

Electrospray ionization mass spectrometry fingerprinting of whisky: immediate proof of origin and authenticity

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Authentic samples of whisky produced in Scotland and USA and counterfeit whisky samples commercialized in Brazil have been directly submitted to electrospray ionization mass spectrometry (ESI-MS) analysis in both the negative and positive ion modes to assess the potential of this technique for simple and rapid quality control and proof of authenticity of whisky samples. ESI in the negative ion mode yields the most characteristic whisky fingerprinting mass spectra in just a few seconds by direct infusion of the samples, detecting the most polar or acidic components of each sample in their deprotonated anionic forms. No pre-treatment of the sample, such as extraction or derivatization or even dilution, is required. The analysis of the ESI(–)-MS data both by simple visual inspection but more particularly by chemometric data treatment enables separation of the whisky samples into three unequivocally distinct groups: Scotch, American and counterfeit whisky, whereas single malt and blended Scotch whiskies are also distinguished to some extent. As indicated by ESI-MS/MS analysis, the diagnostic anions are simple sugars, disaccharides and phenolic compounds. Direct infusion ESI-MS therefore provides immediate chemical fingerprinting of whisky samples for type, origin and quality control, as demonstrated herein for American, Scottish and counterfeit samples, whereas ESI-MS/MS analysis of diagnostic ions adds a second dimension of fingerprinting characterization when improved selectivity is desired.

Introduction

Scotch whisky is one of the UK's major export commodities, having a value of US\$4 billion in 2003 according to the Scotch Whisky Association.¹ Other variants are manufactured around the world, such as in Ireland, USA, Canada and Japan.^{2,3}

In general, whisky is considered a luxury "food product" of high quality, partly owing to the traditional way of distillation and to the prolonged period of production and maturation. Therefore, whisky belongs to a high value-added segment of the market of alcoholic beverages, which is prone to counterfeiting and other manipulations aimed at imitating or copying authentic whiskies. The nature and origins of flavours in various types of whisky are very complex interactions of several steps in the manufacturing process, and sensory tools such as the flavour wheel have been developed to meet the demands of the distillers and the product.⁴

Over the years the unique character of whiskies and the changes occurring during maturation have been studied.⁵ The complex chemical composition of whisky has also been investigated, *e.g.* organic acids and esters,^{6,7} mineral content,⁸ phenolics⁹ and lactones¹⁰ and characteristic low molecular weight fusel alcohols and derived compounds.^{11,12} Various attempts have also been made to develop methods for analysis and quality control of different types of whisky.^{13,14}

Proof of origin and authenticity are major concerns for the distillers, dealers and consumers of genuine whiskies around the world. Attempts have been therefore made to develop standards or methods for quality control and proof of origin

and authenticity. The use of sensory profiling and other organoleptic testing has been valuable to test authenticity, but such methods require a high degree of training.¹⁵ Analysis of copper and other trace elements has been suggested as an indicator of authenticity for Scotch whiskies,¹⁶ whereas the distribution of methyl-branched alcohols (fusel oils) has been found to distinguish Irish whiskies from other variants.¹⁷ The isotopic ratio of ¹³C and ¹²C,¹⁸ and the ratio of furfural and 5-hydroxymethyl-2-furaldehyde have also been proposed as whisky markers.¹⁹

Various mass spectrometric (MS) techniques have also been used to analyse and characterize volatiles in whisky.^{20,21} A MS study applied pyrolysis followed by MS to analyse the effect and quality of oak casks used for maturation of Scotch whisky.²²

ESI-MS with direct infusion of samples has recently been shown to be an efficient, sensitive, and fast "MS-only" technique able to screen the composition of the most polar, acidic or basic components of complex mixtures such as beer,²³ wine,²⁴ and bee propolis.^{25,26} Herein we present results from direct infusion ESI-MS analysis of whisky samples and demonstrate its suitability for quality control and proof of authenticity of whiskies.

Experimental

Whisky samples

A total of fifteen whisky samples were obtained from reliable sources. These include Scotch, American and presumably counterfeit whiskies commercialized in Brazil. Table 1 displays an outline of the type and number of each variant used.

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Table 1 Outline of the types of whisky samples

Type/origin	No. of samples
Scotch:	
Blended	7
Single malt	4
American	2
Counterfeit ^a	2
Total	15

^a Low-price whisky samples commercialized in Brazil.

