced with permission.

In eleven of the eighteen buccal series apomorphine could not be detected in any plasma samples. Plasma apomorphine concentration-time profiles are given for the subcutaneous infusion series in Figure 5-23, and the remaining seven buccal series in Figure 5-24.

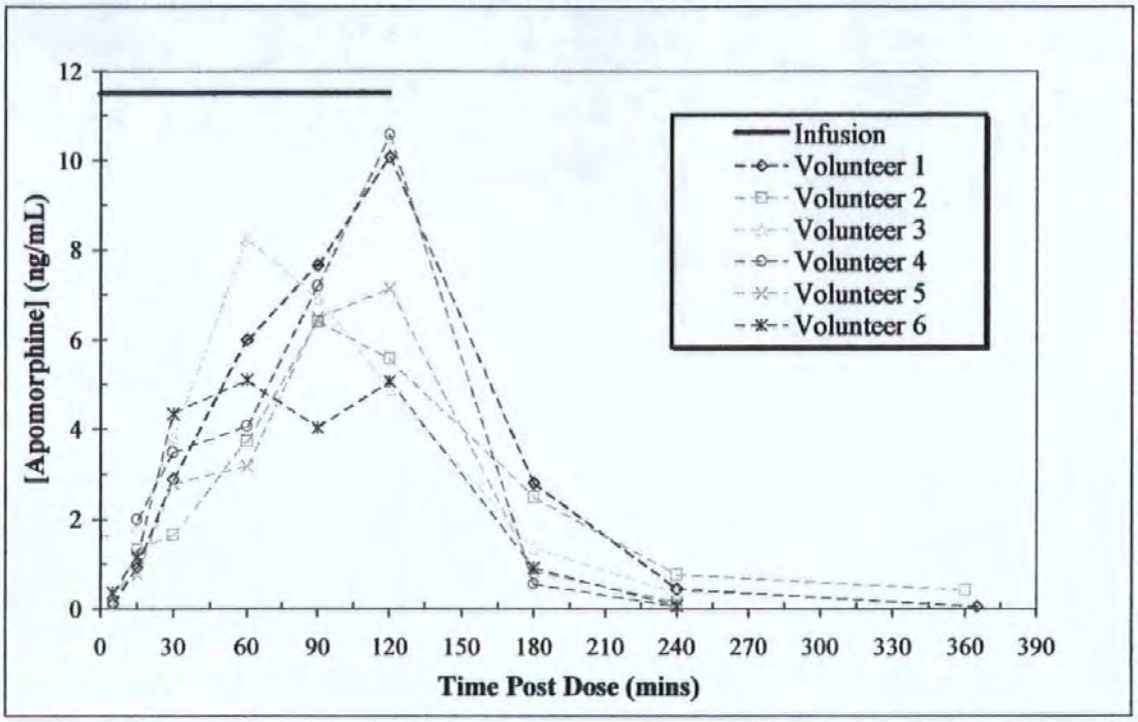

Figure 5-23 Plasma apomorphine concentration following administration of subcutaneous infusion to healthy volunteers (dose = 2mg/h x 2h).

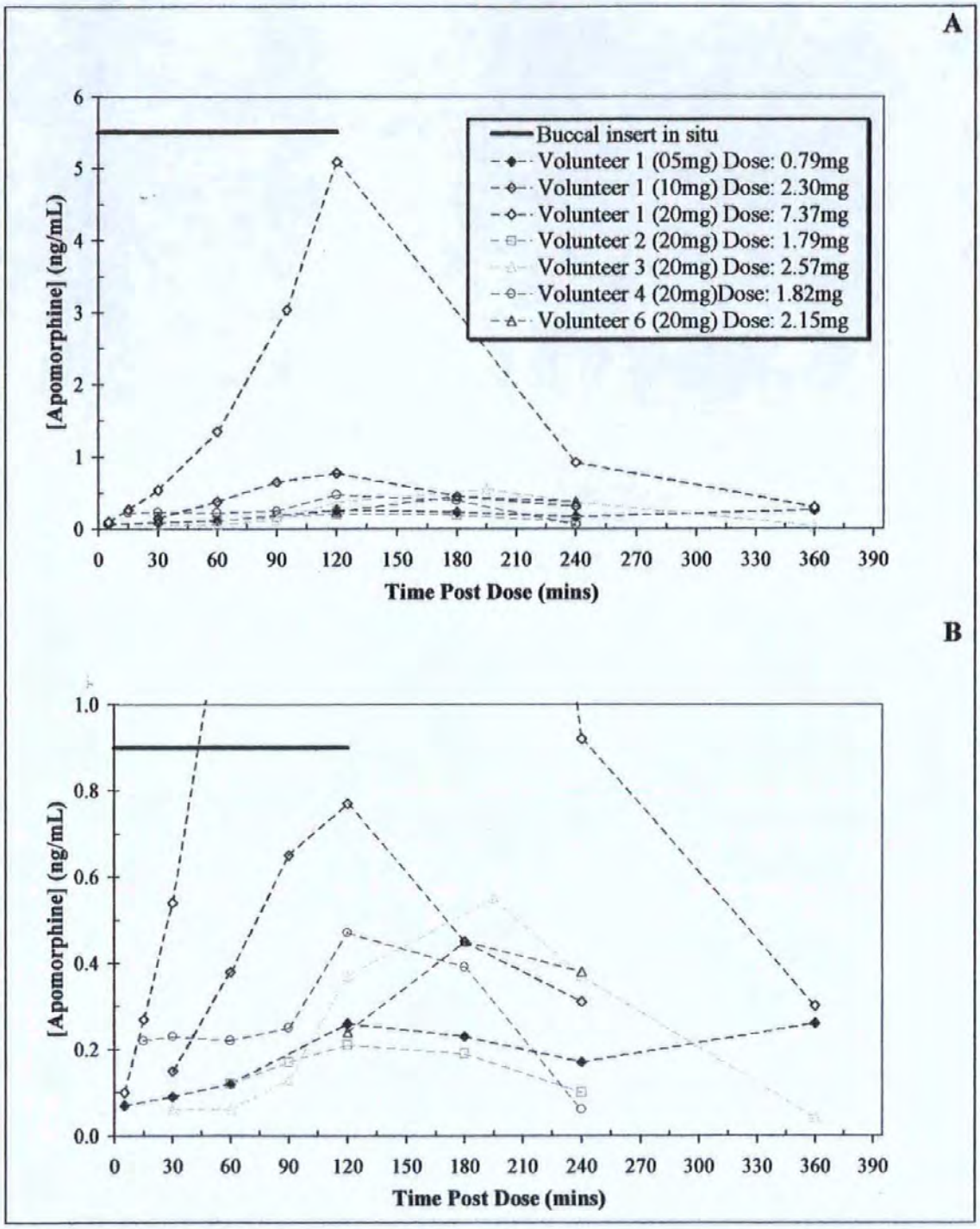

Figure 5-24 Plasma apomorphine concentration following buccal administration to healthy volunteers. Initial potency of buccal insert is given in parentheses. Detail shown in panel B.

In accordance with the *in vivo* swelling characteristics of the hydrogel, there was a lag in the time taken for apomorphine to reach a detectable concentration in the plasma, with the exception of volunteer 1 (all three doses). Volunteer 1 was also exceptional in that this was the only individual for whom reasonable delivery of buccal apomorphine, based on analysis of residual apomorphine in the used buccal inserts (see Figure 5-22, page 5-38), was demonstrated. These two features indicate that there was superior hydration of the insert and/or desorption of apomorphine from the insert in this individual compared to the other volunteers in the study. Factors which might be considered conducive to the superior buccal apomorphine release observed for volunteer 1 are high salivary flow and/or low salivary pH, neither of which were monitored in this study.

In contrast to the predicted performance of the buccal insert *in vivo* (based on the *in vitro* and *in vivo* buccal swelling tests, see Figure 4-4 and 4-5 on page 4-29), there was no evidence of a slowing down in the rate of absorption of apomorphine into plasma in most, i.e. five out of seven, cases. This suggested that drug release rate had not (yet) plateaued in the latter stages of *in situ* exposure to the insert. In the remaining two cases, T_{max} occurred one hour *after* the removal of the insert, i.e. at 180 minutes post-commencement of apomorphine administration.

The incidence of local adverse effects reported by the volunteers was minimal, and where such an event did occur, the effect was mild and transient[4].

Bibliography: Chapter 5.

1. Christmas TJ, Chapple CR and Lees AJ, Role of subcutaneous apomorphine in parkinsonian voiding dysfunction [Abstract]. *Lancet* 1988; 2 : 1451-53.

2. Florentine BD, Frankel K, Raza A, Cobb CJ, Greaves T, Carriere C and Martin S, Local anesthesia for fine-needle aspiration biopsy of palpable breast masses: the effectiveness of a jet injection system. *Diagnostic Cytopathology* 1997; 17 (6): 472-476.

3. Jones M, *Pharmacokinetic Study of Single-Dose Intra-Nasal Apomorphine Powder (3 Doses) in Healthy Volunteers.* Clinical Phase Study Report (Draft 3) , LCG Bioscience, Cambridge, 1999.

4. Lambert P, Britannia Pharmaceuticals Ltd,*personal communication: Clinical Study Documentation*, 27/09/99.

SECTION 6:
DISCUSSION

6. Discussion.

6.1. Pharmacokinetic-Pharmacodynamic Study of Subcutaneous Apomorphine Administration in Patients with Parkinson's Disease.

It is acknowledged that, as a result of difficulties experienced in the recruitment of patients in sufficient numbers for this study, the findings of this investigation are limited by a lack of information regarding intra-patient and inter-variation in pharmacokinetic parameters and pharmacodynamic response following apomorphine administration.

6.1.1. Pharmacokinetic Analysis.

Using the strategy given in Section 4.6, pharmacokinetic modelling was performed on nine plasma concentration-time series. In the majority of series a two-compartment model best described apomorphine pharmacokinetics, whereas a one-compartment model was superior in the remaining series; a finding entirely consistent with published data[1-3] (Table 6-1). In all but one case a lag-time (between drug administration and the onset of absorption) was required to adequately describe the plasma concentration-time profile following bolus dosing (see Appendix 8.15).

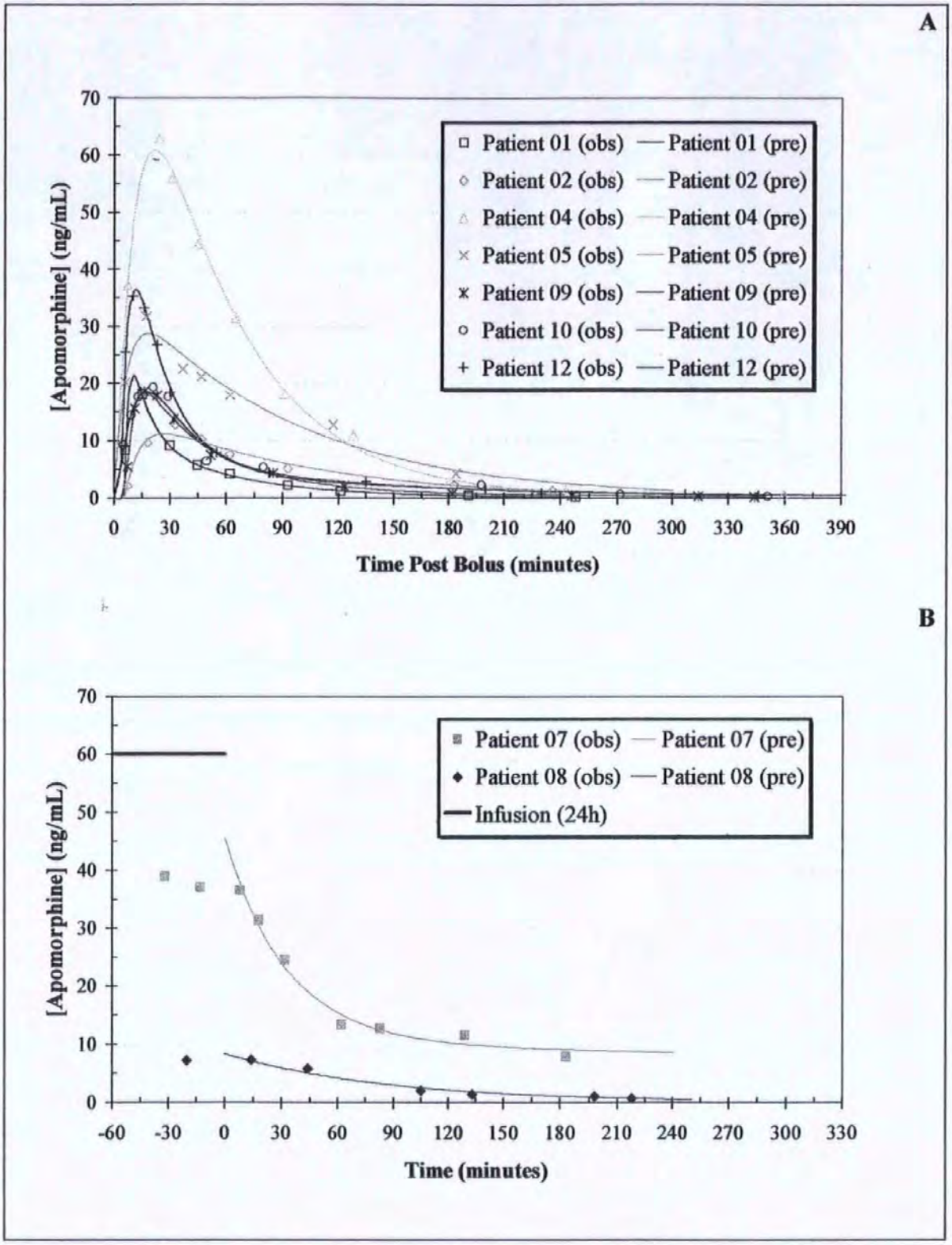

Figure 6-1 **Predicted plasma apomorphine concentration following subcutaneous administration.**

Panel A: Bolus doses were 2mg: patients 01 and 09, 3.5mg: patient 10, 5mg: patients 02, 05 and 12, 10mg: patient 04.

Panel B: Doses were 87μg/kg/h: patient 07, 26μg/kg/h: patient 08.

Patient ID	Pharmacokinetic Model: first order input and output.	
	Compartments	Lag-time?
01	2	Yes
02	1	Yes
04	2	Yes
05	1	No
09	2	Yes
10	2	Yes
12	2	Yes
	(post-infusion) first-order output.	
07	2	NA
08	1	NA

Table 6-1 Structure of models required in the modelling of apomorphine pharmacokinetics following subcutaneous administration.

Where parameter estimates are given in the following discussion, these are expressed as mean (range, number of observations) of values (individual patient parameter estimates can be found in Appendix 8.16).

As documented by previous authors (see Table 6-2), it was demonstrated that apomorphine undergoes rapid absorption, evidenced by the very brief lag-time, i.e. 3.8 minutes (1.8 to 5.9, n=6), short T_{max}, i.e. 17.5 (10 to 27, n=7), and short absorption half-live, i.e. 4.1 minutes (1.4 to 7.3, n=7). A large volume of distribution, i.e. 1.9 L/kg (1.0 to 3.4, n=7) and rapid clearance from plasma, i.e. 2.2L/kg/h (1.5 to 3.3, n=7), was demonstrated, also in accordance with available literature (see Table 6-3). Such properties have been attributed to the relatively high lipophilicity of apomorphine (log P = 2.15)[1, 4, 5].

Publication		Apomorphine Dose	N°. of patients	T_{max} minute		C_{max} ng/mL		C_{max} *normalised to 1mg dose*		AUC (0 to infinity, unless stated) ng/mL.min		AUC *normalised to 1mg dose*	
Gancher[5]	1989	20µg/kg as standard ≈1.4mg[a]	4	5.5	(CV=49%)	14.6[b]	(CV = 8%)	***10.4***[b,c]	***NA***	503[b]	(C.V. = 17%)	***359***[b,c]	***NA***
		30ug/kg as standard ≈2.1mg[a]	12	9.2	(CV=47%)	19.0[b]	(CV = 48%)	***9.0***[b,c]	***NA***	818[b]	(C.V. = 32%)	***390***[b,c]	***NA***
Gancher[6]	1991	20µg/kg as standard 1.45mg (1.4 and 1.5)	2	6.5	(5 and 8)	22[b]	(16 and 28)	***15.0***[b]	***(11 & 19)***	656[b]	(474 to 875)	***461***[b]	***(338 to 583)***
Montastruc[7]	1991	3mg as standard	9	20	(CV=20%)	26	(CV = 19%)	***9***	***NA***	837[d]	(C.V. = 18%)	***279***[d]	***NA***
Grandas[8]	1992	2.3mg (2 to 3)	3 (2 doses each)	12.5[e]	(10 to 25)	25.9[b]	(16 to 39)	***10.8***[b]	***(8 to 13)***	NP	(NP)	***NP***	***(NP)***
Nicolle[1]	1993	40ug/kg as standard ≈2.8mg[a]	8	14	(4 to 30)	15	(7 to 36)	***5.4***[c]	***NA***	802	(585 to 982)	***286***[c]	***NA***
Hofstee[9]	1994	4mg (3 and 5)	2	15	(10 and 20)	34.7	(22 and 48)	***8.4***	***(7 and 10)***	NP	(NP)	***NP***	***(NP)***
Ostergaard[10]	1995	3.4mg (0.8 to 6.0)	16	NP	(5 to 45)	NP	(NP)	***NP***	***(7 to 32)***	NP	(NP)	***NP***	
						nmol/L							
Przedborski[11]	1995[f]	50ug/kg as standard ≈3.5mg[a]	6	20	(NP)	25	(CV = 19%)	***7.1***[c]	***NA***	NP	(NP)	***NP***	***(NP)***
Sam[12]	1995	2.9mg (0.9 to 5.1) 36µg/kg (10 to 58)	6	18.2	(6 to 30)	10.5	(5 to 23)	***4.1***	***(1.3 to 6.4)***	576	(222 to 1190)	***217***[d]	***(80 to 274)***

continued...

[a] assuming a body weight of 70kg.
[b] units were converted, e.g. from pmol/mL.
[c] estimated using approximate dose (in mg).
[d] AUC range was not presented in publication.
[e] median value.
[f] non-parkinsonian subjects with suspected normal pressure hydrocephalus.
[g] AUC range was 0 to 2h.

Publication		Apomorphine Dose	N°. of patients	T_{max} minute	C_{max} ng/mL	C_{max} *normalised to 1mg dose*	AUC (0 to infinity, unless stated) ng/mL.min	AUC *normalised to 1mg dose*
van Larr[13]	1996	2.2 (1.0 to 4.0)	13	14.5 (5 to 30)	19.2 (6 to 53)	*8.7 NA*	NP (NP)	*NP (NP)*
Harder[3]	1998	0.5mg as standard	3	45[e] (15 to 45)	2.9 (CV = 73%)	*5.8 NA*	149[g] (C.V. = 28%)	*299[g] NA*
		1mg as standard	10	30[e] (15 to 90)	4.0 (CV = 43%)	*4.0 NA*	366[g] (C.V. = 74%)	*366[g] NA*
		2mg as standard	10	30[e] (15 to 120)	12.8 (CV = 42%)	*6.4 NA*	982[g] (C.V. = 53%)	*491[g] NA*
		4mg as standard	8	30[e] (15 to 90)	14.1 (CV = 47%)	*3.5 NA*	1297[g] (C.V. = 52%)	*324[g] NA*
This study	1996-1999	4.6mg (2 to 10) 67ug/kg (35 to 167)	7	17.5 (10 to 27) C.V. = 33%	27.8 (11 to 61)	*6.6 (2.2 to 10.7) C.V. = 42%*	2020 (754 to 4861) *n*=7	*430 (257 to 628) C.V. = 30%*

Table 6-2 Mean (range) pharmacokinetic parameters following single-dose apomorphine administration by subcutaneous bolus to patients with Parkinson's disease.

Where the ranges of pharmacokinetic parameter estimates were not available, the C.V. has been calculated using the mean and S.D. given in the publication.

Abbreviations: NA = not applicable, NP = data not present in publication.

[g] AUC range was 0 to 2h.

Publication		Apomorphine dose	N°. of patients	Clearance L/kg/h	(Apparent) Volume of distribution L/kg	t ½ absorption[a] minute	t ½ distribution[b] minute	t ½ elimination[c] minute
Gancher[5]	1989		4	~2.5	~1.5	NP	4.8 (CV=23%) *n*=11	33.6 (CV=12%) n=15
Nicolle[1]	1993	40ug/kg as standard ≈2.8mg[d]	8	3.1 (2.5 to 4.0)	3.5 (CV=17%) *n*=4	NP	15 (7 to 25)	72 (61 to 93)
Hofstee[9]	1994	4mg (3 and 5)	2	NP	≈1.1[d]	6.4 (5.5 and 7.6)	7.2[e]	56.0[e]
Sam[12]	1995	2.9mg (0.9 to 5.1) 36µg/kg (10 to 58)	6	4.2 (3.1 to 7.1)	2.7 (1.2 to 4.1)	5.8 (0.2 to 15.2)	NP	27.4 (CV=14%)
Ostergaard[10]	1995	3.4mg (0.8 to 6.0)	16	NP	NP	NP	NP	~30.0 (14.6 to 68.4)
van Larr[13]	1996	2.2mg (1.0 to 4.0)	13	≈2.4[d]	NP	NP	NP	NP
van Laar[4]	1998	10 to 100µg /kg/h intravenous	10	≈3.9[d]	NP	NP	NP	NP
van dGeest[2]	1998	30µg/kg x 15min intravenous	10	2.4 (1.4 to 4.1)	1.6 (0.7 to 2.2)	NP	NP	41.0 (20.2 to 62.5)
Neef[14]	1999	Population PK analysis: several studies,23 patients, various doses		≈2.1	1.4 (CV =16%)	5.1 (CV=24%)	NP	27.2 (CV=17%)
This study	1996-1999	4.6mg (2 to10) 67ug/kg (35 to 167)	7	2.2 (1.5 to 3.3) C.V. = 24%	1.9 (1.0 to 3.4) C.V. = 44%	4.1 (1.4 to 7.3) C.V. = 55%	13.5 (7.1 to 23.5) C.V. = 52%,*n*=5	69.5 (36.3 to 98.6) C.V. = 30%

Table 6-3 Mean (range) pharmacokinetic parameters following single-dose apomorphine administration by subcutaneous bolus to patients with Parkinson's disease. Abbreviations: NA = not applicable, NP = data not present in publication.

[a] quoted in publication as "absorption half life", or calculated using (ln 2)/K01.
[b] quoted in publication as "distribution half life", or calculated using (ln 2)/alpha.
[c] quoted in publication as "elimination half life", or calculated using (ln 2)/K10 or (ln 2)/beta for one- or two-compartment pharmacokinetics respectively.
[d] assuming a body weight of 70kg.
[e] estimated using micro-constants given in publication.

A linear relationship between C_{max} and bolus dose was demonstrated ($R^2 = 0.8869$, $p<0.001$ over the dose range (2 to 10 mg, 35 to 1667µg/kg, n=8)[a]. Similarly, a linear relationship between AUC and bolus dose was demonstrated ($R^2 = 0.9265$, $p<0.001$) over the dose range (2 to 10 mg, i.e. 35 to 1667µg/kg, n=7).

6.1.1.1. Inter-Patient Variation in Apomorphine Pharmacokinetics.

There was considerable inter-patient variation in T_{max}, dose-normalised $AUC_{0\text{-infinity}}$ and dose-normalised C_{max}, i.e. 33, 30 and 42%, respectively. Inter-patient variation in apomorphine absorption following subcutaneous administration is widely described [1, 2, 5, 15]. It has been shown that factors which alter local blood flow, i.e. temperature of skin in the region of administration, alter apomorphine absorption[5]. Also, local adverse reactions (inflammation) which are induced by chronic apomorphine administration have been known to affect absorption[ ].

On the basis of visual inspection of the $AUC_{0\text{-infinity}}$, and C_{max}, *versus* dose plots it was considered that apomorphine absorption was not affected by administration site (Figure 6-2). This finding is in contrast to the report by Nicolle *et al*, in which a trend towards more complete absorption following injection in the abdominal wall compared to the thigh was observed[1]. T_{max} was not dose-related ($R^2 = 0.0046$, $p=0.8730$, $n=8$) and was also not influenced by administration site.

Dose-normalised $AUC_{0\text{-infinity}}$ was not correlated with age, weight, duration of disease, duration of L-dopa therapy, duration of apomorphine therapy, disease severity (in terms of Hoehn and Yahr score), or related to gender ($n=7$ except Hand Y score where $n=6$).

Dose-normalised Cmax was not correlated with age, duration of disease, duration of L-dopa therapy, duration of apomorphine therapy, disease severity (in terms of Hoehn and Yahr score), or related to gender ($n=7$ except H&Y score where $n=6$).

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[a] This correlation was based on *predicted* C_{max}, with the exception of data from patient 03, which was the *observed* C_{max}. Data from patient 03 was included in the correlation in order to lessen the leverage that may be exerted by the outlying data at 1667µg/kg dose (adjusted for body weight).

