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Electrospray ionization mass spectrometry fingerprinting of perfumes: rapid classification and counterfeit detection

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A fast procedure to classify perfumes and identify counterfeit samples is described. Dilution of a few μL of the sample in a 1:1 methanol/water solution is followed by detection of its major polar components via direct infusion electrospray ionization mass spectrometry (ESI-MS) in the positive ion mode. As proof-of-principle cases, three famous brands of perfumes were used. The ESI(+)-MS fingerprints of authentic samples were very characteristic, showing distinctive sets of polar markers for each sample. Principal component analysis (PCA) placed samples of the three perfume brands in well-defined groups. Counterfeit samples were also clearly detected owing to contrasting ESI-MS fingerprints, with PCA placing these samples far away from the authentic samples. Copyright © 2006 John Wiley & Sons, Ltd.

Forgery of perfumes is very prejudicial both to businesses and to consumers. Because the perfume market is profitable and has been growing steadily, the commercialization of counterfeit fragrances around the world is increasing at a fast rate. Perfume forgery can also pose health problems to users since counterfeit perfumes escape inspection and may contain dangerous chemicals.[1] Efficient analytical techniques are therefore needed to control this illegal, hazardous and widespread activity. For analyses aimed at identification and quantification of all major components of perfumes, gas chromatography/mass spectrometry (GC/MS) has been preferentially used.[1–4]

Our objective in this work was to develop a method capable of detecting perfume forgery without resorting to broad chemical composition analysis or to volatile compound analysis (which is the most common approach),[5] but by using sets of diagnostic components that could distinguish authentic samples from the many counterfeit preparations. For that task, we used direct infusion electrospray ionization mass spectrometry (ESI-MS).[6] This technique is best applied to polar molecules and it requires no chemical derivatization, extraction, or chromatographic separation. Therefore, direct infusion ESI-MS has been applied with success as a fast fingerprint method able to classify and to detect adulteration and aging for samples

such as bee propolis,[7] beer,[8] whisky,[9] wine,[10,11] spices,[12] gasoline,[13] crude oils,[14] vegetable oils,[15] and soybeans.[16]

We report herein our investigation of the suitability of direct infusion ESI-MS for the fast and reliable fingerprint classification and counterfeit detection of perfumes.[17]

EXPERIMENTAL

Chemical reagents and samples
All reagents used were of analytical grade. Thirty-five samples of Eternity by Calvin Klein, Polo Sport by Ralph Lauren and Gabriela Sabatini perfumes, including authentic, counterfeit and 'inspired' samples, were analyzed.

### General experimental procedures
ESI mass spectra were acquired in the positive ion mode[a] on a Q-TOF mass spectrometer (Micromass, Manchester, UK). Typical ESI-MS conditions were: source temperature 100°C, desolvation temperature 100°C, capillary voltage +3.0 kV and cone voltage +40 V. ESI-MS/MS product ion spectra of selected precursor ions were acquired by low-energy (10–20 eV) collision-induced dissociation (CID). The perfume samples (1.0 μL) were homogenized in a flask with a solution containing equal parts of water and methanol, giving a final volume of 1.0 mL. Formic acid (1% v/v) was added to each

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[a]ESI-MS in the negative ion mode was also tested, but it provided inferior results.

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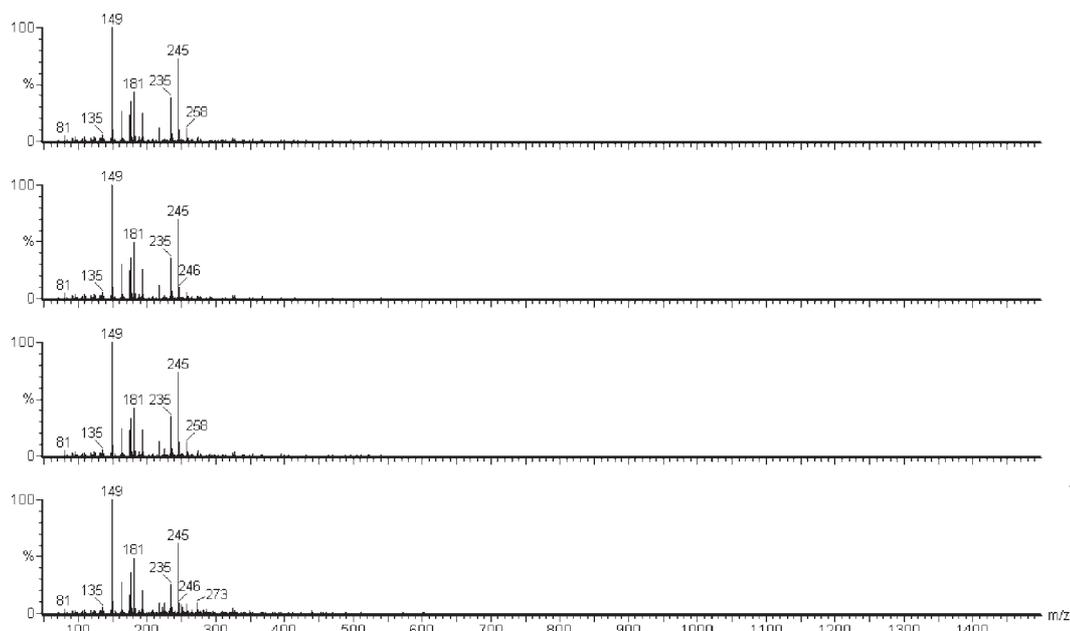
Figure 1. ESI-MS fingerprints in the positive ion mode of methanol/water (1:1) solutions of four different authentic Eternity perfumes.

