

Perfume fingerprinting by easy ambient sonic-spray ionization mass spectrometry: nearly instantaneous typification and counterfeit detection

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Perfume counterfeiting is an illegal worldwide practice that involves huge economic losses and potential consumer risk. EASI is a simple, easily performed and rapidly implemented desorption/ionization technique for ambient mass spectrometry (MS). Herein we demonstrate that EASI-MS allows nearly instantaneous perfume typification and counterfeit detection. Samples are simply sprayed onto a glass rod or paper surface and, after a few seconds of ambient drying, a profile of the most polar components of the perfume is acquired. These components provide unique and reproducible chemical signatures for authentic perfume samples. Counterfeiting is readily recognized since the exact set and relative proportions of the more polar chemicals, sometimes at low concentrations, are unknown or hard to reproduce by the counterfeiters and hence very distinct and variable EASI-MS profiles are observed for the counterfeit samples. Copyright © 2008 John Wiley & Sons, Ltd.

Perfume manufacturing is an intricate art that requires the skillful selection and combination of natural and/or synthetic fragrances by highly trained experts who create trademark products of unique odor and properties and high aggregated value.¹ The most appreciated perfumes become therefore a potential target for counterfeiting. This illegal worldwide practice is prejudicial both to the perfume business and to consumers, who face the risk of being exposed to less carefully prepared mixtures that may contain harmful chemicals.²

Gas chromatography (GC) and GC coupled to mass spectrometry (GC/MS) are currently the techniques most widely used for perfume analysis, providing composition patterns based on chromatographic separation and individual identification and focused on the volatile and semi-volatile constituents.^{3–5} We have recently demonstrated that direct infusion electrospray ionization mass spectrometry (ESI-MS) provides a relatively simple and fast method to typify perfumes and to detect counterfeiting based on characteristic profiles of polar components.⁶ More recently, a faster procedure based on neutral desorption extractive electrospray ionization mass spectrometry (ND-EESI-MS) has been reported for perfume analysis.⁷ The ND-EESI-MS method is interesting since it is fully direct and therefore totally eliminates sample preparation, that is, the need for sample dilution in direct infusion ESI-MS. It also minimizes carry-over problems currently associated with direct infusion ESI-MS. ND-EESI-MS is an attractive method but it does

require the use of a stream of nitrogen gas to desorb the most volatile perfume components remaining in the probe tip. This requirement incorporates, therefore, a set of new experimental variables (in addition to the normal ESI settings) that must be controlled and reproduced with care, such as flux and pressure of the nitrogen carrier gas, gas temperature, and the interval between perfume spraying and analysis. In addition, spectra obtained from the ND-EESI-MS of perfumes are dominated by ions from the major and most commonly used perfume components, that is, the more volatile odorants and solvents. ND-EESI-MS and ESI-MS fingerprints have been compared⁷ and found to complement each other, since ESI-MS is richer in the high m/z range whereas lower m/z ions from the more volatile components of the desorbed vapor are more abundant in ND-EESI-MS.

Recently, several ambient mass spectrometric techniques have been introduced, such as DESI,⁸ DART,⁹ ASAP,¹⁰ ELDI,¹¹ MALDESI,¹² DAPPI¹³ and DeSSI¹⁴ (recently retermed as 'easy ambient sonic-spray ionization', EASI¹⁵). These techniques constitute a welcome advance in modern mass spectrometry since they offer the opportunity to perform desorption and ionization of analytes directly from their natural or auxiliary matrices via a non-sample preparation procedure under ambient conditions (atmospheric pressure and room temperature). Among these techniques, EASI is attractive owing to its simplicity and ease of implementation. An EASI source can be constructed and installed in a few minutes from a few simple MS lab parts (see Fig. 1) and is assisted only by compressed N₂ or air. EASI uses sonic-spray ionization (SSI)¹⁶ to create minute charged droplets with a statistical imbalance of charges. EASI has been applied with success to the analysis of different analytes

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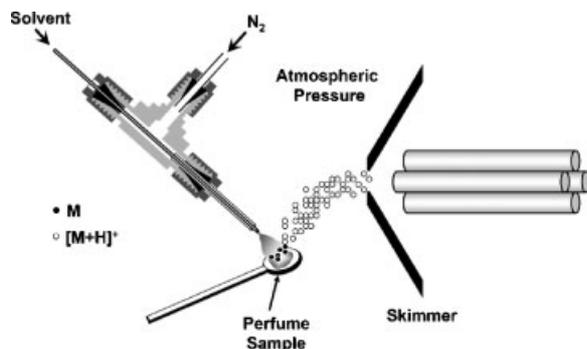