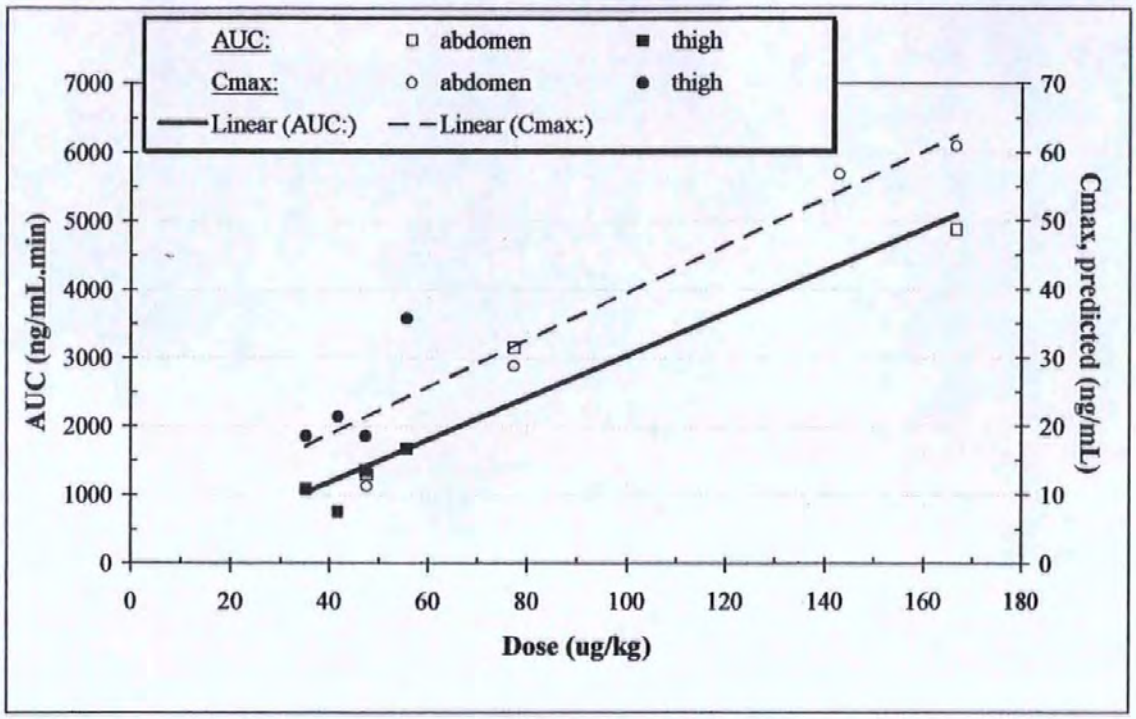

Figure 6-2 **Correlation between dose (adjusted for body weight) and pharmacokinetic absorption parameters following subcutaneous bolus administration of apomorphine to patients with Parkinson's disease.**

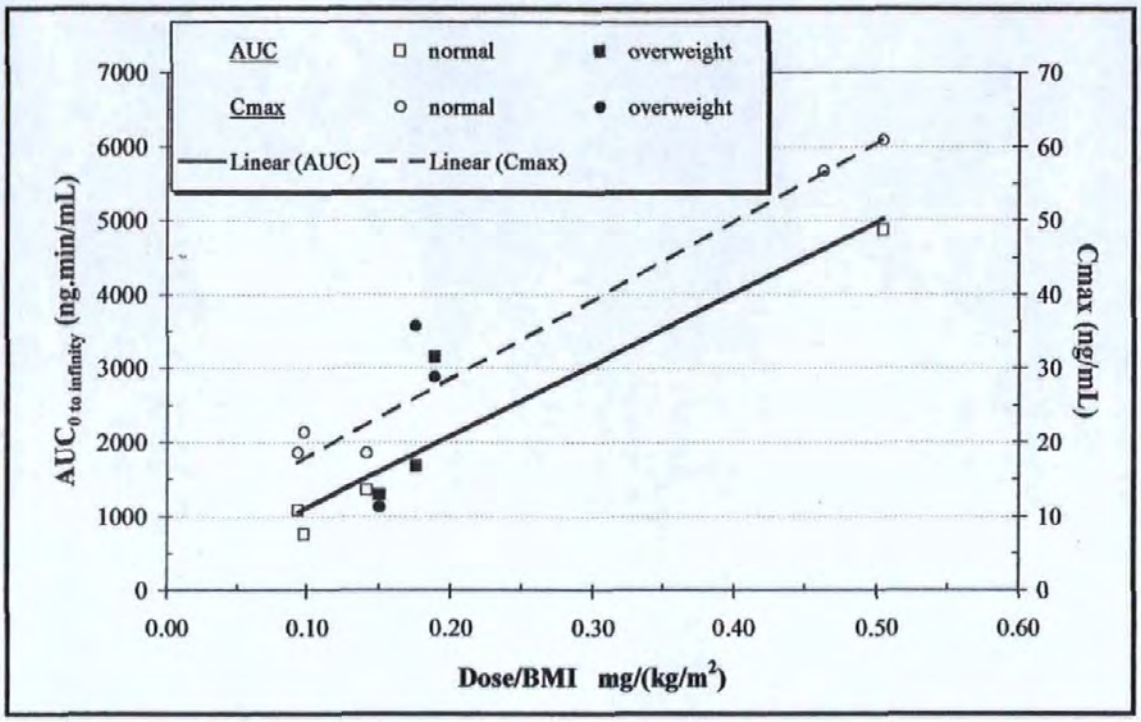

Figure 6-3 **Correlation between dose (adjusted for body mass index) and pharmacokinetic absorption parameters following subcutaneous bolus administration of apomorphine to patients with Parkinson's disease.**

6.1.2. Key Pharmacodynamic Events.

Where response data is given in the following discussion, these are expressed as mean (range, number of observations) of values. Individual data is given in Table 5-1, page 5-23).

6.1.1.2. Latency to Onset of Anti-Parkinsonian Effect.

The latency to onset of effect following apomorphine bolus administration was short, i.e. 12 minutes (4 to 29, n=7) and the duration of effect was brief, i.e. 72 minutes (44 to 144, n=7). (see Table 5.1, page 5-23). These data compare well with published data on apomorphine-induced anti-parkinsonian effect[17-21], and correspond with the rapid absorption and elimination processes observed for apomorphine.

T_{max} occurred after the onset of effect in the majority (5/7) of cases; i.e. between 3 and 14 minutes after the time of onset of effect in individual patients (Figure 6-4). In the remaining two cases, T_{max} preceded the onset of effect, by 2 minutes on both occasions. The short latency to onset of effect, especially given the relation to T_{max}, is indicative of a rapid equilibration of apomorphine between blood and the site of action in the brain[2, 3]]. Again, the high lipid solubility of apomorphine is considered to be an important factor in this[1, 4, 22].

The time of onset of effect was not correlated to T_{max} (R^2=0.0474, p=0.6045, n=7) or to dose adjusted for body weight (R^2 =0.0206, p=0.7591, n=7. Additionally, neither $AUC_{0\text{-}infinity}$ (absolute or dose-normalised) nor C_{max} (absolute or dose-normalised) were correlated to the latency to onset (R^2 =03253, p= 0.1813, n=7, and R^2 =03327, p= 0.1344, n=8, respectively).

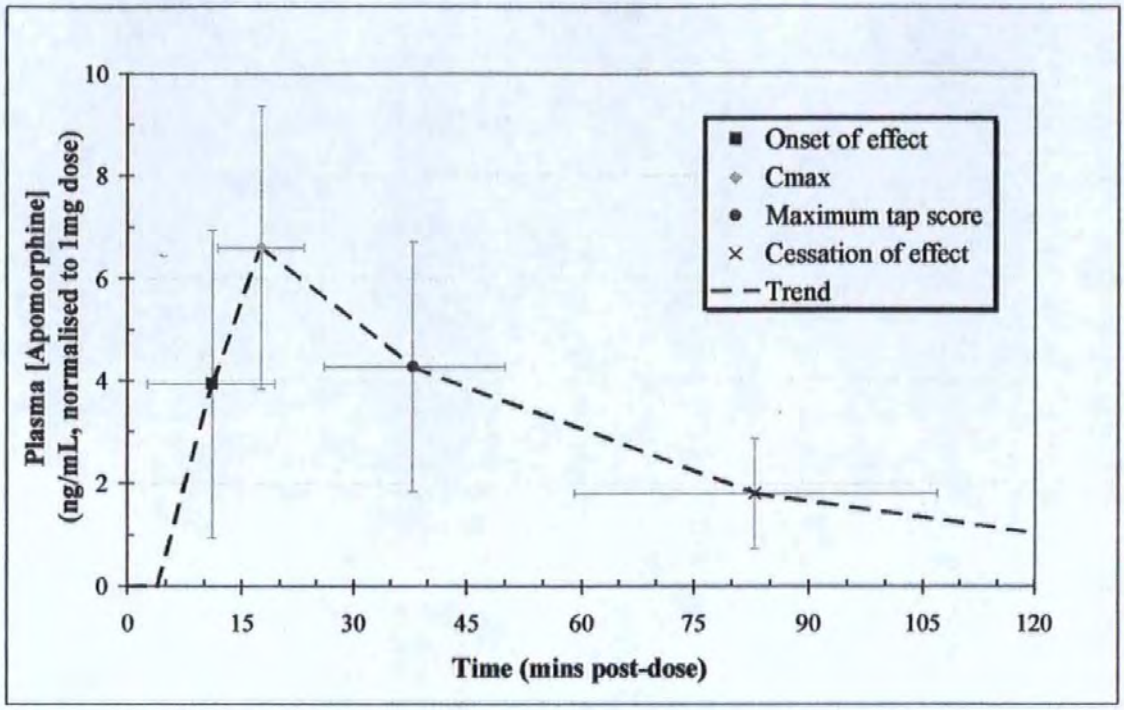

Figure 6-4 **Mean (dose-normalised) plasma apomorphine concentration at key pharmacodynamic events following subcutaneous bolus administration. Error bars show +/- 1 S.D. (*n*=7).**

6.1.1.3. Duration of Anti-Parkinsonian Effect

The duration of effect was not related to dose. Neither $AUC_{0\text{-infinity}}$ (absolute or dose-normalised) nor C_{max} (absolute or dose-normalised) were correlated to the duration of effect (R^2 =0.0049, p= 0.8818, n=7, and R^2 =0.0798, p= 0.5394, n=7). Some investigators have reported that duration of effect was correlated to dose[5, 23].

In accordance with the observations of Sam *et al*, the mean residence time, i.e. 77 minutes (49 to 103, n=7) was similar to the mean duration of effect, i.e. 74 minutes (44 to 121, n=7), suggesting that the onset and termination of effect is determined by the parent drug, and not by a metabolite or second messenger[12]. However if the data is considered for individual patients, it can be shown that the mean residence time was equivalent to the duration of effect in four cases and dissimilar in the remaining three, and therefore this aspect remains inconclusive for this group of patients.

6.1.1.4. Magnitude of Effect.

The magnitude of apomorphine-induced anti-parkinsonian effect (in terms of maximum improvement in tapping test score from baseline) was not dose-related. Additionally, neither $AUC_{0\text{-infinity}}$ (absolute or dose-normalised) nor C_{max} (absolute or dose-normalised) were correlated to the magnitude of effect (R^2 =0.3334, p= 0.1746, n=7, and R^2=0.1408, p= 0.4069, n=8, respectively).

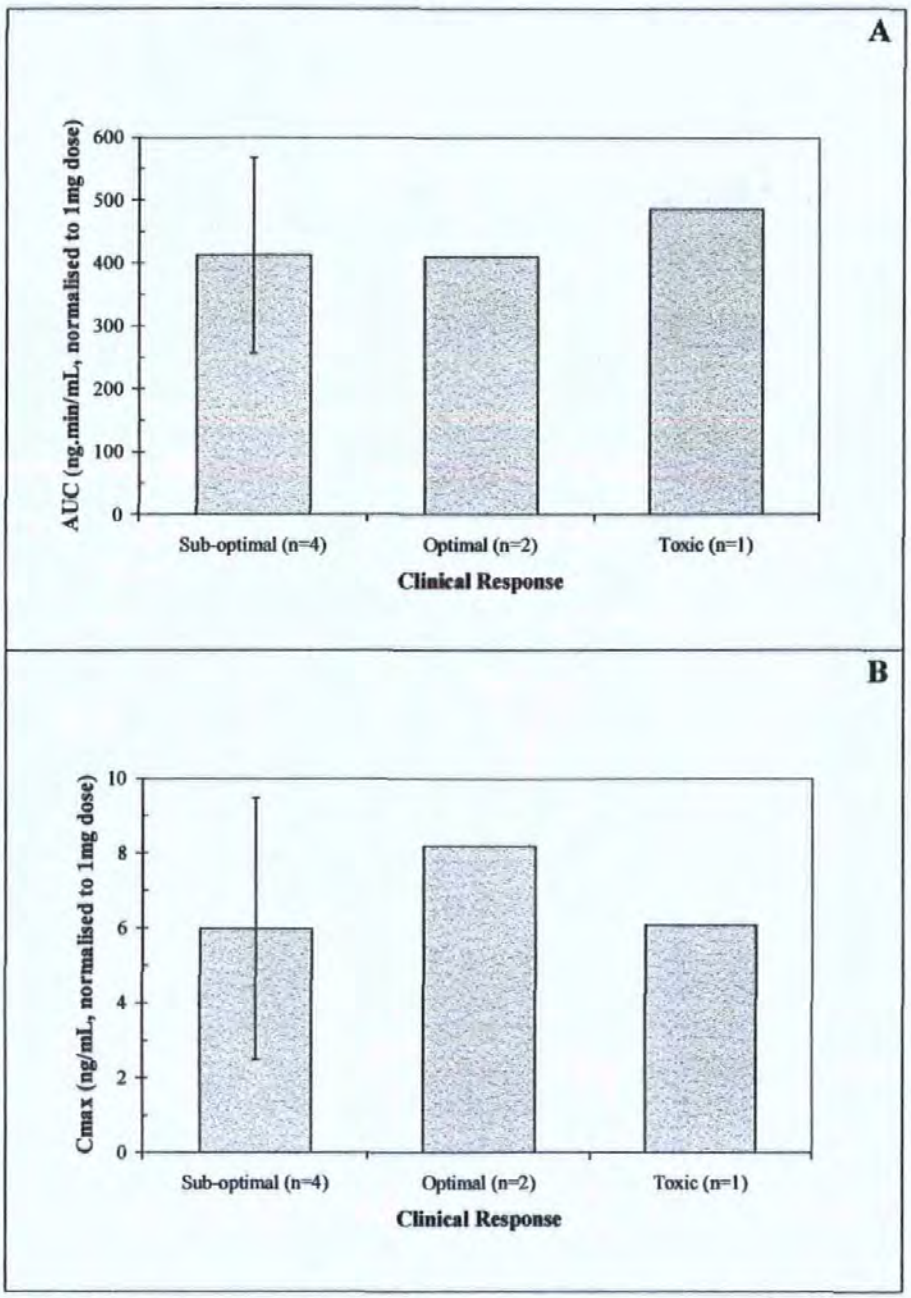

Figure 6-5 Mean dose-normalised $AUC_{0\text{-infinity}}$ (Panel A) and C_{max} (Panel B) following subcutaneous apomorphine bolus administration, grouped by clinical response (relative to typical experience). Error bars show 1S.D.

The maximum improvement in tapping test score from baseline was 24% (11 to 50, n=7) (raw data is given in Appendix 8.14). The threshold for positive apomorphine-induced anti-parkinsonian response is given as 15 % (e.g. in the apomorphine titration protocol[24]), 20 % (e.g. O'Sullivan and Lees[19]) or 25 % (e.g. Danhof *et al*[15]) improvement in tapping test score from baseline. It can be seen from Figure 6-6 (and Table 5.1, page 5-23) that, for this group of patients, a threshold of (at least) 25% improvement in tap score best reflects the threshold for positive response in terms of the patients' rating of the response.

The finding that routine (previously optimised) apomorphine doses elicited sub-optimal or toxic responses was attributed to the effect of wash-out of other anti-parkinsonian drugs. Hutchinson *et al* observed that anti-parkinsonian drug withdrawal for longer than 12 h prolonged the onset of effect of an apomorphine bolus and increased the likelihood of a sub-optimal clinical response to a previously effective dose[25].

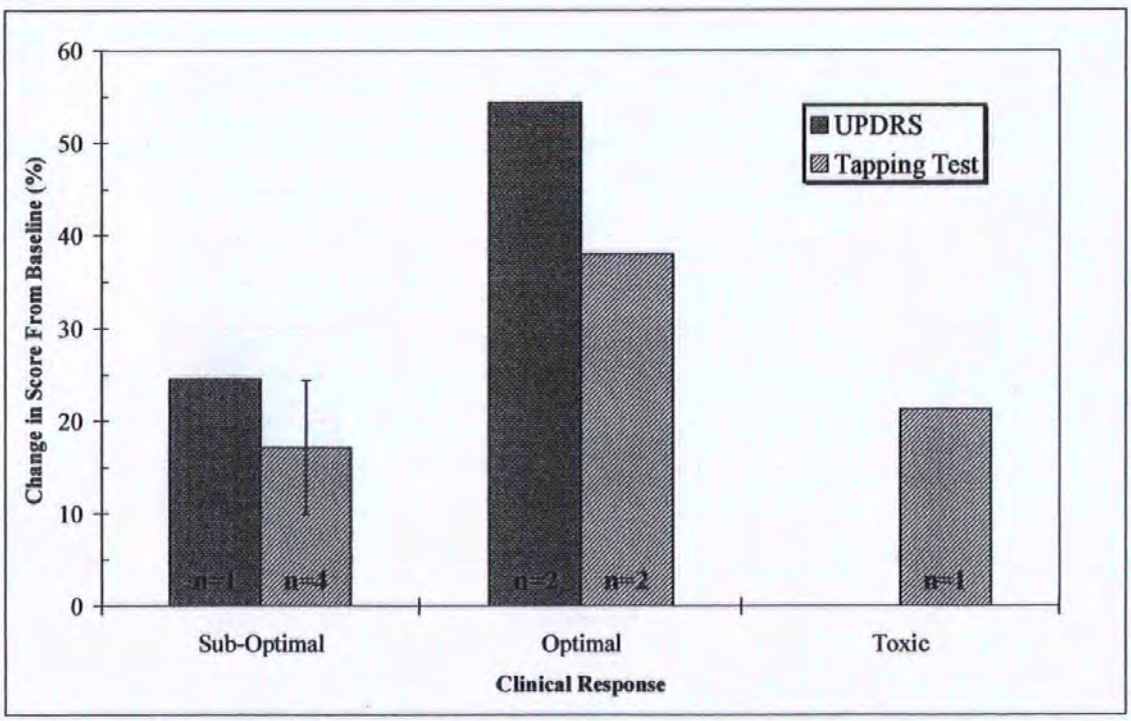

Figure 6-6 Mean apomorphine-induced change in tapping test and UPDRS scores relative to baseline, grouped by clinical response (as rated by the patients).

Error bar shows +/- 1 S.D.

6.1.1.5. Adverse Effects.

Apomorphine-related adverse events, e.g. diaphoresis and dyskinesia, occurred within two minutes of administration of bolus apomorphine to patient 04 and continued until approximately T_{max} (Figure 6-7). Additionally, drowsiness continued throughout the whole period of effect. Given that only a single dose was administered, the threshold for adverse effects could not be determined.

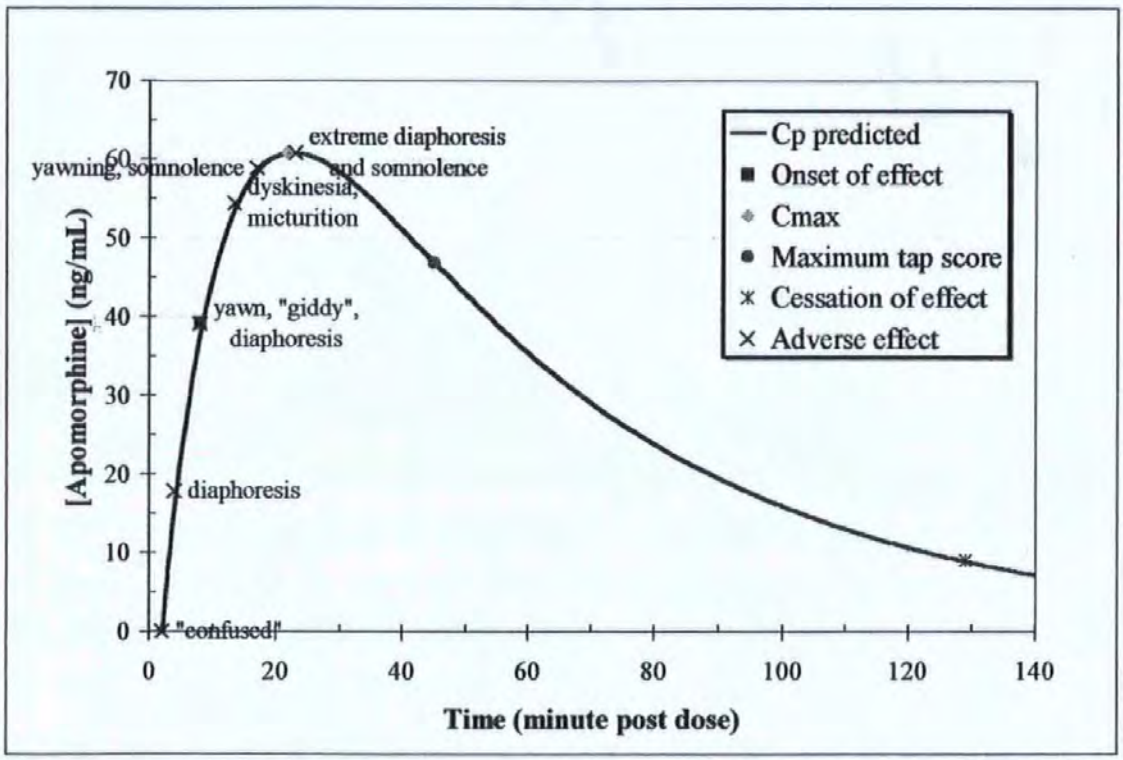

Figure 6-7 Plasma apomorphine concentration at key pharmacodynamic events following subcutaneous bolus administration (10mg) to patient 04.

6.1.1.6. Evaluation of Beta-Phase Intercept in Relation to Anti-Parkinsonian Response.

Values for the beta-phase intercept ranged from 0.4 to 11.3 ng/mL (n=6) (Table 6-4). There was a lack of correlation between the beta-phase intercept and response, in terms of magnitude of effect (R^2=0.2187, p= 0.4270, n=5), and also in the terms of the patients' rating of the response compared to their typical experience (Table 6-4); the lack of a distinction between beta-phase intercept values associated with sub-optimal and optimal apomorphine-induced anti-parkinsonian responses is illustrated in Figure 6-8. It was therefore demonstrated that the beta-phase intercept did not represent a threshold concentration for effect, and as such was shown to be of no predictive value. Thus it is likely that the consistency in this parameter observed in the historical data (Tables 2-1 and 2-2, page 2-3) was coincidental.

Patient ID	Apomorphine dose			Beta-phase intercept (ng/mL)	Improvement in Tap Score (%)	Quality of "on" phase
	mode	mg	µg/kg			
01	bolus	2	41.7	11.3	11	Sub-optimal
04	bolus	10	166.7	0.4	21	Toxic
07	infusion	87.2µg/kg/h		10.5	(-55)	Optimal
09	bolus	2	35.1	3.5	50	Optimal
10	bolus	3.5	47.3	5.8	22	Sub-optimal
12	bolus	5	55.6	7.7	26	Optimal

Table 6-4 Beta-phase intercept values and apomorphine-induced response.

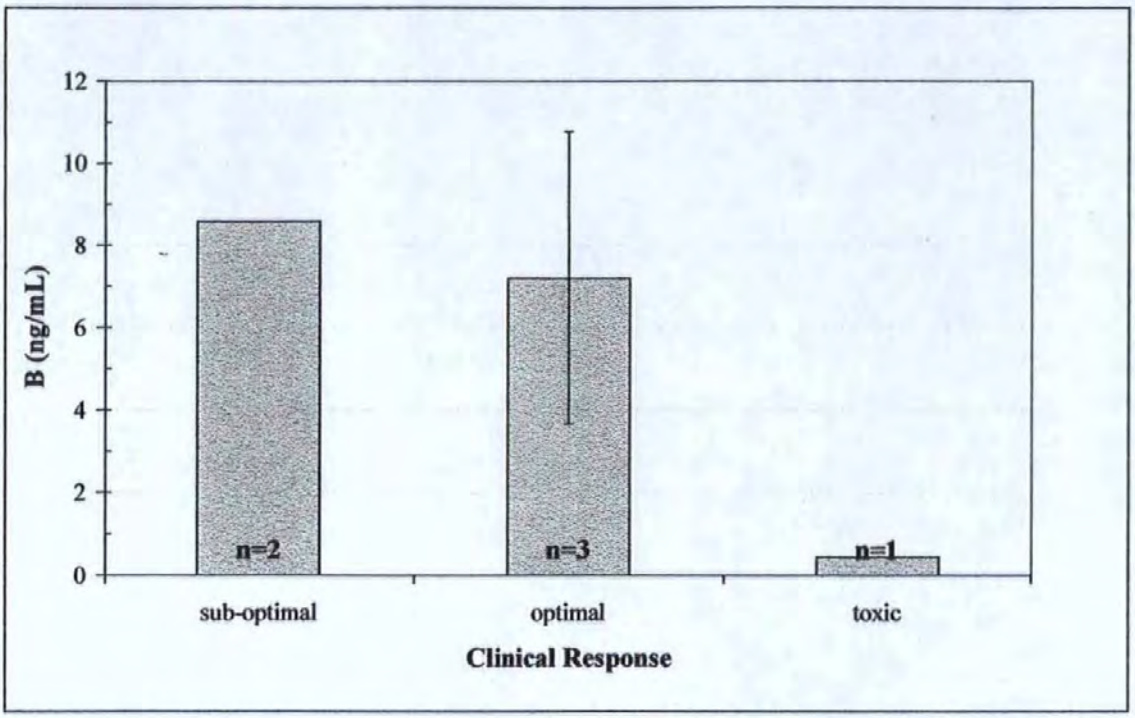

Figure 6-8 **Mean beta-phase intercept value obtained following apomorphine administration, grouped by clinical response (relative to patients' typical experience). Error bars show $^{+}$/-1S.D.**

6.1.3. Pharmacokinetic-Pharmacodynamic Analysis.

Having explored the relationship between post-distributional apomorphine pharmacokinetics and anti-parkinsonian effect, another approach was employed in the investigation of apomorphine pharmacokinetic-pharmacodynamic relationships. This approach was based on the findings of the initial exploration of the relationship between observed plasma apomorphine concentration and observed effect (see Section 6.1). To summarise these findings, in the case of patients 01, 04 and 10 it was found that no clear relationship existed between effect and plasma concentration. Two of these patients (patients 01 and 10) experienced a bi-phasic "on" phase which could not be described using standard pharmacodynamic models. In the case of patients 09 and 12, there was evidence of counterclockwise hysteresis, indicating the existence of an indirect relationship between plasma apomorphine concentration and effect. In contrast to this there was little or no hysteresis in the case of patients 02 and 05, the implication here being that a direct relationship between plasma apomorphine concentration and effect was involved.

Given the variation in the plasma concentration-effect relationship of apomorphine in this group of patients, it was considered necessary to investigate both direct (i.e. time-independent) and indirect (i.e. time-dependent) pharmacokinetic-pharmacodynamic models.

6.1.1.7. Direct Pharmacokinetic-Pharmacodynamic Models.

Initially basic pharmacodynamic models, i.e. logarithmic and hyperbolic effect models, were fitted to the concentration-effect data, and discrimination between competing models was undertaken using the criteria given in Section 4.6, page 4-103). The sigmoid E_{max} model was identified as the appropriate model by these criteria. The predicted change in tapping test score following apomorphine administration according to this model is illustrated for patients 02, 05, 09 and 12 in Figure 6-9.

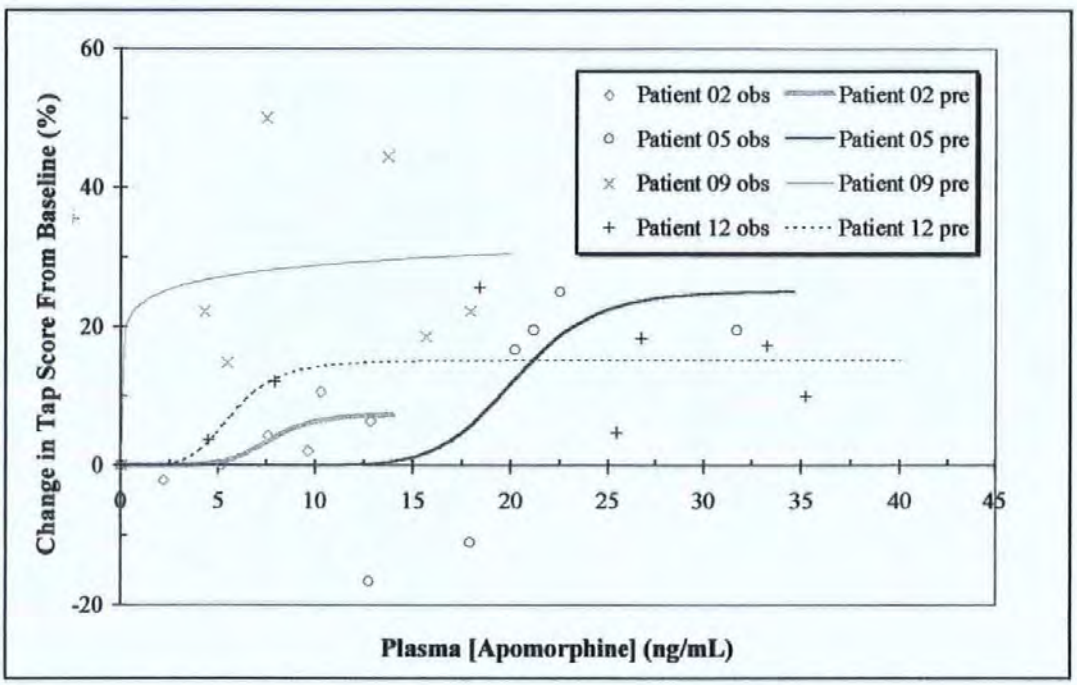

Figure 6-9 Predicted apomorphine-induced effect following subcutaneous bolus administration according to the sigmoid E_{max} model[a].

