

2011

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<http://hdl.handle.net/10026.1/3701>


10.1136/ejhpharm-2012-000112

Annales Pharmaceutiques Francaises

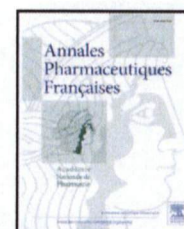
BMJ

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ORIGINAL ARTICLE

Guidelines for the practical stability studies of anticancer drugs: A European consensus conference[☆]

Recommandations pour les essais de stabilité pratique des médicaments anticancéreux : une conférence de consensus européenne

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Received 29 June 2011; accepted 7 July 2011

KEYWORDS

Practical stability;
Guidelines;
Anticancer drugs;
Consensus
conference;
Europe

Summary Stability studies performed by the pharmaceutical industry are only designed to fulfill licensing requirements. Thus, post-dilution or -reconstitution stability data are frequently limited to 24 h only for bacteriological reasons regardless of the true chemical stability which could, in many cases, be longer. In practice, the pharmacy-based centralized preparation may require infusions to be made several days in advance to provide, for example, the filling of ambulatory devices for continuous infusions or batch preparations for dose banding. Furthermore, a non-justified limited stability for expensive products is obviously very costly. Thus, there is a compelling need for additional stability data covering practical uses of anticancer drugs. A European conference consensus was held in France, May 2010, under the auspices of the French Society of Oncology Pharmacy (SFPO) to propose adapted rules on stability in practical situations and guidelines to perform corresponding stability studies. For each anticancer drug, considering their therapeutic index, the pharmacokinetics/pharmacodynamics (PK/PD) variability, specific clinical use and risks related to degradation products, the classical limit of 10%

[☆] This article presents the outcomes from the SFPO First European workshop on methodology in drug stability studies in the field of oncology, 14–15 May, 2010, Abbaye-des-Vaux-de-Cernay, France.

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of degradation can be inappropriate. Therefore, acceptance limits must be clinically relevant and should be defined for each drug individually. Design of stability studies has to reflect the different needs of the clinical practice (preparation for the week-ends, outpatient transportations, implantable devices, dose banding...). It is essential to use validated stability-indicating methods, separating degradation products being formed in the practical use of the drug. Sequential temperature designs should be encouraged to replicate problems seen in daily practice such as rupture of the cold-chain or temperature-cycling between refrigerated storage and ambient in-use conditions. Stressed conditions are recommended to evaluate not only the role of classical variables (pH, temperature, light) but also the mechanical stress. Physical stability such as particles' formation should be systematically evaluated. The consensus conference focused on the need to perform more studies on the stability of biotherapies, including a minimum of three complementary separating methods and a careful evaluation of submicron aggregates. The determination of the biological activity of proteins could be also useful. A guideline on the practical stability of anticancer drugs is proposed to cover current clinical and pharmaceutical practice. It should contribute to improved security of use, optimization of centralized handling and reduced costs. Finally, we have attempted to establish a new drug stability paradigm based on practical clinical needs, to complement regulatory guidelines which are essentially orientated to the stability of manufactured drugs.

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MOTS CLÉS

Stabilité pratique ;
Recommandations ;
Médicaments
anticancéreux ;
Conférence de
consensus ;
Europe

Résumé Les études de stabilité réalisées par l'industrie pharmaceutique sont uniquement orientées sur les exigences de l'autorisation de mise sur le marché. De ce fait, les durées de stabilité postreconstitution ou après dilution sont souvent limitées à 24 heures uniquement pour des raisons bactériologiques sans tenir compte de la stabilité chimique réelle qui peut être beaucoup plus longue. En pratique, les unités centralisées de préparation des chimiothérapies ont besoin de préparer à l'avance pour plusieurs jours, de remplir les dispositifs d'administration ambulatoires ou préparer des lots pour le *dose banding*. De plus, des données non justifiées de stabilité sont très coûteuses pour des médicaments chers. Il y a donc un besoin urgent de disposer d'études de stabilité sur les anticancéreux qui couvrent les pratiques quotidiennes. Une conférence de consensus européenne s'est tenue en France en mai 2010, sous les auspices de la Société française de pharmacie oncologique (SFPO) pour proposer des règles adaptées de stabilité et des recommandations pour réaliser les essais afférents. Pour chaque anticancéreux, en considérant son index thérapeutique, la variabilité pharmacocinétique/pharmacodynamie, ses utilisations cliniques spécifiques et les risques liés aux produits de dégradation, la limite classique de 10% maximum de dégradation peut être inappropriée. De ce fait, les limites d'acceptation doivent être cliniquement justifiées et définies médicament par médicament. Le design des études de stabilité doit refléter les différents besoins de la pratique clinique (préparation pour un week-end, transport pour patients externes, chambres implantables, *dose banding*). Il est essentiel d'employer des méthodes indicatrices de stabilité validées séparant les produits de dégradation. Les études séquentielles de température devraient être encouragées pour mimer les problèmes pouvant intervenir en pratique quotidienne comme les ruptures de chaîne du froid. Les conditions stressées sont recommandées pour évaluer non seulement les paramètres classiques (température, lumière...) mais aussi les stress mécaniques. La stabilité physique (formation de microparticules) doit être systématiquement recherchée. La conférence de consensus s'est focalisée sur le besoin de promouvoir les études de stabilité sur les biothérapies qui doivent inclure au moins trois méthodes séparatives complémentaires et évaluer la formation de micro-agrégats. La détermination de l'activité biologique peut être intéressante. Des recommandations sur la stabilité pratique des anticancéreux ont été proposées pour couvrir les besoins actuels, tant cliniques que pharmaceutiques. Elles devraient contribuer à améliorer leur sécurité d'emploi, optimiser le fonctionnement des unités de reconstitution et réduire les coûts. In fine, nous proposons d'établir un nouveau paradigme sur la stabilité des médicaments et les études afférentes qui tiennent plus compte des besoins en pratique et complémentaire des exigences réglementaires essentiellement orientées sur les demandes d'enregistrement.

