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Rapid classification of perfumes by extractive electrospray ionization mass spectrometry (EESI-MS)

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Extractive electrospray ionization mass spectrometry (EESI-MS) was applied to rapid fingerprinting of various perfumes for quality classification. Unique EESI-MS fingerprints of ten famous brands were obtained. This technique was shown to be applicable to rapid forgery detection on the example of an authentic and a counterfeit 'Miss Dior' fragrance by Christian Dior. We believe that the high throughput and simplicity of this sample-preparation-free method can be advantageous in the perfume industry, for instance when applied to online quality control. Copyright © 2008 John Wiley & Sons, Ltd.

Perfumes, mixtures of fragrant essential oils, aroma compounds, fixatives and matrices have been used by mankind for centuries. The quality of perfumes relies heavily on the fragrance profile, thus the composition of the perfume controls the price and usage of the products. Theoretically, either quantitative analysis of each component of a perfume or chemical fingerprinting can be used for quality control.

For quantitative component analyses of perfumes, gas chromatography combined with mass spectrometry (GC/MS) has been preferentially used.[1–4] However, in cases where time is a factor, e.g. for online quality control of products in the perfume industry, this strategy may turn out to be unacceptably slow.

Recently, direct infusion electrospray ionization mass spectrometry (ESI-MS)[5] has been applied in order to classify perfumes and identify counterfeit samples.[6] This technique requires no chemical derivatization, extraction, or chromatographic separation which makes it attractive when fast perfume classification is important. The authors were able to detect perfume forgery directly from differences in the mass spectral fingerprints of authentic and counterfeit samples without resorting to detailed chemical composition analysis. However, using direct infusion of a complex mixture introduces certain memory effects caused by accumulation of sticky ingredients (e.g. the fixatives, oils) in transfer lines, which can slow down the analytical workflow. Moreover, the need for dilution of samples sometimes also limits the speed of the analyses, and thus the practical applications of the method. The carry-over issue associated with the direct infusion can be eliminated by using automated sample injection systems based on micro-fluidic ESI chips.[7] The throughput of such systems is however typically limited to about one run per minute, let alone the high cost of such automated systems and microchips that cannot be reused.

Similar to desorption electrospray ionization (DESI),[8–10] extractive electrospray ionization (EESI) tolerates complex matrices, allowing real-time, online monitoring of complex liquid samples[11] (e.g. milk, raw urine and waste water), and in vivo fingerprinting of breath.[12,13] In EESI, analytes are charged by infusing them into an ionizing electrospray of a pure solvent; the ions produced are detected by MS to give an EESI-MS fingerprint of a sample. The power of EESI-MS has been further demonstrated by successfully applying it to studies of ion-ion reactions,[14] manipulation of charge states of biopolymers at atmospheric pressure ambient,[15] and in the field of metabolomics.[16,17] Recently, EESI has been extended to rapid analysis of volatile and semivolatile compounds sampled from various surfaces after desorption by a neutral gas jet.[14,18] Coupling this neutral desorption (ND) sampling to EESI results in a method for rapid, sensitive real-time detection of analytes on surfaces of living objects without chemical contamination. In ND-EESI, the desorption process is separated from the ionization process in both space and time; thus, as a fringe benefit, ion suppression effects are significantly decreased.

Here we present a sample-preparation-free approach based on ND-EESI for ultra-rapid classification of perfumes by extractive electrospray ionization mass spectrometry. The high throughput and simplicity of this sample-preparation-free method can be advantageous in the perfume industry, for example, for online quality control.

EXPERIMENTAL

The experiments were run in positive ion mode on a commercial electrospray ionization (ESI) quadrupole-time-of-flight (Q-TOF) mass spectrometer QTOF Ultima™ (Micromass, Manchester, UK). Briefly, the capillary voltage was 3 kV and the cone voltage was 40 V. Other parameters

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were default values of the instrument. No further optimization was performed.

The commercially available perfumes were deposited onto a paper strip (1 cm wide, 5 cm long) by nebulizing from the original bottle – the same way as a salesperson in a perfume shop would do to let customers smell a product. The distance from the bottle sprayer to the paper strip edge was 2 cm. One short dose was always sprayed. The samples were allowed to evaporate for 10 s, so that no liquid was left before the neutral gas sampling. The smelling strip was then brought close (about 3 cm) to the capillary end of the ESI source (Z-spray, Micromass, UK) running a pure solvent mixture (methanol/water/acetic acid 40%/40%/20%) at a flow rate of 5 µL/min. A stream of nitrogen gas at a flux of 0.3–3 L/(min × mm²) was directed at the probe strip surface. The high neutral gas flow (3 L/(min × mm²)) needed to extract low volatility compounds also provides a large yield of high volatility chemicals. This can lead both to overload of the instrument's detector and to certain memory effects since a longer time would be needed in order to clear the perfume aroma from the ambient air. For these reasons some chemical information that could otherwise be obtained by using high neutral gas flow rates was sometimes sacrificed in most experiments by lowering the flow down to 0.3 L/(min × mm²).