General experimental procedures

A Q-TOF mass spectrometer (Micromass, Manchester, UK) was used for fingerprinting ESI-MS analysis. The general conditions were: source temperature of 80 °C, capillary voltage of 2.1 kV and cone voltage of 40 V. For ESI(+)-MS analysis, 0.5 µl of formic acid was added to the whisky sample (500 µl) to yield 0.1% as the final concentration. Likewise, for ESI(-)-MS analysis, ammonium hydroxide was added to a concentration of 0.1%. ESI-MS was performed by direct infusion with a flow rate of 10 µl min⁻¹ using a syringe pump (Harvard Apparatus). Mass spectra were acquired and accumulated over 60 s (much shorter accumulation times can also be used for high-throughput screening) and spectra were scanned over the 50 to 3000 *m/z* range.

ESI-MS/MS was performed by mass-selecting the ion of interest using the first quadrupole Q1, which was in turn subjected to 15–55 eV collisions with argon in the second rf-only collision quadrupole (Q2) while scanning the orthogonal TOF mass analyser to acquire its tandem mass spectrum. The collision gas pressure was optimized to produce extensive dissociation with minimal loss of ion current.

Data handling

All data obtained from ESI-MS of the various whisky samples were extracted using MassLynx 3.5 (Waters, Manchester, UK). Mass spectral data was accumulated over 60 s, centered and aligned to generate a final data matrix of 15 samples and 290 mass signals (variables) ranging from *m/z* 80 to 550 (a range that contained all ions of interest as judged by visual inspection), while the recorded intensities ranged from 5 000 to 820 000. To classify the whisky samples, a discriminant partial least squares (D-PLS) regression was performed in Unscrambler v. 8.0 (CAMO Process A/S, Oslo, Norway) using MS data as X and a dummy matrix of known classes as Y. As pretreatment, data in the X-matrix were mean centered and normalized prior to analysis.

Results

Although ESI(+)-MS is also able to distinguish the three types of whisky samples unambiguously, ESI(-)-MS produces by far the most characteristic mass spectra for whisky fingerprinting; hence only the ESI(-)-MS data will be presented and discussed.

Mass spectra in the negative ion mode

Fig. 1 exhibits ESI(-)-MS spectra of three samples of Scotch whisky. Such spectra are very characteristic and similar owing to nearly equal composition of the most polar or acidic components of each sample. Some small and important variations are observed, however, which likely result from particular Scotch whisky variants, *e.g.* single malt *vs.* blended or degree of peaty character. Clearly, a number of diagnostic anions, such as those of *m/z* 143, 171 and 199 are always

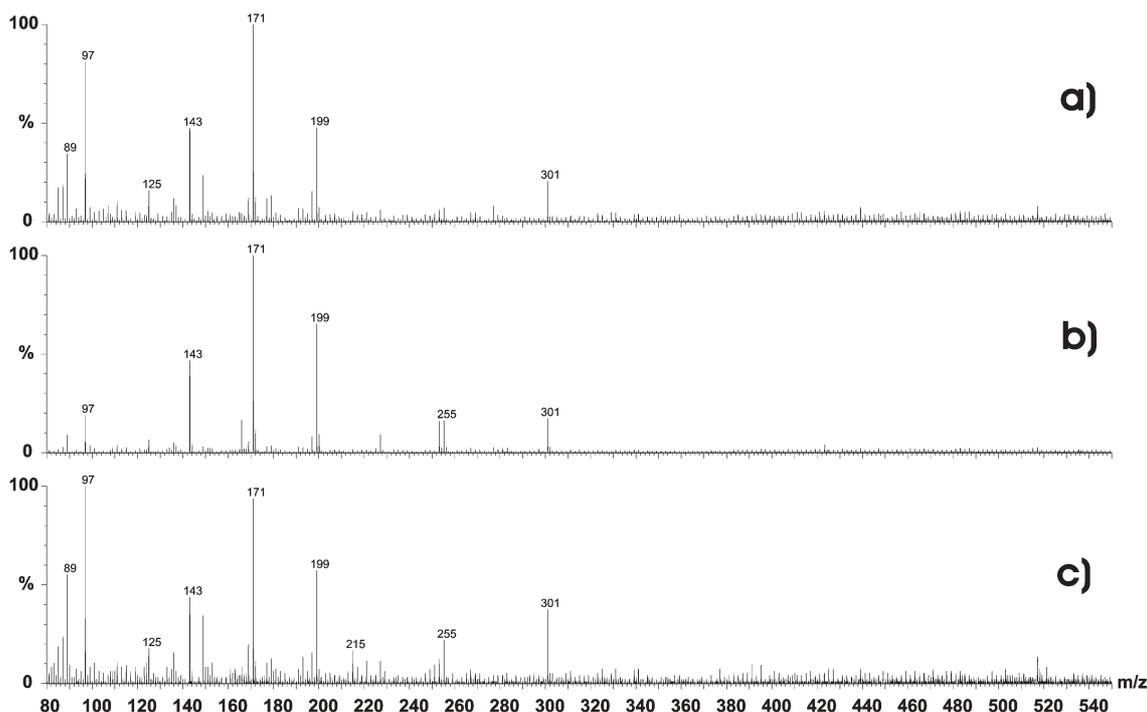


Fig. 1 (a)–(c) ESI(-)-MS for three Scotch whisky samples: (a) blended, and (b/c) single malts.

