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Spectral and chromatographic characterisation of the onoceroid, ambrane: a potential novel biological marker

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ABSTRACT

The gas chromatography-mass spectrometry (GC-MS) characteristics, including GC retention indices and mass spectra, of isomers of the tricyclic onoceroid C₃₀ hydrocarbon, ambrane (1,1,4a,6-tetramethyl-5-{4-methyl-6-[2,2,6-trimethylcyclohexyl]hexyl}decahydronaphthalene) are reported. Ambrane is yet to be reported in sediments or petroleum: other biochemically-related onoceroids, such as onoceranes and serratanes, have been. Now that GC-MS data are available, a search for ambranes in geochemical samples may be both feasible and warranted.

Keywords: Ambrane; Onoceroids; Biological marker; Retention index

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1. Introduction

Onoceroids are triterpenes biosynthesised from squalene or oxidosqualene by cyclisation from both termini (Ueda et al., 2013; Shinozaki et al., 2016). They include compounds with ambrane (I; 1,1,4a,6-tetramethyl-5-{4-methyl-6-[2,2,6-trimethylcyclohexyl]hexyl}decahydronaphthalene), onocerane (II), serratane (III) and colysane (IV) skeletons (Figure 1).

The mass spectra of onoceranes and serratanes have long been known (e.g. Kimble, 1982; Wang et al., 1988). These alkanes have also subsequently been reported in a number of oils and sediments or rocks (e.g. Curiale, 1988; Fu et al., 1988; Wang et al., 1988; Pearson and Obaje, 1999; reviewed by Peters et al., 2007). So far as we are aware, ambrane (and colysane) have not. This may be because the gas chromatographic and mass spectral characteristics (the most commonly used criteria for identification of such biological marker hydrocarbons), have yet to be published for ambrane and colysane.

However, the geological preservation of onoceranes, the biosynthesis of the onoceroids by cyclase enzymes from bacteria (Ueda et al., 2013; Shinozaki et al., 2016) and the occurrence of numerous onoceroids, particularly in ferns are all known (e.g. reviewed by Murakami and Tanaka 1988). It would thus seem feasible that compounds such as ambranes eventually will be found in the geosphere.

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We therefore decided to synthesise ambrane and to obtain the mass spectra and GC retention indices of the synthesised isomers. Future identifications of ambrane in geological samples might provide information on geostatigraphy or be useful in source rock or oil-oil correlations.

There is precedent for such an approach. Synthesis and characterisation of the biological marker, crocetane (2,6,11,15-tetramethylhexadecane) by Robson, (1987), a compound which was then unknown as a biological marker, antedated by many years, the identification of crocetane as a molecular indicator for the anaerobic oxidation of methane and photic zone euxinia (e.g. Maslen et al., 2009, and references therein). Arguably the earlier acceptance for publication of analytical data for crocetane produced in 1986 and submitted for publication around that time, but only eventually published in 1993 (Robson and Rowland, 1993), may have led to earlier identification and use of crocetane by geochemists (e.g. Bian et al., 2001; Greenwood and Summons, 2003; Maslen et al., 2009; Spaak et al., 2016 and references therein).

2. Experimental

A sample of modern jetsam ambergris of unknown age was supplied from an unknown beach location in New Zealand. A sample of authentic ambrein (V) was provided by Ueda et al., (2013). A dichloromethane (DCM) soluble extract (10 mg mL⁻¹ in DCM) of the ambergris was transferred through a pre-extracted (DCM Soxhlet; 24 h) cotton wool plugged Pasteur pipette to a pre-rinsed (DCM)

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round bottom flask (50 mL). Solvent was removed under a gentle stream of nitrogen and the residue weighed (9.5 mg). Catalyst, platinum (IV) oxide (PtO₂; Sigma-Aldrich; 59.1 mg), solvent (n-hexane, 10 mL) and a Teflon flea were added to the flask and an adapter fitted. A cotton wool plugged Pasteur pipette was fed through the adapter until close to the base of the flask. The gap at the adapter connection was gently wrapped in foil and hydrogen gas gently bubbled through the liquid with stirring (3 h).