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Background

Stability studies performed by the pharmaceutical industry are only designed to fulfill licensing requirements. When medicines are being licensed, little attention is given to the practical use of these drugs and there is no recognition that pharmaceuticals start a new life once they are prepared for patient administration. When reviewing package inserts, the general assumption is that a drug will be reconstituted, if necessary, and administered on a clinical ward. But increasingly the situation for hospital compounded drugs may be different, and preparing a drug ready for administration to a patient may be achieved in a variety of ways. Given that clinical needs may deviate from licensing requirements, we have identified a need to fill a gap between available data in a package insert or in the Summary of Product Characteristics (SPC) and practical needs [1].

Thus, postdilution or -reconstitution stability data are frequently limited to 24 h only for bacteriological reasons or the fact that stability tests were only conducted over very short periods regardless of the true chemical stability which could potentially be much longer. This is obviously insufficient for most practical situations [2]. As an example, the stability of the antibody trastuzumab reconstituted by bacteriostatic water is given as 28 days by the manufacturer. However, the same product reconstituted with water for injection is only 48 h, demonstrating that this proposed stability limit is only based on possible risk of biological contamination and not on true physicochemical stability [3].

Nowadays, in most hospitals, reconstitution and preparation of anticancer drugs takes place in centralised compounding units in a controlled and validated environment with expert staff. When compounding medicines for patients in such units several aspects are taken into account: dose accuracy, sterility assurance, occupational exposure and stability under practical clinical conditions.

This leads to safe products from a bacteriological, dosage and contamination point of view, as these patient ready preparations have been prepared under Good Hospital Pharmacy Manufacturing Practice, in which the principles of GMP have been applied to hospital pharmacy compounding. Therefore, the only relevant issue is the actual chemical and physical stability.

The new requirements which can be covered by centralized preparation units are:

- preparation in advance for a whole treatment cycle of a particular patient, for several days;
- preparation in advance to cover 7 days/24 h availability (e.g. spanning weekends and holidays);
- increase in the efficient use of existing dose strengths and reduced waste;
- filling of ambulatory devices for continuous infusions over extended periods;
- preparation in advance to optimize workload and to reduce time pressure and rush for pharmacy and nursing staff;
- batch preparations for dose banding.

Dose banding is a relatively new concept in cancer treatment, where the pharmacy produces predefined strengths of ready-to-use product that can be administered to a patient at any required moment. The dose strengths are selected

such, that with these products most dosage needs can be met with acceptable accuracy (e.g. $\pm 5\%$) [4,5]. Obviously, safe dose banding requires extended infusion stability to be efficient (28–84 days) [6,7].

We also have to take into consideration that drugs can be prepared in practice in a variety of containers, ambulatory devices and tubing and subsequently we need data to support the absence of possible drug-container interactions, optimal storage conditions and also the stability profile when the recommended temperature chain was not adhered to, such as during the rupture of the cold-chain. Indeed, this problem can often occur in many practical situations such as refrigerator failure during a week-end. Obviously, it is of crucial importance to determine if exposure to room temperature for 48 h can induce enough degradation to justify discarding a high cost new anticancer drugs such as antibodies. These data are never furnished by the manufacturer, considering that the cold chain should be fully complied with. However, it could be reasonably considered that a drug exhibiting a shelf-life of more than 2 years may not be affected by an excursion at 25 °C over 2 days. Therefore, to demonstrate this assumption, some recent stability studies used a "sequential temperature" design where the same drug infusions are stored at different temperatures in sequence to replicate some practical situations: infusion bag removed from its refrigerated storage, transported to the ward, and sometimes returned to the pharmacy unused because of an unforeseen delay in patient's treatment and then refrigerated for later re-issue [8].

With the emerging use of costly monoclonal antibodies and more generally therapeutic proteins in the field of oncology, stability issues become of paramount importance. Indeed, if physical instability is rarely observed for low molecular weight molecules, proteins can undergo a variety of structural modifications independent of chemical modifications because of their polymeric nature. Moreover, these sensitive products could undergo more complex degradation pathway during the various manipulation steps than classical drugs. Therefore, the specific physical-chemical properties of proteins and their complex instability behaviours such as aggregation require specific assays, relevant analytical approaches and appropriately designed studies [9–11].

More generally, stability limits for expensive medicines based on short-time periods or studies using non-practical conditions can prove financially costly. A pertinent example concerns the stability of diluted bortezomib which was claimed by the manufacturer to be less than 24 h. However, independent stability studies published after marketing bortezomib have demonstrated stability for at least one week, thus allowing very important cost savings [12–15]. Considering these arguments, stability data furnished by the manufacturer for marketing authorization purpose is obviously insufficient and more practical stability data are thus required.