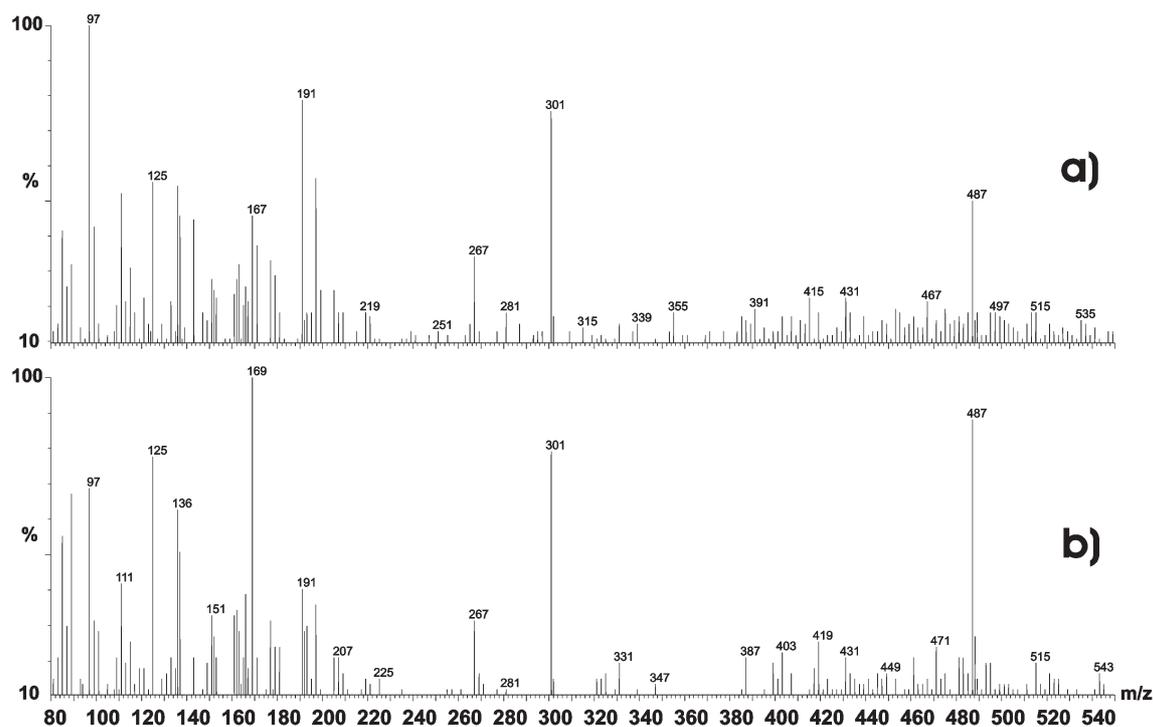


Fig. 2 (a)–(b) ESI(–)-MS of two samples of American whisky.

detected as major ions and this set appears therefore to constitute the three most characteristic diagnostic anions for ESI(–)-MS fingerprinting identification of Scotch whisky.

Fig. 2 exhibits ESI(–)-MS spectra of two samples of American whisky. Some anions with relatively higher abundances appear to be diagnostic for American whisky such as those of 136, 169, 191, and 267, as well as a great number of not so abundant but heavier anions of m/z around 300–450. But by far the most characteristic ion for the American whisky is clearly that of m/z 487. The anion of m/z 136 indicates a N-containing compound of 137 Da, and the origin of this is most likely the cereal source used in the fermentation since the N-content of wood is known to be very low.

Fig. 3 exhibits ESI(–)-MS spectra of two counterfeit whisky samples. Although similarities, mainly with Scotch whisky, regarding diagnostic anions are observed, it is clear that a variety of diagnostic anions such as those of m/z 179, 341, 377, 387, 405, 439, 485, 503, 529 and 549 clearly distinguish the counterfeit whisky samples.