Whilst satisfactory fit (based on the criteria given in Section 4.6) was achieved in the cases of patients 02, 05 and 12, model mis-specifiation was apparent in the case of patient 09, based on the magnitude of the residuals and the large C.V. associated with the final parameter estimates (C.V. < 20000%). The use of a direct pharmacokinetic-pharmacodynamic model may appear to be in conflict with the observation that the maximum effect occurred after T_{max} (approximately 20 minutes later), however the lack of hysteresis observed for patents 02 and 05 can be explained by the existence of a (very) rapid

equilibrium of apomorphine with the effect site[3, 26]. The pharmacokinetic-pharmacodynamic parameters of apomorphine according to the sigmoid E_{max} model[a] are summarised in Table 6-5.

Publication/ Patient I.D.	E_{max} (change in tap score, %)	EC_{50} (ng/mL)	Hill co-efficient (γ)	K_{E0} (min^{-1})
Harder (n=12)[3]	NA	6.0 (2.3 to 11.2)	9 (3 to 25)	0.10 (0.05 to 0.14)
This study				
01	No relationship detected.			
02	7.4	7.7	6.6	
04	No relationship detected.			
05	25.2	20.3	10.0	
09	58.8	12.6	0.2	
10	No relationship detected.			
12	15.1	5.8	4.9	

Table 6-5 Pharmacokinetic-pharmacodynamic parameters of apomorphine according to the sigmoid E_{max} model.

Parameter estimates reproduced from Harder[3] are expressed as the median (range) according to sigmoid E_{max} model.

Abbreviation: NA = not applicable.

Values for the Hill co-efficient were large (excluding patient 09), thus indicating that the relationship between plasma apomorphine concentration and effect was an "all or nothing" (dichotomous) phenomenon[27].

Where a steep sigmoidal concentration-effect relationship exists, EC_{50} is considered to be a threshold concentration for effect[3]. In the cases of patients 02 and 05, EC_{50} was a reasonable approximation of the plasma apomorphine concentration at onset of effect, i.e. 11 and 26 ng/mL respectively. In contrast, there was little agreement between EC_{50} and the plasma apomorphine concentration at onset of effect for patient 12, i.e. 34 ng/mL. Due to the small number of patients involved it was not possible to make a judgement on this issue.

[a] $E = (Emax \cdot C^{\gamma}) / (C^{\gamma} + EC_{50}^{\gamma})$.

6.1.1.8. Indirect Pharmacokinetic-Pharmacodynamic Models.

Sigmoid E_{max} Pharmacodynamic Model.

This model was comprised of the previously identified pharmacokinetic model which best described the time course of apomorphine concentration in plasma for a given patient, linked via an effect compartment to the sigmoid E_{max} pharmacodynamic model. An example is given in Figure 6-10.

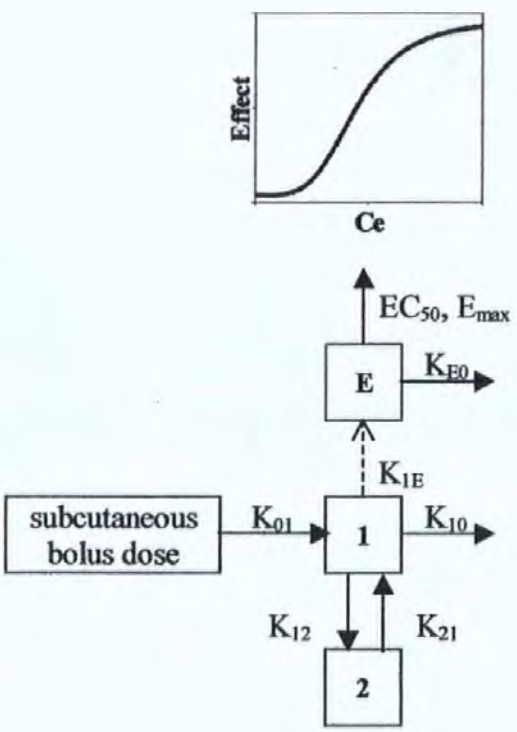

Figure 6-10 Schematic representation of the effect compartment model used for patient 09 and 12: two-compartment, first order input (lagtime) pharmacokinetic model with an effect compartment, whereby effect is described according to the sigmoid E_{max} model.

Abbreviations: 1 = central compartment , 2 = peripheral compartment, Ce = drug concentration in effect compartment, E = effect compartment, K01, K10, K12, K21 = compartment rate constants.

However this particular solution was rejected for each patient dataset on the basis that predicted data did not reflect trends in observed data, i.e. estimates of E_{max} were up to 100 times the observed maximum effect, and model parameters estimates were associated with

unacceptably large C.V.s. This outcome indicated that model mis-specification has occurred, i.e. the Emax model had been applied to effect data which did not exhibit saturable behaviour (in the observed concentration range).

The effect compartment (sigmoid E_{max} pharmacodynamic) model has previously been applied to subcutaneous bolus administration of apomorphine in patients with Parkinson's disease by Harder *et al*[3] and consequently information on E_{max} has in fact been successfully derived. The contrast in outcome, between the study by Harder *et al* and the study presented in this thesis, following application of the effect compartment (sigmoid E_{max} pharmacodynamic) model might be expected due to differences in the (mean) apomorphine pharmacokinetics of the patients in each of the two studies; whilst the mean C_{max} was similar for each, it appeared that there was a difference in clearance between the two, i.e. the mean concentration of apomorphine at 120 minutes post-dose was approximately 74% of that at C_{max} in the case of Harder *et al*, and only 17% for the patients presented in this thesis.

The absence of a clearly defined E_{max} is likely to be the result of a deficiency in the experimental design, i.e. the non-steady state design. The study of apomorphine under non-steady state conditions (and in the absence of continuous pharmacodynamic monitoring), was problematic in that, as a consequence of the short absorption and elimination half-lives and the brief period of effect, the observed plasma apomorphine concentration range was not sufficiently large enough nor sustained for long enough to efficiently sample the pharmacodynamic response. For these reasons it would be more appropriate to conduct the pharmacokinetic and pharmacodynamic sampling under multiple plasma pseudo-steady state conditions since this strategy allows more time, compared to the administration of a single bolus dose, for multiple measurements of response to be performed[28]. Consequently a more reliable definition of the concentration-effect relationship is likely to result. If applied

correctly this approach is considered to be the "gold standard" in the elucidation of concentration-effect relationships *in vivo*[29]. This approach was used by van Laar *et* al in the characterisation of the therapeutic window of apomorphine in Parkinson's disease[4].

A further contributory factor to the absence of a clearly definable E_{max} may be one of insufficient pharmacodynamic sampling during the response. Although, generally, pharmacokinetic and pharmacodynamic sampling is performed simultaneously, the pharmacodynamic sampling scheme could have been optimised independently of the plasma sapling scheme in an attempt to characterise peak effect[30]. It should, however, be taken into consideration that the use of a more intensive pharmacodynamic sampling scheme might induce fatigue and/or loss of motivation on the part of the patient which in turn may increase pharmacodynamic variability[31].

The choice of pharmacodynamic tool may also have contributed to the inability to model E_{max} in that the tapping test lacks a definitive maximum score, in contrast to the gradation of response that a rating scale e.g. Webster rating scale, offers. In the light of this, attempts were made to re-define the range of effect, i.e. (i) the maximum tapping test score for each patient was assigned as 100% improvement from baseline, and other tapping test scores were redefined accordingly, and (ii) a simple scoring system was constructed whereby 0 = "off", 1 = between 0 and 20% improvement in score, and 2 = greater than 20% improvement in tap score. However, there remained an absence of a plateau in response with increasing plasma concentration and so such data transformations were proved not to be useful.

Exponential Pharmacodynamic Model.

In the light of the mis-specification of the E_{max} model, an exponential pharmacodynamic model was substituted for the sigmoid Emax model (Box 6-1 and Appendix 8.17.1). This was based on the observation that, according to the effect compartment (sigmoid E_{max} pharmacodynamic) model, an approximately linear relationship existed between $\log_e$ (effect) and apomorphine concentration in the effect compartment (Figure 6-11).

$$E = E_0 \cdot e^{\eta.Ce}$$

where

- E_0 is the baseline effect,
- η is the slope factor for the relationship between the concentration of drug in the effect compartment and $\log_e$ (effect),
- Ce is the drug concentration in the effect compartment.

Box 6-1 Exponential pharmacodynamic model.

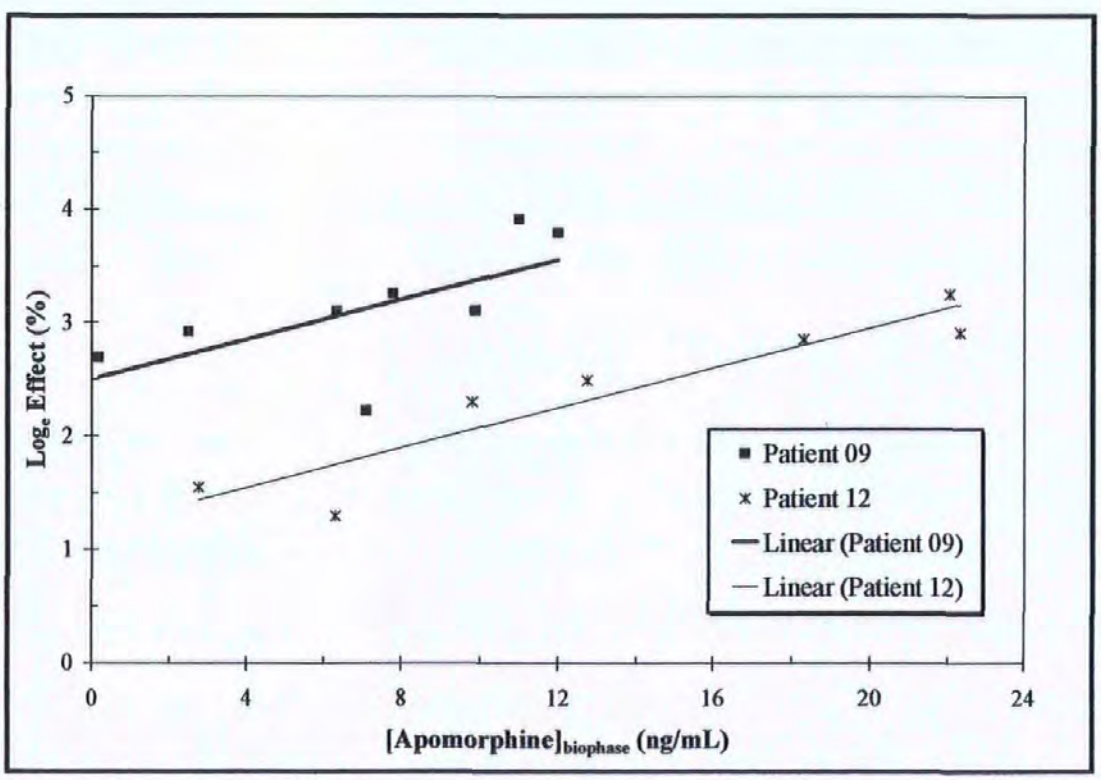

Figure 6-11 Relationship between $\log_e$-transformed pharmacodynamic data and predicted apomorphine concentration in the effect compartment, according to the effect compartment (sigmoid E_{max}) model.

The application of an effect compartment model which incorporated exponential pharmacodynamics allowed a satisfactory fit of the concentration-effect relationship observed for patients 09 and 12 (only). The predicted apomorphine concentration-effect relationship according to this model is illustrated using data from patient 09 in Figure 6-12 and Figure 6-13.

The inherent limitation of this approach was the absence of information relating to maximal apomorphine-induced anti-parkinsonian effect. An additional constraint was that the exponential pharmacodynamic model did not allow a meaningful definition of E_0. Using patient 09 as an example in order to elaborate on this issue; the final estimate for E_0 was given as 6% improvement from baseline according to the exponential dynamic model. There was an obvious disparity between the estimate of baseline effect and actual baseline response, the latter having been defined as zero improvement from baseline. Since this model did not allow a value of zero for E_0 this parameter was fixed at a value of 1%, as an approximation of baseline effect.

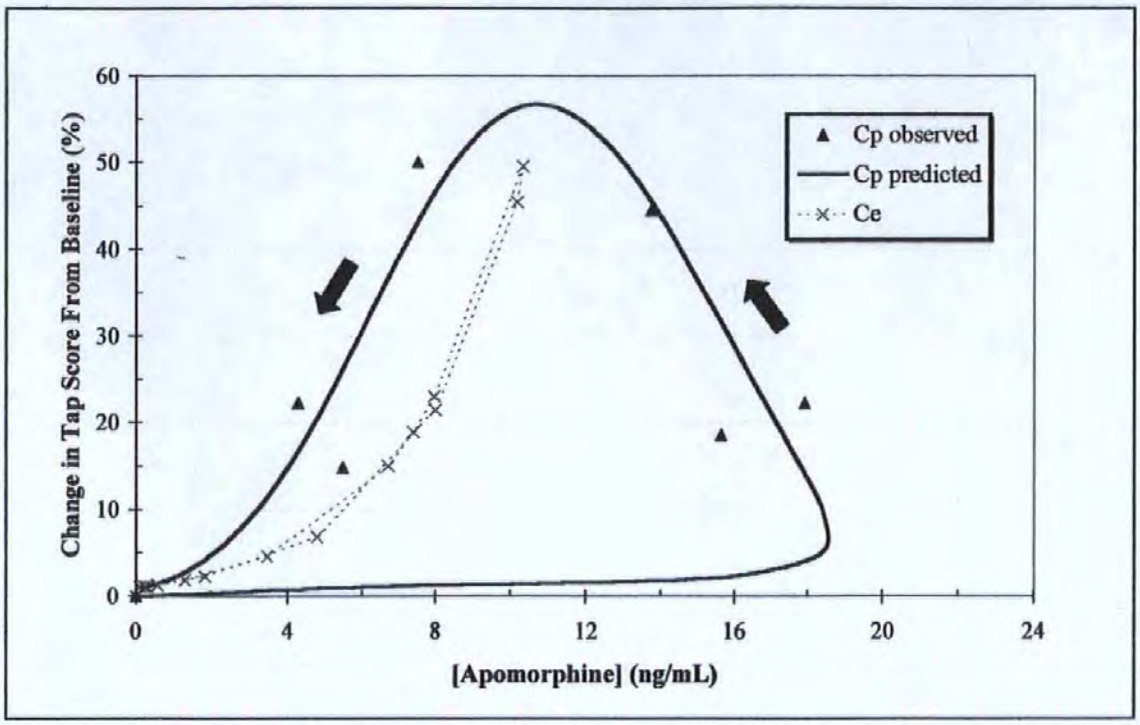

Figure 6-12 Predicted apomorphine-induced effect following subcutaneous bolus administration according to the effect compartment (exponential pharmacodynamic) model (Patient 09).

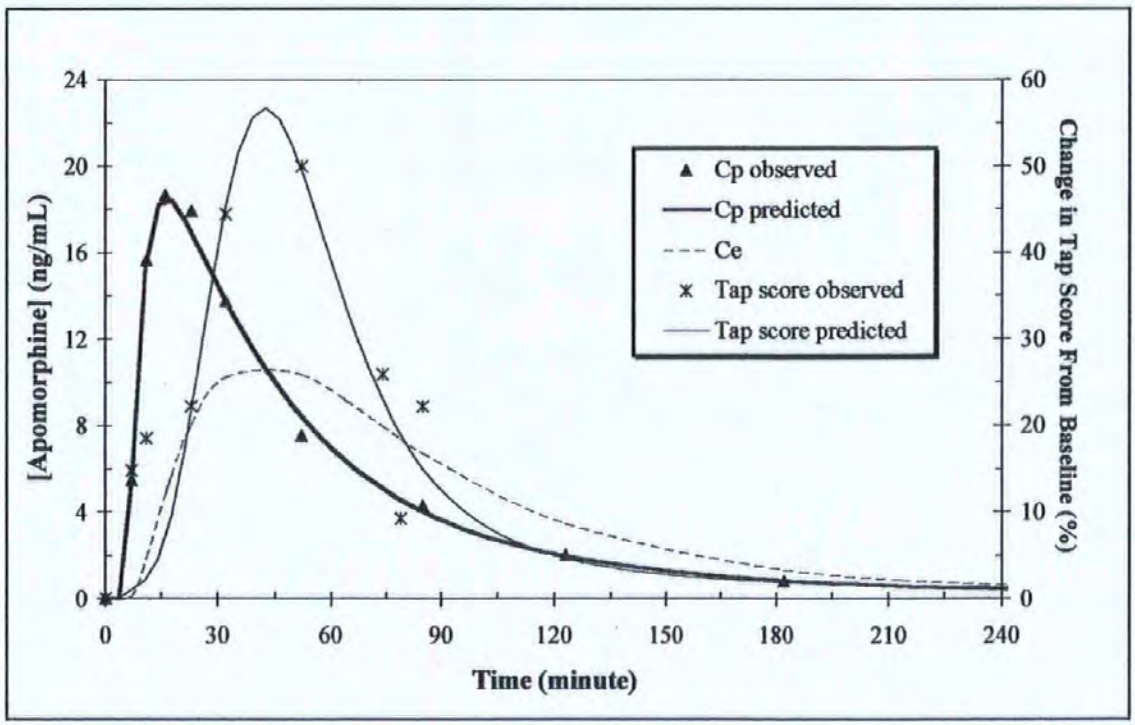

Figure 6-13 Relationship of apomorphine and anti-parkinsonian effect described using the effect compartment (exponential pharmacodynamic) model (patient 09).

In the cases of patients 02 and 05, the predicted time course of effect compartment apomorphine concentration and plasma apomorphine concentration were essentially superimposed, indicating that a direct pharmacokinetic-pharmacodynamic model was indeed the appropriate solution.

The pharmacokinetic-pharmacodynamic parameters of apomorphine according to the effect compartment model are summarised in Table 6-6.

Publication/ Patient I.D.	**K_{e0} (min^{-1})**	**$t_{½}K_{e0}$ (T_{eq}) (min)**	**Slope factor (η)**
Harder (n=12)[3]	0.10 (0.05 to 0.14)	7 (5 to 13) n=7	NA
This study			
01	No relationship detected.		
02	NA	NA	NA
04	No relationship detected.		
05	NA	NA	NA
09	0.042	16.5	0.38
10	No relationship detected.		
12	0.084	8.3	0.13

Table 6-6 Pharmacokinetic-pharmacodynamic parameters of apomorphine according to the (exponential pharmacodynamic) effect compartment model.

Parameter estimates reproduced from Harder[3] are expressed as the median (range) according to the sigmoid E_{max} model.

Abbreviation: NA = not applicable, T_{eq} = equilibration half-time.

The short equilibration half-time (T_{eq}), i.e. 16.5 and 8.3 minutes in patients 09 and 12 respectively, accounts for the rapid onset of anti-parkinsonian effect and reflects the rapid passage of apomorphine across the blood-brain-barrier[3]. This feature of the concentration-effect relationship of apomorphine has been attributed to the high lipophilicity of apomorphine[3].

6.1.4. Considerations in the Use of the Tapping Tester.

The strategy taken for the study of apomorphine pharmacokinetic-pharmacodynamic relationships presented in this thesis would likely have benefited from the incorporation of more rigorous pharmacodynamic monitoring. Whilst the tapping test reflected well the global clinical response (evidenced, for example, by the agreement between maximum improvement in tap score and the patients' rating of response), the tool is lacking in a number of the features that are considered to be desirable in a pharmacodynamic monitoring system[28, 30, 32, 33]. These limitations of the tapping test include:-

(i) The range of outcome values.

- The tapping test does not possess an explicit minimum and maximum value. This potentially introduced problems in pharmacokinetic-pharmacodynamic modelling, especially since the number of observations was low. (The lack of a pre-defined range does, however, allow flexibility in the application of the tapping test as a pharmacodynamic tool, i.e. the task can be applied to patients with different baseline and/or peak motor function, since each patient acts as their own control.)
- The absence of a direct relationship between tapping test performance and anti-parkinsonian response, i.e. adverse effects that occur at supra-threshold levels of dopaminergic stimulation (e.g. dyskinesia, postural hypotension, neuropsychological effects such as confusion, somnolence, nausea – the latter being specific to apomorphine) have the potential to impair performance in the tapping test. In this respect, the tapping rate at "toxic" apomorphine levels may not be distinguishable from that at baseline or "sub-optimal" levels.

(ii) The invalidity of the test.

Tapping test performance has been shown to correlate well with other measures of parkinsonism[34], e.g. the rigidity rating of the Columbia University Rating Scale[31], and change in score for the modified Webster scale[35]. That having been said, the (restored) ability to execute repetitive precise movements of the upper extremity due to administration of apomorphine may not necessarily translate to an improvement in other, perhaps more troublesome, parkinsonian symptoms, e.g. painful dystonia of the lower extremity, in individual cases. In this respect the tapping test may be considered to be lacking in clinical relevance.

(iii) The influence of psychological status.

Motor function at any given time is clearly dependent on the physiological and biochemical status of the motor centre of the CNS, however tapping test performance is potentially influenced by changing psychological status during the sampling time course. As with other performance tasks, the tapping test relies heavily on the active co-operation of the subject. It is likely that the patients' motivation varied during the sampling time course, as that pharmacodynamic variability was introduced as a direct consequence of this. It is noteworthy that the baseline tapping test performance was assessed at the point where patients were undergoing a protracted parkinsonian "off" state as a result of the prolonged drug wash-out period. It was at this point that a lack of motivation, and feelings of depression and anxiety were likely to be at their highest level (resulting in the case of one patient in the withdrawal from the study). Clearly it might be expected that a general improvement in psychological status may result from the amelioration of parkinsonian symptoms following administration of apomorphine, and with that, a different approach to the tapping task.

(iv) Learning effects.

There is evidence to show that (at least) two attempts at the task are required to establish a stable baseline motor performance[31, 34, 35]. It must, however, be taken into consideration that substantial repetition of the task at baseline may be difficult for some patients in the "off" state[31, 34].

6.1.5. Summary of outcomes in the study of the pharmacokinetic-pharmacodynamics of subcutaneous apomorphine in patients with Parkinson's disease.

The investigation of apomorphine pharmacokinetics and pharmacodynamics in patients with Parkinson's disease was designed primarily as an exploration of the relationship between the beta-phase intercept and clinical response. This was a novel approach in the study of apomorphine pharmacokinetic-pharmacodynamic relationships and was based on observations of an apparent correlation between this particular pharmacokinetic parameter and the anti-parkinsonian effect in general terms.

However it was demonstrated that the beta-phase intercept was unrelated to apomorphine pharmacodynamics (in terms of improvement in tapping test score from baseline) and therefore was of no predictive value in the patients studied (n=6). The correlation between the beta-phase intercept and response initially observed in the literature was therefore interpreted as co-incidental.

According to the traditional two-stage approach to data analysis, there was a short absorption half-life, i.e. mean (S.D.) of 4.1 (2.1) minutes, short elimination half life, i.e. 69.5 (21.1) minutes, rapid clearance from plasma, i.e. 2.2 (0.5) L/kg/h, and the volume of distribution was 1.9 (0.8) L/kg . The typical features of apomorphine-induced anti-parkinsonian effect following subcutaneous bolus were observed, e.g. the short latency to onset of effect, i.e. 12 (8) minutes, and the brief duration of effect, i.e. 72 (25) minutes. Simultaneous pharmacokinetic-pharmacodynamic modelling was performed, using an effect compartment (exponential pharmacodynamics) model to account for counterclockwise hysteresis in a sub-group of patients (n=2). The equilibration half-life was short, i.e. 8.3 and 16.5 minutes. The limitations of this approach were that neither baseline nor maximal apomorphine-induced anti-parkinsonian response could be predicted.

A direct pharmacodynamic (sigmoid E_{max}) model was applied to series where hysteresis in the plasma concentration-effect plot was not evident (n=2). The absence of counterclockwise hysteresis in these patients was attributed to a (very) rapid equilibrium of apomorphine with the effect site. The drug concentration which produced 50% of maximum effect (EC_{50}) was 7.7 and 20.3 ng/mL. Values for the Hill co-efficient were large, i.e. 7 and 10, indicating that the relationship between plasma apomorphine concentration and effect was an "all or nothing" (dichotomous) phenomenon.

Whilst the experimental design, i.e. single (bolus) dose of apomorphine (at the patients' routine dose), was appropriate for the investigation of the beta-phase intercept in individual patients, the design was not entirely compatible with the application of the aforementioned pharmacodynamic modelling techniques, i.e. the observed plasma apomorphine concentration range was not sufficiently large enough nor sustained for long enough to efficiently sample the pharmacodynamic response. This was a direct consequence of performing the study under non-steady state conditions, confounded by the short absorption and elimination half-lives of apomorphine, the brief period of anti-parkinsonian effect, and the absence of continuous pharmacodynamic monitoring.

6.2. *Preliminary Study of Needle-Free Subcutaneous Injections of Apomorphine in Parkinson's Disease.*

It must be stated that the findings of this investigation are limited by a lack of information regarding intra-patient variation in pharmacokinetic parameters and pharmacodynamic response following either conventional or needle-free delivery of apomorphine, and by the small number of patients involved. Therefore, whilst trends in the data are reported here, the statistical significance of any differences in the pharmacokinetics, pharmacodynamics or tolerability between the two treatments could not be established.

In the interpretation of the comparison of the two treatments, it is important to note the following issues:-

(i) The target time period for administration of apomorphine was between 08:00 and 10:00, however due to various practical and logistical difficulties, e.g. resistant "on" phase (patient 10, needle-free trial 1) and additional procedure required in the manipulation of the pallidal stimulator (patient 12), the time of administration did in fact fall outside the stated range in these patients (see Table 4-5, page 4-20). Nevertheless, consistency in the times of administration of apomorphine was maintained for both trials of the needle-free device in patient 10.

(ii) The site of subcutaneous administration was, in each case, the anterior aspect of the thigh. The posture of the patients during apomorphine administration varied, i.e. standing up versus sitting on a chair, depending on the patients' usual routines, however a consistency in posture was maintained within each patient for both treatments (see Table Table 4-5, page 4-20)

(iii) Blood sampling times for each patient were well matched across the two treatments, with particular attention given to initial samples. There was more variation in the timing of later samples collected from patient 12 due to time constraints (see Appendix 8.18).