sample for ESI(+)-MS analysis. These solutions were then injected into the ESI source at a flow rate of 10 μL min^{-1} using a syringe pump (Harvard Apparatus, Holliston, MA, USA) and mass spectra were acquired over the m/z 50–1500 range.

Data handling

All data obtained from ESI-MS fingerprints of the perfume samples were extracted using MassLynx 3.5 (Waters, Manchester, UK). Mass spectrometric data were accumulated over 60 s, centered and aligned to generate a final data matrix of 12 samples and 41 m/z values (variables) ranging from m/z 90 to 930 (for the Eternity samples), 12 samples and 57 m/z values (variables) ranging from m/z 90 to 930 (Gabriela

Sabatini), and 10 samples and 39 m/z values ranging from m/z 100 to 815 (Polo Sport). To classify the perfume samples after ESI-MS fingerprinting, principal component analysis (PCA) was performed on the ESI-MS data using the Statistica v. 6.0 program.

RESULTS AND DISCUSSION

Figure 1 shows the ESI(+)-MS fingerprinting of authentic perfume samples of the Eternity brand. The samples were obtained from known stores, from different batches, and contained the seal of authenticity provided by the producers. It is evident that all the ESI-MS spectra of the Eternity
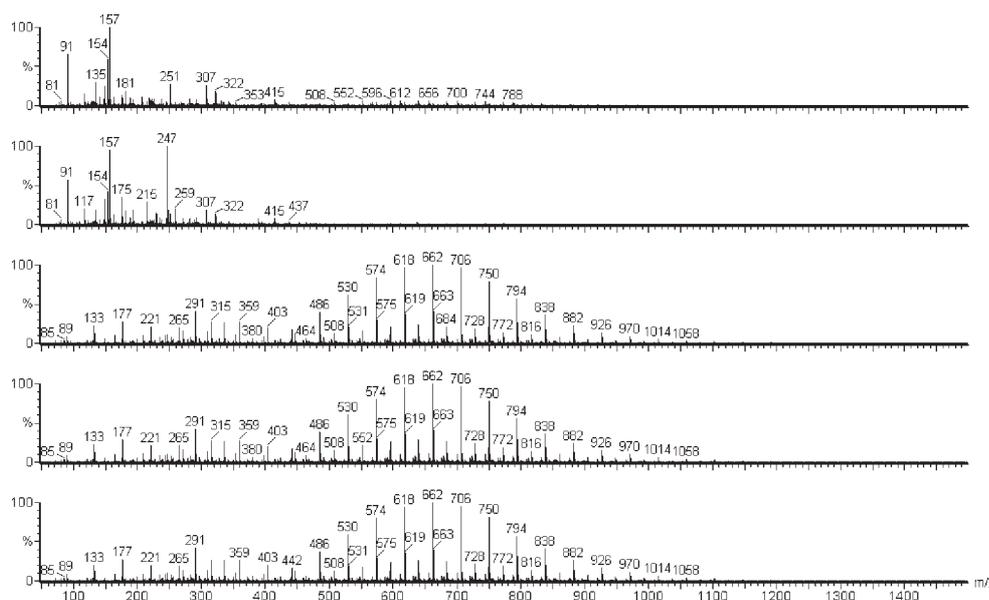
Figure 2. ESI-MS fingerprints in the positive ion mode of methanol/water (1:1) solution of five different counterfeit Eternity samples.
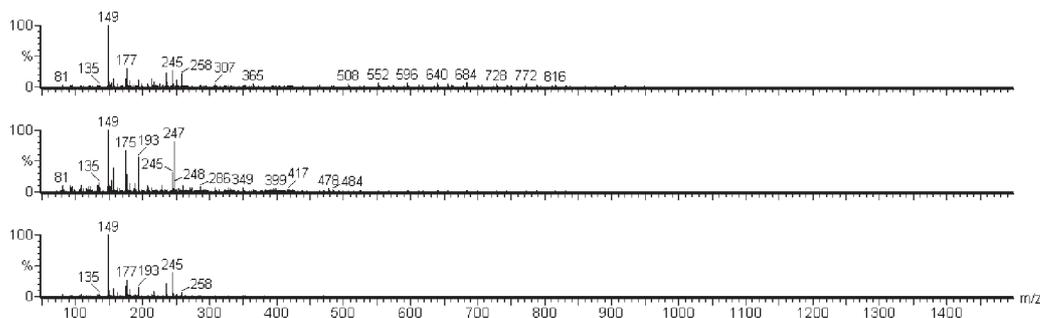
Figure 3. ESI-MS fingerprints in the positive ion mode of methanol/water (1:1) solutions of three different 'inspired' Eternity samples.