To facilitate comparison, Fig. 4 shows characteristic ESI(–)-MS of a single sample of each of the three major types of whisky investigated herein. It is evident that Scotch (Fig. 4a), American (Fig. 4b) and counterfeit (Fig. 4c) whisky samples can be easily differentiated. For the Scotch whisky, the three major diagnostic anions are those of m/z 143, 171, and 199. The American whisky is clearly characterized mainly by a single diagnostic anion of m/z 487. The counterfeit whisky shares the same set of three diagnostic ions as those of the Scotch whisky (m/z 143, 171, and 199) but many other diagnostic ions are seen. Note that, interestingly, two such anions of m/z 179 and 341 correspond to the

deprotonated molecules $[M - H]^-$ of simple sugars, likely of a monosaccharide such as glucose (180 Da) and a disaccharide such as sucrose (342 Da), respectively.

MS/MS

To improve selectivity by offering an additional MS dimension of whisky fingerprinting and to have, at the same time, an indication of their chemical natures, we performed ESI-MS/MS analysis of the most diagnostic anions. Anions of the same m/z values detected in different whisky samples were selected to investigate whether they arise from ionization of the same components. Fig. 5a and 5b exhibit the ESI(–) tandem mass spectra for the anion of m/z 179 present in counterfeit and American whiskies, respectively. Similar fragmentation patterns characteristic of a hexose (glucose for instance) for both samples are observed, and thereby prove that they have identical or at least isomeric structures. A series of fragment ions arising from water loss (18 Da) is observed, which is typical for $[M - H]^-$ ions of sugars.²⁷ Fig. 5c and 5d display ESI-MS/MS for the diagnostic anion of m/z 487 from two samples of American whiskies. The spectra are nearly identical indicating the same origin and that ESI(–)-MS/MS of the diagnostic anion of m/z 487 provides enhanced and highly selective identification of American whisky.

Fig. 6 presents ESI-MS/MS of the anion of m/z 143 for both Scotch and counterfeit whiskies. For the anion from the counterfeit sample, little dissociation occurs even when considerably increasing the collision energy up to 55 eV. In great contrast, the ion of m/z 143 from Scotch whisky dissociates extensively therefore indicating different chemical compositions for both mass-selected anions.

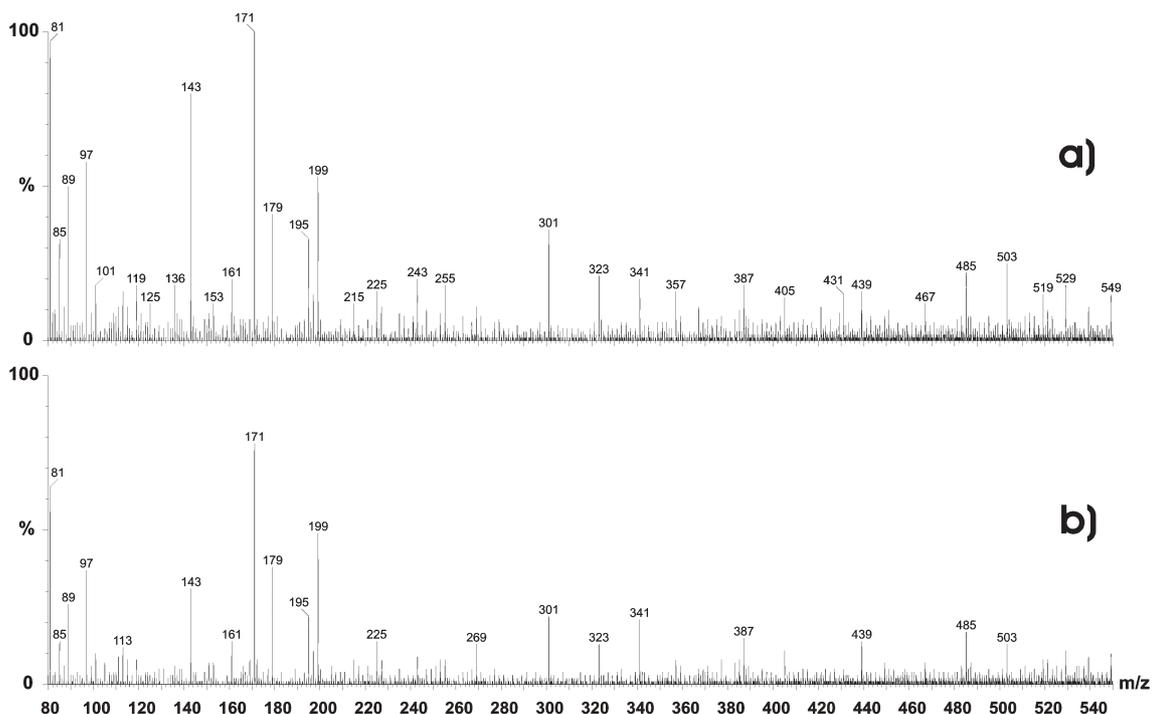


Fig. 3 (a)–(b) ESI(–)-MS of two samples of counterfeit whisky commercialized in Brazil.