Figure 1. Schematic of EASI-MS fingerprinting of perfumes. Desorption/ionization of the more polar perfume components from the glass surface is performed by the minute charged droplets produced by sonic-spraying an acidic water/methanol solution in a N_2 (or air) assisted-only process. EASI requires only a Swagelok[®] T-element, ferrules and gas tubing, and a fused-silica capillary.

and matrices such as drug tablets,¹⁴ oils,¹⁷ biodiesel¹⁸ and wine,¹⁹ and has been recently coupled to membrane introduction mass spectrometry (EASI-MIMS)¹⁵ and thin-layer chromatography (TLC-EASI-MS).²⁰ EASI-MS is based on SSI, which has been described as the softest ionization technique.²¹ This gentleness is an advantage for EASI fingerprinting due to its tendency to suppress ion dissociation and hence to form a single ion (most often protonated, sodiated or deprotonated molecules) for each semi or polar component. Herein we report on the use of EASI-MS to perform ultra-fast, nearly memory free, simple and highly reproducible (few experimental variables) typification and counterfeit detection of perfume samples.

EXPERIMENTAL

Chemical reagents and samples

Formic acid and methanol of HPLC grade was purchased from Merck SA (Rio de Janeiro, Brazil) and used without further purification. Deionized water was obtained from a MilliQ (Millipore, Sao Paulo, Brazil) purification unit. Twenty-nine samples of the perfumes Eternity from Calvin Klein, Polo Sport of Ralph Lauren and Gabriela Sabatini, including the authentic and counterfeit, were analyzed. Authentic samples were obtained from the formal market and contained the expected seal of authenticity whereas counterfeit samples were purchased from the informal market at much lower prices. These latter samples also failed to display the seal of authenticity present on the authentic samples.

General experimental procedures

MS experiments were performed on a Q-Trap[®] triple-quadrupole mass spectrometer (Applied Biosystems, Sao Paulo, Brazil) equipped with a homemade EASI source mounted on the commercial nano-ESI source. Note that we mounted the EASI source on the Applied Biosystems nanospray ESI source for convenience only, as the Q-Trap instrument would not work properly without one of its

commercial sources connected to it. However, any simple holder should allow proper EASI operation. The EASI-MS system is described in detail elsewhere.¹⁴ The main experimental parameters were: flow rate of the 7:3 acidic (0.01% formic acid) methanol/water solution of $20 \mu\text{L min}^{-1}$, nebulizing gas backpressure of ca. 30 bar, curtain gas pressure of 5 bar, de-clustering potential of 100 V, tip-glass rod distance of ca. 2 mm, and capillary-glass rod entrance angle of ca. 30° .

To perform EASI-MS analysis of perfumes, ca. $5 \mu\text{L}$ of the sample was sprayed directly onto the glass rod (probe tip) and the solvent was allowed to evaporate for a few seconds in air. EASI-MS was then performed immediately in the positive ion mode.

Chemometric analysis

Data from EASI-MS fingerprints of the perfumes were extracted using Analyst 4.1 software (Applied Biosystems). Mass spectral data were accumulated over 60 s to generate a final data matrix (m/z versus ion abundances) of 13 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 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 EASI-MS fingerprinting, principal component analysis (PCA) was performed on the EASI-MS data by the Unscrambler v. 6.0 program (CAMO A/S, Trondheim, Norway).

RESULTS AND DISCUSSION

Figure 2(A) shows a representative EASI-MS fingerprint of an authentic perfume sample of the Eternity brand. A characteristic set of ions, mainly those of m/z 149, 177, 193, 223, 235, 245, 301 and 307, constitute an EASI-MS chemical signature for this perfume brand, which was seen for all the authentic samples with high reproducibility (see the section 'Chemometric analysis' below). As Fig. 2(B) exemplifies, the EASI-MS fingerprints of counterfeit samples are different from those of the genuine samples and also very variable as the result of the many different compositions used by counterfeiters when trying to reproduce the correct aroma. In the profile shown in Fig. 2(B), the use of a polyethoxylated emulsifier is suggested by a series of oligomeric ions separated by 44 m/z units. When the EASI-MS fingerprints are compared with those obtained by direct infusion ESI-MS,⁶ quite similar profiles are seen with sometimes slightly simpler spectra owing probably to reduced ion dissociation or enriched composition of semi-polar and polar components after air drying.