Pharmacokinetics were best described by a first order input, two compartment model which included a lag-time. See Figure 6-14 to Figure 6-16, and Table 6-8.

There was one exception to this outcome in that a lag-time was not required in the case of patient 10 needle-free trial 2. In this particular case the lag-time model was rejected in favour of a non-lag-time function on the basis that the lag-time was estimated (as 0.013 minutes) with great imprecision (C.V. > 80000%), in addition to the fact that the predicted apomorphine concentration-time profile using this model did not mimic the trend in the observed data, i.e. the predicted T_{max} occurred after the observed T_{max}. This was attributed to the fact that from the first sampling point the concentration was in decline, thus there was an absence of data describing the increase in concentration directly following dosing.

The repeat subcutaneous administration of apomorphine to patients 09, 10 and 12 in the investigation of needle-free delivery of apomorphine allowed an intra-patient comparison of beta-intercept values to be made. In accordance with the previous findings (Section 6.1.1.6, page 6-18), the beta-phase intercept was not predictive of anti-parkinsonian response, and furthermore it was found that the beta-phase intercept was of no predictive value even within a given patient (Table 6-7).

Patient ID		Beta-phase intercept	Quality of "on" phase.
No.	Treatment	(ng/mL)	
9	CON	3.5	Optimal
	NF	0.4	Optimal
10	CON	5.84	Sub-optimal
	NF 1	NA	Optimal
	NF 2	12.4	Optimal
12	CON	7.7	Optimal
	NF	10.2	Sub-optimal

Table 6-7 **Beta-phase intercept values following administration of subcutaneous apomorphine.**

Abbreviations: CON = conventional, NF = needle-free.

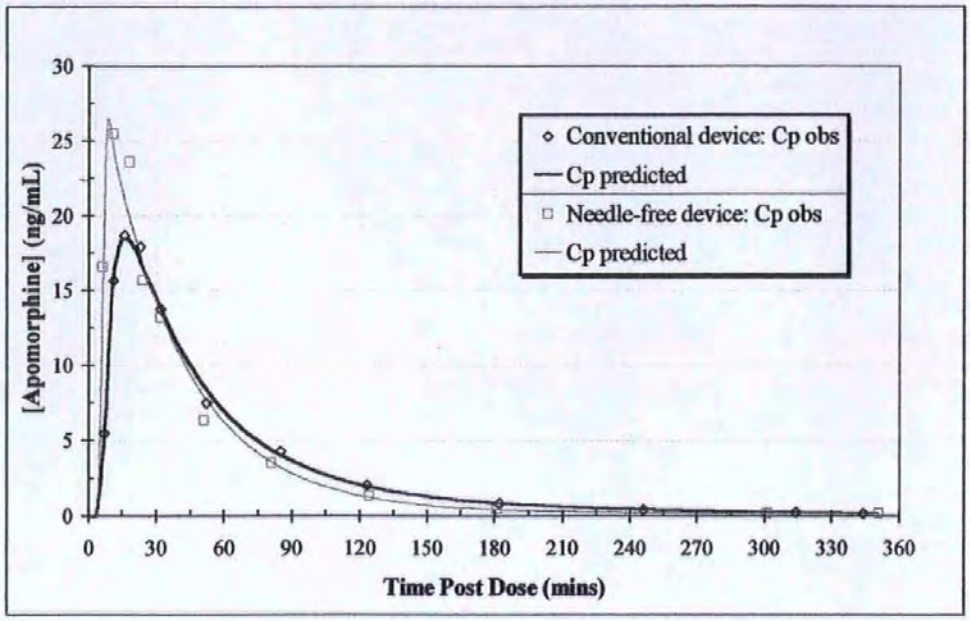

Figure 6-14 Predicted plasma apomorphine concentration: Patient 09 (dose = 2mg).

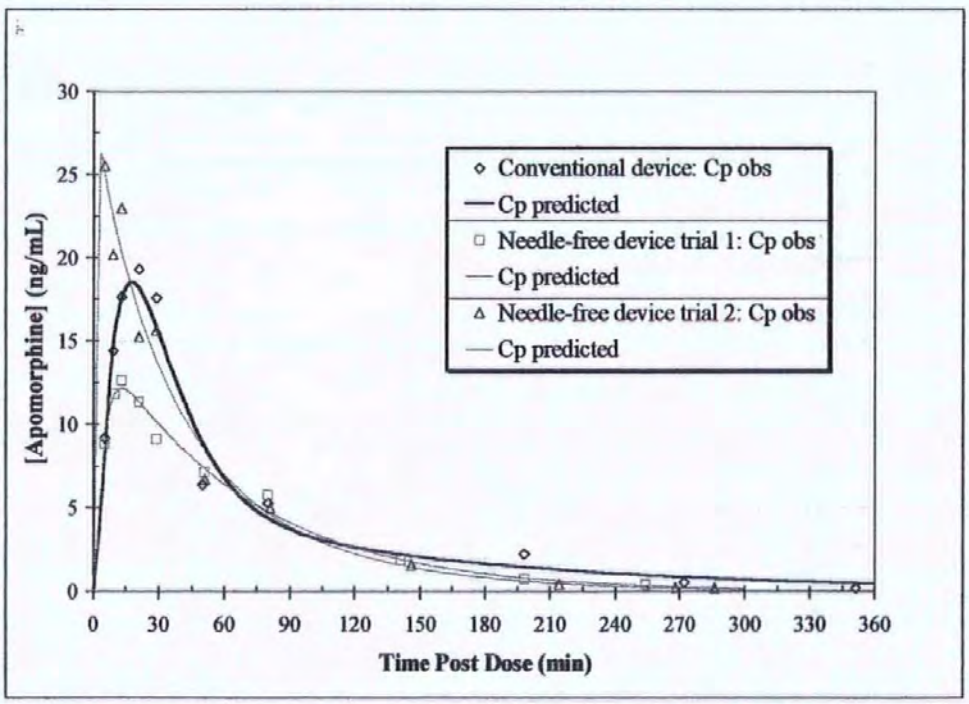

Figure 6-15 Predicted plasma apomorphine concentration: Patient 10 (dose = 3.5mg).

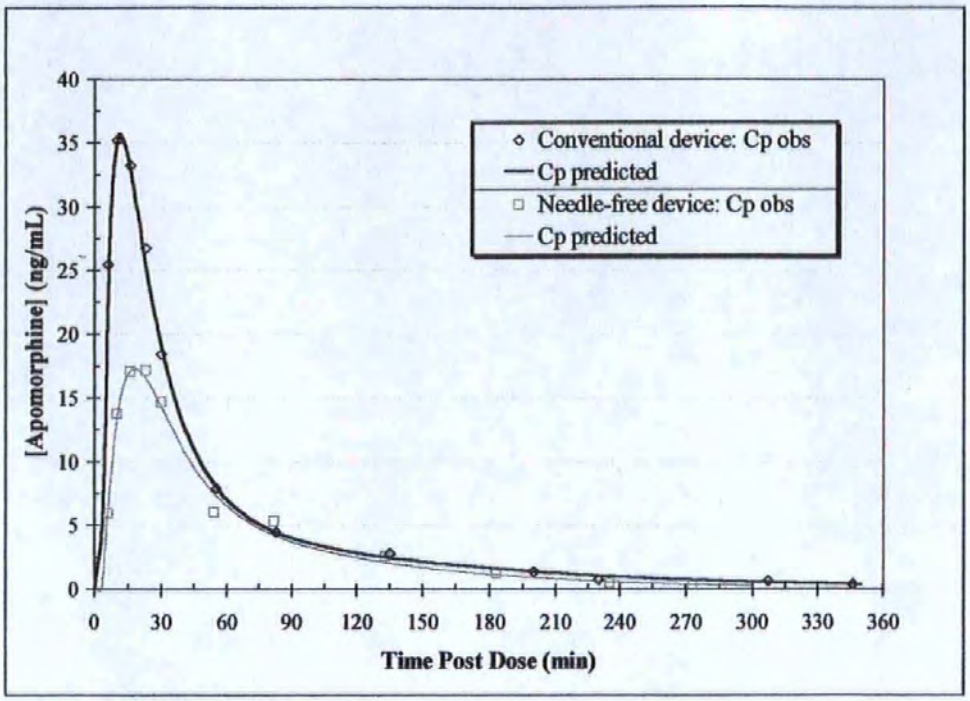

Figure 6-16 Predicted plasma apomorphine concentration: Patient 12 (dose = 5mg).

Patient ID No.	Treatment	C_{max} *observed* (ng/mL)	C_{max} *predicted* (ng/mL)	Dose-normalised C_{max} *predicted* (μg/mL)	T_{max} *observed* (minute)	T_{max} *predicted* (minute)	$AUC_{0\text{-infinity}}$ (ng.min/mL)	Dose-normalised $AUC_{0\text{-infinity}}$ (μg.min/mL)	Bioequivalence[a] (%)
9	CON	18.9	18.5	9.3	16	16.2	1083	542	100
	NF	25.4	25.9	12.9	11	10.0	1031	516	95
10	CON	19.3	18.5	5.3	21	17.6	1350	386	100
	NF 1	12.6	12.2	3.5	13	13.0	977	279	72
	NF 2	25.5	26.3	7.5	5	2.3	1263	361	94
12	CON	35.2	35.7	7.2	10	11.5	1667	333	100
	NF	17.2	17.3	3.5	23	18.1	1102	220	66

Table 6-8 Pharmacokinetics of apomorphine injection by conventional (needle) and novel (needle-free) devices.

Abbreviations: CON = conventional, NF = needle-free.

[a] Estimated using intra-patient $AUC_{0\text{-infinity}}$ relative to conventional delivery.

When the dose was delivered efficiently by needle-free injection, i.e. where there was an absence of a significant local tissue reaction (n = 2: patient 09 and patient 10 trial 2), then C_{max} was greater than that estimated for conventional delivery in those patients (by a mean of 41%), T_{max} was shorter (mean of 63%) and $AUC_{0\text{-infinity}}$ was essentially equivalent, being on average 95% of that observed for conventional delivery.

Conversely, when an adverse local tissue reaction occurred in response to needle-free administration of apomorphine (n = 2: patient 10 trial 1 and patient 12), there was a reduction in C_{max} (mean of 43%) and $AUC_{0\text{-infinity}}$ (mean of 31%) as compared to conventional delivery in those patients. T_{max} varied, being quicker than conventional delivery on one occasion and slower on the other occasion.

These findings indicate an association between absorption and local tissue effects in this study, whereby damage to the subcutaneous tissue compromised absorption of drug into the bloodstream. However there remains the possibility that absorption was reduced (relative to conventional delivery) in the two cases described *independently* of local tissue effects.

An alternative explanation for the comparatively low C_{max} and $AUC_{0\text{-infinity}}$ that occurred on two occasions following needle-free injection is that there was incomplete drug delivery as a result of poor technique or a design failure.

It is possible that a proportion of the apomorphine solution may have flowed back through the epidermis immediately after injection in the case of patient 10, since it was reported that the skin surface at the administration site was wet following the first trial of the needle-free device. Indeed the experience of Cooper *et al*[36] with needle-free injection of lidocaine to the human forearm showed that it was important to press the J-TIP® both firmly and perpendicularly against the skin and to maintain the applied pressure for a few seconds after discharging the device to limit back-flow of the drug solution through the skin.

However in the case of patient 12, in which relatively incomplete absorption was also demonstrated, the skin surface was absolutely dry. Therefore in *this* case it may be that a proportion of the apomorphine solution was ejected into the air, and not across the epidermis.

A further variable which may theoretically be relevant to both absorption and bruising following needle-free injection is the posture adopted by the patient at the time of injection. However, given that the two trials of needle-free apomorphine delivery in patient 10 resulted in opposing outcomes with respect to absorption and bruising (and pain) *despite* the fact that the two trials were carried out under the same conditions regarding posture, it is unlikely that posture contributed greatly to the results.

In fact the posture that is thought to give an optimum absorption/pain profile is one whereby the availability of subcutaneous tissue is maximised, but where muscle contraction is minimised. For subcutaneous injection to the thigh this would entail the patient lying down with the leg outstretched but relaxed[37]. This information became available after the completion of the clinical study.

The development of an adverse local tissue reaction was independent of bleeding on administration of apomorphine.

Reports of local side effects resulting from jet injection of drug solutions are given in the literature. Approximately 50% of patients experienced mild local reaction (redness and swelling) following fine-needle aspiration biopsy of breast lesions, which was performed following application of local anaesthetic via a jet injector[38]. It was stated that such complications could have been related to the anaesthetic method, biopsy, or indeed to both. Local side effects, including bruising[39], have been reported as a result of using needle-free technology for insulin administration[40]. Verrips *et al* compared the use of a jet-injector

with a multi-dose injection pen in human growth hormone therapy, the incidence of bruising occurring as a consequence of using the jet injector was higher that that for the pen system[41].

There are, however, reports to the contrary: Hardison *et al* states that the use of a needle-free device for induction of local anaesthesia was significantly free of side effects[42], and in a separate investigation of the application of local anaesthetic using a jet injector there were no adverse local effects in the 206 patients studied[43].

Considering the high incidence of local tissue complications in subcutaneous apomorphine therapy, it was not surprising that local tissue complications did occur, however the severity of the bruising following needle-free delivery was somewhat unexpected. It appeared that, when rupture of capillaries *did* occur, the process occurred extensively. It could be suggested that this was a direct result of the relatively wide dispersal of solution into the subcutaneous tissue as compared to a needle injection. Bruising occurred in combination with local induration, the latter being a characteristic complication of apomorphine therapy.

Thus it is likely that the manifestation of local tissue complications was a result of a Britaject®-specific reaction *and* the properties of the needle-free device. Whilst both factors were implicated, it was not possible in this study to establish the relative contributions of each. The inclusion of needle-free subcutaneous injections of saline and sodium metabisulphite (the latter being the additive used in the Britaject® preparation) in the study protocol as controls for needle-free injection and apomorphine-induced effects, respectively, might have served to resolve this issue.

The needle-free treatment compared unfavourably with the conventional treatment in respect of the pain experienced on administration. Familiarisation with the use of needles for injection is potentially a factor here since both of the patients currently receiving

apomorphine by subcutaneous bolus injection rated the needle-free device as relatively *more* painful. In a study of lidocaine administration to the forearm in which 72 patients were randomised to receive the local anaesthetic either by the J-TIP® or from a standard needle and syringe, the authors reported that the needle free group had experienced significantly *less* pain on injection than the conventional treatment group[36]. There are other examples in the literature which state that the pain or discomfort of needle-free injection was less than[38, 41, 44, 45] or equal to[40] that experienced due to drug delivery through a needle.

The quality of the "on" phase as reported by the patients, and also in terms of magnitude of effect (maximum improvement in tapping test score from baseline) and onset and duration of effect, was independent of pharmacokinetic parameters (C_{max}, T_{max} and AUC) and independent of local tissue reaction.

It was not possible to establish any trends relating to differences in pharmacodynamic response, e.g. onset or magnitude of effect, between the treatments. This was due to (i) a lack of information regarding intra-patient variation in the quality of the apomorphine-induced "on" phase, and (ii) the inter-patient variation in response following the control, i.e. conventional, administration of apomorphine.

It was considered that the differences in the pharmacodynamic response between the two treatments were probably within normal intra-patient variation under the study conditions. In future similar investigations, intra-patient variation must be determined in order to interpret the differences in response between the treatments.

The patient commentary was useful in the comparison of anti-parkinsonian responses in a given patient. However it was recognised that the extent to which details regarding changes in clinical symptoms were volunteered by the patients was somewhat dependent on the individuals' motivation at the time.

Factors which had the potential to influence pharmacokinetic and pharmacodynamic outcome but were independent of delivery device included the temperature of the skin at the administration site[1] and the effect of changes in circulation to the subcutaneous tissue, e.g. reduction in blood flow as a result of feeding. Such factors were not controlled in this investigation.

To summarise, it was demonstrated that a potentially desirable pharmacokinetic profile (in terms of a quicker T_{max} and greater C_{max}) *could* be achieved, but that this did not necessarily translate to an improved pharmacodynamic response. Whilst this represents an advantage for those who are adverse to needle injection, the unfavourable outcome in terms of adverse local tissue effects and/or pain of administration may actually preclude the use of the device. It was not possible in this preliminary study to identify the cause of the unacceptable tissue reaction, only to predict that both the delivery mechanism and the drug solution (Britaject®) were implicated, and that poor technique may have contributed to the outcome. If the risk of local tissue damage could be reduced then the needle-free device has the potential to be developed as a viable alternative to the existing needle system for intermittent apomorphine therapy.

The study was designed as a preliminary investigation of the utility of a needle-free injector system as a delivery device for apomorphine in Parkinson's disease, combined with an investigation of pharmacokinetic-pharmacodynamic relationships which included the beta phase intercept as an outcome measure. Given these objectives, a sampling protocol over (approximately) 300minutes post-dose was employed. However, since the novel feature of needle-free delivery is based on the potential for rapid absorption (as a consequence of the increased surface area of drug solution made available to subcutaneous vasculature), it would be a requirement that in a future investigation of this technique, a more intensive

sampling strategy in the immediate period after dosing should be employed in order to characterise C_{max} with greater accuracy.

6.3. *Pharmacokinetic Study of Single-Dose Intranasal Apomorphine (Three Doses) in Healthy Volunteers.*

The observed concentration-time series were best described by a first-order input, one-compartment pharmacokinetic model, with the exception of five out of the twenty-two series for which a two compartment model was superior. In the majority of cases, i.e. sixteen out of twenty-two series, a lag-time was required to adequately reflect trends in the observed data. Predicted plasma apomorphine concentration-time profiles are given in Figures 6-17 to 6-22. A comparison of the salient pharmacokinetic parameters for the two routes is presented in Table 6-10 (page 6-59).

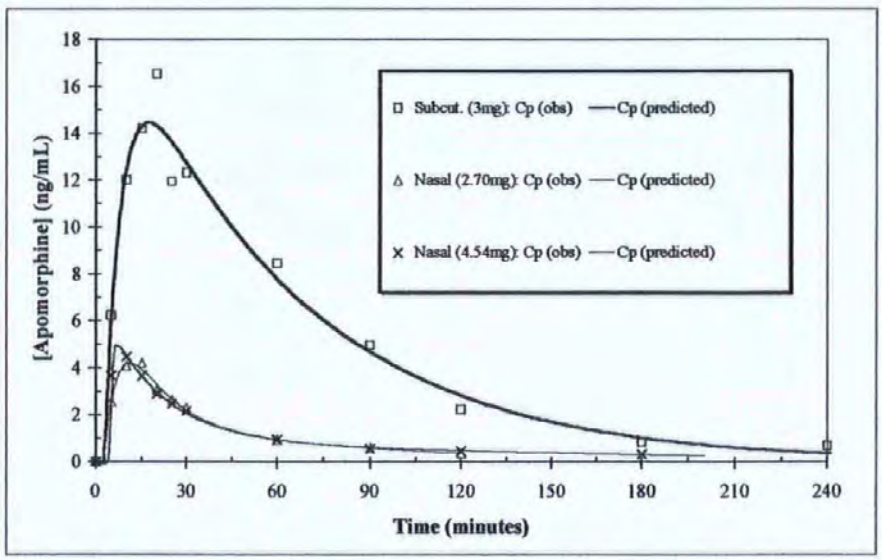

Figure 6-17 Predicted plasma apomorphine concentration following subcutaneous and intranasal administration to volunteer 1 (doses are given in parentheses).

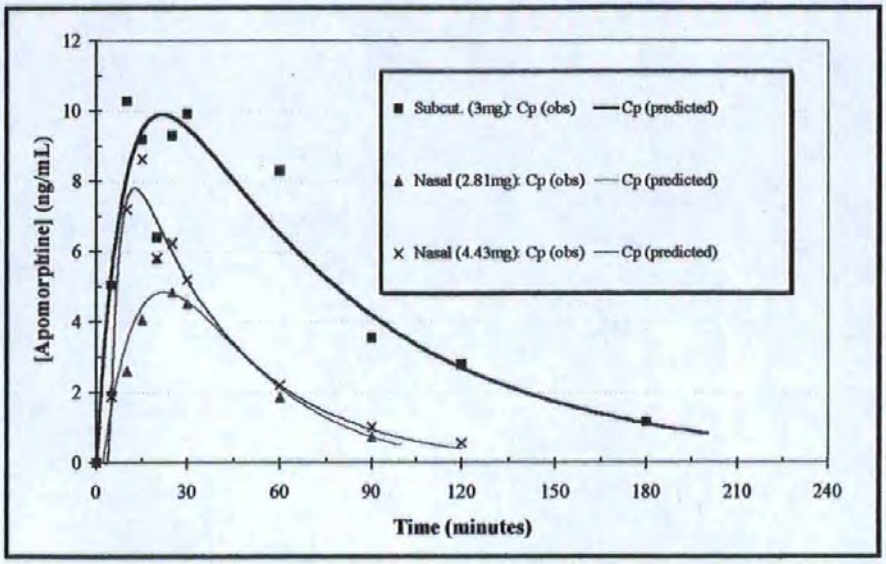

Figure 6-18 Predicted plasma apomorphine concentration following subcutaneous and intranasal administration to volunteer 2 (doses are given in parentheses).

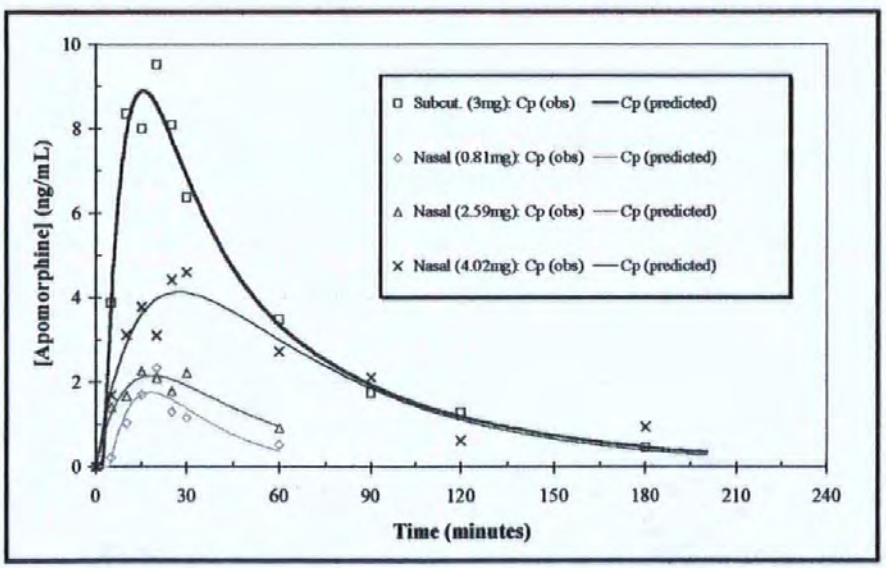

Figure 6-19 Predicted plasma apomorphine concentration following subcutaneous and intranasal administration to volunteer 4 (doses are given in parentheses).

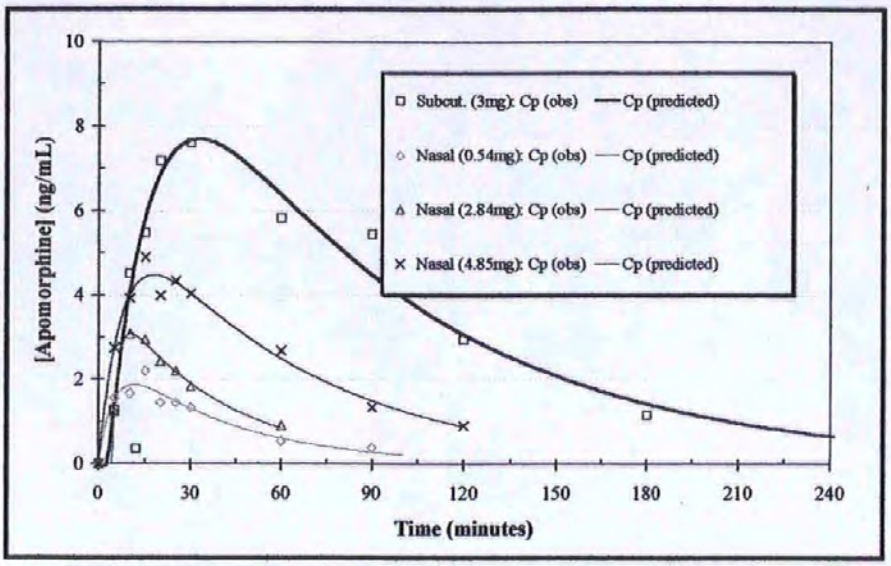

Figure 6-20 Predicted plasma apomorphine concentration following subcutaneous and intranasal administration to volunteer 5 (doses are given in parentheses).

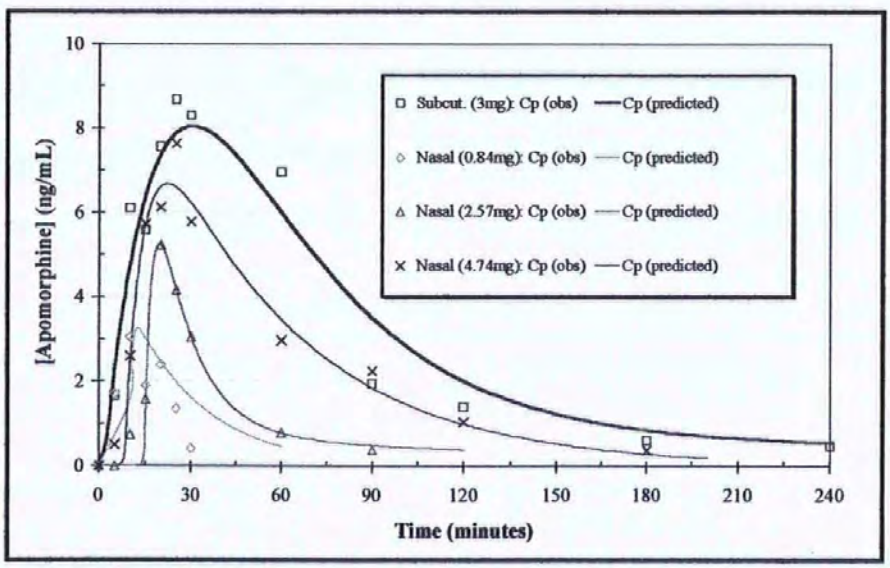

Figure 6-21 Predicted plasma apomorphine concentration following subcutaneous and intranasal administration to volunteer 6 (doses are given in parenthesis).