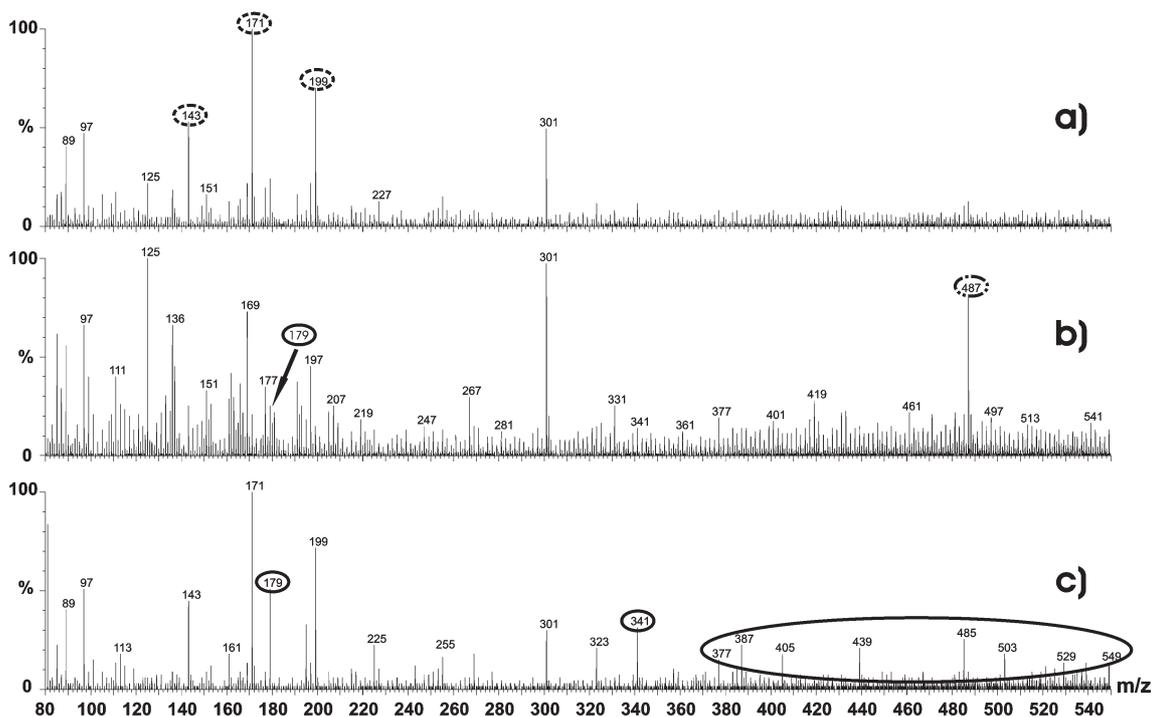


Fig. 4 (a)–(c) Typical ESI(–)-MS of (a) Scotch, (b) American and (c) counterfeit whisky samples. Major diagnostic anions are highlighted with circles.

Multivariate data analysis

Fig. 7 shows results from a D-PLS regression performed with whisky classes *versus* m/z ratios of anions in the range from 100 to 550. A PLS regression model with 4 principal components is

found to be optimal, as principal components 1 and 2 describe 74% and 4% of the total variation in the X-data, respectively, whereas 23% and 24% can be accounted for in the Y-data structure. The algorithm for PLS regression was originally presented four decades ago,²⁸ and the technique has been