One might argue that sufficient guidelines are available to perform stability studies such as ICH guidelines or Pharmacopoeia monographs [16]. However, they have been designed for purposes not entirely covering the practical needs. Indeed, ICH guidelines have the objective to regulate quality of marketed drugs in an international context and pharmacopoeia monographs often refer to raw materials and offer no solution when applied to marketed

products under practical conditions. However, the methodologies proposed in the official literature may be helpful in developing methodology for practical situations. Thus, systematic research programs should be promoted to support the practical challenges faced everyday by oncology pharmacists and there is a compelling need for additional stability data covering practical uses of anticancer drugs and adapted guidelines for stability studies.

Methods

A European conference consensus was held in France, Abbaye des Vaux de Cernay, May 2010, under the auspices of the French Society of Oncology Pharmacy (SFPO) to define adapted rules on stability in practical situations and to propose guidelines to perform the corresponding stability studies. A panel of ten European experts shared their specific and "practical" experience and worked during two days to produce guidelines. This panel is referred as "consensus group". Conference consensus was limited to drugs used in the field of oncology.

Primary goals were to identify unresolved questions in methodology for stability studies and specific hospital needs for clinical and compounding pharmacists.

Work was based on ICH (ICH Harmonised tripartite guideline) particularly ICH Q1A (evaluation for stability data), ICH Q1A(R2) (stability testing of new drug substances and products), ICH Q2A (test on validation of analytical procedures), ICH Q1B (stability testing: photostability testing of new drug substances and products), Q3B (impurities in new drug products), Q5C (stability testing of biotechnological/biological product), European Pharmacopeia 6th and 7th editions, and most relevant literature [16–26]. Obviously, the main goal was not to rewrite complete guidelines but to revise or adapt ICH guidelines or general reviews [16] when inappropriate for anticancer drugs in a clinical environment.

Results and discussion

Unresolved questions

After examination of the literature, the initial postulate was that many unresolved questions remained, such as:

- The relevant stability limits for practical purposes, including the question of those for degradation products.
- The kind of methods to be used when ICH guidelines are not adapted both for physical and chemical evaluation.
- The evolutions of protocols of stressed conditions.
- The need of more relevant design for stability studies (i.e. sequential cycling and non isothermal studies).
- The specific requirement in the stability studies of biotherapies (physical instability, orthogonal methods).
- The relevance of the determination of pharmacological activity as marker of stability.

Thus, it was decided to propose general recommendations and specific approaches for stability studies of biopharmaceutics.

General recommendations on the stability limits

Generally speaking, the stability is the property of a drug to retain its physical, chemical, microbiological and biological properties within specified limits. However, the concept of practical stability (or in-use stability) is more extended, referring to the stability of a drug not only determined under conventional situations but also taking in account variations observed in clinical practice both voluntarily defined or unexpected. Chemotherapy agents are generally considered as exhibiting a very narrow therapeutic range and being very toxic by themselves, although this is not always strictly true. Thus, practical stability limits should be only defined for each drug on an individual basis after consideration of its therapeutic index, clinical use, safety and potency and its pharmacodynamic / pharmacokinetic variability, and the total cumulative dose. Indeed, considering the general rule that a drug remains stable in clinical practice (i.e. at recommended dilution and vehicle) until 90% in assay from its initial value (T_{90} value), this 10% of degradation as stability limit has been widely used in the published stability studies. However, depending of the drug, this limit could lead to an acceptable or unacceptable loss of efficiency. As an example, for a same dose of 5-FU by infusion, the AUC between patients can vary of about 500% [27]. Thus, the administration of only 90% of the theoretical amount of 5FU may not be clinically relevant in terms of efficacy. Moreover, increased risks associated to the degradation products (DPs) must be also considered (active, toxic or inert product) and the question of DPs is of major importance in stability studies, especially for anticancer drugs since a low percentage of instability could lead to the appearance of a highly toxic DP. For example, it has been suggested that the increased cardiotoxicity in patients receiving high doses of 5-FU could be caused by the presence of small quantities of DPs (fluoromalonaldehyde and fluoroacetaldehyde) resulting from storage in basic medium to solubilize the drug [28]. Even if 5-FU can be considered as stable for over 14 days at 33 °C in EVA and PVC reservoirs on the basis of its remaining concentration, a three-day storage at 37 °C leads to acute increase of total DPs [29].

Thus, the consensus group considers that the classical and dogmatic T_{90} reported in a majority of stability studies could be modified in T_{95} , T_{85} or any stability limit depending on the drug. However, in all cases, it is strongly recommended that the chosen stability limits must be justified and clinically relevant. In a general guideline, it is recommended that limits for anticancer drugs with low therapeutic index (e.g. drugs inducing hematological or neurological toxicity such as busulfan, vincristine, carboplatine...) should be not less than T_{95} [30–33]. Stability limits lower than T_{95} could be acceptable for very unstable products but only in the absence of any toxic DPs and in cases of significant interindividual variability in metabolism and activity. Specific routes of administration must be also considered: e.g. the intrathecal route should involve stricter and more rigorous acceptance criterion. The consensus group recommends less than 5% of degradation but also a careful examination of any sign of physical instability such as aggregation or precipitation, which is potentially very harmful by this route. The same rule should be also followed for ocular route and

even with the IV route, small precipitates are a possible risk to the patient.