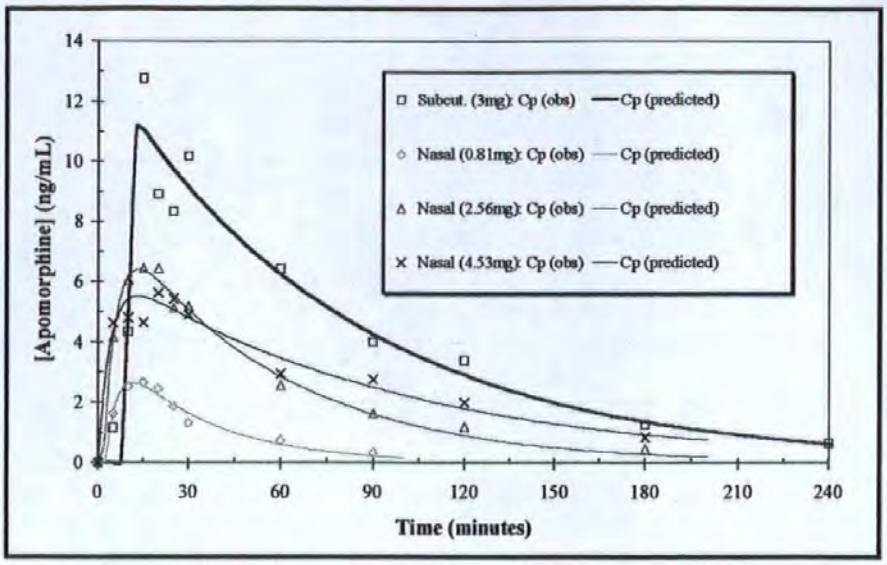

Figure 6-22 Predicted plasma apomorphine concentration following subcutaneous and intranasal administration to volunteer 7 (doses are given in parentheses).

	Subcutaneous	Intranasal, initial potency:-		
		1mg	3mg	5mg
	n=6	*n*=4	*n*=6	*n*=6
Dose (mg)	3.00 (3.00 to 3.00)	0.75 (0.54 to 0.84)	2.68 (2.56 to 2.84)	4.52 (4.02 to 4.85)
Lag-time (minute)	3.3 (0.0 to 9.6)	4.2 (0.0 to 9.6)	4.2 (0.0 to 14.4)	2.8 (0.0 to 7.9)
K01 $t_{½}$ (minute)	7.7 (3.9 to 15.8)	3.3 (0.6 to 6.7)	5.2 (1.35 to 14.4)	4.2 (0.5 to 10.5)
T_{max} *predicted* (minute)	21.8 (13.3 to 32.6)	13.3 (11.2 to 18.0)	15.8 (10.2 to 22.3)	16.6 (6.8 to 27.3)
Dose-normalised C_{max} *predicted* (ng/mL[a])	3.0 (2.6 to 4.8) C.V.= 25%	3.2 (2.2 to 3.9) C.V.= 23%	1.6 (0.8 to 2.5) C.V.= 38%	1.1 (0.9 to 1.8) C.V.= 25%
Dose-normalised AUC (ng.min/mL[a])	269 (178 to 372) C.V.= 21%	133 (94 to 182) C.V.= 33%	96 (49 to 174) C.V.= 51%	84 (37 to 123) C.V.= 30%
Relative Bioavailability[b] (%)	100 (100 to 100)	57 (49 to 70)	38 (19 to 65)	34 10 to 56)
Elimination $t_{½}$ [c] (minute)	46.1 (35.6 to 54.5)	19.5 (13.5 to 27.0)	41.1 (13.4 to 99.3)	50.5 (24.2 to 102)

Table 6-9 Mean (range) pharmacokinetic parameters for the subcutaneous and intranasal administration of apomorphine to healthy volunteers.

[a] Per 1mg dose.
[b] Estimated using intra-patient $AUC_{0\text{-infinity}}$ relative to subcutaneous delivery.
[c] Calculated using (ln 2)/K10 or (ln 2)/beta for one-or two-compartment models respectively.

As demonstrated in previous investigations, apomorphine is as rapidly absorbed via the intranasal route as the subcutaneous route[12, 23, 46], and substantial inter-individual variation in pharmacokinetic parameters exists.

The pharmacokinetic parameters derived for volunteers 1 and 2, i.e. for subcutaneous mid- and high-dose nasal treatments, were excluded from the ANOVA due to the absence of pharmacokinetic parameters for the low intranasal dose.

There was a short absorption half life following intranasal dosing, the means of which were less than that for subcutaneous administration, but were not statistically different ($p=0.163$). The time-lag was brief for both administration routes and, with a C.V. of approximately 100% for each of the treatment groups, was not statistically different across the treatments ($p=0.736$). In contrast to Sam *et al*[12], the means for T_{max} following intranasal administration were shorter than that estimated for the subcutaneous route, indicating that satisfactory deposition of apomorphine powder into the nasal cavity was achieved, but as with the published comparative T_{max} data[12], the difference in T_{max} between the treatments was not statistically significant ($p=0.178$).

C_{max} and $AUC_{0\text{-infinity}}$ were correlated to administered intranasal dose ($p<0.01$ in each case, dose range = 0.54 to 4.85mg, $n=16$), see Figure 6-23.

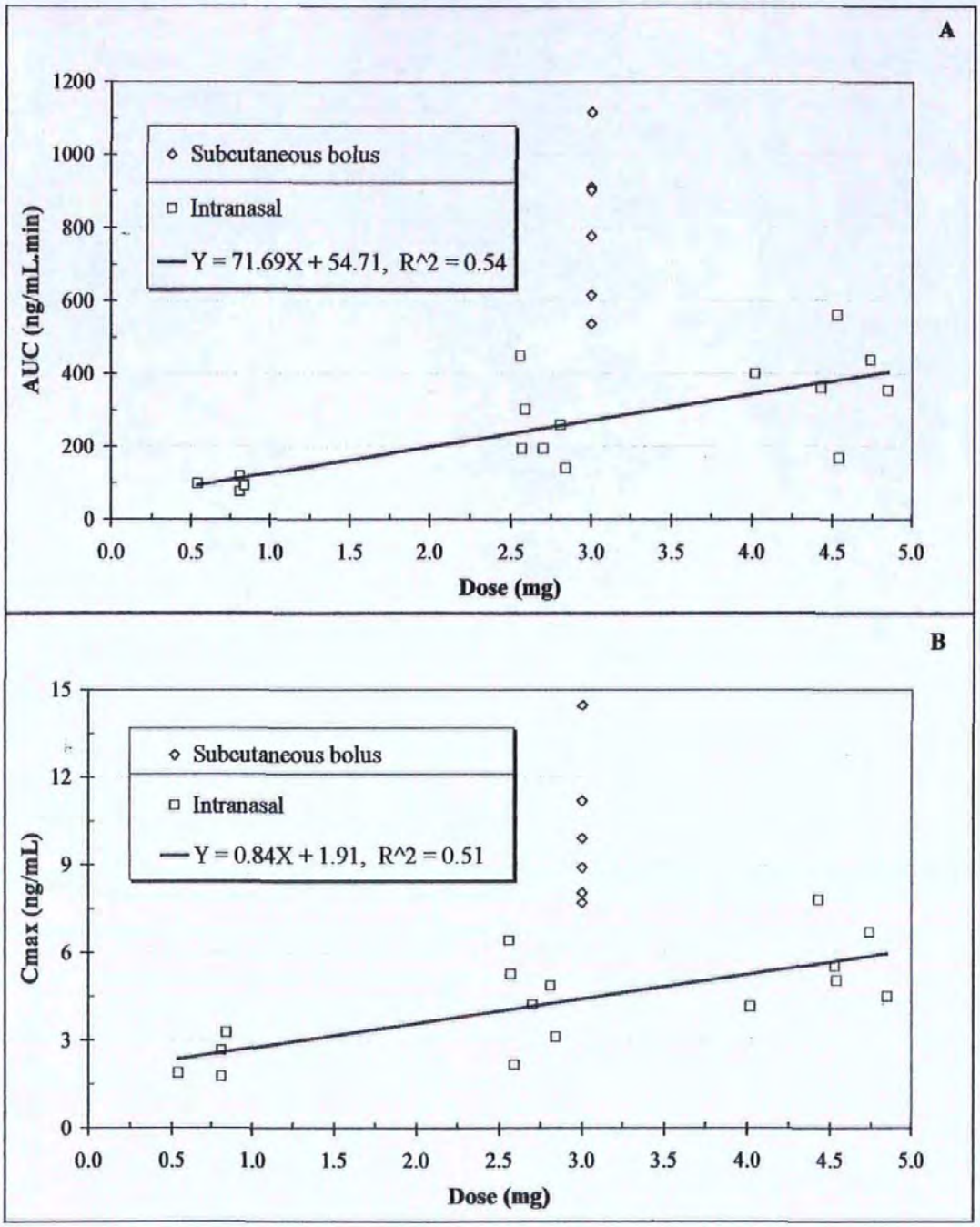

Figure 6-23 Correlation between dose and $AUC_{0\text{-infinity}}$ (panel A), and dose and C_{max} (panel B), following intranasal administration of apomorphine (three doses) to six healthy volunteers.

Both the dose-normalised C_{max} and $AUC_{0\text{-infinity}}$ obtained for the intranasal route were reduced relative to those obtained for subcutaneous administration in the same volunteer. This was represented in the mean relative bioavailability of the intranasal route, which was 41% (range was 10 to 70%, n=16) of the control subcutaneous route (defined as 100%), in

terms of intra-individual $AUC_{0\text{-infinity}}$ normalised for dose. The mean relative bioavailability of the intranasal system used in this preliminary study compares closely with that reported by Sam *et al*[12]. There was a significant difference in mean relative bioavailability between the subcutaneous treatment and each of the three intranasal treatments, but no significant differences between the three intranasal treatments themselves ($p=0.001$). That having been said, there was an apparent decrease in mean relative bioavailability with increasing intranasal dose. This can be explained by a consideration of the rates of apomorphine dissolution and mucociliary clearance *in situ*, whereby the percentage of an administered dose that was unavailable for intranasal absorption, i.e. cleared to the nasopharynx and swallowed, would increase with increasing dose. Evidence for this was in the form of the reporting of a bitter taste in the mouth following intranasal administration, and the short T_{max} following intranasal administration relative to subcutaneous administration.

Factors which are likely to have contributed to the poor relative bioavailability of intranasal apomorphine administration include the limited solubility of apomorphine in nasal mucus, the incompatibility of local pH and pKb of apomorphine with regard to the predominance of the ionised, i.e. non-absorbable, form of apomorphine, and the residence time of apomorphine powder at the nasal mucosa, which is limited by the mucociliary clearance rate[47].

Having taken account of the variation in amount of apomorphine released from the insufflator, it was possible that an inter- or intra-individual variation in the volume of nasal secretions contributed to the large variation in relative bioavailability (since this would alter the proportion of the administrated dose that was allowed to dissolve). Other proposed contributory factors are: (i) a variation in the pH of nasal mucus, which affects the percentage of ionised form of apomorphine present (pH of nasal secretion was not

measured), and (ii) a variation in sniff effort, which in turn has the potential to result in variation in the rate of clearance of particulates to the nasopharynx[47].

The local environment at the administration site can be modified in order to enhance the partitioning of the drug into the mucosal tissue. One strategy is to reduce the mucosal barrier function by the incorporation a penetration enhancer into the drug delivery process. Examples of penetration enhancers are surfactants, which act to perturb membrane integrity and thus facilitate drug diffusion[48, 49], and inclusion complexes with cyclodextrins, which enable the transient opening of tight junctions between epithelial cells[48, 49] and can improve the solubility and stability and tolerability of the drug[50] [51]. In addition, alteration of the pH of the local environment in order to favour the non-ionised form potentially allows for more comprehensive absorption of drug[52]. In the case of intranasal and absorption of apomorphine, a lowering of the pH *in vivo* would increase the percentage of the non-ionised form.

It may be desirable to increase the residence time of intranasal formulations. This can be achieved by the use of a mucoadhesive intranasal formulation, whereby mucociliary clearance is impeded[48].

Local tissue adverse reactions to intranasal apomorphine dosing have the potential to compromise absorption[23]. The incidence of adverse local effects reported by volunteers was minimal and those that were reported were mild and transient[53]. This is very much in agreement with the findings of other acute studies of intranasal administration of apomorphine[54, 55]. Chronic use of the dosage form was not the subject of this investigation. This remains an important issue to be addressed in the future, given that there is considerable evidence in the literature associating significant local tissue damage with the chronic use of intranasal apomorphine[23, 46, 54, 55].

See Section 6.4.1 for a general summary of outcomes in the study of intranasal and buccal delivery of apomorphine compared to conventional (subcutaneous) apomorphine delivery.

6.4. *Pharmacokinetic Study of Single-Dose Buccal Apomorphine (Three Doses) in Healthy Volunteers.*

The pharmacokinetics of the subcutaneous infusions of apomorphine were best described by a one compartment model with constant input and first-order output (Figure 6-24). Apomorphine pharmacokinetics following buccal administration were described using the same model, but with a lag time (between drug administration and the onset of drug absorption) incorporated into the pharmacokinetic model to account for the time lag in swelling of the insert (see Figure 6-25 and Appendix 8.17.2).

It is acknowledged that this is a basic representation of the pharmacokinetics of the buccal formulation of apomorphine, in that the model does not fully describe drug input, the latter being a complex function of the swelling of the hydrogel, dissolution of apomorphine and release of drug from the insert. The buccal drug input function might be better described by a Weibull function [56-58]. A comparison of salient pharmacokinetic parameters is given in Table 6-10 (page 6-59).

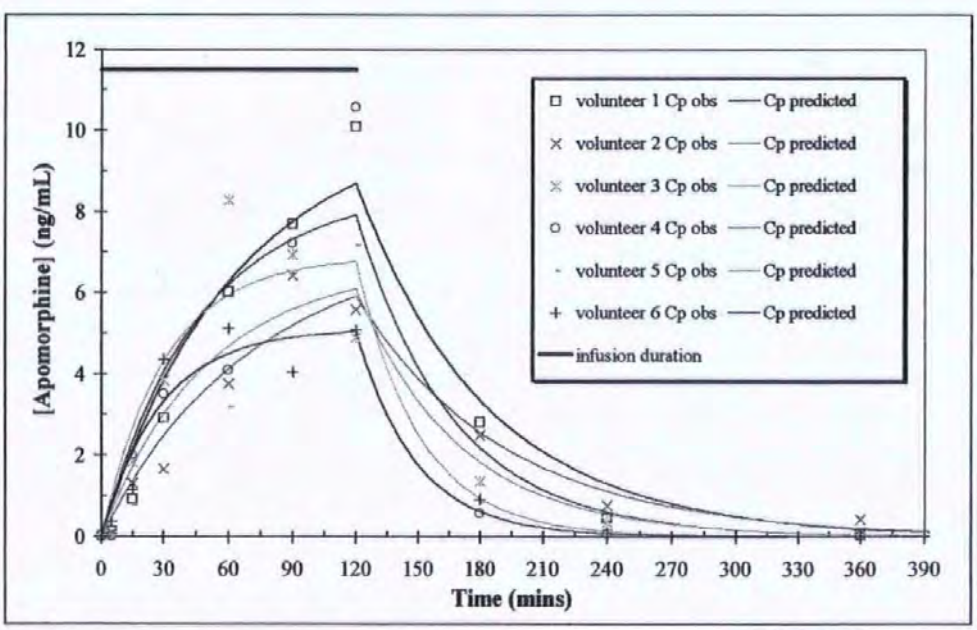

Figure 6-24 Predicted plasma concentration following subcutaneous infusion to healthy volunteers (dose = 2mg/h x 2h).

Abbreviations: Cp = concentration in the plasma, obs = observed.

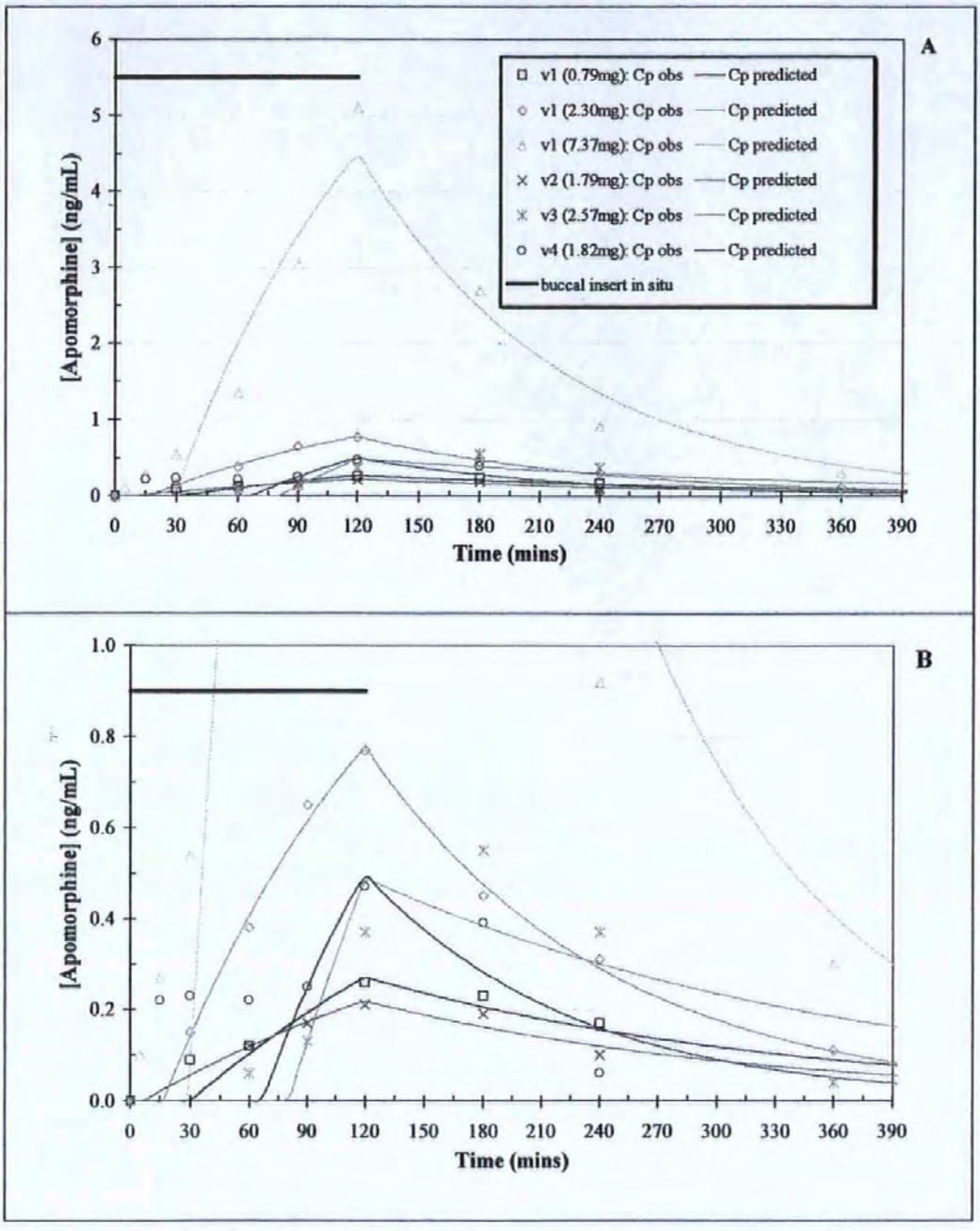

Figure 6-25 Predicted plasma apomorphine concentration following buccal administration to healthy volunteers. Detail shown in panel B. Doses are given in parentheses.

Abbreviations: Cp = concentration in the plasma, obs = observed,v = volunteer ID.

	Subcutaneous	Buccal, initial potency:-		
		5mg	10mg	20mg
	n=6	*n*=1	*n*=1	*n*=4
Dose (mg)	4.00 (4.00 to 4.00)	0.79 NA	2.30 NA	2.94 (1.59 to 7.37)
Lag-time (minute)	NA NA	30.0 NA	16.5 NA	45.6 (6.6 to 80.0)
T_{max} *observed*[a] (minute)	120 (60 to 120)	120 NA	120 NA	120 (120 to 180)
Dose-normalised C_{max} *predicted* (ng/mL[b])	1.7 (1.3 to 2.2)	0.3 NA	0.3 NA	0.3 (0.1 to 0.5)
Dose-normalised AUC (ng.min/mL[b])	224 (154 to 306)	95 NA	61 NA	53 (32 to 93)
Relative Bioavailability[c] (%)	100 (100 to 100)	30 NA	20 NA	21 (14 to 30)
$t_{½}$ K10 (minute)	33.6 (19.8 to 49.9)	150.6 NA	84.5 NA	112.4 (69.2 to 170.0)

Table 6-10 Mean (range) pharmacokinetic parameters for the subcutaneous and buccal administration of apomorphine to healthy volunteers.

The relationship between dose and both C_{max} and $AUC_{0\text{-infinity}}$ is illustrated in Figure 6-26. Estimates of C_{max} and $AUC_{0\text{-infinity}}$ for the buccal apomorphine treatment appear to be dose-related, however, since the outlying data point exerted considerable leverage on the linear relationship between said pharmacokinetic parameters and apomorphine dose, it was considered inappropriate to present such correlation statistics.

[a] Median (range).
[b] Per 1mg dose.
[c] Estimated using intra-patient $AUC_{0\text{-infinity}}$ relative to subcutaneous delivery.

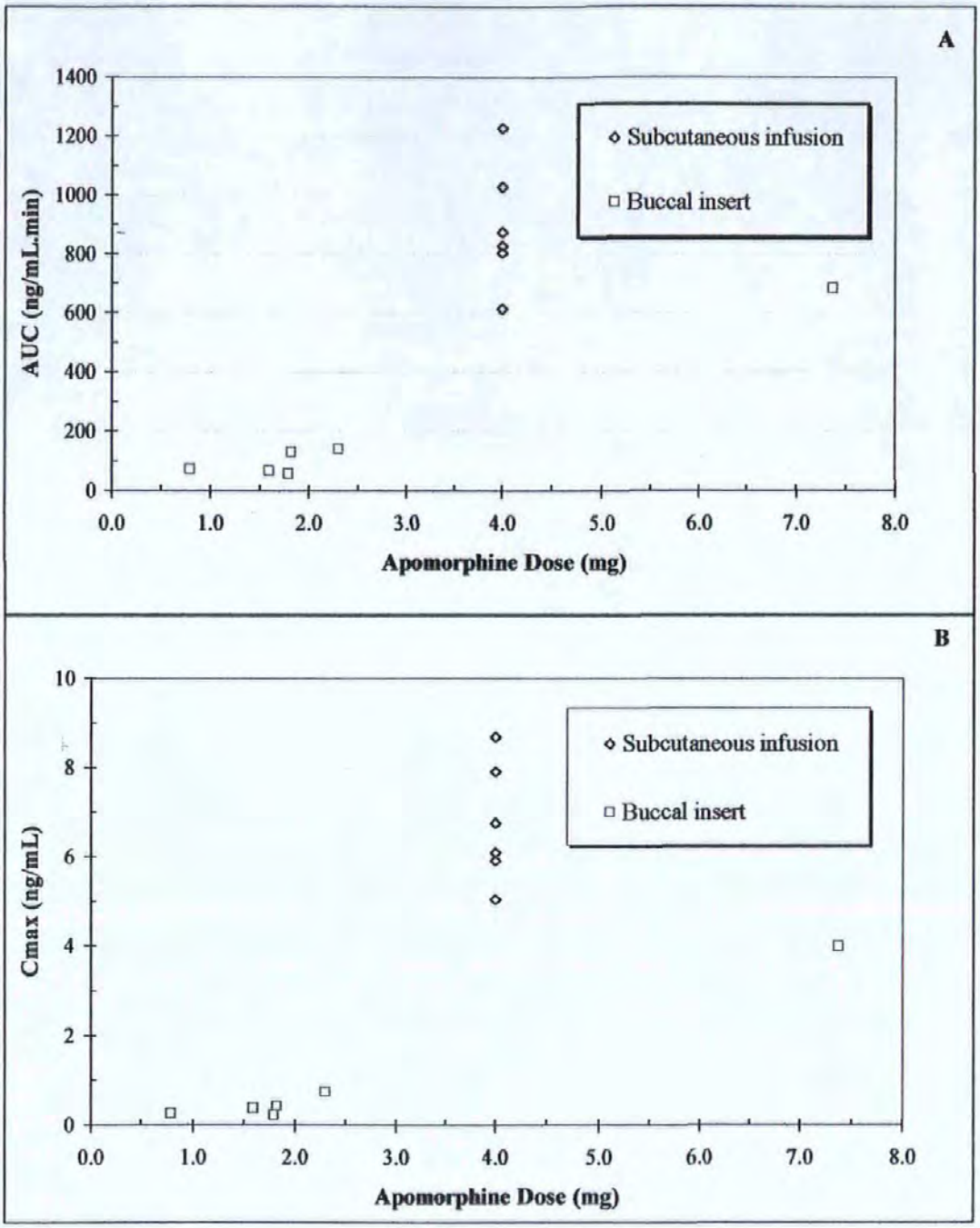

Figure 6-26 Relationship between dose and $AUC_{0\text{-infinity}}$ (panel A), and dose and C_{max} (panel B), following buccal administration of apomorphine to healthy volunteers.

In comparing the means of pharmacokinetic parameter estimates between subcutaneous and buccal administration of apomorphine, it was considered appropriate to treat all six buccal series as a single group. This approach was used due to the limited observations of such parameters in the case of the low and mid-dosage buccal apomorphine insert. Thus single factor ANOVA was performed.

Both the dose-normalised C_{max} and $AUC_{0\text{-infinity}}$ obtained for the buccal apomorphine formulation were very much reduced compared to those obtained for subcutaneous administration in the same volunteer. There was a significant difference between the two treatments in both mean C_{max} and $AUC_{0\text{-infinity}}$ ($p<0.001$ in each case).

In accordance with this the relative bioavailability of the buccal route was low (mean of 22%, range 14 to 30%, n=6), although improved from published sublingual studies (means of 10 to 18%[6, 7, 13, 59]).

The factors which may have contributed to the poor relative bioavailability of buccally administered apomorphine are of a similar nature to those identified with regard to the poor relative bioavailability following intranasal apomorphine administration (See Section 6.3). Thus the likely factors include the limited solubility of apomorphine in saliva, i.e. approximately 0.8 mg/mL (Controlled Therapeutics (Scotland), East Kilbride, UK) compared to 20mg/mL in water[60], and the ionization state of apomorphine under *in vivo* conditions.

The variation in relative bioavailability could have resulted from an inter- or intra-individual variation in rate of salivation, thereby influencing such factors as the desorption of apomorphine from the insert and the proportion of the administered dose that was swallowed. Additionally, a variation in salivary pH might have affected the percentage of ionised form of apomorphine present. The pH of the volunteers' saliva was not measured. The variation in relative bioavailability may be related to the absorption of apomorphine at oral mucosa *other than* buccal mucosa, i.e. gingival and/or sublingual mucosae. It was reported that, on some occasions, the insert had detached from the surface of the gingiva prior to the end of the 120 minute duration of apomorphine administration. On these

occasions the insert was retained in the mouth until the 120 minute period had elapsed. Details of volunteer(s)/buccal apomorphine dose(s) that were implicated were not recorded.

General strategies to improve the absorption of apomorphine at buccal mucosa are similar to those given in Section 6.3 for intranasal apomorphine, e.g. incorporation of a penetration enhancer.

Strategies to increase the solubility of the drug *in vivo* with regard more specifically to buccal administration include: (i) the positioning of the insert in relation to salivary flow, i.e. at rest the salivary flow in the lower buccal region is greatest (the submandibular and sublingual glands produce 75% of the total amount of saliva produced), however upon stimulation, the parotid glands in the upper oral cavity produce double the amount of saliva than do the submandibular and sublingual glands[49], (ii) the modification of the composition of the hydrogel in order to optimise the mechanisms which control the release of water soluble drugs from the hydrogel[61] (or, by the incorporation of a laminated surface on the insert, in order to ensure uni-directional release from the hydrogel[49]), and (iii) attention to teeth cleaning, since this procedure could have the effect of raising the pH of the local environment to the extent that apomorphine solubility is reduced.