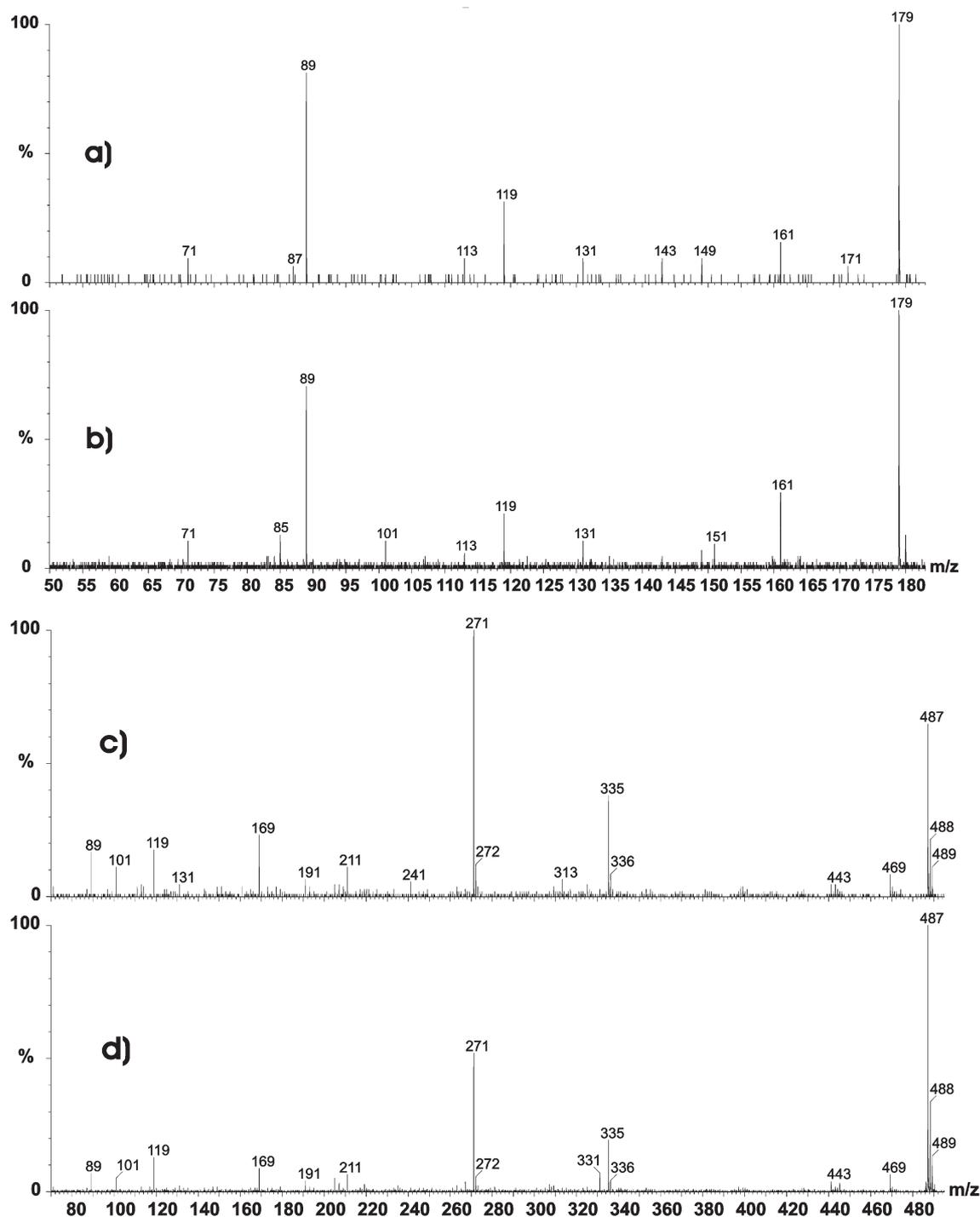


Fig. 5 (a)–(d) ESI(–)-MS/MS of diagnostic anions from different whisky samples. Anions of m/z 179 from (a) counterfeit whisky and (b) from American whisky. Anions of m/z 487 (c and d) from two American whisky samples.

further developed and refined for various types of multivariate data.²⁹ The developments in chemometrics in analytical chemistry have been the subject of many reviews.³⁰ The sample scores in Fig. 7a exhibit separate grouping for Scotch, American, and counterfeit whiskies, whereas the Scotch variants of blended or single malt whiskies could also be differentiated except only for a single sample of blended malt. In Fig. 7b, the m/z ratios of anions responsible for the

grouping of samples can be observed in a plot of the correlation loading in which sample classes are also indicated. The closer the variables are located to the outer circular line (–1; 1) the higher percentage of correctly modelled variance they explain. It can also be seen that counterfeit samples correlate well with the anions of m/z 179 and 255, whereas American samples are related to numerous components making it difficult to pin-point single m/z combinations with

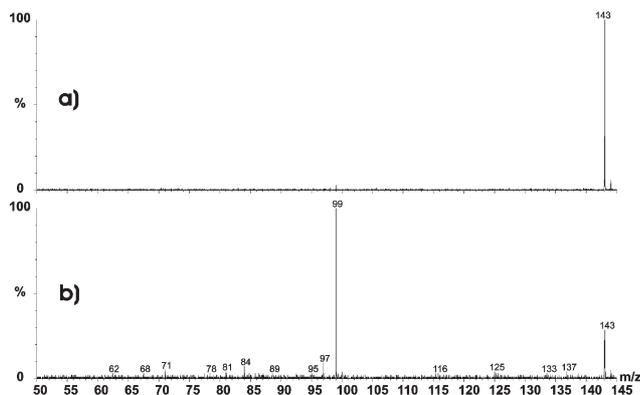


Fig. 6 (a)–(b) ESI(–)-MS/MS of the anion of m/z 143 from (a) counterfeit and (b) Scotch whisky samples.