samples display the same set of characteristic polar compounds. In these spectra, the major ions are m/z 149, 181, 235 and 245. These ions are probably the protonated molecules of the minor components of the perfume mixture, providing therefore interesting diagnostic ESI-fingerprinting ions that will be difficult to imitate for this brand of perfume. The ESI-MS/MS product ion spectra for these ions were collected (not shown) for comparison with data from reference compounds, since these spectra could be used to provide even more selective perfume classification via ESI-MS/MS fingerprinting with structural assignment of the diagnostic ions. The exact knowledge of their chemical composition is, however, not relevant for fast ESI-MS fingerprinting classification.

Figure 2 shows the ESI(+)-MS fingerprinting of counterfeit samples of Eternity. These samples were purchased from the informal market at much lower prices and fail to show the seal of authenticity present on the authentic samples. The polar compounds present in these samples, as identified by their ESI-MS protonated molecules, are quite different from each other, indicating the various components used in these illegal formulations. Also very evident in some spectra is an oligomeric distribution of ions separated by 44 m/z units,

characteristic of a polyethoxylated polymer, probably polyethylene glycol (PEG), used as an emulsifier for the low-quality perfume preparations.

Figure 3 shows the ESI(+)-MS fingerprints of perfume samples sold as fragrances inspired by the Eternity brand. Note that these 'inspired' perfumes cannot be classified as counterfeits since their labels do not claim the samples to be authentic but the salesperson will indicate that the aroma composition is inspired by a famous brand, that is, it tries to imitate the fragrances of a specific brand. Often, the 'inspired' perfumes do display aromas nearly undistinguishable (by the non-expert) from the authentic samples. The ESI-MS fingerprints of these samples (Fig. 3) are indeed close to those of the authentic samples but major differences can certainly be noticed, and these differences allow unequivocal distinction. Note in Fig. 3 what is probably a series of PEG ions separated by 44 m/z units (m/z 508–816).