The mass spectra were accumulated for only 10–20 s, and followed by background subtraction over the 50–800 *m/z* range (MassLynx 4.0, Waters, Manchester, UK).

Ten authentic fragrances were subjected to EESI-MS fingerprinting: 'Weekend' by Burberry, 'Relaxing fragrance' by Shiseido, 'Be delicious' by DKNY, 'Beautiful' by Estee Lauder, 'Hugo XY' by Hugo Boss, 'L'Homme' and 'Opium' by Yves Saint Laurent, 'le Male' by Jean Paul Gaultier, 'Miss Dior' by Christian Dior, 'ETH Zurich 150' by Givadaun.

Except for the last one three samples of each brand were obtained from different stores. 'Inspired' and counterfeit samples for 'Opium' and 'Miss Dior' fragrances, respectively, were also obtained.

Methanol (>99.8% pure), UHP water, acetic acid (>99.8% pure), (+)-limonene (95%) and (±)-beta-citronellol (95%) were purchased from Fluka (Switzerland).

RESULTS AND DISCUSSION

Figure 1 shows an EESI-MS fingerprint of authentic 'Opium' fragrance obtained with the help of a neutral gas flow of 3 L/(min × mm²). It can be clearly seen that the most abundant peaks appear in the 100–300 *m/z* range, corresponding to highly volatile compounds which are easily desorbed from the surface of a smelling strip. When looking at a narrow mass window, one can, however, see that the actual fingerprint is much more complex than at first glance.

EESI-MS/MS spectra were collected (not shown) for the four major peaks in the fingerprint, *m/z* 137, 157, 173, 219. Two of them, *m/z* 137 and 157, are supposed to be protonated species of limonene and citronellol that can be found in the ingredients list for 'Opium'. In order to validate this, the reference EESI-MS/MS spectra of pure (+)-limonene and (±)-beta-citronellol were obtained (not shown). The same sets of characteristic fragments were observed for *m/z* 137 ions and limonene, as well as for *m/z* 157 ions and citronellol, respectively. Thus a structural assignment of the diagnostic ions is feasible. Chemical identification by MS/MS is, however, not relevant for rapid online fingerprinting and was not tried hereafter in this study.