This flexible and more clinical approach of the stability limits of anticancer agents is central to the dose banding of cancer chemotherapy. In this approach, it is considered that some flexibility of chemotherapy dosing is possible, permitting both the patient and the health-care system to benefit from the advantages of the batch preparation of chemotherapy: better quality assurance, cost saving, or dispensing of medication for administration without delay. Dose banding is a pragmatic approach. After agreement between prescribers and pharmacists, doses of intravenous cytotoxic drugs (generally chosen among those with low toxicity or large variability) calculated on an individualized basis are fitted to defined ranges, or bands. A predetermined standard dose, usually the mid-point of the band, is administered using premade infusions, either singly or in combination. The maximum variation of the adjustment between the standard dose and the doses constituting each band is 5% or less [4]. Obviously, given the maximum error of 5% that could be introduced by dose banding, it is sensible to restrict drug degradation to less than 5%.

Recommendations on the stability studies

The stability study should include testing of those attributes susceptible to change during storage that are likely to influence quality, safety, and/or efficacy. The rationale for attributes to be tested in the stability study should be clearly stated and a systematic approach should be followed to conduct well-designed stability-indicating studies, as suggested by Bakshi and Singh [17]. However, stability studies on anticancer drugs deserve specific recommendations beyond general guidelines. This was the main goal of the consensus group to define those specific requirements. Thus, the essential aspects of physical, chemical and biological stabilities have been considered.

Physical stability

Physical stability is often neglected in many stability studies. Only gross change of colour or appearance of precipitate are followed, without any quantification. The consensus group recommends that physical stability should be more systematically evaluated, particularly particle formation. Indeed, it may be the main determinant of shelf-life of a formulation (e.g. microprecipitation in paclitaxel pseudo solution) and may restrict storage conditions (e.g. high strength 5-FU, 50 mg/mL stored at 25 °C). Indeed, any thermodynamically instable formulation such as micellar pseudo-solution or nearly saturated solution can form subvisible aggregates and/or precipitate, due to underestimated and various causes (temperature, shaking, interaction with devices such as needles. . .) and, thus, induces severe side-effects such as patient embolism after intravenous infusions. The physical evaluation of the solution is of particular importance for intrathecal, ocular and intra-arterial routes. The classical visual inspection is important but not sufficient and should be supplemented by a subvisual evaluation. A more refined physical evaluation, using turbidimetry, light obstruction, dynamic light scattering or microscopic analysis, is particularly important for therapeutic proteins to evaluate their

kinetic profiles of aggregation. However, these analytical methods could be difficult to validate in practice as compared to chemical stability methods such as HPLC and should necessitate more evaluation work.

Visual examination

The visual examination permits to detect formation of particles or change in the initial colour of the solution. The examination should be well defined and standardized, and as a minimum should accord to the corresponding Pharmacopoeia monographs. For visible particle formation (i.e. > 0.2 µm), the widely used optical examination method must be performed according to the European Pharmacopoeia; 7th Ed (tests 2.2.1 or 2.9.20). The particle counter (light obstruction) and microscopic analysis or with image analysis can also be used and these are useful predictors of physical stability (evolution of size, shape and count of particles). However these methods are not readily available in most hospital laboratories. Colour changes could be also difficult to assess since specifications in drug monographs may give indications such as "colorless to slight yellow" for the solubility test. Moreover, differences in coloration between commercial batches are not uncommon. European Pharmacopoeia (7th Ed, test 2.2.2) methods for colour assessment of liquids in the range yellow to brown are based on a visual comparison with liquid standards formulated from dilutions of primary red, yellow and blue. However this method is only semi-quantitative and not really designed for stability studies. The consensus group considers that it should urgently initiate collaborative studies to define more standardized methodologies and to specify limits for a colour change test.

Nevertheless, it is recommended that any stability study of solution includes at least a visual examination for opalescence and colour change following the pharmacopoeia methods. Alternatively, microscopic examination and turbidimetry, which are relatively easy to perform and not too expensive, are encouraged. For colour change, it is recommended to furnish some elements of quantification such as comparison of visible absorption spectra during the experiment course.

Subvisual evaluation

The evaluation of subvisual particulates in the solution is particularly important since a microprecipitate can appear (but remain visibly undetectable), increase with time and induce formation of a visible precipitate especially at low temperatures, as described for pemetrexed [34]. The particle counter (light obstruction) and microscopic analysis following European Pharmacopoeia (7th Ed, test 2.9.19) are good predictors of physical stability (evolution of size, shape and count of particles) but these methods are not readily available in most hospital laboratories.

Following the Lumry-Eyring model of the nucleation theory, adsorption of a molecule, especially a protein, on the surface of contaminating microparticles (glass, stainless steel, silica), which can be present in diluting vehicles or introduced during the dilution steps, could induce the subsequent formation of microaggregates [35–39]. Even though no visible precipitate is seen in the infusion, the formation of microaggregates during the storage is nevertheless the sign of an instability of the solution which can precipitate later or in the infusion set during the administration. Moreover, this microaggregation, which can strongly affect

stability, represents only a very small percentage of the total amount of drug (< 0.1%) and may not be detected during HPLC analysis because it is less than the intrinsic variability of the method. Moreover, submicron particles can pass freely through the chromatographic column since the diameter of the stationary phases are 3 to 5 μm , or alternatively can re-dissolve in the mobile phase.