The ion abundances in EASI-MS are quite high allowing major ions to be selected and dissociated by collision-induced dissociation (CID) via EASI-MS/MS experiments (not shown). Although ion characterization is not necessary for fingerprinting, EASI-MS/MS may also be applied for enhanced selectivity when suspicious samples are found to display similar EASI mass spectra or counterfeit samples are suspected to contain hazardous chemicals.

Figure 3 shows representative EASI-MS fingerprints of authentic and counterfeit samples of perfume samples of the

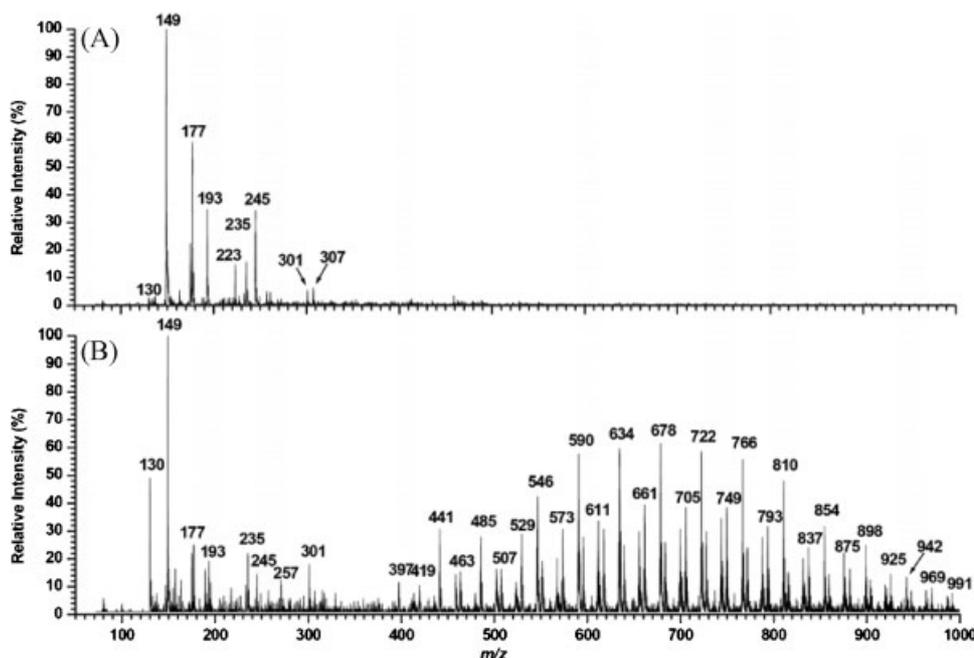


Figure 2. EASI-MS fingerprints of representative samples of (A) authentic and (B) counterfeit perfumes of the Eternity brand.

Gabriela Sabatini brand. The characteristic and reproducible set of chemical markers for this brand are mainly the ions of m/z 157, 175, 229, 247, 299, 315, 277, 458, 463 and 479 (Fig. 3(A)). The EASI mass spectra of the counterfeit samples are again different from those of the genuine samples and also highly variable. In the EASI mass spectrum of a counterfeit sample shown in Fig. 3(B), a less complex set of polar compounds is detected.

Figure 4 shows the EASI-MS fingerprints of authentic and counterfeit samples of perfume samples of the Polo Sport brand. Note again in Fig. 4(A) the characteristic (and highly reproducible) set of chemical markers for the authentic sample (main ions of m/z 149, 177, 245, 299, 307 and 339) whereas all the counterfeit samples that we tested show again different and variable sets of polar components. For the EASI mass spectrum shown in