The incidence of local adverse effects reported by the volunteers was minimal, and where such an event did occur, the effect was mild and transient[62]. Chronic use of the dosage form was not the subject of this investigation, and thus remains an issue to be addressed in the future.

As an exploration of the pharmacokinetics of multiple buccal insert usage, repeated buccal administration every three hours was simulated (WinNonlin version 1.5, Pharsight, USA) using the user-defined pharmacokinetic model described previously in this Section (see Appendix 8.17.2. The pharmacokinetic parameter estimates used in the simulation were the

mean values for the six buccal apomorphine pharmacokinetic series described previously, i.e. volume = 3402L, k10 = 0.0074 minute, lag-time = 39.7 minute. The dose used was the mean dose of the six buccal apomorphine pharmacokinetic series, i.e. 3.2mg (Figure 6-27). This particular schedule was investigated as it represented a practical schedule for buccal usage, i.e. after two hours *in situ* the insert tends to detach from the mucosa and since the insert cannot be in place whilst eating, intermissions are required. Apomorphine administration was simulated for an eleven hour period, a duration which represented the use of buccal inserts during waking hours, since buccal delivery of apomorphine during sleep is precluded due to the risk of swallowing the insert.

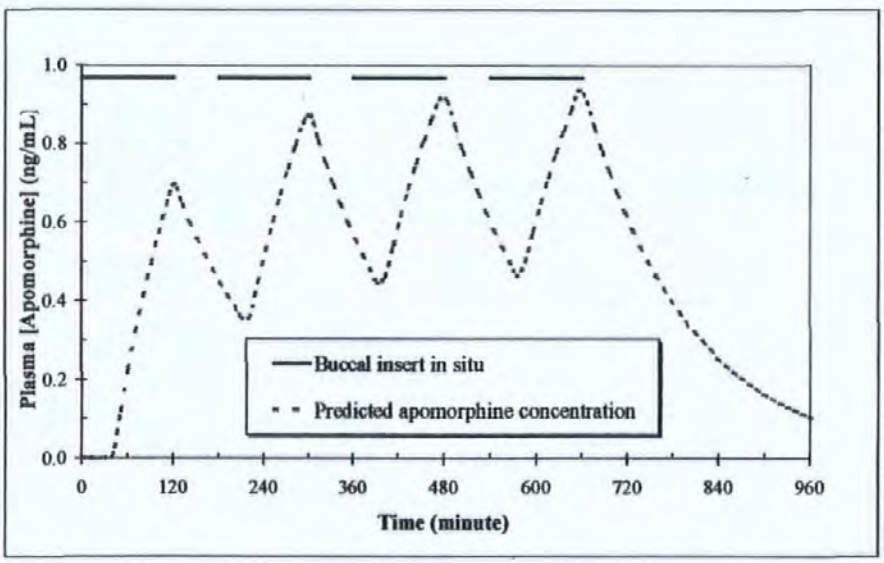

Figure 6-27 Predicted plasma apomorphine concentration on repeated dosing: buccal administration (for 2h duration) every 3 hours.

A plateau effect was observed after application of the second buccal insert. The steady state plasma apomorphine concentration range was 0.5 to 0.9 ng/mL.

6.4.1. General summary of outcomes in the study of intranasal and buccal delivery of apomorphine compared to conventional (subcutaneous) apomorphine delivery.

Apomorphine release from the buccal hydrogel and, to a lesser extent, the nasal insufflator was low and variable, indicating the need to optimise the delivery mechanisms of the devices used in order to proceed with the development of these novel systems. When drug release did occur, dose-related plasma concentrations were observed, but the bioavailability was low in comparison with the subcutaneous control apomorphine administration, suggesting that formulation issues must be addressed in order to achieve therapeutic plasma concentrations of apomorphine.

It is likely that the two delivery systems would achieve high patient acceptability in comparison to the conventional subcutaneous system. Patient acceptability, and therapeutic outcome, will depend on the local adverse effects profile resulting from chronic use of the novel apomorphine formulations.

It was considered by the investigators (Section 4.2.2.1 and Section 4.2.3.1) that the open label, non-randomised, ascending dose protocol was an efficient approach in the exploration of new formulations of apomorphine. However it must be stated that apomorphine pharmacokinetics following subcutaneous administration are subject to considerable inter-subject variation and, to a lesser degree, intra-subject variation[1]. Thus the inclusion of repeated dosing of the intranasal and buccal formulations with a matched number of subcutaneous control doses may have afforded a more robust investigation of the novel apomorphine formulations. Nevertheless, the objective of deriving information on the pharmacokinetics and relative bioavailability of the novel formulations/delivery modes was met. Such information allowed the investigators to proceed with development of the novel apomorphine delivery modalities.

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SECTION 7:
FINAL SUMMARY AND FUTURE WORK.

7. Final Summary and Future Work.

There were two aspects to the study of apomorphine in the treatment of Parkinson's disease presented in this thesis. These were: (i) a clinical pharmacokinetic-pharmacodynamic study which was undertaken in view of the challenges of apomorphine dose-titration in Parkinson's disease, and in response to the scarcity of available literature on the pharmacokinetic-pharmacodynamic relationships of apomorphine in Parkinson's disease, and (ii) exploratory pharmacokinetic (and tolerability) studies of apomorphine administration using novel delivery/formulation combinations, which were undertaken in view of the inherent limitations associated with the conventional (subcutaneous) route of administration of apomorphine (e.g. cutaneous nodule formation, needle-phobia).

An HPLC assay was developed which allowed the detection of apomorphine in plasma at suitably low levels of the analyte (LOQ was 0.05ng/mL), could distinguish apomorphine (forced) degradation products and potential apomorphine metabolites (apomorphine orthoquinone and isoapocodeine), and was not compromised by the concomitant administration of the commonly prescribed anti-parkinsonian drugs. The success of apomorphine quantification in clinical samples depended heavily on the use of anti-oxidants in the collection, storage and extraction procedures.

It was noted that the analytical method could be further developed to include the quantification of other (proposed) apomorphine metabolites, such as apomorphine glucuronide and *N*-demethylated apomorphine.

With regards to the first aspect of the research, focus was given to investigating the relevance of a potential correlation between the beta-phase intercept of a two-compartment pharmacokinetic model and apomorphine-induced anti-parkinsonian response in patients

with Parkinson's disease. It was hypothesised that this particular correlation, which was based on a review of plasma apomorphine concentration-time profiles in the literature, may be of use in a dose-optimisation scheme. However it was demonstrated that the beta-phase intercept was unrelated to apomorphine pharmacodynamics (in terms of improvement in tapping test score from baseline) and therefore was of no predictive value in the patients studied (n=6). The correlation between the beta-phase intercept and response initially observed in the literature was therefore interpreted as being co-incidental.

The characteristic features of apomorphine pharmacokinetics were clearly demonstrated in the seven patients treated with subcutaneous bolus apomorphine; according to the traditional two-stage approach to data analysis, there was a short absorption half-live, i.e. mean (S.D.) of 4.1 (2.1) minutes, short elimination half live, i.e. 69.5 (21.1) minutes, rapid clearance from plasma, i.e. 2.2 (0.5) L/kg/h, and the volume of distribution was 1.9 (0.8) L/kg . Similarly, the typical features of apomorphine-induced anti-parkinsonian effect following subcutaneous bolus were observed, e.g. the short latency to onset of effect, i.e. 12 (8) minutes, and the brief duration of effect, i.e. 72 (25) minutes.

Simultaneous PK-PD modelling was performed, using an effect compartment (exponential pharmacodynamics) model to account for counterclockwise hysteresis in a sub-group of patients (n=2). The equilibration half-life was short, i.e. 8.3 and 16.5 minutes. The limitations of this approach were that neither baseline nor maximal apomorphine-induced anti-parkinsonian response could be predicted.

A direct pharmacodynamic (sigmoid E_{max}) model was applied to series where hysteresis in the plasma concentration-effect plot was not evident (n=2). The absence of counterclockwise hysteresis in these patients was attributed to a (very) rapid equilibrium of apomorphine with the effect site. The drug concentration which produced 50% of maximum effect (EC_{50}) was 7.7 and 20.3 ng/mL. Values for the Hill co-efficient were large,

i.e. 7 and 10, indicating that the relationship between plasma apomorphine concentration and effect was an "all or nothing" (dichotomous) phenomenon.

Whilst the experimental design, i.e. single (bolus) dose of apomorphine (at the patients' routine dose), was appropriate for the investigation of the beta-phase intercept in individual patients, the design was not entirely compatible with the application of the aforementioned pharmacodynamic modelling techniques in that the observed plasma apomorphine concentration range was not sufficiently large enough nor sustained for long enough to efficiently sample the pharmacodynamic response. This was a direct consequence of performing the study under non-steady state conditions (and in the absence of continuous pharmacodynamic monitoring), given that apomorphine has short absorption and elimination half-lives and a brief period of effect.

The research highlighted the difficulties involved with the study of pharmacokinetic-pharmacodynamic relationships of a drug which is associated with high inter- and intra-patient variability in pharmacokinetics, pharmacodynamics and narrow therapeutic window. In particular the importance of appropriate experimental design on the success of modelling concentration-effect relationships was demonstrated, i.e. the disadvantages of the non-steady state approach, the detrimental effect of drug withdrawal (wash-out) on anti-parkinsonian response, and the requirement for a pharmacodynamic tool which is capable of continuously monitoring apomorphine-induced anti-parkinsonian effects.

In order to further elaborate on the relationship between apomorphine pharmacokinetic and pharmacodynamics, a departure from standard two stage modelling techniques to a more direct population modelling approach, i.e. mixed effects modelling, is warranted.

The novel delivery systems under scrutiny were: (i) Britaject® (Britannia Pharmaceuticals Ltd.) apomorphine formulation administered subcutaneously using a needle-free (jet) injector (J-TIP®, National Medical Products Inc.), (ii) an intranasal apomorphine powder formulation delivered using a turbospin insufflator (CDFS), and (iii) an apomorphine hydrogel co-polymer produced as a dosage-form for buccal delivery (Controlled Therapeutics (Scotland) Ltd.).

The pharmacokinetics, pharmacodynamics and tolerability of subcutaneous needle-free delivery of apomorphine (Britaject®, Britannia Pharmaceuticals Ltd) in comparison with the conventional apparatus, i.e. needle and syringe or Penject® (Britannia Pharmaceuticals Ltd) were determined in three patients with Parkinson's disease. It was demonstrated that the pharmacokinetics of needle-free administration using the J-TIP® injector (National Medical Products Inc.) depended on the "efficiency" of dosing.

On two occasions, a local adverse tissue reaction, i.e. bruising and induration, occurred in response to administration of apomorphine by needle-free injection. On these occasions, C_{max} and $AUC_{0\text{-infinity}}$ were reduced (by means of 43% and 31% respectively, n=2) as compared to conventional delivery in those patients. T_{max} varied, being quicker than conventional delivery on one occasion and slower on the other occasion.

Conversely, in the absence of adverse reaction at the injection site, C_{max} was greater than that estimated for conventional delivery in those patients (by a mean of 41%, n=2), T_{max} was shorter (mean of 63%, n=2) and $AUC_{0\text{-infinity}}$ was essentially equivalent, being on average 95% of that observed for conventional delivery.

It was not possible to establish any trends relating to differences in pharmacodynamic response, e.g. onset or magnitude of effect, between the treatments. This was due to (i) a lack of information regarding intra-patient variation in the quality of the apomorphine-induced "on" phase, and (ii) the inter-patient variation in response following the control, i.e.

conventional, administration of apomorphine. It was considered that the differences in the pharmacodynamic response between the two treatments were probably within normal intra-patient variation under the study conditions. In future similar investigations, intra-patient variation must be determined in order to interpret the differences in response between the treatments.

The implication of these findings was that, whilst it was demonstrated that a desirable pharmacokinetic profile could be achieved (in terms of an equivalent AUC, shorter T_{max} and greater C_{max}), the unfavourable outcome in terms of adverse local tissue effects and/or pain of administration, which was not patient-specific, negates the use of the device, despite the obvious advantage of the novel system for those who are adverse to needle injection. It was proposed that the manifestation of local tissue complications was a result of a Britaject®-specific reaction *and* the actual delivery mechanism, i.e. pressurised jet injection. Whilst both factors were implicated, it was not possible in this study to establish the relative contributions of each due to the absence of administration of apomorphine-free solutions as a control in the experimental design.

A comparison of apomorphine pharmacokinetics following conventional subcutaneous administration and either intranasal dosing with a powder formulation (CDFS), or buccal administration using a hydrogel formulation (Controlled Therapeutics Scotland Ltd), was performed in healthy volunteers.

Apomorphine release from the buccal hydrogel and, to a lesser extent, the nasal insufflator was low and variable, indicating the need to optimise the delivery mechanisms of the devices used in order to proceed with the development of these novel systems. When drug release did occur, dose-related plasma concentrations were observed, but the bioavailability was low in comparison with subcutaneous control apomorphine administration, i.e. mean (S.D, number of observations) was 41% (18, n=16) for the intranasal system and 22% (7,

n=6) for the buccal system. This suggests that formulation issues must be addressed in order to achieve therapeutic plasma concentrations of apomorphine.

It is likely that the two delivery systems would achieve high patient acceptability in comparison to the conventional subcutaneous system. Both patient acceptability and therapeutic outcome will depend on the local adverse effects profile resulting from chronic use of the novel apomorphine delivery systems. Development of the intra-nasal and buccal systems has proceeded based on the pharmacokinetic (and tolerability) data generated in these studies, with the intranasal route proving the more promising of the two.

A recurring issue in the (chronic) administration of apomorphine (Britaject®, Britannia Pharmaceuticals Ltd) is the occurrence of local adverse reactions, i.e. inflammatory events, at the site of administration, be that the subdermis and epidermis, nasal mucosa, or sublingual mucosa. The cause of the adverse reaction, in terms of the contribution of apomorphine and/or the Britaject® excipient, remains to be established. It is suggested in the literature that diluting the apomorphine solution with normal saline prior to subcutaneous administration reduces the risk of nodule formation, however, in the absence of apomorphine stability data and storage guidelines in such solutions, there may be some difficulties in supplying patients with such. In this respect, a study on the stability of apomorphine in saline, with a combined assessment of the effect of on cutaneous nodule formation is warranted.

SECTION 8:
APPENDICES

8. Appendices.

8.1. Unified Parkinson's Disease Rating Scale (UPDRS).

Part I: MENTATION, BEHAVIOUR AND MOOD

Rate items 1-4 by interview.

1. Intellectual impairment: ☐

- **0** = None
- **1** = Mild: consistent forgetfulness with partial recollection of events and no other difficulties
- **2** = Moderate memory loss, with disorientation and moderate difficulty handling complex problems; mild but definite impairment of function at home, with need of occasional prompting
- **3** = Severe memory loss with disorientation for time and often for place, severe impairment in handling problems
- **4** = Severe memory loss with orientation preserved to person only; unable to make judgements or solve problems; requires much help with personal care; cannot be left alone at all

2. Thought disorder (due to dementia or drug intoxication): ☐

- **0** = None
- **1** = Vivid dreaming
- **2** = 'Benign' hallucinations with insight retained
- **3** = Occasional to frequent hallucinations or delusions without insight; could interfere with daily activities
- **4** = Persistent hallucinations, delusions, or florid psychosis; not able to care for self

3. Depression: ☐

- **0** = Not present
- **1** = Periods of sadness or guilt greater than normal but never sustained for days or weeks
- **2** = Sustained depression (1 week or more)
- **3** = Sustained depression with vegetative symptoms (insomnia, anorexia, weight loss, loss of interest)
- **4** = Sustained depression with vegetative symptoms and suicidal thoughts or intent

4. Motivation / initiative: ☐

- **0** = Normal
- **1** = Less assertive than usual; more passive
- **2** = Loss of initiative or interest in elective (non-routine) activities
- **3** = Loss of initiative or interest in day-to-day (routine) activities
- **4** = Withdrawn, complete loss of motivation

Part II: ACTIVITIES OF DAILY LIVING (in the past week)

Rate items 5-17 by interview.

5. Speech:

0 = Normal
1 = Mildly affected, no difficulty in being understood
2 = Moderately affected, sometimes asked to repeat statements
3 = Severely affected, frequently asked to repeat statements
4 = Unintelligible most of the time

6. Salivation:

0 = Normal
1 = Slight but definite excess of saliva in the mouth; may have night-time drooling
2 = Moderately excessive saliva; may have minimal drooling
3 = Marked excess of saliva; some drooling
4 = Marked drooling; requires constant use of tissue or handkerchief

7. Swallowing:

0 = Normal
1 = Rare choking
2 = Occasional choking
3 = Requires soft food
4 = Requires nasogastric tube or gastrostomy feeding

8. Handwriting:

0 = Normal
1 = Slightly slow or small
2 = Moderately slow or small; all words are legible
3 = Severely affected; not all words are legible
4 = The majority of words are not legible

9. Cutting food and handling utensils:

0 = Normal
1 = Somewhat slow and clumsy, but no help needed
2 = Can cut most foods, although clumsy and slow; some help needed
3 = Food must be cut by someone but can still feed slowly
4 = Needs to be fed

10. Dressing:

0 = Normal
1 = Somewhat slow, but no help needed
2 = Occasional assistance needed with buttoning, getting arms into sleeves
3 = Considerable help required, but can do some things alone
4 = Helpless

11. Hygiene:

- **0** = Normal
- **1** = Somewhat slow, but no help needed
- **2** = Needs help to shower or bathe; very slow in hygiene care
- **3** = Requires assistance for washing, brushing teeth, combing hair, going to bathroom (toilet)
- **4** = Needs Foley (bladder) catheter or other aids

12. Turning in bed and adjusting bedclothes:

- **0** = Normal
- **1** = Somewhat slow and clumsy, but no help needed
- **2** = Can turn alone or adjust sheets, but with great difficulty
- **3** = Can initiate attempt but cannot turn or adjust sheets alone
- **4** = Helpless

13. Falling (unrelated to freezing):

- **0** = None
- **1** = Rare falling
- **2** = Occasional falls, less than once daily
- **3** = Falls an average of once daily
- **4** = Falls more than once daily

14. Freezing when walking:

- **0** = None
- **1** = Rare freezing when walking; may have start hesitation
- **2** = Occasional freezing when walking
- **3** = Frequent freezing; occasionally falls because of freezing
- **4** = Frequently falls because of freezing

15. Walking:

- **0** = Normal
- **1** = Mild difficulty; may not swing arms or may tend to drag leg
- **2** = Moderate difficulty, but requires little or no assistance
- **3** = Severe disturbance of walking; requires assistance
- **4** = Cannot walk at all, even with assistance

16. Tremor:

- **0** = Absent
- **1** = Slight and infrequently present
- **2** = Moderate; bothersome to patient
- **3** = Severe; interferes with many activities
- **4** = Marked; interferes with most activities

17. Sensory complaints related to parkinsonism:

0 = None
1 = Occasionally has numbness
2 = Frequently has numbness, tingling, or aching; not distressing
3 = Frequent painful sensations
4 = Excruciating pain

Part III: MOTOR EXAMINATION

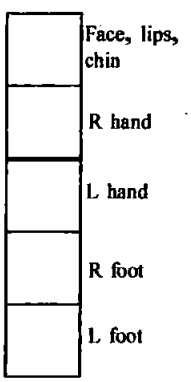
18. Speech:

0 = Normal
1 = Slight loss of expression, diction and/or volume
2 = Monotone, slurred but understandable; moderately impaired
3 = Marked impairment, difficult to understand
4 = Unintelligible

19. Facial expression:

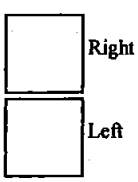
0 = Normal
1 = Minimal hypomimia; could be normal 'poker face'
2 = Slight but definitely abnormal diminution of facial expression
3 = Moderate hypomimia; lips parted some of the time
4 = Masked or fixed facies, with severe or complete loss of facial expression; lips parted ¼ inch (6 mm) or more

20. Tremor at rest:



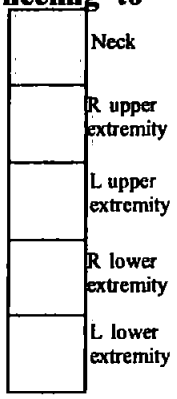
0 = Absent
1 = Slight, and infrequently present
2 = Mild in amplitude and persistent, or moderate in amplitude but only intermittently present
3 = Moderate in amplitude and present most of the time
4 = Marked in amplitude and present most of the time

21. Action or postural tremor of hands:



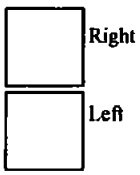
0 = Absent
1 = Slight; present with action
2 = Moderate in amplitude; present with action
3 = Moderate in amplitude; present with posture-holding as well as with action
4 = Marked in amplitude; interferes with feeding

22. Rigidity:
Judged on passive movement with patient relaxed in sitting position; 'cogwheeling' to be ignored.



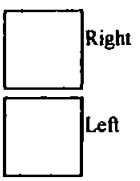
0 = Absent
1 = Slight or detectable only when activated by mirror or other movements
2 = Mild to moderate
3 = Marked, but full range of motion easily achieved
4 = Severe, range of motion achieved with difficulty

23. Finger taps:
Patient taps thumb with index finger in rapid succession with widest amplitude possible,
each hand separately.



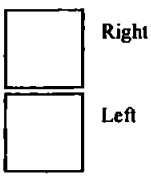
0 = Normal
1 = Mild slowing and/or reduction in amplitude
2 = Moderately impaired; definite and early fatiguing; may have occasional arrests in movement
3 = Severely impaired; frequent hesitation in initiating movements or arrests in ongoing movement
4 = Can barely perform task

24. Hand movements:
Patient opens and closes hands in rapid succession with widest amplitude possible, each hand separately.



0 = Normal
1 = Mild slowing and/or reduction in movement
2 = Moderately impaired; definite and early fatiguing; may have occasional arrests in movement
3 = Severely impaired; frequent hesitation in initiating movements or arrests in ongoing movement
4 = Can barely perform task

25. Rapid alternating movements of the hand:
Pronation-supination movements of hands, vertically or horizontally, with as large an amplitude as possible, both hands simultaneously.



0 = Normal
1 = Mild slowing and/or reduction in movement
2 = Moderately impaired; definite and early fatiguing; may have occasional arrests in movement
3 = Severely impaired; frequent hesitation in initiating movements or arrests in ongoing movement
4 = Can barely perform task

26. Leg agility:
Patient taps heel on ground in rapid succession, picking up entire leg; amplitude should be about 3 inches (75 mm).

Right ☐
Left ☐

0 = Normal
1 = Mild slowing and/or reduction in movement
2 = Moderately impaired; definite and early fatiguing; may have occasional arrests in movement
3 = Severely impaired; frequent hesitation in initiating movements or arrests in ongoing movement
4 = Can barely perform task

27. Arising from chair:
Patient attempts to arise from a straight-backed wood or metal chair, with arms folded across chest.

☐

0 = Normal
1 = Slow, or may need more than one attempt
2 = Pushes self up from arms of seat
3 = Tends to fall back and may have to try several times but can get up without help
4 = Unable to arise without help

28. Posture:

☐

0 = Normal erect
1 = Not quite erect, slightly stooped posture; could be normal for older person
2 = Moderately stooped posture, definitely abnormal; can be slightly leaning to one side
3 = Severely stooped posture with kyphosis; can be moderately leaning to one side
4 = Marked flexion, with extreme abnormality of posture

29. Gait:

☐

0 = Normal
1 = Walks slowly, may shuffle with short steps but no festination or propulsion
2 = Walks with difficulty, but requires little or no assistance; may have some festination, short steps, or propulsion
3 = Severe disturbance of gait; requires assistance
4 = Cannot walk at all even with assistance

30. Postural stability:
Response to sudden posterior displacement produced by a pull on shoulders while patient is erect, with eyes open and feet slightly apart; patient *is* prepared.

☐

0 = Normal
1 = Retropulsion but recovers unaided
2 = Absence of postural response, would fall if not caught by examiner
3 = Very unstable, tends to lose balance spontaneously
4 = Unable to stand without assistance

31. Body bradykinesia and hypokinesia:
Combining slowness, hesitancy, decreased arm swing, small amplitude and poverty of movement in general.

☐

- **0** = None
- **1** = Minimal slowness giving movement a deliberate character, could be normal for some persons; possibly reduced amplitude
- **2** = Mild degree of slowness and poverty of movement that is definitely abnormal; alternatively some reduced amplitude
- **3** = Moderate slowness; poverty or small amplitude of movement
- **4** = Marked slowness; poverty or small amplitude of movement

Part IV: COMPLICATIONS OF THERAPY (in the past week)

A. Dyskinesias

32. Duration:
What proportion of the waking day are dyskinesias present? (Historical information)

☐

- **0** = None
- **1** = 1-25% of day
- **2** = 26-50% of day
- **3** = 51-75% of day
- **4** = 76-100% of day

33. Disability:
How disabling are the dyskinesias? (Historical information, may be modified by office examination)

☐

- **0** = Not disabling
- **1** = Mildly disabling
- **2** = Moderately disabling
- **3** = Severely disabling
- **4** = Completely disabling

34. Painful dyskinesias:
How painful are the dyskinesias?

☐

- **0** = No painful dyskinesia
- **1** = Slightly
- **2** = Moderately
- **3** = Severely
- **4** = Markedly

35. Presence of early morning dystonia. (Historical information):

☐

- **0** = No
- **1** = Yes

B. Clinical Fluctuations

36. Are there any 'off' periods predictable as to timing after a dose of medication?

0 = No
1 = Yes

37. Are there any 'off' periods unpredictable as to timing after a dose of medication?

0 = No
1 = Yes

38. Do any 'off' periods come on suddenly (i.e. within a few seconds)?

0 = No
1 = Yes

39. What proportion of the waking day is the patient 'off' on average?

0 = None
1 = 1-25% of day
2 = 26-50% of day
3 = 51-75% of day
4 = 76-100% of day

C. Other Complications

40. Does the patient have anorexia, nausea, or vomiting?

0 = No
1 = Yes

41. Does the patient have any sleep disturbances (for example, insomnia or hypersomnolence)?

0 = No
1 = Yes

42. Does the patient have symptomatic orthostasis?

0 = No
1 = Yes

Part V: MODIFIED HOEHN AND YAHR STAGING

Stage 0 = No signs of disease

Stage 1 = Unilateral disease

Stage 1.5 = Unilateral plus axial involvement

Stage 2 = Bilateral disease without impairment of balance

Stage 2.5 = Mild bilateral disease with recovery on pull test

Stage 3 = Mild to moderate bilateral disease; some postural instability; physically independent

Stage 4 = Severe disability; still able to walk or stand unassisted

Stage 5 = Wheelchair-bound or bedridden unless aided

Part VI: SCHWAB AND ENGLAND ACTIVITIES OF DAILY LIVING SCALE

Ask patient and relative to score patient's ability over the preceding week to the nearest 5 per cent using the following guidelines.