After cooling, the liquid phase (with 3 x washings) was transferred to a pre-weighed 7 mL vial through a pre-extracted (DCM Soxhlet; 24 h) cotton wool plugged Pasteur pipette. Solvent was removed (N₂/60°C) and the product weighed (9.4 mg; 99%). The products were reconstituted in n-hexane prior to analysis using GC-MS. The re-dried product, in a small aliquot of n-hexane, was separated on a silica column (30 mm fully activated pre-ashed silica in a cotton wool plugged Pasteur pipette) previously conditioned with n-hexane (2 mL) and eluted with n-hexane (2 mL; S1, 'aliphatic'; ~75%) and DCM (2 mL; S2, 'polar'; ~25%). Solvent was removed from collected fractions (N₂; 60°C) and extracts reconstituted in n-hexane (1 mL).

GC-MS analyses were carried out using an Agilent 7890A gas chromatograph with autosampler interfaced with an Agilent 5975C mass selective detector. Sample (1 µL) was injected in splitless mode (250°C) onto a Rx1®-1ms column (30 m x 0.25 mm x 0.25 µm; Thames Restek UK Ltd., UK) with helium as carrier gas (constant flow mode; 1 mL/min) and the oven programmed from 40 - 210°C at

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10°C/min and from 210 - 300°C at 5°C/min with a final 10 minute hold. The transfer line was maintained at 280°C, the ion source at 230°C and the quadrupole at 150°C. Ionisation was performed at 70 eV.

3. Results and discussion

GC-MS of a sample of jetsam (beached) ambergris from an unknown location in New Zealand, revealed one major component with a mass spectrum consistent with that published many times (e.g. Ueda et al., 2013) for the diunsaturated C₃₀ alcohol, ambrein (Figure 1, V). The compound also co-eluted (GC-MS) with a sample of authentic ambrein provided by Ueda et al., (2013). Attempted hydrogenation herein of an extract of the ambergris (essentially nearly pure V) to the corresponding saturated alcohol, (which trivially might be named 'ambranol'), unexpectedly also resulted in formation of isomers of an alkane. The latter were tentatively assigned from interpretation of mass spectra of three components of the total reaction mixture (Figure 2b), as isomers of ambrane (Figure 1, I), presumably formed by hydrogenolysis. Ambranol and some ambrenes were also present (Figure 2b). Elution of the total reaction products with hexane through a small column of silica, produced a fraction with the same three major peaks in the GC-MS chromatogram (Figure 2c), consistent with their assignment as alkanes, with retention indices, 2914, 2941 and 2946 on Rx1®-1ms stationary phase. Electron impact mass spectra obtained at 70eV are

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shown in Figure 3 and were characterised by molecular ions (m/z 416), ions attributed to loss of a methyl group from the latter (m/z 401) and a base peak ion in each case of m/z 123, attributed to the well-known fragmentation of terpenoid bicyclic alkanes. Whilst other minor differences in the spectra were observed, including minor ions at m/z 179, 271 and 303, (Figure 3), the above constituted the major diagnostic features and were interpreted as due to the formation of isomers at the original sites of unsaturation and hydroxylation in ambrein (V), from non-stereospecific hydrogenolysis and reduction.

The above features, including use of selected ion mass chromatography by GC-MS of m/z 123 and 416/401 at the appropriate retention positions (i.e. ca 2900-2950 RI), should allow a search for ambranes in petroleum and sediment extracts in future. Given the initial discovery of onoceranes in Miocene angiosperm leaf fossils of *Pseudofagus*, (Giannasi and Niklas, 1981) and the occurrence of ambrein in ambergris, a reasonable substrate to examine for the presence of ambrane might be the fossil ambergris *Ambergrisichnus alleronae* from the Early Pleistocene, Gelasian to Calabrian (1.95-1.55 Ma; Baldanza et al., 2013; Monaco et al., 2014). Such studies are underway.

4. Conclusions

The GC retention indices and mass spectra of isomers of the tricyclic onoceroid C_{30} hydrocarbon, ambrane (1,1,4a,6-tetramethyl-5-{4-methyl-6-[2,2,6-trimethylcyclohexyl]hexyl}decahydronaphthalene) are reported. Future studies may reveal whether ambrane is a novel biological marker.