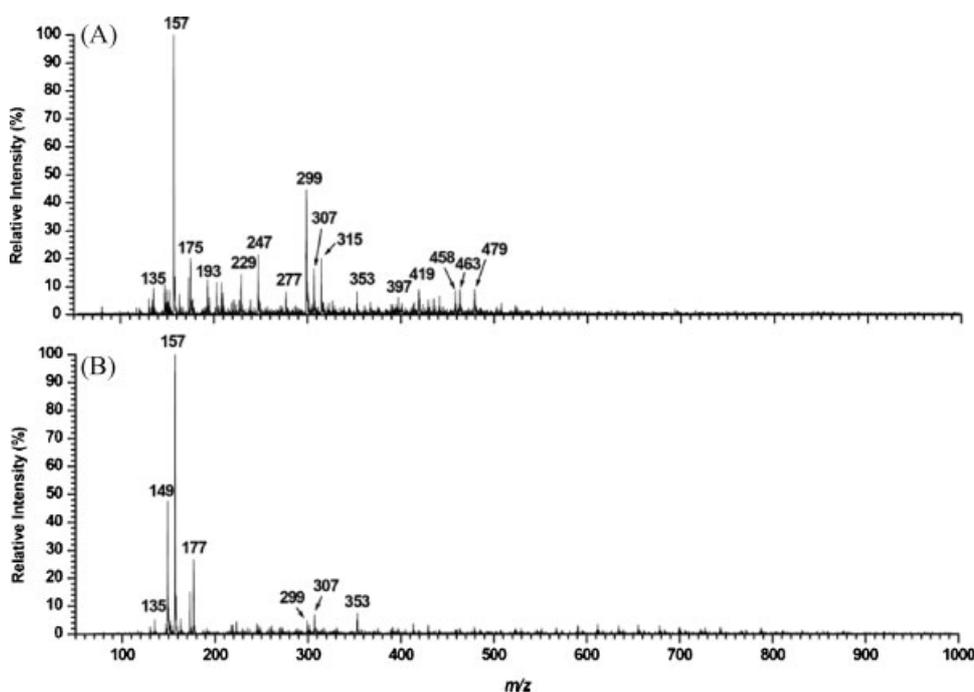


Figure 3. EASI-MS fingerprints of (A) authentic and (B) counterfeit samples of perfumes from the Gabriela Sabatini brand.

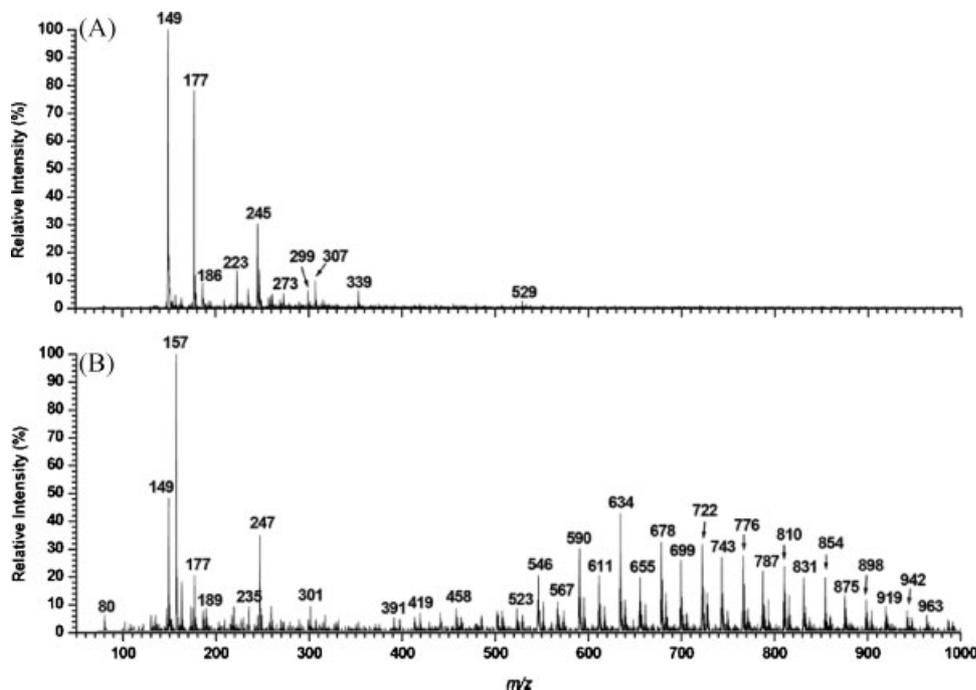


Figure 4. EASI-MS fingerprints of (A) authentic and (B) counterfeit samples of perfumes of the Polo Sport brand.

Fig. 4(B), note again the presence of a polyethoxylated emulsifier.

Chemometric analysis

To statically test the performance of EASI-MS to typify perfumes, PCA data treatment was performed. Figure 5 shows a scatter plot of PC1 versus PC2 for the EASI mass spectra of authentic samples of Eternity, Gabriela Sabatini and Polo Sport. Note that all brands are closely packed with little variance and that each group is clearly separated from

the others. Figure 6 shows a scatter plot of PC1 versus PC2 for the EASI mass spectra of samples of the Eternity perfumes. The samples are again clearly grouped in their respective authentic (A) and counterfeit (C) categories. Similar results were observed for the Gabriela Sabatini and Polo Sport perfumes (data not shown). Note that whereas the authentic samples are closely grouped, due to reproducible sets of polar components (chemical signatures of each perfume), the counterfeit samples are greatly dispersed as the result of variable counterfeiting procedures and compositions.