Figure 4 shows, as examples, the ESI(+)-MS fingerprints of one authentic, one counterfeit and one 'inspired' perfume sample of the Gabriela Sabatini brand. For authentic samples, the major ions are m/z 225, 229, 247 and 299. For the counterfeit sample, a different set of diagnostic ions is detected. For the 'inspired' Gabriela Sabatini perfume
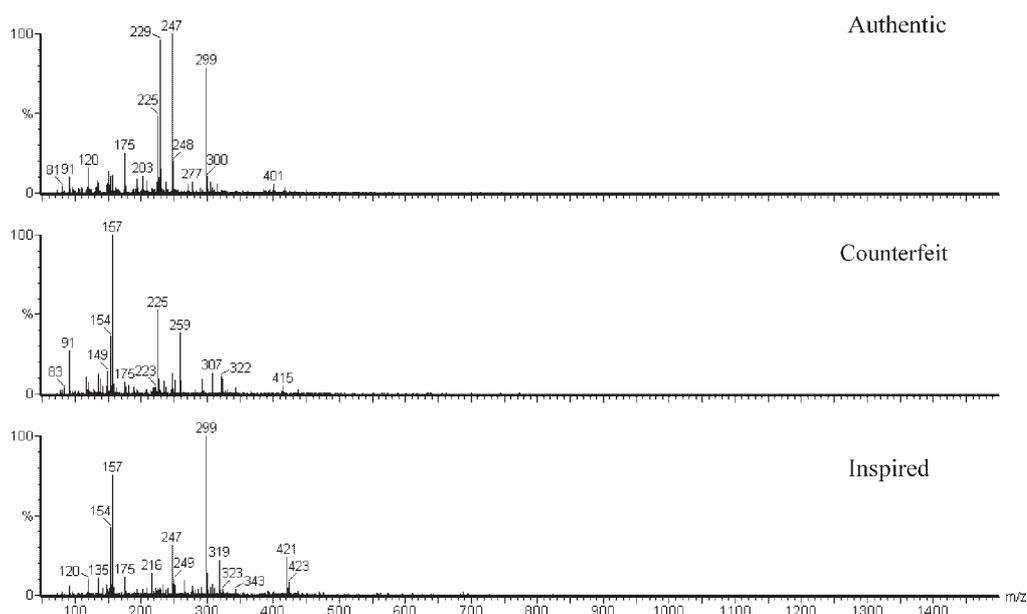
Figure 4. ESI-MS fingerprints in the positive ion mode of methanol/water (1:1) solution of authentic, counterfeit and 'inspired' Gabriela Sabatini perfumes.
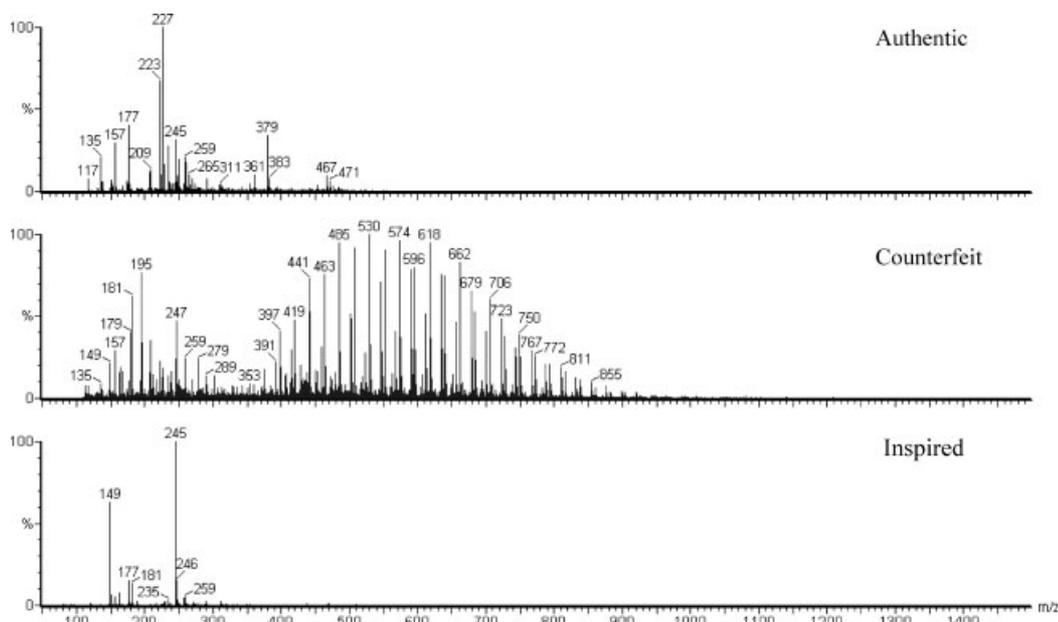
Figure 5. ESI-MS fingerprints in the positive ion mode of methanol/water (1:1) solutions of authentic, counterfeit and 'inspired' Polo Sport perfumes.

sample, the major ions are m/z 157, 247, 299 and 421. Note that this sample does indeed share some diagnostic ESI-fingerprinting ions with the authentic perfume sample, but the 'inspired' perfume still displays a characteristic set of ions that allows clear distinction.

Figure 5 shows the ESI(+)-MS fingerprints of one authentic, one counterfeit and one 'inspired' perfume sample of the Polo Sport brand. For the authentic sample, the major diagnostic ions are m/z 223, 245, 261 and 467. Again, high reproducibility is obtained for ESI-MS among the authentic samples as well as a very characteristic set of diagnostic ions for this brand. For counterfeit samples of Polo Sport, different diagnostic ions are detected in which the characteristic PEG

ions (44 m/z units separation) found in some of the other counterfeit perfumes are also detected. For the 'inspired' Polo Sport perfume sample, two major diagnostic ions at m/z 149 and 245 are detected. Note that one of these ions, m/z 245, is also detected in the ESI-MS of the authentic Polo Sport samples, but the spectrum of the 'inspired' sample is still clearly distinguishable.