The particle counter (light obscuration) and microscopic analysis following European Pharmacopoeia (7th Ed, test 2.9.19) are good predictors of physical stability (evolution of size, shape and count of particles) but these methods are not easily available in most hospital laboratories. Turbidimetry at three wavelengths (350, 410 and 530 nm) is easy to perform and is a non-destructive method to evaluate the formation of microparticles. If the microparticles formed are of the same order of magnitude for size, turbidimetry can provide a continuous quantitative estimation of the number of microparticles. This method is widely used in the study of protein aggregation [11,40,41]. Although this method cannot determine neither size profile nor particle count, it is very precise and useful to evaluate subvisible aggregation and correlates well with the discontinuous opalescence test described by the European Pharmacopoeia (7th Ed., test 2.2.1) using reference suspensions of hydrazine/hexamethylentetramine (Table 1). Thus, the consensus group recommends the use of turbidimetry as a continuous method to evaluate the formation of particles over time, both visible and subvisible, in all stability studies of anticancer drugs in solution if other methods such as light obstruction are not possible.

Stressed conditions (accelerated tests) could be performed to test the potential physical instability prior to conducting real-time stability studies. Stirring or shaking tests seem useful to evaluate the instability of proteins or thermodynamically unstable solutions such as concentrated or pseudo micellar [11,40,41].

Chemical evaluation and validation of analytical method

General tests

The search for any pH variation is a classical test which could be a simple indicating method for chemical stability. The pH should be monitored and reported throughout the study period. Variations in pH values must be interpreted carefully. Indeed pH variations can be observed at the end of a study even though there have been no degradation of the drug as demonstrated by separative methods such as HPLC. This discrepancy could reflect CO_2 diffusion through the wall of the plastic bag and subsequent acidification, particularly in non-buffered solution but without any consequence if the drug is not acid sensitive. However, pH is a logarithmic scale; a decrease of one unit on the pH scale means a 10-fold increase in proton concentration. Thus, a modification of one or two pH units should not be considered as a "slight modification in pH values" and should be explained.

For solutions stored in plastic bags, the determination of water loss due to diffusion of water vapour through the plastic wall must be always performed to obtain the correct concentrations of the drug and DPs. Each tested bag must be weighed at all sample times. Significant losses (more than few mg per week for 500 ml polyethylene bags) should be

considered as major concern, indicating leaks or problem of permeability. Clearly, if over-wraps are applied to infusion bags in practice to protect from light and reduce water loss, these should also be applied in stability studies.

Methods for evaluation of chemical instability

General. As previously claimed, it is essential to use validated stability-indicating assay methods (SIAMs) which are able to separate DPs being formed in the practical use of the drug infusion [17,24]. A stability-indicating assay is a validated quantitative analytical method that can detect the changes with time in the chemical, physical, or microbiological properties of the drug substance, and that are specific so that the contents of active ingredients, DPs, and other components of interest can be accurately measured without interference [17]. It must be established that there is no interference on the assay by vehicles or degradation by-products, and normally forced degradation studies (stressed conditions) are carried out on the parent drug to determine nature and chromatographic peak of degradation by-product and other excipients [18]. Careful examination of the chemical structure of the tested drug and its possible degradation pathway, as detailed in the previously published literature, should guide the choice of the most relevant analytical method. However, it should be emphasized that, for long-established drugs, published analytical methods, even based on HPLC, are often non SIAM or not well validated according to the current guidelines [17,18,24,26]. Similarly, the analytical methods described in the Pharmacopoeia were primarily developed to find impurities arising from synthesis in the corresponding raw chemical and not to evaluate DPs. The use of a relevant separating method is essential. HPLC is the method of choice but other methods such as high performance thin layer chromatography (HPTLC) or capillary electrophoresis (CE) can be employed. Determination of the peak spectral purity by on-line photodiode array allows for more opportunity to detect DPs and to evaluate purity of compounds. Alternatively, a mass spectrometry detector can be used but are generally not readily available in most laboratories. Regardless of the method used, the verification of the purity of peaks under stressed conditions is strongly recommended by the consensus group for all stability studies.

Methods which cannot separate the intact drug from its DPs or excipients such as titrimetry or spectrophotometry are not suitable for evaluation of chemical stability, except in particular cases. Indeed, the previous example of 5-FU, using magnetic nuclear resonance of fluoride demonstrated that the determination of the parent compound by HPLC alone, albeit by a precise HPLC method, was not sufficient and that appearance of any DP must be also carefully assessed [26].

Interpretation of the variation in the concentration of drug should be clearly discussed since it can be due to different causes such as physical degradation, absorption or adsorption onto the container walls, or chemical alteration with the formation of one or several DPs. Any increase of the initial concentration should be interpreted a priori as evaporation of water from the wall of the container. Therefore, weighting of containers is essential as previously discussed.

The exact determination of the concentration of DP is only useful if its structure, activity (or toxicity), especially for new drugs, is known. If these criteria are known, adapted

Table 1 Absorbance (mean \pm SD) of reference suspensions for the limpidity test according to European Pharmacopoeia 7th Ed, test 2.2.1.
Absorbance (moyenne \pm SD) des suspensions de référence du test de limpidité suivant la Pharmacopée européenne 7^e Ed, test 2.2.1.