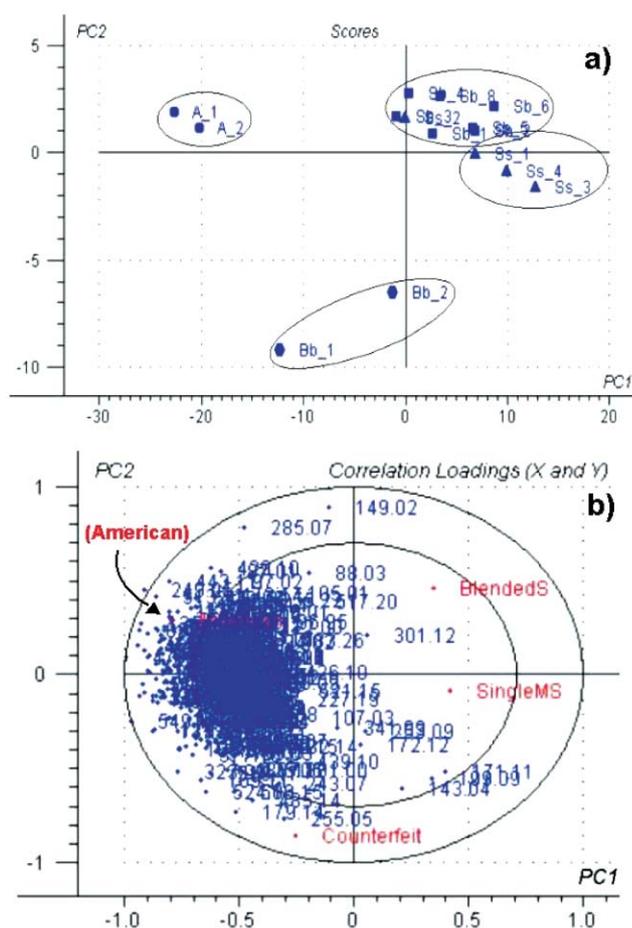


Fig. 7 (a)–(b) D-PLS regression analysis of ESI(–)-MS data in relation to the three types of whisky investigated. Plot (a) shows scores for principal components 1 and 2 for samples with names: A_{1/2} = American whisky, Bb_{1/2} = counterfeit whisky, Ss_{1–4} = Scotch single malt and Sb_{1–8} = Scotch blended whisky. Plot (b) shows correlation loadings for principal components 1 and 2 with m/z values and whisky classes; BlendedS: blended Scotch; SingleMS: single malt Scotch; location of American indicated with arrow.

higher degrees of significance than others. In contrast, the two kinds of Scotch whisky, blended or single malt, are characterized by being negatively correlated to these numerous ions of

specific m/z values. However, compounds located in the right half of the correlation loadings plot are positively correlated to the Scotch whiskeys, but a single component cannot fully account for the MS fingerprints of these samples. Nevertheless, the trio of compounds having anions of m/z 143, 171 and 199, respectively, all have some degree of positive correlation to Scotch whiskeys and mostly to single malts as well as counterfeit samples.

Discussion

The present results show that direct insertion ESI-MS analysis in the negative ion mode produces unique fingerprinting mass spectra with diagnostic anions which enable precise identification of Scotch or American whiskeys. Counterfeit whisky, as exemplified herein by samples commercialized in Brazil, are also easily characterized particularly for diagnostic anions arising from deprotonation of simple sugars. A second MS dimension of fingerprinting selectivity is obtained by acquiring ESI tandem mass spectra of diagnostic anions. Therefore, direct infusion ESI(–)-MS constitutes a rapid and reliable technique to typify whiskeys, to probe authenticity, and to control its quality and maturation particularly at distilleries. These aspects will be discussed briefly in the following section.

Chemical characteristics of whisky

The effect of cask maturation may differ considerably for Scotch whisky and American bourbon as varying regulations are applied.³¹ This effect means that casks used for Scotch whisky gradually will reduce the amount of extractables and thus affect the flavour and character of the spirit. This circumstance may explain why heavier polar compounds in particular produced the diagnostic anion (m/z 487) for American whisky in comparison with Scotch whisky.