100% = Completely independent; able to do all chores without slowness, difficulty, or impairment; essentially normal; unaware of any difficulty

90% = Completely independent; able to do all chores with some degree of slowness, difficulty, or impairment; may take twice as long as usual; beginning to be aware of difficulty

80% = Completely independent in most chores; takes twice as long as normal; conscious of difficulty and slowness

70% = Not completely independent; more difficulty with some chores; takes three to four times as long as normal in some; must spend a large part of the day with some chores

60% = Some dependency; can do most chores, but exceedingly slowly and with considerable effort and errors; some chores impossible

50% = More dependent; needs help with half the chores, slower, etc.; difficulty with everything

40% = Very dependent; can assist with all chores but does few alone

30% = With effort, now and then does a few chores alone or begins alone; much help needed

20% = Does nothing alone; can be a slight help with some chores; severe invalid

10% = Totally dependent and helpless; complete invalid

0% = Vegetative functions such as swallowing, bladder and bowels are not functioning; bedridden

Source: Wade [1].

8.2. Current Threshold Dose-Finding Protocol.

Provoking and Assessing an "Off" State: After at least three days of hospitalisation all anti-parkinsonian therapy is withheld overnight to provoke an "off" state in motor performance and to undertake a baseline motor assessment as follows:

a) Alternate, unilateral hand tapping for 30 seconds on mounted digital counters (preferably 20cm apart)[2]
b) Time taken to walk 12 metres.
c) Clinical assessment of tremor and dyskinesia according to a four point scale (0=nil, 1=mild, 2=moderate, 3=severe).
d) Scoring on a modified Webster disability scale to assess 12 features of parkinsonism (maximum disability score of 36)[3]

Determination of the Threshold Dose: Following baseline motor assessment the patient is challenged for apomorphine responsiveness according to the following schedule;

- 1.5mg apomorphine HCl is injected subcutaneously and the patient is observed over 30 minutes for motor responsiveness.
- If no or poor response is obtained, a second dose of 3mg apomorphine HCl is given 40 minutes after the first dose, and the patient observed for a further 30 minutes.
- The dosage is increased in an incremental fashion every 40 minutes and the patient observed carefully for an unequivocal motor response. The third dose is 5mg subcutaneously, and the forth dose is 7mg subcutaneously. If the patient shows no response to the 7mg dose then the patient must be classified as a non-responder to apomorphine HCl and no further attempts to provoke a motor response should be made. If the patient shows only a mild response to the 7mg dose, a maximum dose of 10mg can be used to see if an unequivocal motor response is possible.
- The lowest dose producing an unequivocal motor response is called the "threshold dose". For the majority of patients the threshold dose is less than 7mg apomorphine HCl, although very occasionally it can be made up to 10mg apomorphine HCl.

Motor response is judged to be positive if 2 or more of the following are seen:

a) More than 15% increase in tapping score.
b) More than 25% improvement in walking time.
c) An improvement of at least 2 points of tremor score.
d) An improvement of Webster's score of 3 or more.

Initiation of Treatment: Following establishment of an acceptable threshold dose of apomorphine HCl injection, the patient should be restarted on conventional anti-parkinsonian therapy. A subcutaneous injection of the established threshold dose may then be given into the lower abdomen or outer thigh at the first signs of an "off" episode. The patient should then be observed over the following hour and the quality of the "on" period noted. It may be appropriate to modify the dose of apomorphine HCl according to the patient's response.

Close monitoring of therapeutic benefits and side effects under specialist supervision is required after initiation of treatment. The daily dose can vary between patients and will typically be in the range of 3mg up to 30mg per day in divided doses. The frequency of injection will also vary between patients and may be between 1 to 10 per day but in rare cases may be up to 12 times per day.

Patients who have shown a good "on" period response during the initiation stage, but whose overall control remains unsatisfactory using intermittent injections, or who require many and frequent injections (more than 10 per day), may be commenced on or transferred to continuous subcutaneous infusion by minipump as follows;
Continuous infusion is started at a rate of 1mg apomorphine HCl per hour then increased according to the individual response. Increases in the infusion rate should not exceed 0.5mg per hour at intervals of not less than 4 hours. Hourly infusion rates may change between 1mg and 4mg, equivalent to 0.015-0.06 mg/kg/hour.

Source: Britaject® Data Sheet[4]

8.3. UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria

STEP 1 Diagnosis of parkinsonian syndrome

- Bradykinesia and at least one of the following:
  - muscular rigidity
  - 4-6Hz rest tremor
  - postural instability not caused by primary visual, vestibular, cerebellar or proprioceptive dysfunction

STEP 2 Exclusion criteria for Parkinson's disease

- History of repeated strokes with stepwise progression of parkinsonian features
- History of repeated head injury
- History of definite encephalitis
- Oculogyric crises
- Neuroleptic treatment at onset of symptoms
- More than one affected relative
- Sustained remission
- Strictly unilateral features after three years
- Supranuclear gaze palsy
- Cerebellar signs
- Early severe autonomic involvement
- Early severe dementia with disturbances of memory, language and praxis
- Babinski sign
- Presence of cerebral tumour or communicating hydrocephalus on CT scan
- Negative response to large doses of levodopa (if malabsorption excluded)
- MPTP exposure

STEP 3 Supporting prospective positive criteria for Parkinson's disease.
Three or more required for diagnosis of definite Parkinson's disease.

- Unilateral onset
- Rest tremor present
- Progressive disorder
- Persistent asymmetry affecting the side of onset most
- Excellent response (70-100%) to levodopa
- Severe levodopa-induced chorea
- Levodopa response for 5 years or more
- Clinical course for 10 years or more

Source: Gibb and Lees[5]

8.4. Patient Demographics.

Patient ID	Gender	Age (years)	Body weight (kg)	Height (m)	Body Mass Index (kg/m^2)
01	female	51	48.2	1.5	20.5
02	male	74	105.4	1.8	33.2
03	male	55	69.9	1.8	21.6
04	male	73	60.1	1.7	19.8
05	female	73	65.1	1.6	26.5
06			NA		
07	male	49	85.8	1.8	26.5
08	female	76	72.5	1.6	28.5
09	female	55	57.1	1.6	21.5
10	male	70	74.3	1.7	24.7
11	male	69	65.3	1.7	
12	male	57	90.4	1.8	28.47

Table 8-1 Patient demographics. Abbreviation: NA = not applicable.

Patient ID	Disease duration (years)	Duration of L-dopa therapy (years)	Duration of apomorphine therapy (years)	Current apomorphine regimen	UPDRS total score ("on")	Hoehn and Yahr score ("on")
01	17	17	5	bolus: 2mg x5 / week	35	NR
02	11	11	1	bolus: 5mg x5 / day	NR	4
03	11	9	5	(infusion: 50mg/24h)	68	4
04	13	12	1	infusion: 30mg/15h	71	4
05	14	12	2	bolus: 5mg x2 / day	74	4
07	13	12	<1	infusion: 180mg/24h	36	4
08	10	9	<1	infusion: 45mg/24h	40	3
09	28	20	3	bolus: 2mg x 5 / day	36	2
10	10	10	3	bolus: intermittent	42	2
12	16	5	9	not currently taking	48	2

Table 8-2 Parkinson's disease status of study population. Abbreviation: NR = not recorded

8.5. Effect of Dantron-Based Laxatives on Apomorphine Assay.

Introduction.

Quantification of apomorphine in plasma sampled from patient 11, using the analytical method given in Section 4.4 (page 4-60), was precluded by the presence of two components in the plasma extract which co-eluted with apomorphine (Figure 8-1A and B).

Case History: Patient 11.

A 69 year old male with Parkinson's disease had onset of right arm tremor 22 years previously and 15 years previously he underwent left thalamotomy complicated by hemorrhagic stroke with residual dysarthria, right hemiparesis and right hemidystonia. His parkinsonian symptoms responded to levodopa/carbidopa but were complicated by motor fluctuations. He did not tolerate benzhexol or pergolide and was commenced on subcutaneous apomorphine injections 4 years previously. Continuous infusion of apomorphine was commenced 1 year later, but significant abdominal nodules and erratic control lead to its discontinuation after 1 year and ropinirole was commenced. He continued to obtain significant benefit from intermittent subcutaneous injections of apomorphine however. His condition was complicated by long-standing constipation for which he had been taking regular co-danthramer and co-danthrusate for more than 5 years.

Experimental 1.

Concomitant drugs administered to patient 11 (listed on the study day as current medication) were investigated as potential sources of the interferences. Of these, levodopa/carbidopa, ropinirole and domperidone had previously been investigated during assay validation and found not to compromise apomorphine determination using the described HPLC assay (see Section 4.5.1.4, page 4-78). Hence solutions of the remaining drugs in the current drug regimen were prepared in water and assayed according to the method described. The drug solutions were: amitriptyline tablet (0.3% w/v), co-danthramer (0.01% w/v), co-danthrusate (0.6% w/v), diazepam emulsion I.V. injection (0.1% w/v), vitamin and mineral supplement (Ketovite® tablet, substituted for Forceval® which was not available locally, 1 tablet dissolved in 8mL water), senna tablet (substituted for Manevac®, which was not available locally, 0.1% w/v).

Results 1.

On the basis of chromatographic retention time alone, co-danthrusate and co-danthramer emerged as potential sources of the interference (Figure 8-1C and D).

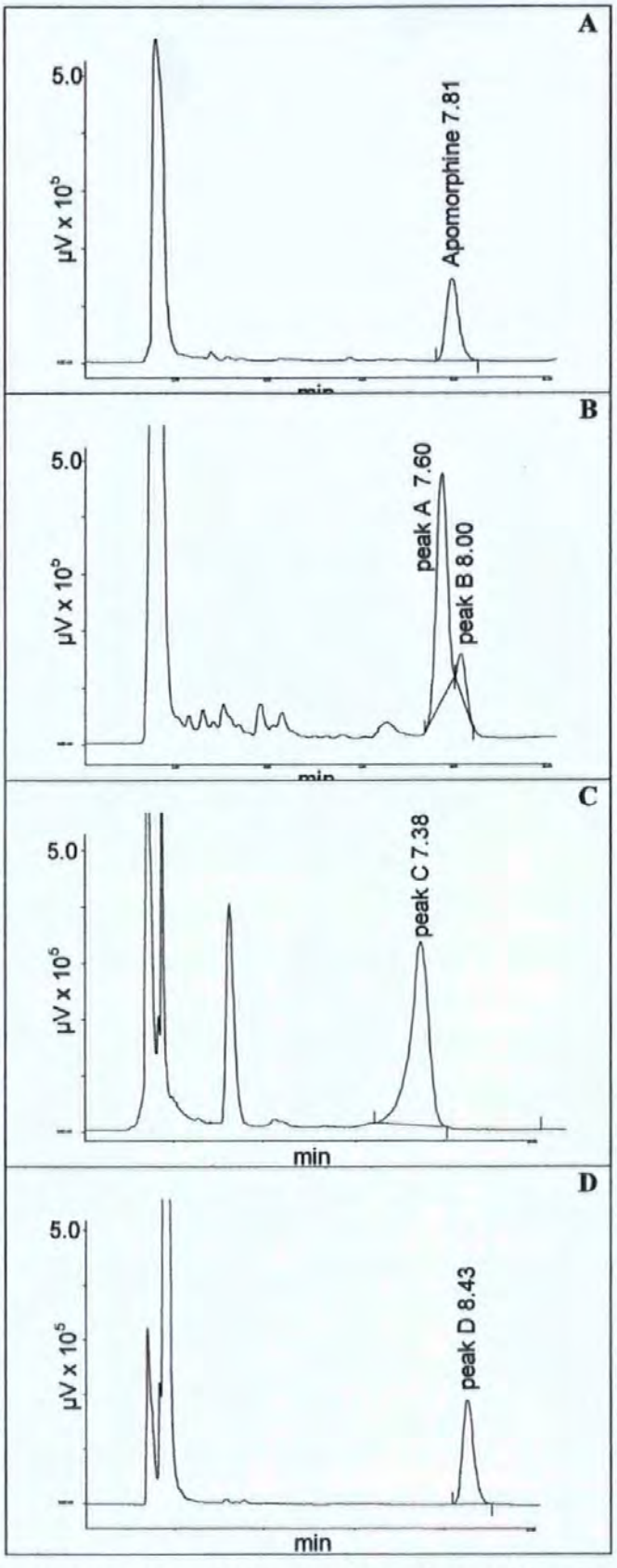


Figure 8-1 Determination of co-eluting analytes observed in plasma of patient 11.

Panel A: Apomorphine (20ng/mL) extracted from pooled control plasma.
Panel B: Baseline (pre-apomorphine) plasma extract from patient 11.
Panel C: Co-danthrusate powder (0.6% w/v) in water.
Panel D: Co-danthramer suspension (0.01% w/v) in water.

Experimental 2.

A spectral library search was performed whereby the peak spectra of components A and B were compared to reference peak spectra contained in a user-defined library (see Appendix 8.12). The spectral library contained apomorphine and NPA standards; products of the forced degradation of apomorphine and NPA in water under acidic, alkaline and oxidative conditions, and on heating to 60°C (see Section 4.5.1.3, page 4-69); and co-danthrusate and co-danthramer standards. For each comparison a spectral similarity index (SSI) was obtained, where SSI = 1 represented a complete match.

Results 2.

Comparison of peak spectra of components A and B with reference drug spectra for co-danthrusate and co-danthramer resulted in high (>0.98) SSI (Table 8-3).

Interference in baseline plasma extract	**Reference spectrum**		**SSI**
	Peak	Origin	
Peak A	C	co-danthrusate powder	0.991
	D	co-danthramer suspension	0.983
Peak B	D	co-danthramer suspension	0.993
	C	co-danthrusate powder	0.990

Table 8-3 Outcome following peak spectra library search routine.

Abbreviation: SSI = spectral similarity index.

Discussion.

On the basis of spectral analysis it was concluded that peaks A and B were derived from co-danthrusate and co-danthramer, medication which had been administered (as indicated) up to and including the study day.

Co-danthramer and co-danthrusate are stimulant laxatives based on dantron (i.e. 1,8-dihydroxyanthraquinone, see Figure 8-2). Dantron is formulated with a surfactant laxative, i.e. docusate sodium and poloxamer "188" in co-danthrusate and co-danthramer, respectively.

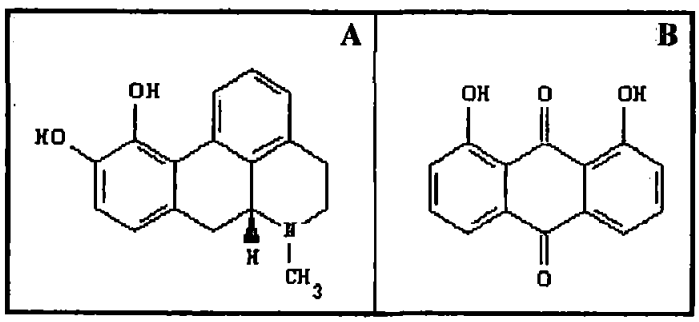


Figure 8-2 Chemical structures of apomorphine (Panel A) and dantron (Panel B).

Whilst the definitive identification of peaks A and B has not been attempted in this investigation, peak retention times suggest that peak A is due to co-danthrusate and peak B is due to co-danthramer. The difference in retention times observed for the two dantron preparations might be attributable to the different surfactant laxatives in the formulations.

Co-administration of co-danthramer and co-danthrusate prevented the quantification of apomorphine by the HPLC method described. This has implications for the determination of plasma apomorphine concentration, using the described assay (or similar), in subjects whose drug regimen includes these, or other, anthraquinone-based preparations, especially given that such laxatives are commonly prescribed for (elderly) Parkinson's disease patients[6].

Preliminary work which aimed to remove the interferences from the plasma extract was performed. It was demonstrated that increasing the 50% (v/v) methanol:water washes from 1 x 1mL to 3 x 1mL had the effect of removing the interfering compounds from the solid phase extraction column (to waste), without detriment to the isolation and subsequent elution of apomorphine. It was therefore recommended that , for future work, the HPLC assay be validated incorporating the additional 50% (v/v) methanol:water washes.

Acknowledgement.
Dr. J. O'Sullivan provided the case history.

8.6. Tapping Test Instructions to Patient.

Investigator: Your task is to tap as fast as you can between the two counters using your left/right[a] hand only (give brief demonstration). You should start with your tapping hand on the table in line with the middle of the tapping tester. I will ask if you are ready to begin. When you are, I will say "Start", and you will begin tapping. You should keep tapping until you hear the beep, which signals the end of the 30s test period.

8.7. Walking Test Instructions to Patient.

Investigator: Your task is to walk as quickly as you can up to the mark, turn, and walk back to the start point. I will ask if you are ready to begin. When you are, I will say "Start", and you will begin walking.

[a] The side most affected with parkinsonian symptoms.

8.8. Patient Information Leaflet (for Subcutaneous Bolus Study).

University of Plymouth, Derriford Hospital

We are a research group who are interested in the use of Britaject apomorphine in Parkinson's disease. We would like to find out more about the way the level of apomorphine in the blood changes as you switch "on" and "off".

We hope that this information will help us to tailor apomorphine therapy to individual patients. If we could do this, it would mean that each individual would be getting the most from their apomorphine injections or infusion.

As one of Dr. ..... patients who is being treated with apomorphine, we would like to ask if you would be willing to participate in one of our studies

What will happen on the study day?

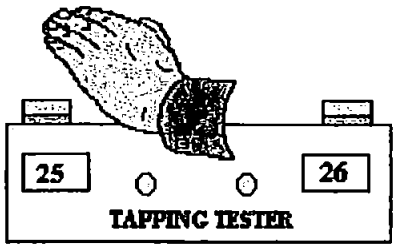
You will need to come to..... Hospital. We will ask you **not** to take your early morning anti-parkinsonian medication so that you will be "off" at the start of the study day.

Between 8am and 10am you will receive a **single** apomorphine injection at your normal dose so that you will switch "on". This will be the **only** apomorphine you will receive for the following **six hours**. We ask this because we want to look at the effects of a single apomorphine dose. All the other medication that you usually take will be available to you.

We want to take blood samples over the six-hour period that follows the apomorphine injection. We will use a sampling needle known as a "venflon" to do this. Once the venflon is inserted into a vein in the arm, blood can be taken without pricking the skin. Normally five samples will be taken in the first hour and another five will be taken over the following five hours.

As well as taking blood samples, we will ask you questions about your symptoms. We will make notes on your answers and also on things like which medication you take during the study day and the times that you have something to eat.

We will also ask you to complete some straightforward tests i.e. a tapping test (on seven occasions during the study) and a walking test (three times during the study).



With your consent, you will be videoed as you take some of these tests. The purpose of the video is to make sure that we have recorded all your symptoms correctly. The video will not be shown to anybody other than the members of the research team.

Your blood pressure and pulse will be checked on occasions during the study.

The study finishes when the last blood sample is collected (six hours after the apomorphine injection).

General Information.

- During the day you can eat, drink, rest or move around as you wish.
- We are happy to pay your transport costs to and from the hospital on the study day.

If you agree to take part we will let your GP know.

Do you have to take part?
No, taking part is voluntary.

You may decline to participate in this study without giving your reasons or incurring displeasure. If you agree to participate you will need to sign a consent form.

You are free to withdraw from the study at any stage without affecting your subsequent care or treatment in any way. You do not have to give a reason for your withdrawal from the study.

Britannia Pharmaceuticals does not believe that you will suffer injury by participating in this study. You should know however that, in the event that you *do* suffer injury as a result of any procedure carried out in accordance with the study protocol, Britannia Pharmaceuticals will compensate you without you having to prove that they are at fault.

Britannia Pharmaceuticals will not compensate you where injury results from any procedure carried out not in accordance with the protocol for the study.

Your right at law to claim compensation for injury where you can prove negligence is not affected.

If you have **any** questions, please do not hesitate to contact:

.....

at.....

on .....

8.9. Patient Consent Form.

Please ask the patient to complete the following:-

	Please cross out as necessary
Have you read the Patient Information Sheet?	Yes / No
Have you had the chance to ask questions and to discuss the study?	Yes / No
Have you received satisfactory answers to your questions?	Yes / No
Have you received enough information about this study?	Yes / No

Who has spoken to you about what is involved in this study? .....

Do you understand that you are free to withdraw from the study:
at any time?
without having to give a reason for withdrawing?
and without affecting your future medical care? Yes / No

Do you agree to take part in this study? Yes / No

Do you consent to be videoed during the study? Yes / No

Signed: ..... Date: .....

Name (in block letters) : .....

Signed (investigator) : ..... Date: .....

8.10. Example of Variance Inflation Factor Analysis.

Output generated for patient 09.

Parameter	Estimate	$VIF_{original}$	$VIF_{restructured}$
A (ng/mL)	24	373022	2899.17
Alpha (min)	0.03	0.0042	0.0026
B (ng/mL)	3.5	1147	203.2
Beta (min)	0.009	0.000187	0.000161
K01 (min^{-1})	0.24	379	3.21
Tlag (min)	6	606477	844.961

8.11. The Development of Apomorphine Extraction Methods.

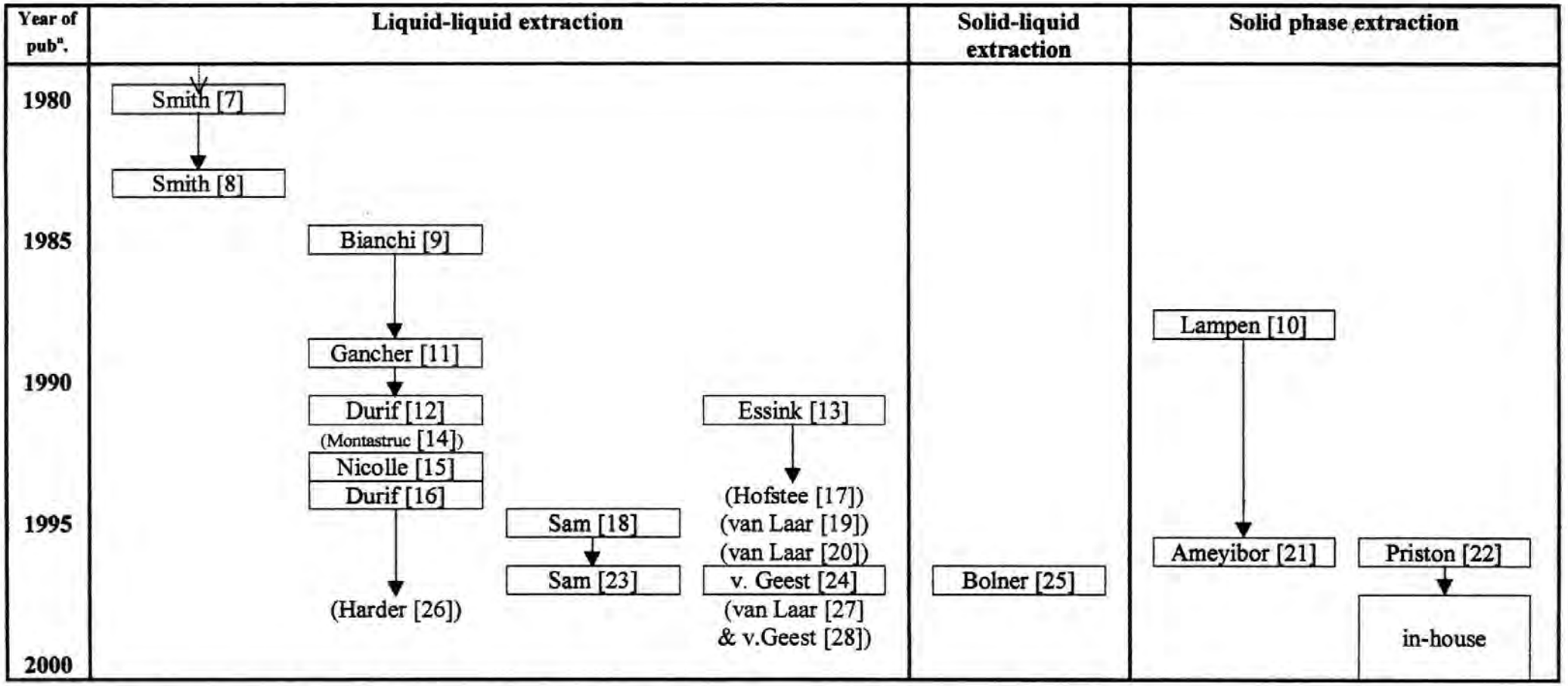


Figure 8-3 Summary of the development, modification (and use) of assay methods for apomorphine. The extraction methods given in the highlighted articles were evaluated (refer to Section 4.3.5, page 4-52).

8.12. User-Defined Spectral Library.

Library Definition Report Page 1 of 2

User : System
Printed : 8/17/01 11:51:38 AM

Spectral Library: E:\ChromQuest\public\DATA\Wendy's Data\Apomorphine.lib
Library Notes: 0.25M NaH2PO4, 0.25% HSA, 0.003% EDTA, 40% MeOH, pH3.3 C Columbus col #21C1

Component Name	Lambda Max From (nm)	Lambda Max To (nm)	Lambda Max (nm)	Time (min.)	Comment
Apomorphine in EA	192	798	209	7.67	
Apomorphine in EA	192	798	209	7.67	
Apomorphine in H2O	192	798	209	7.13	
NPA in EA	192	798	208	10.90	
Apomorphine in formalin	192	798	209	7.10	
Apo Oxidative Degradation Product 6.4min	192	798	221	6.38	
Apo Oxidative Degradation Product 8.9min	192	798	246	8.90	
Apo Alkaline Degradation Product 6.88min	192	798	201	6.88	
Apo Acid Degradation Product 2.60min	192	798	208	2.60	
Apo Acid Degradation Product 3.39min	192	798	205	3.37	
Apo Alkali Degradation Product 2.52min	192	798	210	2.52	
Apo Alkali Degradation Product 3.38min	192	798	205	3.38	
Apo Alkali Degradation Product 4.25min	192	798	211	4.25	
Apo Acid Degradation Product 4.27min	192	798	206	4.27	
thrombus in H2O neat 12.3min.spc	192	798	205	12.30	
Black-green Apo 4.93mins	192	798	203	4.93	
Black-green apo 3.10min	192	798	211	3.10	
Black-green apo 11.27min	192	798	219	11.27	
Black-green apo 2.72min	192	798	211	2.73	
Black-green apo 3.70min	192	798	211	3.70	
Quinone in EA 11.75min	192	798	219	11.75	
Quinone in mobile phase 8.3m	192	798	229	8.28	
Quinone in EA 8.2min	192	798	229	8.20	
Quinone in EA 6.2min	192	798	246	6.20	
Quinone in EA 9.3min	192	798	222	9.30	
NPA in EA 14min	192	798	209	14.10	
NPA extracted from plasma	192	798	206	12.62	
Apo extracted from plasma	192	798	205	8.07	
Apo extracted from plasma	192	798	205	8.07	
Senna in H2O 1	192	798	209	7.70	
Senna in H2O 2	192	798	208	8.18	

Library Definition Report Page 2 of 2

User : System
Printed : 8/17/01 11:51:38 AM

Senna in H2O 3	192	798	207	11.70	
Senna in H2O 4	192	798	222	15.50	
Dantron powder in H2O 1	192	798	228	8.15	
Dantron powder in H2O 2	192	798	256	8.95	
Dantron suspension in H2O 1	192	798	256	9.20	
Dantron suspension in H2O 2.	192	798	228	8.33	
Apo in EA SPE	192	798	209	7.40	
	0	0	0	0.00	
	0	0	0	0.00	

8.13. Pharmacodynamic Response Following (Conventional) Subcutaneous Apomorphine Administration to Patients: Raw Data.

Patient ID	Mean baseline score (n=2) (no. taps/30s)	Maximum score (no. taps/30s)	Time of maximum score (mins post-dose)	Change in score: baseline to peak "on" score[a] (% improvement)
1	44 (n=1)	49	17 and 57	11.4
2	47 (n=1)	52	49	10.6
4	52	63	47	21.2
5	36	45	42	25.0
9	54	81	51	50.0
10	54	65	39	21.5
12	96	120	32	26.0

Table 8-4 **Pharmacodynamic effect of apomorphine: assessed using the tapping test.**

[a] Calculated as ((peak "on" score – baseline score) / baseline score) x 100.