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Figure Legends

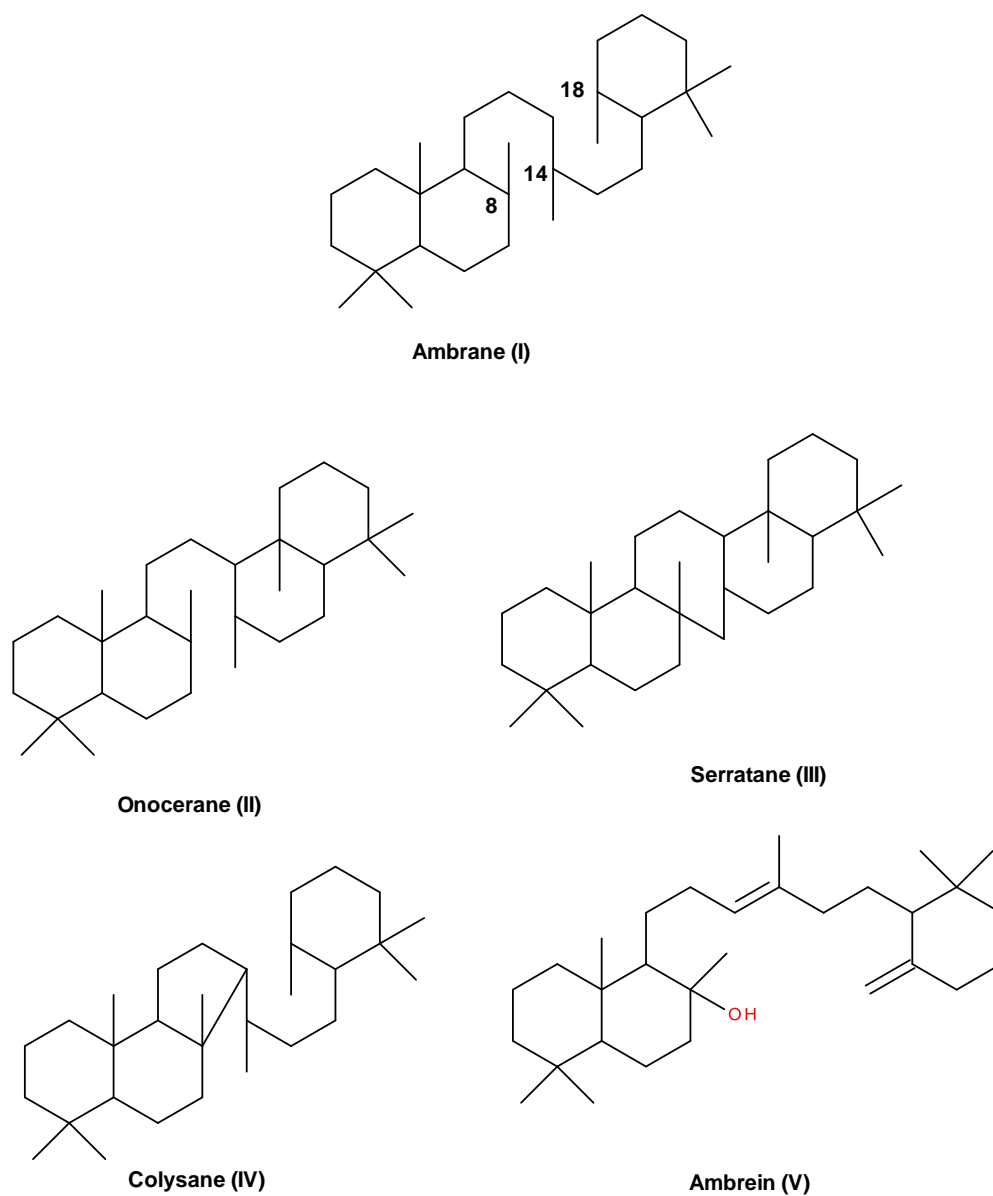
Figure 1 (I-V). Structures of ambrane and related onoceroids.

Figure 2 GC-MS total ion chromatograms of (a) mixture of reference n-alkanes (C₂₇₋₃₂) (b) total hydrogenation and hydrogenolysis products of ambrein (c) ambranes (A,B, C) isolated from the hydrogenolysis products of ambrein by column chromatography and elution with hexane (S1 fraction; ~75%) (d) ‘ambranol’ isolated from the hydrogenation products of ambrein by column chromatography and elution with dichloromethane (S2 fraction; ~25%)

Figure 3 Electron impact mass spectra of ambranes A-C isolated from the hydrogenolysis products of ambrein. Retention indices A 2914, B 2941 and C 2946 on Rx1®-1ms.