Category of reference suspension	Absorbance at (nm)		Aspect of solution
	350	550	
I	0.017 \pm 0.002	0.007 \pm 0.001	Clear
II	0.032 \pm 0.003	0.014 \pm 0.002	Slightly opalescent
III	0.085 \pm 0.001	0.035 \pm 0.001	Opalescent
IV	0.144 \pm 0.005	0.059 \pm 0.003	Very opalescent

From reference [39].

limits must be defined. However, it must be kept in mind that some degradation species may be transient, especially in case of successive degradation steps. However, it is not easy to determine structure of DP, to obtain pure standard if the structure is known or to propose relevant limits. This aspect should be discussed in regard of the toxic potential of the DP as previously mentioned and is a subject for further research.

Analytical aspects. In general good laboratory practices recommend the use of pure compounds to validate an analytical method. This practice is highly supported by the consensus group. However, in many cases, a pure compound is not easily available, especially for new drugs such as antibodies and the handling and weighing of pure cytotoxic powders in a lab can be very dangerous. Therefore, it could be acceptable that the commercially available form be used as reference to construct the standard curve and to validate the analytical method. Moreover, the stability limits are based on the remaining percentage of the initial concentration and knowledge of the exact concentration is not required since peak area normalization at T₀ of the tested drug, with or without internal standard (IS), is usually sufficient. Albeit IS is not generally required for simple solutions (if no extraction is needed), it should be verified that potential DPs do not interfere with its peak. The linearity of the method should be performed from 60 to 140% of the central value (60, 80, 100, 120 and 140%) as classically accepted.

Robustness of the method should be tested and it should be ascertained that the practical chromatography conditions cannot modify the results. In particular, the stability of diluted samples should be checked during a run since the diluted samples for HPLC analysis can be very unstable and could be degraded within a few hours in the autosampler. This artificial degradation must not be confused with the real degradation process.

Stressed conditions. Use of stressed conditions has two primary goals: (1) to develop a relevant stability-indicating assay; (2) to evaluate rapidly the influence of different parameters on drug stability (e.g. pH, temperature, light...). The stability-indicating capacity of the HPLC method must be demonstrated by degrading the samples under various conditions. The conditions must be aggressive enough to produce primary DPs but ideally should not destroy the drug entirely. Indeed, extremes such as pH 1 or 12 should not be selected since the formed DPs could be

completely different from those observed in practice where only limited variation of the pH can be observed. Moreover, the conditions should not induce the progression of further breakdown products from the initial DP, which would not be observed during usual storage in practice. The ideal situation is to degrade about 20 to 30% of the drug and to obtain DPs clearly separated from the intact drug. Thus, a stepwise progression of stressing conditions should be recommended: HCl to obtain pH 3 and NaOH to pH 9 for beginning, temperature starting at 50 °C and increase by 10 °C steps, UV light, oxidative conditions (H₂O₂: 3 to 30%).

As previously discussed, stressed conditions are also recommended to evaluate the role of specific conditions which can occur in practical situations such as the mechanical stress, especially for proteins.

Design of the stability study

Number and analysis of samples

ICH guidelines indicate that stability studies should be performed on three different batches in the final containers. For practical stability studies, it could be argued that only one batch need be tested if it is a licensed drug since it is a condition of the licence that there is no batch to batch variation, so there is no point in testing multiple batches.

Therefore, in practical stability studies, one batch could be sufficient but, at minimum, each point must be determined in triplicate. Moreover, in order to obtain more data, it could be acceptable to perform simplified assays in small volume and then extrapolate to the final volume. Therefore, the stability study could be performed in two steps. The first one (stressed experiments) can be performed in glass vials to study the intrinsic stability of the solution and to determine the relevant experimental stability conditions to be used. This approach, using small quantities of drug, permits an easy and economical testing of multiple stress conditions. The second one should be carried out in the final containers. For very unstable drugs, three different batches should be used, but with one assay for each point to minimize artificial degradation during the analytical process. Each solvent used in clinical practice must be evaluated during the stability studies (in general, normal saline and isotonic dextrose). The constituting material of the container, the manufacturer and the batch number must be indicated. In specific cases such as adsorption of drug onto container walls, it

could be also useful to test several brands of solvent bags since variability in the plastic composition can occur. The final product should be clearly defined (concentration, final volume, conditions of storage) and reflect the real clinical practice.

The samples can be analysed in real time if a reproducible and well-defined reference standard is available. The practice to freeze all the samples of the stability study to analyse them a same day later must be validated. Indeed, this approach implies that the solution is stable when frozen which is not proven for each product and the process of thawing can also influence the results. If samples are frozen and stored prior to analysis, data should be furnished to prove the stability of the samples, not only from a chemical point of view but also physical since a freezing/thawing cycle can induce unexpected aggregation.

Temperature

The storage temperature should be clearly defined and concordant with the real life of the compounded product. Refrigeration is classically defined at $5 \pm 3^\circ\text{C}$. The term "ambient temperature" should be avoided. The room temperature in hospital is around 25°C , but can vary country to country depending of the weather and air-conditioning facilities. The use of a temperature-controlled incubator at 25°C can be recommended to standardize the "room temperature" stability studies between 22 and 28°C . Since this facility is uncommon in most hospital laboratories, the consensus group considers that a precise control of "room temperature" is not critical for drugs with low degradation rate, but recommends that the true ambient temperature is recorded throughout the study. However, for drugs very sensitive to small differences of temperature (e.g. melphalan or azacytidine), a more controlled storage temperature is required. A higher temperature should be also used to mimic drugs infused by portable or implantable devices. In this case, 37°C should be preferred than 40°C (ICH). Although hygrometry-controlled incubators are not generally available in some laboratories, their use is strongly recommended for long-term stability studies using storage of plastics bags to minimize water loss, especially at elevated temperatures. Alternatively, storage of bags in desiccators containing water in a classical low-cost dry incubator or refrigerator could be suggested or alternatively infusions could be over-wrapped to reduce moisture loss if this is also done in clinical practice.