Patient ID	Part III baseline score (off")	Total score when "on"		Part III score when "on"		Time of "on" phase rating	Change in pIII score: "off" to "on"[a]
		following apomorphine administration	Obtained prior to study day	following apomorphine administration	Obtained prior to study day	(mins post-dose)	(% improvement)
1	57	NR	35	NR	4	NA	NA
2	NR	NR	NR	NR	NR	NA	NA
4	58	NR	71	NR	39	NA	NA
5	69	NR	74	NR	29	NA	NA
9	52	36	NR	19	NR	41	63
10	39	42	NR	28	NR	43	28
12	42	48[b]	NR	23	NR	33	45

Table 8-5 **UPDRS assessment: Total scores of zero (representing normal mobility) to 206 (representing maximum motor disability) are possible. Pharmacodynamic effect of apomorphine (assessed using the UPDRS part III motor evaluation): Scores of zero (representing normal mobility) to 108 (representing maximum motor disability) are possible.**

[a] Calculated as (("off" score – "on" score) / 108) x 100.
[b] Assessed following needle-free apomorphine dose.

8.14. Pharmacodynamic Response Following (Conventional and Needle-free) Subcutaneous Apomorphine Administration to Patients: Raw Data.

Patient ID No.	Treatment	Baseline ("off") score	"On" phase score	Time of "on" phase rating (mins post-dose)	Change in score: "off" to "on"[a] (% improvement)
9	CON	52	19	41	31
	NF	40	9	26	29
10	CON	39	28	43	10
	NF 1	43	28	33	14
	NF 2	42	20	36	20
12	CON	42	23	33	18
	NF	33	23	38	9

Table 8-6 **Pharmacodynamic effect of apomorphine: assessed using the UPDRS part III (motor evaluation). Scores of zero (representing normal mobility) to 108 (representing maximum motor disability) are possible.**

Abbreviations: CON = conventional, NF = needle-free.

[a] Calculated as (("off" score – "on" score) / 108) x 100.

Patient ID No.	Treatment	Hand used for tapping test	Mean baseline score (n=2) (no. taps/30s)	Peak "on" score (no. taps/30s)	Time of peak "on" score (mins post-dose)	Change in score: baseline to peak "on" score[a] (% improvement)
9	CON	Right	54	81	51	50
	NF		66	85	48	29
10	CON	Right	54	65	39	22
	NF 1		50	69	30	38
	NF 2		62	68	31	11
12	CON	Left	96	120	32	26
	NF		88	103	31	17

Table 8-7 Pharmacodynamic effect of apomorphine: assessed using the tapping test.

Abbreviations: CON = conventional, NF = needle-free.

[a] Calculated as ((peak "on" score – baseline score) / baseline score) x 100.

8.15. Diagnostic Features Used in Pharmacokinetic Model Discrimination.

Patient 01.

Diagnostics	1 Compartment		2 Compartments
	minus lag-time	plus lag-time	plus lag-time
WSSR	4.61E-01	3.91E-01	4.38E-02
S	0.28	0.28	0.12
Degrees of freedom	6	5	3
Correlation (obs Y vs pre Y)	0.9256	0.9491	0.9997
Akaike Information Criteria	-0.97	-0.46	-16.16
Condition No.	45	12659	993
Rank	3	4	6
Number of sign changes in consecutive residuals	3	3	5

Patient 02.

Diagnostics	1 Compartment		2 Compartments
	minus lag-time	plus lag-time	plus lag-time
WSSR	4.53E-01	6.15E-02	error (see below)
S	0.30	0.12	
Degrees of freedom	5	4	
Correlation (obs Y vs pre Y)	0.9398	0.9816	
Akaike Information Criteria	-0.33	-14.31	
Condition No.	7	13	
Rank	3	4	
Number of sign changes in consecutive residuals	3	4	

** WARNING ***

VARIANCE - COVARIANCE MATRIX IS NOT OF FULL RANK OR IS ILL-CONDITIONED. PARAMETER ESTIMATES AND THEIR ASSOCIATED STANDARD ERRORS SHOULD BE INTERPRETED WITH CAUTION.

Patient 04.

Diagnostics	1 Compartment		2 Compartments	
	minus lag-time	plus lag-time	minus lag-time	plus lag-time
WSSR	5.01E-01	4.60E-01	9.63E-02	9.59E-02
S	0.25	0.28	0.14	0.15
Degrees of freedom	7	6	5	4
Correlation (obs Y vs pre Y)		0.9972		0.9984
Akaike Information Criteria	0.92	0.23	-13.40	-11.44
Condition No.	15	496	402	201
Rank	3	4	5	6
Number of sign changes in consecutive residuals	3	3	4	6

Patient 05.

Diagnostics	1 Compartment		2 Compartments
	minus lag-time	plus lag-time	minus lag-time
WSSR	1.05E-01	1.03E-01	error (see below)
S	0.16	0.19	
Degrees of freedom	4	3	
Correlation (obs Y vs pre Y)	0.9714	0.9717	
Akaike Information Criteria	-9.77	-7.91	
Condition No.	12	7427	
Rank	3	4	
Number of sign changes in consecutive residuals	4	4	

ERROR 10203 *** AN ERROR OCCURRED DURING CURVE STRIPPING.
ERROR 10201 *** INITIAL ESTIMATES CANNOT BE DETERMINED FOR THIS MODEL.
WARNING
VARIANCE - COVARIANCE MATRIX IS NOT OF FULL RANK OR IS ILL-CONDITIONED. PARAMETER ESTIMATES AND THEIR ASSOCIATED STANDARD ERRORS SHOULD BE INTERPRETED WITH CAUTION.

Patient 07.

Diagnostics	1 Compartment	2 Compartments	3 Compartments
WSSR	6.73E-01	6.25E-02	1.11E-01
S	0.29	0.11	0.17
Degrees of freedom	8	5	4
Correlation (obs Y vs pre Y)	0.8742	0.9941	0.9890
Akaike Information Criteria	0.43	-16.95	-7.81
Condition No.	3	26	81927
Rank	2	4	6
Number of changes in sign of residuals	2	4	3

Patient 08.

Diagnostics	1 Compartment	2 Compartments
WSSR	1.71E-01	1.09E-01
S	0.19	0.23
Degrees of freedom	5	2
Correlation (obs Y vs pre Y)	0.9894	0.9853
Akaike Information Criteria	-6.58	-5.31
Condition No.	4	188
Rank	4	4
Number of changes in sign of residuals	2	3

Patient 09.

Diagnostics	1 Compartment		2 Compartments
	minus lag-time	plus lag-time	plus lag-time
WSSR	error (see below)	1.17E+00	3.12E-02
S		0.36	0.07
Degrees of freedom		9	6
Correlation (obs Y vs pre Y)		0.9554	0.9984
Akaike Information Criteria		9.83	-29.60
Condition No.		1920	27
Rank		4	6
Number of sign changes in consecutive residuals		3	7

*** ERROR 10203 *** AN ERROR OCCURRED DURING CURVE STRIPPING.
*** ERROR 10201 *** INITIAL ESTIMATES CANNOT BE DETERMINED FOR THIS MODEL.

Patient 10.

Diagnostics	1 Compartment		2 Compartments
	minus lag-time	plus lag-time	plus lag-time
WSSR	5.74E-01	5.70E-01	1.33E+01
S	0.27	0.29	1.63
Degrees of freedom	8	7	5
Correlation (obs Y vs pre Y)	0.9452		0.9876
Akaike Information Criteria	0.45	2.40	37.88
Condition No.	10	47	3348
Rank	3	4	6
Number of sign changes in consecutive residuals	4	4	5
Notes	Cmax missed	Cmax missed	Cmax fit

Patient12.

Diagnostics	1 Compartment		2 Compartments
	minus lag-time	plus lag-time	plus lag-time
WSSR	5.74E-01	5.70E-01	1.33E+01
S	0.27	0.29	1.63
Degrees of freedom	8	7	5
Correlation (obs Y vs pre Y)	0.9452		0.9876
Akaike Information Criteria	0.45	2.40	37.88
Condition No.	10	47	3348
Rank	3	4	6
Number of sign changes in consecutive residuals	4	4	5
Notes	Cmax missed	Cmax missed	Cmax fit

8.16. Pharmacokinetic Parameter Estimates.

Patient	A (ng/mL)		Alpha (min^{-1})		B (ng/mL)	
	Estimate	CV (%)	Estimate	CV (%)	Estimate	CV (%)
01	22.4	416.8	0.0982	137.8	11.3	31.5
02	NA	NA	NA	NA	NA	NA
04	118.1	38.3	0.0207	19.2	0.4	52.2
05	NA	NA	NA	NA	NA	NA
09	23.9	9.4	0.0295	11.6	3.5	32.1
10	136.2	2775.5	0.0597	451.2	5.8	256.2
12	61.8	58.5	0.0576	30.3	7.7	23.2

Patient	Beta (min^{-1})		K10 (min^{-1})		Lag-time (min)	
	Estimate	CV (%)	Estimate	CV (%)	Estimate	CV (%)
01	0.0191	5.2	0.5064	1485.8	5.3	185.7
02	NA	NA	0.1250	36.3	5.7	8.6
04	0.0011	1287.3	0.0952	76.2	1.2	340.3
05	NA	NA	0.1550	31.1	NA	NA
09	0.0089	11.6	0.2438	22.2	5.9	3.7
10	0.0070	173.7	0.0807	388.7	1.8	85.8
12	0.0086	10.4	0.2360	81.8	3.1	72.4

Table 8-8 Primary pharmacokinetic parameter estimates.

Patient	t ½ Alpha (min)		AUC (ng/mL.min)		t ½ Beta (min)		CL/F (mL/min)		Cmax (ng/mL)		t ½ K01 (min)	
	Estimate	CV %	Estimate	CV %	Estimate	CV %	Estimate	CV %	Estimate	CV %	Estimate	CV %
01	7.1	137.7	754	50.4	36.3	5.2	2651	50.4	21.3	312.1	1.4	1484.3
02	NA	NA	1287	4.7	NA	NA	3885	4.7	11.2	6.9	5.5	36.3
04	33.4	19.2	4861	71.7	42.7	1286.0	2057	71.8	60.8	14.3	7.3	76.2
05	NA	NA	3143	6.8	NA	NA	1591	6.8	28.7	8.0	4.5	31.1
09	23.5	11.6	1083	2.6	77.5	11.6	1847	2.6	18.5	3.9	2.8	22.2
10	11.6	450.7	1350	30.6	98.6	173.5	2592	30.8	18.5	5.8	8.6	388.3
12	12.0	30.2	1667	5.0	80.2	10.4	3000	5.0	35.7	12.2	2.9	81.7

Patient	K10 (min^{-1})		t ½ K10 (min)		K12 (min^{-1})		K21 (min^{-1})		Tmax (min)		Volume (mL)	
	Estimate	CV %	Estimate	CV %	Estimate	CV %	Estimate	CV %	Estimate	CV %	Estimate	CV %
01	0.0384	59.6	18.1	59.5	0.0301	243.5	0.0489	83.7	10.0	354.4	69107	27.5
02	0.0110[a]	6.8	63.0	6.8	NA	NA	NA	NA	27.0	17.2	352977[a]	7.9
04	0.0191	84.5	36.3	84.5	0.0015	834.5	0.0012	1220.7	21.7	22.6	107735	16.3
05	0.0112[a]	11.4	61.7	11.4	NA	NA	NA	NA	18.3	20.0	141678[a]	10.9
09	0.0224	6.2	30.9	6.2	0.0042	27.8	0.0118	17.7	16.2	7.2	82339	6.7
10	0.0301	363.2	23.0	362.8	0.0227	611.8	0.0139	252.0	17.6	12.2	86039	35.5
12	0.0325	22.5	21.3	22.5	0.0185	47.4	0.0153	20.3	11.5	15.6	92384	23.6

Table 8-9 Secondary pharmacokinetic parameter estimates.

[a] Primary parameter.

8.17. User-Defined Models.

8.17.1. Subcutaneous Apomorphine Bolus Link Model (Exponential Pharmacodynamics).

```
MODEL 9
remark*****************************************************
remark   Developer: Ingram
remark   Model Version:  1.0
remark*****************************************************
remark    filename: userlnk1
remark    WNL model 412 modified to include exponential PD
remark    (two compartment model,first order input and
remark    output, Tlag, defined in terms of micro-constants)

rema   no.    parameter      constant       secondary parm.
rema   ---    ---------      --------       ---------------
rema    1     volume       stripping dose    auc
rema    2     k01          # doses           k10 half life
rema    3     k10          dose 1            k01 half life
rema    4     k12          time of dose 1    alpha
rema    5     k21                            beta
rema    6     tlag                           alpha half life
rema    7     keo                            beta half life
rema    8     eta                            a
rema    9                                    b
rema   10                                    keo half life
rema   11                                    cl

comm
nparm 8
nsec 11
pnames 'vc', 'k01', 'k10', 'k12', 'k21', 'tlag' &
         'keo','eta'
snames 'auc', 'k10_hl', 'k01_hl', 'alpha', 'beta',
         'alpha_hl', & 'beta_hl', 'a', 'b', 'keo_hl', 'cl'
nfun 2
end

temp
E0=1
vc=p(1)
r1=dsqrt((k12+k21+k10)**2 - (4*k21*k10))
alpha=((k12+k21+k10) + r1)/2
beta=((k12+k21+k10) - r1)/2
end

func 1
j=1
ndose = con(1)

do i=1 to ndose
```

```
j=j+2
if x <= con(j) then goto red
endif
next

red:
ndose = i-1
sum=0
j=1

do i=1 to ndose
j=j+2
t=x - con(j) - tlag
d=con(j-1)
a1=(d/vc)*k01*(k21-alpha)/(alpha-beta)/(alpha-k01)
b1=-1*(d/vc)*k01*(k21-beta)/(alpha-beta)/(beta-k01)
c1=(d/vc)*k01*(k21-k01)/(beta-k01)/(alpha-k01)
if t le 0 then
  amt = 0
  else
  amt=max(0,a1*dexp(-alpha*t) + b1*dexp(-beta*t) + c1*dexp(-
     k01*t))
endif
sum=sum + amt
next
f=sum
end

func 2
j=1
ndose = con(1)

do i=1 to ndose
j=j+2
if x <= con(j) then goto blue
endif
next

blue:
ndose = i-1
sum=0
j=1

do i=1 to ndose
j=j+2
t=x - con(j) - tlag
d=con(j-1)
coef= keo*d*k01/vc
ce0=((k21-k01)/((alpha-k01)*(beta-k01)*(keo-k01)))*exp(-
k01*t)
ce1=((k21-alpha)/((k01-alpha)*(beta-alpha)*(keo-
alpha)))*exp(-alpha*t)
ce2=((k21-beta)/((k01-beta)*(alpha-beta)*(keo-beta)))*exp(-
beta*t)
```

```
ce3=((k21-keo)/((k01-keo)*(alpha-keo)*(beta-keo)))*exp(-
keo*t)
if t <= 0 then
   amt = 0
   else
   amt=coef*(ce0+ce1+ce2+ce3)
endif
sum=sum + amt
next
ce=sum
if ce > 0 then
  f=E0*exp(eta*ce)
  else
  f = 0
endif
end

seco
d=con(2)
auc=d/vc/k10
k10_hl=-dlog(.5)/k10
k01_hl=-dlog(.5)/k01
alpha_hl=-dlog(.5)/alpha
beta_hl=-dlog(.5)/beta
a=(d/vc)*k01*(k21-alpha)/(alpha-beta)/(alpha-k01)
b=-1*(d/vc)*k01*(k21-beta)/(alpha-beta)/(beta-k01)
keo_hl=-dlog(.5)/keo
cl=d/auc
end
EOM
```

8.17.2. Buccal Apomorphine Pharmacokinetic Model.

```
MODEL 7
remark*********************************************************
remark   Developer: Ingram
remark   Model Version:  1.0
remark one compartment model - constant iv input, first order
remark output, including lag-time.
rema
rema   no.    parameter      constant       secondary parm.
rema   ---    ---------      --------       ---------------
rema   1       volume         # doses            auc
rema   2       k10            dose 1             k10 half life
rema   3       Tlag           start time         cmax
rema   4                      end time, etc.     cl
rema   5                                         aumc
rema   6                                         mrt
rema   7                                         vss
rema*********************************************************

comm
nparm 3
nsec 7
pnames 'VOLUME', 'K10', 'Tlag'
snames 'AUC', 'K10HL', 'CMAX', 'Cl', 'AUMC', 'MRT', 'Vss'
end

func 1

j = 0
ndose = con(1)

rema  Count up the number of doses administered up to time x
do i = 1 to ndose
j=j+3
if x <= con(j) then goto red
endif
next

rema  Perform superposition
red:
ndose = i-1
sum=0
j=0
do i = 1 to ndose
j = j + 3
t = x - con(j) - Tlag
d = con(j-1)
ti = 120 - tlag
del = t - ti
tstar = max(0, del)
coef = d / (ti * volume * k10)
if t le 0 then
```

```
  amt = 0
  else
amt = coef * (exp(-k10 * tstar) - exp(-k10 * t))
endif
sum = sum + amt
next

f = sum
end

seco
dose = con(2)
ti = con(4) - con(3)
auc = dose / (volume * k10)
k10hl = -loge(.5)/k10
coef = dose / (ti * volume * k10)
cmax = coef * (1 - exp(-k10 * ti))
cl = dose / auc
aumciv = dose / (volume * k10 * k10)
aumc = aumciv + (ti * auc / 2)
mrt = (aumc/auc) - ti / 2
vss = dose * ((aumc/auc) - ti / 2) / auc
end

EOM
```

8.18. Needle-Free Study: Raw Concentration-Time Data.

Blood Sample		Patient 09				Patient 10						Patient 12			
		conventional		needle-free		conventional		needle-free trial#1		needle-free trial#2		conventional		needle-free	
no.	mins	time (mins)	[apo] ng/mL	time (mins)	[apo] ng/mL	time (mins)	[apo] ng/mL	time (mins)	[apo] ng/mL	time (mins)	[apo] ng/mL	time (mins)	[apo] ng/mL	time (mins)	[apo] ng/mL
1	pre		ND		ND	-7	ND	-57	ND	-3	ND		ND		ND
2	5	7	5.5	6	16.6	5	9.2	5	8.8	5	25.5	6	25.5	6	5.9
3	10	11	15.7	11	25.4	9	14.4	10	11.8	9	20.2	10	35.2	10	13.8
4	15	16	18.7	18	23.6	13	17.6	13	12.6	13	23.0	16	33.3	16	17.1
5	22	23	17.9	24	15.7	21	19.3	21	11.4	21	15.2	23	26.8	23	17.2
6	30	32	13.7	32	13.2	29	17.6	29	9.1	29	15.6	30	18.4	30	14.7
7	50	52	7.5	51	6.4	50	6.4	51	7.1	51	6.7	55	7.9	54	6.0
8	80	85	4.3	81	3.5	80	5.3	80	5.8	81	5.0	83	4.5	82	5.3
9	125	123	2.0	124	1.3	133	<3N	141	1.9	146	1.6	135	2.8	133	2.6
10	180	182	0.8	181	0.4	198	2.3	198	0.7	214	0.4	200	1.4	183	1.3
11	240	246	0.4	240	0.2	272	0.5	254	0.4	268	0.2	230	0.8	235	0.5
12	310	314	0.2			NR1		287	ND	286	0.2	307	0.7	273	0.4
13	360	344	0.2			351	0.2	354	ND	346	<3N	346	0.4		

Table 8-10 Needle-Free Study: Raw Concentration-Time Data

Abbreviations: <3N = apomorphine peak area < three times the baseline noise, ND = no apomorphine detected, NR1 = not recorded: problem with venflon.

8.19. Contribution to Publications.

WM Ingram, TJL Malone, VR Pearce, MJ Priston & GJ Sewell, Pharmacokinetic-pharmacodynamic study of subcutaneous apomorphine in Parkinson's disease. *Age and Ageing* 2001; **30S1**: 47.

Presented at the British Geriatrics Society Autumn Meeting 2001 (London).

Introduction
Subcutaneous apomorphine is a potent dopamine agonist and a useful agent in Parkinson's disease for patients experiencing unpredictable 'off' periods. High interpatient variability in apomorphine pharmacokinetics and pharmacodynamics indicates the need for dose optimisation to be based on individual handling of the drug. A pilot pharmacokinetic study involving patients on optimised apomorphine therapy identified a consistency in post-distributional pharmacokinetics. The significance of this relationship was explored.

Methods
Five patients optimised on intermittent subcutaneous apomorphine had antiparkinsonian medication withheld overnight and were given a single subcutaneous apomorphine bolus. Two patients optimised on 24 hour subcutaneous apomorphine infusions were also recruited and their infusions stopped. During the following six hours blood samples were taken for apomorphine assay from both groups. The tools used for pharmacodynamic monitoring were (1) the tapping test and (2) individualised qualitative markers of response.

Results
An apomorphine bolus following overnight wash-out produced atypical 'on' periods in four out of five patients, i.e. three exhibited a sub-optimal response, one experienced adverse effects. The pharmacodynamic effect was best described by the sigmoid E_{max} model. The quality of the "on" period was not related to post-distributional pharmacokinetics or to EC_{50} (drug concentration required to produce 50% of maximal effect).

Conclusions
Apomorphine post-distributional pharmacokinetics were not correlated to antiparkinsonian response. No other significant pharmacokinetic predictors of pharmacodynamic effects could be identified.

WM Ingram, MJ Priston & GJ Sewell, Pharmacokinetic-pharmacodynamic study on apomorphine in patients with idiopathic Parkinson's disease. *Journal of Pharmacy and Pharmacology* 1999; **51S**: 160.

Presented at the 136th British Pharmaceutical Conference (Cardiff).

Idiopathic Parkinson's disease is common amongst the elderly population. Parkinsonism is caused by degeneration of the dopaminergic neurones of the nigrostriatal system. Loss of function at this region accounts for all the motor manifestations of the disease; these being akinesia, rigidity, postural instability and tremor. Levodopa preparations are the treatment of choice, being particularly effective in alleviating akinesia and rigidity. However, approximately 10% of patients per treatment year develop fluctuations in motor function. The response to levodopa becomes increasingly brittle. Ultimately some patients develop the "on-off" response, in which clinical state fluctuates abruptly between periods of relative mobility ("on") and severe parkinsonian disability ("off") despite optimally timed doses of levodopa.

Apomorphine, a potent dopamine receptor agonist, is used in anti-parkinsonian drug regimes to reverse refractory motor fluctuations. The drug is administered subcutaneously by intermittent injections, usually in the role of a rescue therapy, or by prolonged continuous infusion.

Peripheral PK studies have documented the large inter-patient variation in drug absorption, indicating the need for dose optimisation to be based on individual handling of the drug (Gancher 1989, Nicolle 1993).

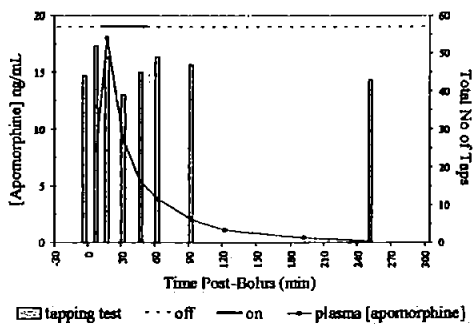
A review of published PK profiles revealed a potential PK-PD relationship which is based on post-distribution elimination PK. It is envisaged that a rapid dose optimisation protocol for apomorphine therapy can be developed around this relationship.

A PK-PD study to determine the significance of this relationship is in progress. This study involves blood sampling over an extended period. PD monitoring is individualised by the identification of key symptom changes in each subject prior to the study day. These changes are used as "qualitative markers" of drug response. The quantitative measurement employed is the "tapping test". See Fig. 1 for typical results. Blood sample analysis incorporates propyl-norapomorphine as an internal standard.

Apomorphine is extracted from plasma under vacuum using Bond-Elut C18 1mL/100mg columns and subsequently analysed by reverse phase liquid chromatography. The column used is a Phenomenex Columbus (C18, 5μm, 150mm x 4.6mm ID). The

mobile phase consists of (0.25M NaH_2PO_4, 0.25% heptane sulphonic acid (w/v), to pH 3.3 with orthophosphoric acid) containing 0.003% (w/v) EDTA and 40% (v/v) methanol, and is run at 1mL/min. Apomorphine is detected using a Jasco spectrofluorometer at λex 270nm, λem 450nm. The assay is stability indicating and has proven linearity. Intra-day precision is 8.2% and 2.1% at 0.5 and 20ng/mL, respectively (n=7). Inter-day precision is 4.9% and 5.7% at 0.5 and 20ng/mL, respectively (n=7). The LOQ is 43pg/mL.

Fig. 1 Plasma Apomorphine Concentration and Clinical Status Profile (Subject 01)



Findings will be discussed

Nicolle E *et al* (1993) Fund Clin Phar 7 (5): 245-52

Gancher ST *et al* (1989) Ann Neur 26 (2): 232-38

WM Ingram, MJ Priston & GJ Sewell, Dose optimisation scheme for apomorphine in Parkinson's disease: preliminary pharmacokinetic-pharmacodynamic studies. *European Journal of Pharmaceutical Sciences* 1999; 8 (2): x.

Presented at the 5th Congress of the European Federation of Pharmaceutical Sciences (Jerusalem).

Apomorphine, a potent dopamine receptor agonist, is used in anti-parkinsonian drug regimes to reverse refractory motor fluctuations. The drug is administered subcutaneously, by intermittent injections or prolonged continuous infusion. Peripheral PK studies have documented the large inter-patient variation in drug absorption[1], indicating the need for dose optimisation to be based on individual "handling" of the drug. A review of published PK profiles revealed a potential PK-PD relationship, based on post-distribution elimination PKs. It is envisaged that a rapid dose-optimisation protocol for apomorphine therapy can be developed around this relationship. A PK-PD study to determine the significance of this relationship is in progress. This study involves blood sampling over an extended period and the use of the "tapping tester" as the primary PD tool. Plasma apomorphine is measured using a novel solid phase extraction procedure and highly sensitive LC assay employing fluorescence detection. Preliminary PK results are given:-

Patient	Route & Dose	AUC ng.h/mL	$t_{½\alpha}$ (min)	$t_{½\beta}$ (min)	B (ng/mL)
1	bolus: 2mg	11.2	7.4	36.1	9.1
2	bolus: 5mg	28.6	21.7	127.0	6.9
3	bolus: 10mg	67.3	39.1	103.8	7.9
4	infusion: 35mg/12h	236.1	8.2	76.4	10.9
5	infusion: 141mg/12h	405.2	46.6	166.5	10.8

Preliminary findings will be discussed.

[1]Essink A.W.G *et al* Journal of Chromatography 1991; 570: 419-424

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For Joyce.