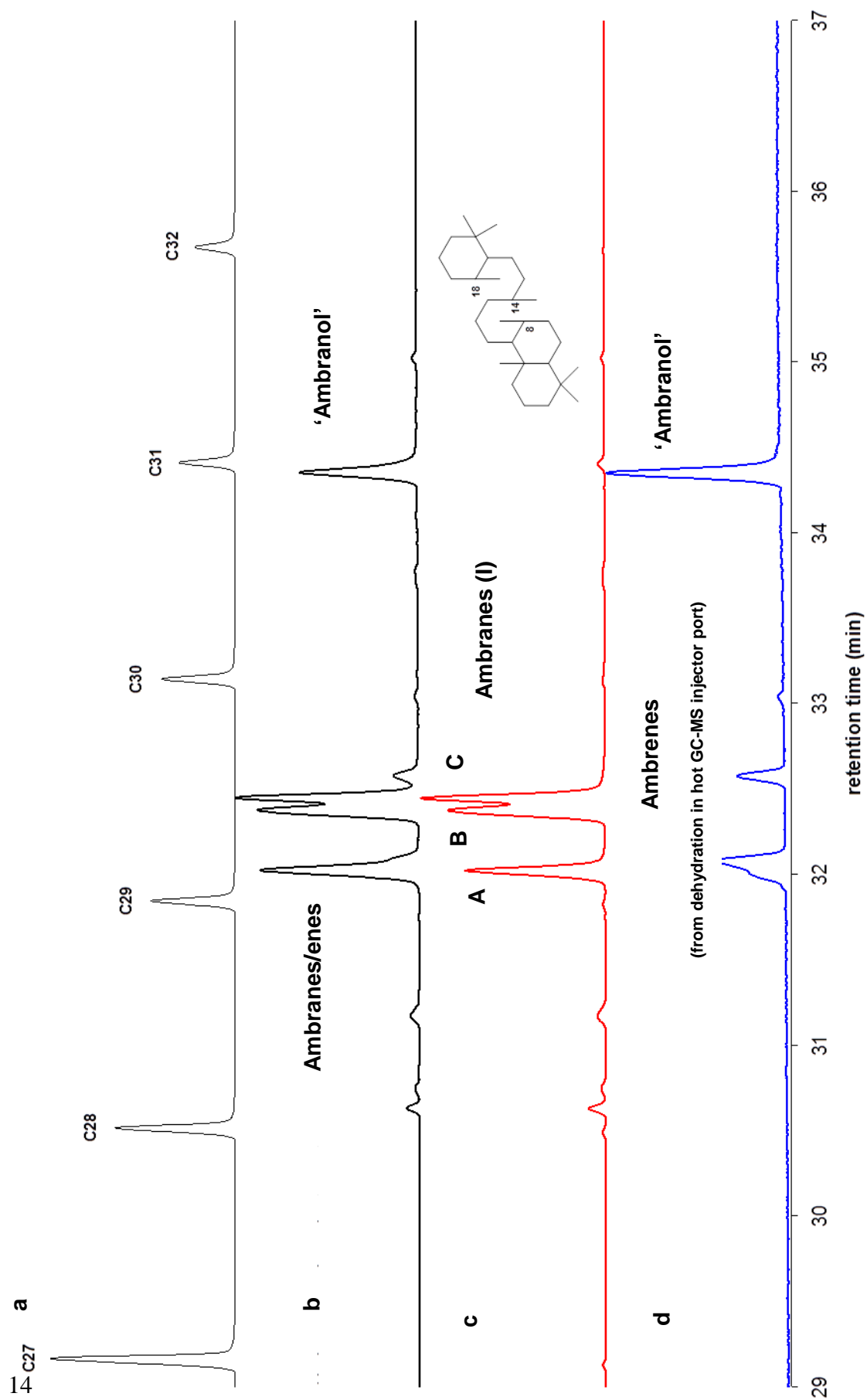
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Figure 1.