Freezing of drugs in their final bags can improve long-term stability of many drugs [42,43] and could be very interesting for technical and economical reasons. Thus, considering the high cost of many anticancer drugs and the strong need to improve workload in centralized units, the consensus group would like to encourage more research in this field to ensure freeze-thaw cycles are properly validated and are reproducible.

Thermal excursions and sequential temperature cycling studies

Sequential temperature designs have been used by some authors to replicate several problems frequently observed in daily practice such as unexpected rupture of the cold-chain, refrigerator failure during a week-end, bags stored in the ward at room temperature or return to unused bags in the pharmacy without temperature control [7,8,44].

Thus, to produce validated stability data corresponding to these situations may significantly help pharmacists to avoid unnecessary wastage especially for expensive drugs. The consensus conference wishes encourage the development of more stability studies using this very interesting and practical approach.

Light

Generally speaking, the stability study should be designed in ambient room light that mimic the practical conditions in pharmacy or clinical wards. It is only the case for poorly designed stability studies performed at "room temperature on the lab bench". However, except for laboratories disposing of special temperature-controlled incubator equipped by illumination tubes, reproducible conditions of illumination are difficult to obtain. However, most of anticancer drugs are not very light-sensitive. Therefore, it could be considered that the use of better controlled temperature and humidity conditions is more important for the relevance of stability studies than to keep bags on the lab bench (e.g. without control of these critical parameters) only to have an approximate exposure to the light. For drugs known to be highly sensitive to light, an excursion outside the specification of the light protection should be encouraged to appreciate the importance of the degradation and if the protection from light is practically relevant [45]. However, since in many countries all infusions and prefilled syringes are automatically over-wrapped in light-protecting plastic bags, testing the influence of light in practical stability studies could be not really necessary.

Special conditions

The consensus group considers that more experimental stressed-conditions studies (excursion stability studies) which evaluate practical situations such as exceeding temperature limits for short time period, exposure to light for light sensitive product, stressed conditions of transportation (pneumatic network) or accidental freezing, should be encouraged. These data, albeit very useful in practice, are almost never available in manufacturer drug information files or only under generic sentences such as "avoid shaking" or "do not expose to light" which give no useful information.

Sterility

The sterile conditions during the manufacturing process and the initial sterility of the final product depend on the application of Good Manufacturing Practice in the centralized unit (validation of the handling environment, closed systems, staff training and competency, operator validation, process validation, in-process media-fills). It is expected that these conditions are respected to ensure the quality of the manufacturing process and thus, the validity of the stability data. However, the preservation of the sterility in the final administration device also depends on the nature of the container and the storage conditions (especially important for syringes with luer lock closing systems or bags with clamped infusion set). The secondary packaging is also important (sealed polyethylene bags for example). However, it is classically considered that many anticancer drugs such as antibiotics-derived structure (anthracyclines or bleomycine) do not facilitate bacterial growth although

some contradictory data are available on this topic [46]. Therefore, the consensus group considers that in most of cases, the evaluation of the sterility of the final product is not generally required in the stability studies. However, the evaluation of long-term preservation of sterility is mandatory for preparations expected to be stored for a long-term such as batches preparation, drug diluted in dextrose or infusions with components considered as promoters of bacterial growth such as proteins. In these cases, any "microbiological stability" study should be designed taking in account the specificity of the final product.

Specific aspects concerning pharmaceutical proteins

General background

Although many pharmaceutical proteins are also used in non-cancer pathologies such as autoimmune diseases, another important challenge in the field of anticancer drug is the difficulty of assessing the stability of new biotechnology-issued drugs such as antibodies. These sensitive products can undergo more complex degradation pathways during the various manipulation steps than classical drugs. Indeed, *in vivo* activity of proteins depends not only on their primary structure (sequence) but also on their structure in 3-dimensional space (secondary, tertiary and quaternary structures). Thus, the conformation of a protein could change subtly when exposed to mild chemical or physical stresses such as shaking, small temperature change, variations in ionic strength, light, exposure to oxygen or to traces of metals [2, 10, 47, 48]. Finally, as with low molecular weight chemical drugs, proteins should be characterized not only in terms of identity and impurity content but also in terms of heterogeneity, which is a specific trait of this type of drug.

Protein instability includes two main types of alteration with several possible pathways: (1) physical instability: aggregation, denaturation or adsorption on surfaces; (2) chemical instability: desamidation, disulfide bond breakage, hydrolysis, isomerization, non-disulfide crosslinking, deglycosylation or Maillard reaction. The main causes of instability include temperature (elevation or freezing), formulation pH, adsorption, salt effects, oxidation (associated with metal ions and chelating agents), shaking and shearing and concentration. Therefore, stability assays for therapeutic proteins must involve specific studies and represent a real analytical challenge [10, 48]. Although, most authors agreed that several complementary (orthogonal) methods must be used in stability study including at least three complementary separating methods, no clear guideline or recommendation is currently available.