Exploratory analysis

To test the performance of ESI-MS for exploratory classification of perfumes, PCA data treatment was performed. Figure 6 shows a scatter plot of PC1 versus PC2 for the ESI(+)-MS spectra of authentic samples of Eternity, Gabriela Sabatini and Polo Sport. All the brands are clearly grouped and each group is far away from others. Figure 7 shows a
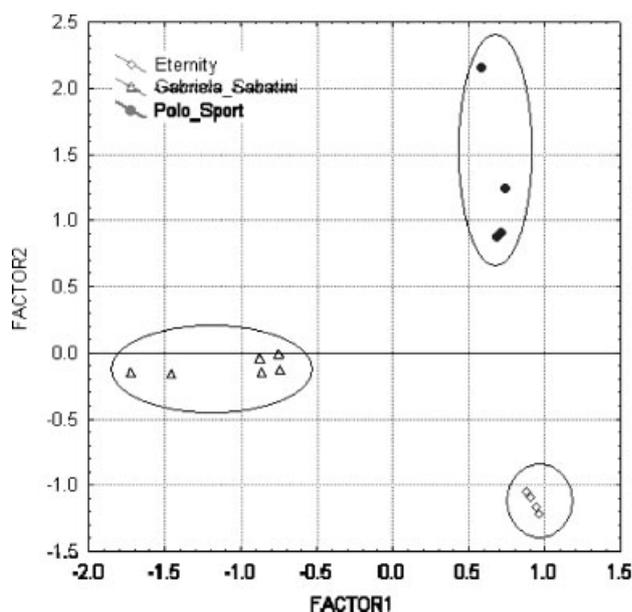
Figure 6. PCA of ESI(+)-MS data of the three different brands of authentic perfumes: Eternity, Gabriela Sabatini and Polo Sport.
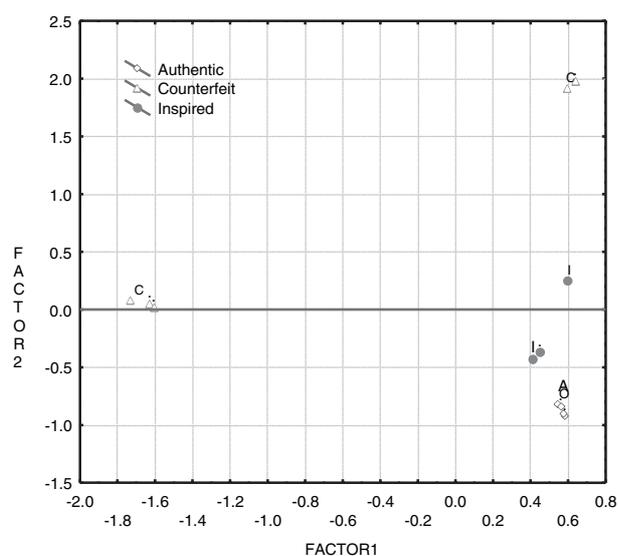
Figure 7. PCA of ESI(+)-MS data of three categories of Eternity perfumes: authentic (A), counterfeit (C) and 'inspired' (I).

scatter plot of PC1 versus PC2 for the ESI(+)-MS spectra of Eternity perfumes. The samples are clearly grouped in their respective authentic (A), counterfeit (C) and inspired (I) categories. For C samples, two groups are formed; one that contains and the other that lacks the oligomeric cluster of PEG ions. Note that, as already discussed, I samples are closer to the A samples but they are still clearly separated. PCA analysis was also applied to the ESI(+)-MS spectra of Gabriela Sabatini and Polo Sport perfumes samples (not shown), and similar results were obtained.

CONCLUSIONS

A new approach based on ESI-MS fingerprinting for the fast and reliable detection of perfume forgery has been demonstrated. Sample preparation is minimal and methanol/water dilution permits the detection, as protonated molecules, of characteristic diagnostic compounds present in authentic, counterfeit and 'inspired' perfumes. The method can also be easily automated particularly when using robotized automated sample injection systems based on micro-fluidic ESI chips that eliminate cross-contamination and permit high-throughput analysis.[18] We envisage that the method can be used to establish ESI-MS fingerprinting libraries of perfumes for comparison with those from samples under investigation, and that such libraries could be continuously updated by the addition of the ESI-MS spectra of new perfumes even before they are commercially released.

Acknowledgements

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