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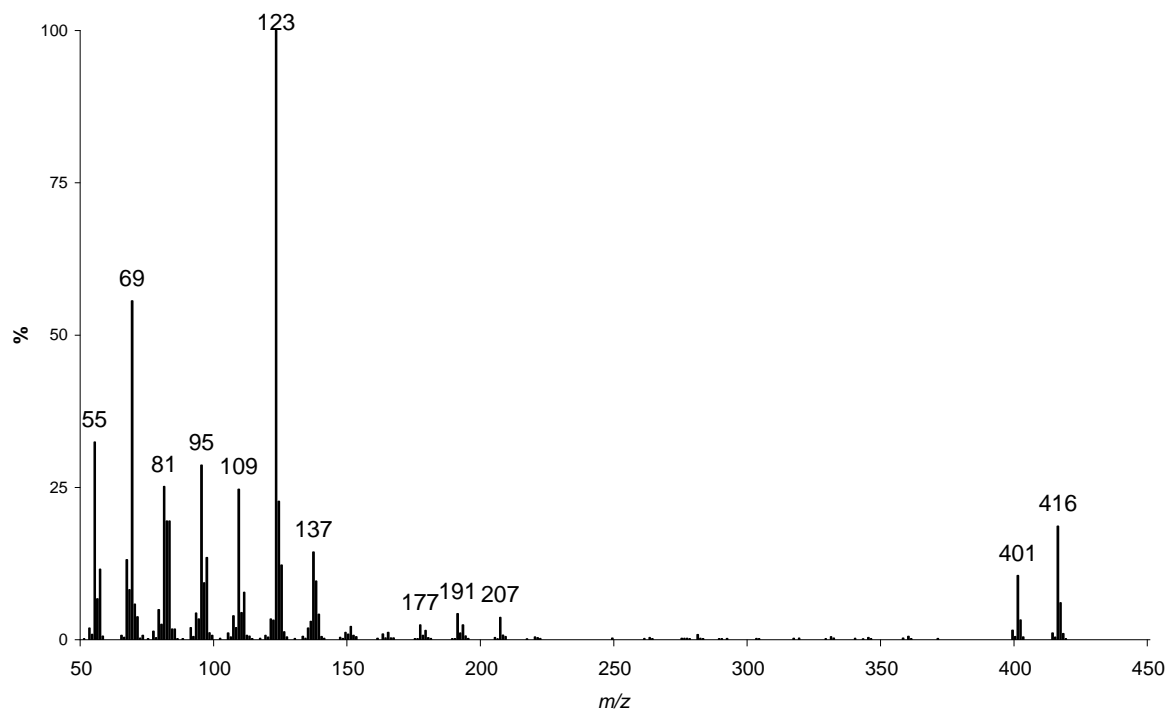
Figure 2.



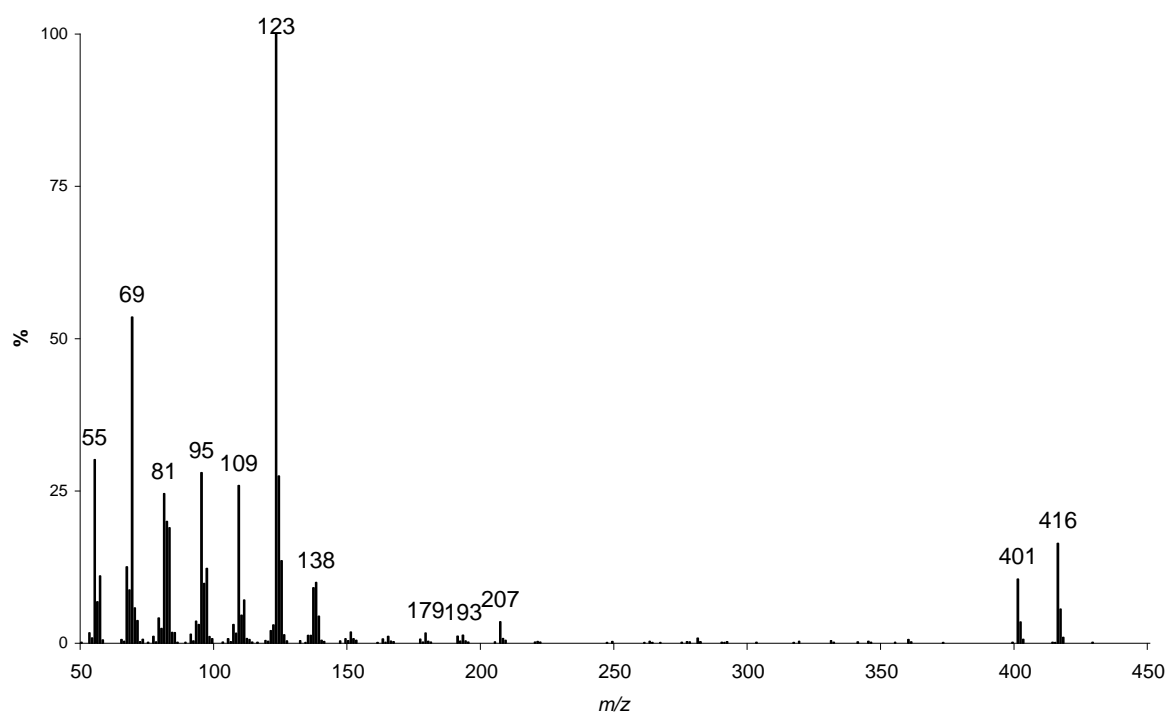
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Figure 3.

A



B



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C