Physical instability

The aggregation of proteins is a major and underestimated physical instability which could have major implications in terms of efficacy or toxicity [3, 9, 10, 38]. Aggregates formed may be strongly antigenic and therefore loss of efficacy could result from the appearance of neutralizing antibodies or the patient could suffer severe immunological reactions. In particular, one of the most underestimated causes of aggregation is mechanical stresses: shaking or stirring, shearing (e.g. caused by rapid sampling by syringe),

exposure to hydrophobic gas interface (bubbling or filtration). As previously discussed, turbidimetry can easily determine the formation of microaggregates. However, other complementary methods should be used to estimate more closely the physical stability of a protein. Dynamic light scattering (DLS) method is able to evaluate both soluble and non soluble aggregates and can describe time-dependant profiles of particle size-distribution. Size exclusion chromatography (SEC) can measure level of monomeric protein and soluble polymeric aggregates.

By direct UV spectroscopy after centrifugation, the determination of non-aggregated protein content (absorbance at 279 nm) readily permits to calculation of aggregation and second-derivative spectra can be useful to detect small modification of its tertiary structure. Lalou et al. reported the association of these different complementary methods in a study focusing on the mechanically-induced aggregation of the monoclonal cetuximab [11]. Fluorescence spectrometry can be also used to evaluate structural changes due to photooxidation [45].

Due to multiple causes of physical instability, the evaluation of the stability of biotherapies should ideally be performed by including stressed conditions typical of the "daily practice": rapid injection and rinsing with the production of bubbles into the infusion bag, accidental shaking, and transportation by pneumatic network. As previously discussed, simple experimental design such as the stirring test can be done to generally mimic mechanical stresses.

The consensus group recommends that physical stability of proteins, especially antibodies, should be evaluated by several complementary methods including at minima turbidimetry and SEC.

Chemical instability

Deamidation is considered as a common degradation pathway for proteins and peptides, strongly depending on the pH. Deamidation generates DPs and may contribute to immunogenicity. As for the evaluation of the physical instability, several complementary methods must be used to assess chemical degradation of proteins.

To evaluate chemical degradation of proteins, several chromatographic methods have been used [48]. Ionic exchange chromatography (IEC), particularly cation exchange chromatography, is the gold standard for protein analysis, since its main thermal-dependent degradation pathway desamidation (asparagine residue giving aspartic acid residue by hydrolysis and loss of ammoniac), is readily visualized by the appearance of acidic peaks. SEC can identify chain scission and peptide mapping after reverse-phase HPLC separation of peptides formed by enzymatic treatment and reveals subtle modifications of the primary structure of proteins.

Therefore, the consensus group recommends that the chemical stability of antibodies must be assessed by a minimum of three separation methods, i.e. IEC, SEC and peptide-mapping, but complementary or alternative methods such as CE or MS can also be used.

Biological stability

Due to the particular structure of proteins and its activity/3D-structure relationship, the assessment of the

biological activity during stability studies could be useful as an ultimate test. Obviously, the most relevant method to test the pharmacological activity should be chosen. ELISA could be a useful method for monoclonal antibodies [49]. However, complementary test such as the determination of the cytotoxic activity on cell lines could be also used as, for example, in the case of rituximab. Nevertheless, the consensus group considers that the determination of the remaining pharmacological activity by a biological assay, albeit specific, is complementary of a full physicochemical analysis and should not be considered alone as a stability indicating method, taking in account its inherent analytical variability and its inability to detect low-level of DPs or aggregates which can induce serious anaphylactic reactions or renal failure [50–52].

Conclusion

All of the drugs used in modern medicine are licensed with very limited stability data which are insufficient to fulfil the new ways of drugs being handled in the 21st century clinical environment. As a consequence, there is an urgent need for additional data to support the pharmaceutical quality of these practices. Ideally, the drug development programs of pharmaceutical industry should generate enough stability data to allow for a more flexible clinical application, or would make available to the community of pharmacists data that have been generated beyond the official package insert. Unfortunately, the full access to the stability experiments furnished by manufacturers to registering authorities is not allowed, as for other data obtained during preclinical experiments or clinical trials. This paucity of suitable information is obviously detrimental to public health, as pointed out very recently by several authors [53]. Despite the paramount importance of relevant stability data for oncology pharmacists, faced with the centralized preparation of anticancer drugs exhibiting narrow therapeutic range, the access to useful and practically adapted information is not easy. Some databases such as *Trissel's Handbook on injectable drugs*, *King Guide to parenteral admixtures* [54] and the Infostab website, are invaluable [55] but published results are often dated, very heterogeneous in terms of quality and relevance. Indeed, until now, there is no consensual approach about the best protocols to evaluate the stability of anticancer drugs in practical situations. However, as long as such data are lacking, hospital pharmacists should take the responsibility to initiate systematic research programs to support their practical needs as pointed out by Vigneron [20,21]. It means that oncology pharmacy practitioners need to establish a wide range of validated assays to test the different ways to prepare and to store drugs for periods extending the stability limits indicated in package inserts or SPC.

Following a European consensus conference, a guideline on assessing the practical stability of anticancer drugs has been tentatively proposed in this paper to help direct the current clinical and pharmaceutical practice. Thus, we propose the establishment of a new drug stability paradigm issued from practical clinical needs, to complement regulatory guidelines essentially oriented on the stability of manufactured drugs, to allow safer, more flexible

centralized compounding and cost-effective care for our patients. Finally, the open discussions needed to establish these guidelines have encouraged us to identify new research fields in oncology pharmacy.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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