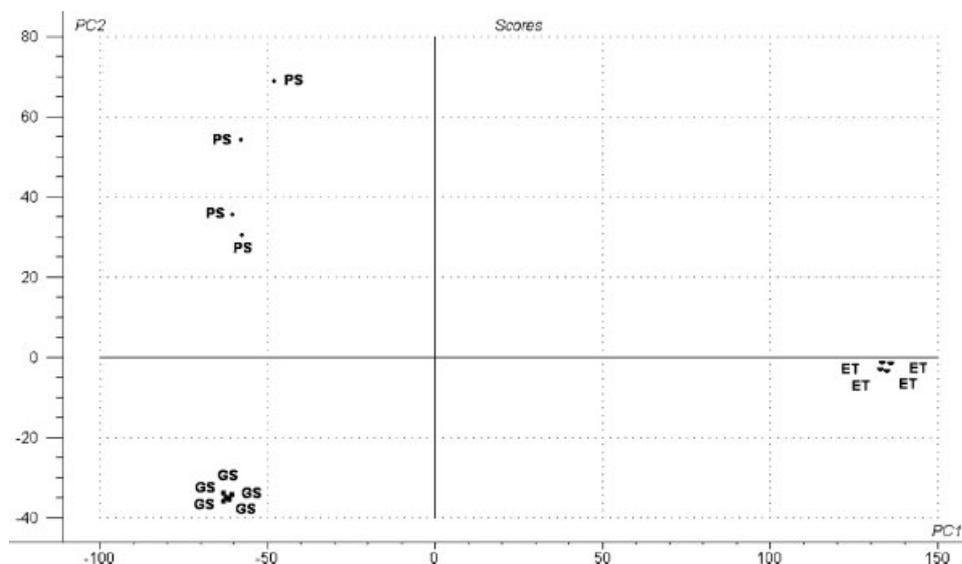


Figure 5. PCA of EASI-MS data of the three different brands of authentic perfumes: Eternity (ET), Gabriela Sabatini (GS) and Polo Sport (PS).

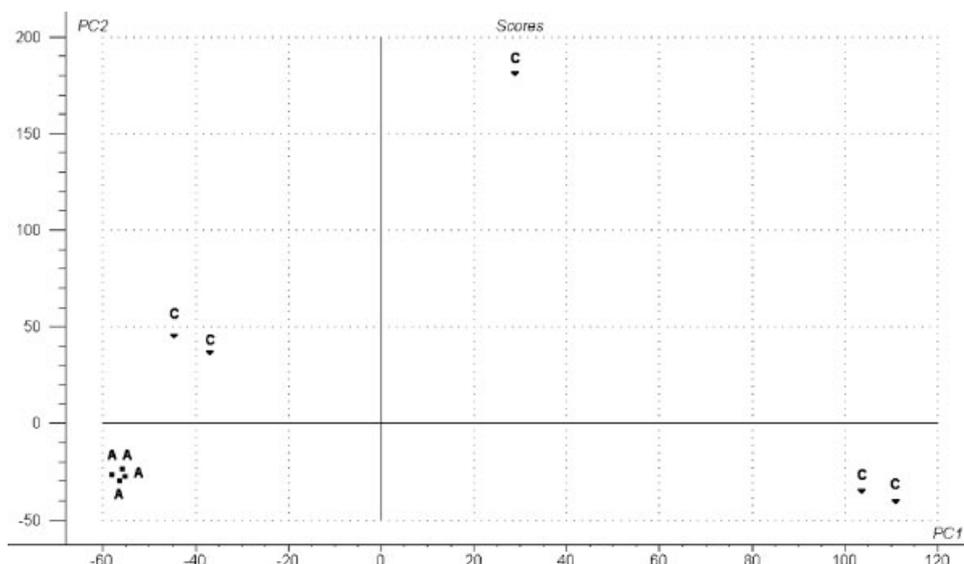


Figure 6. PCA of EASI-MS data of Eternity perfumes: authentic (A) and counterfeit (C).

CONCLUSIONS

EASI-MS fingerprinting provides an efficient method for the simple, direct, ultra-fast, nearly memory free, and highly reproducible typification of perfumes and the detection of counterfeit samples. Samples are sprayed onto a glass rod and characteristic profiles of ions, arising from the desorbed and ionized more polar components remaining after a few seconds of drying in air, are detected. These reproducible profiles function as fingerprints for each brand of perfume. EASI-MS/MS may also be applied for enhanced selectivity when suspicious samples are found to display similar EASI mass spectra or are suspected to contain hazardous chemicals. Reference EASI-MS fingerprint libraries of perfumes could be developed for quality control and forensic investigations.

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