Although visual inspection of the ESI-MS data seems to be highly effective, this may be regarded as a subjective classification approach. Hence, multivariate analysis can be applied to provide statistical separation of different types of whiskeys. The American whisky is strongly correlated to a high number of negative anions, whereas anions of m/z 485, 255 and 179 are well correlated with counterfeit whisky. Furthermore, it is clear that the three anions of m/z 143, 171 and 199 are all characteristic for Scotch whisky but they are also present in counterfeit whisky. The ion of m/z 301 is present in all samples investigated and is therefore located in the centre of the chemometric plot shown in Fig. 7b.

The component producing the negative anion of m/z 301 in all samples may be a polyphenolic component such as ellagic acid (302 Da). The presence of such an acid has previously been identified in various distilled spirits³² and this may account for its detection in the counterfeit samples. Ellagic acid can act as a strong radical scavenger and as an effective antioxidant in alcoholic drinks,³³ protecting the stomach against oxidative stress.^{34,35}

The appreciable amount of mono- and disaccharides found in the counterfeit whisky samples as revealed by the ions of m/z 179 and 341 indicates the use of caramel to adjust colour and flavour. Significant amounts of monosaccharides have previously been identified in both whiskeys of low quality and in

American bourbon.³⁶ Monosaccharides such as mannose and xylose have been proposed to originate from the new casks used in the maturation of American bourbon. Monosaccharides may also be present in genuine blended and malt whisky, but at low concentrations, owing to gradual release due to acid-catalysed hydrolysis of tannin from the wood. The monosaccharide amount is an indication of the end of the required 3 year maturation period for genuine Scotch whiskies.³⁷ Thus, rapid and sensitive identification of traces of monosaccharides by ESI(-)-MS seems also to be a powerful approach to monitor maturation stages of whisky.

ESI-MS and whisky classification

As already mentioned, over the years many methods have been proposed to control quality or to classify whiskies.^{15–22} We envisage that ESI-MS and ESI-MS/MS particularly in the negative ion mode are powerful techniques for quality control and for rapid, sensitive and selective investigation of many attributes of various whisky types.

Previous analytical methods for authenticity analysis have used higher-alcohol profiles for specific brands of Scotch whisky, and this has been found to be very consistent over many production batches. Therefore, gas chromatographic (GC) determination of such alcohols together with the presence of other specific congeners offers an effective but more laborious and time consuming approach to authenticity analysis.³⁸ In addition, pyrolysis-MS combined with multivariate analysis of the resulting mass spectra enable non-authentic samples to be discriminated from the authentic product. Current standards of authenticity control in the industry of blended Scotch whisky is built around GC and is applied to analyse and quantify volatile congeners. Recently, a rapid but not so selective portable method (for detecting the presence of peat or wood derived phenolic compounds and/or colourant added to adjust final colour intensity) was proposed for pin-pointing suspect whisky samples, based on UV/visible absorbance.³⁹ Hence, direct ESI(-)-MS(/MS) fingerprinting of whisky samples nicely adds to the sophisticated portfolio of analytical strategies available to probe whisky authenticity and quality control, offering clear advantages as a rapid, simple, direct and highly selective alternative. Whereas the consistency of the ESI-MS method herein introduced is yet to be verified over many production batches, the present results point unequivocally towards superior performance.

The use of GC, GC-MS, and UV/visible spectrophotometry for identifying the falsification of strong alcoholic beverages (vodka, gin, cognac and whisky) has also been considered, and these techniques in combination may be able to detect markers or impurities present in a certain ratio. Furthermore, this multicomponent analysis applied to cognacs and related liquors can reveal alcohol produced from non-grape raw materials or determine whether the cognac spirit has been in contact with oak wood, plus how long this aging has lasted.²¹ In this context, the application of fast ESI-MS fingerprinting together with more elaborate GC(-MS) analysis will provide a wider picture of the components present in the whisky as both the low molecular weight volatile compounds (by GC-MS) and the more polar or acidic components (by ESI-MS/MS) will be

identified. This combination will certainly enhance counterfeit detection. Additionally, we are confident that ESI-MS can provide a general fingerprinting method applicable not only to whisky but to many other fine alcoholic drinks that are subject to falsification, *e.g.* cognac and other liquors of high quality such as cachaça. This generality is currently under investigation in our laboratory.

Conclusion

ESI-MS(/MS) fingerprinting provides a direct, simple, rapid, sensitive, and highly selective method for origin and authenticity certification of whisky samples. It is also likely to be useful for product quality control and profile characterization particularly at distilleries. Another application with major benefits would certainly be demonstrated if ESI-MS profiles are found to correlate well with sensory profiles of samples of variable qualities.⁴⁰

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