Remarkably, sampling of high molecular weight, low volatility compounds can be achieved by means of neutral
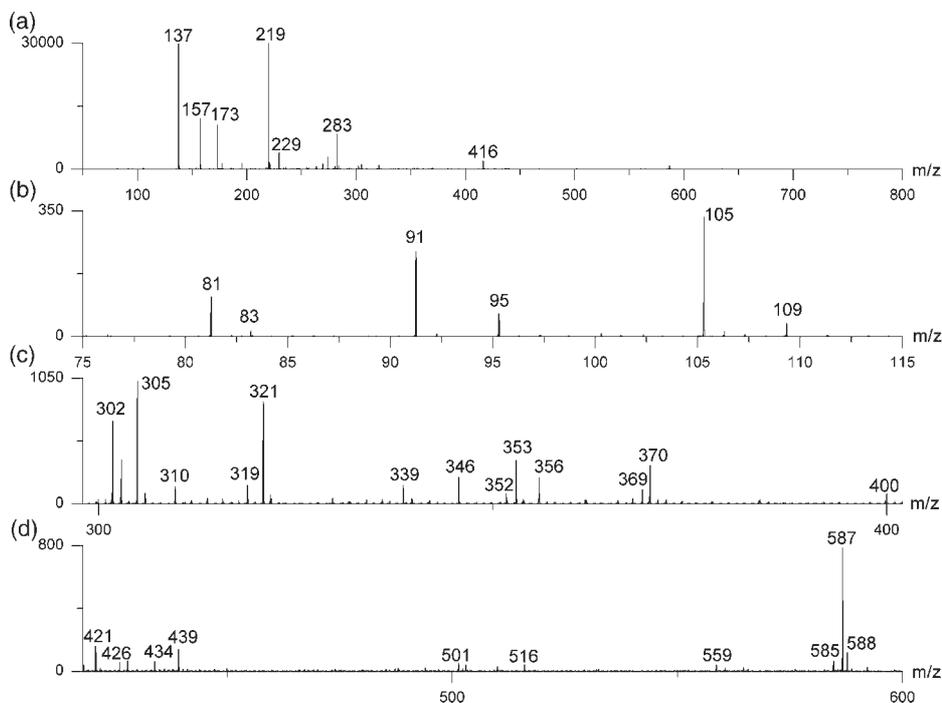
Figure 1. (a) Chemical fingerprint of 'Opium' fragrance recorded in positive ion mode by EESI-MS. (b–d) Mass windows showing minor components which are not noticeable in the wide *m/z* range.
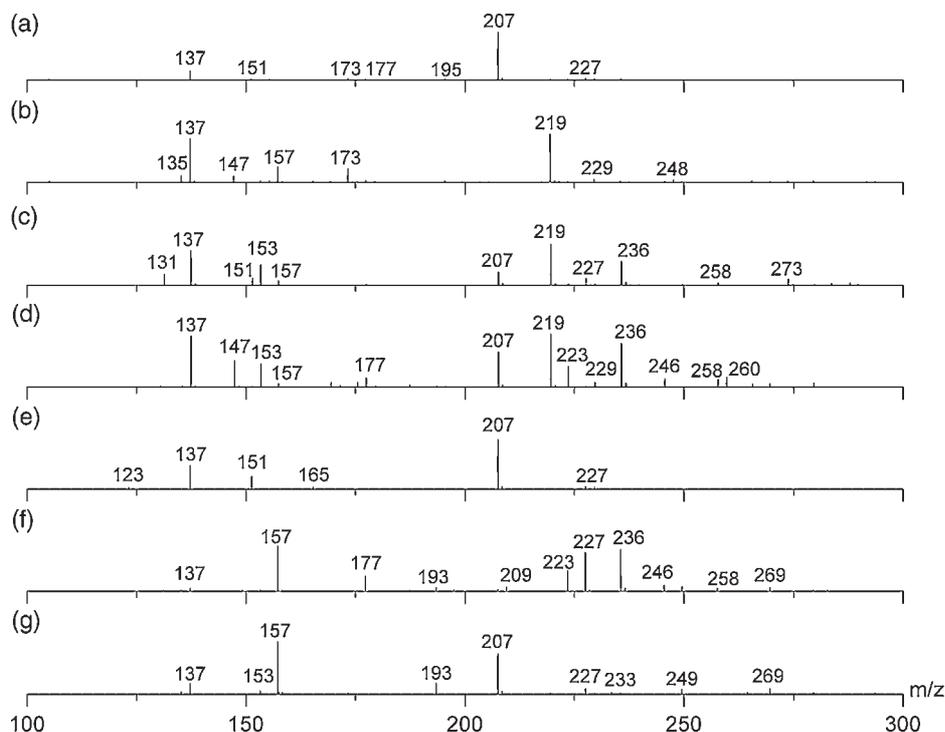
Figure 2. Chemical fingerprints of seven famous fragrances, recorded in positive ion detection mode by EESI-MS: (a) 'Beautiful' by Estee Lauder, (b) 'Opium' by Yves Saint Laurent, (c) 'Hugo XY' by Hugo Boss, (d) 'le Male' by Jean Paul Gaultier, (e) 'Be delicious' by DKNY, (f) 'Weekend' by Burberry, and (g) 'Natural fragrance' by Shiseido.

desorption. In order to do so, quite a strong nitrogen gas flux is needed ($3\,L/(min \times mm^2)$). The highest detected m/z value for 'Opium' was m/z 659 (not shown). The peaks at m/z 105, 280 and 305 in Fig. 1 are chemical noise. Discrimination between perfume ingredients and chemical noise in the mass spectrometer can be achieved by looking at selected ion time traces ('chromatograms') – if a compound originates from the smelling strip then its ion current will show an increase at the time when the smelling strip is brought close to the mass spectrometer.

Figure 2 presents EESI-MS fingerprints of seven famous fragrances ('Weekend' by Burberry, 'Relaxing fragrance' by Shiseido, 'Be delicious' by DKNY, 'Beautiful' by Estee Lauder, 'Hugo XY' by Hugo Boss, 'Opium' by Yves Saint Laurent and 'le Male' by Jean Paul Gaultier) showing the

$100–300\,m/z$ range that contains the most abundant peaks. For the reasons stated in the Experimental section, the neutral gas flow was lowered to $0.3\,L/(min \times mm^2)$ in these experiments. Distinctive sets of characteristic compounds unique for each sample are clearly observed. Thus the fragrances can be rapidly qualitatively differentiated by directly comparing EESI survey spectra. Tailing of the perfume compounds was typically only $\approx 10\,s$. During this time, the ion current for every detected compound returned to the background level.

Figure 3 compares an EESI-MS fingerprint of the 'ETH Zurich 150' fragrance with a fingerprint of the same fragrance obtained by direct infusion ESI-MS. In the latter case the sample was diluted 1000 times in a solution of water/methanol/formic acid (49.5%/49.5%/1% v/v/v)
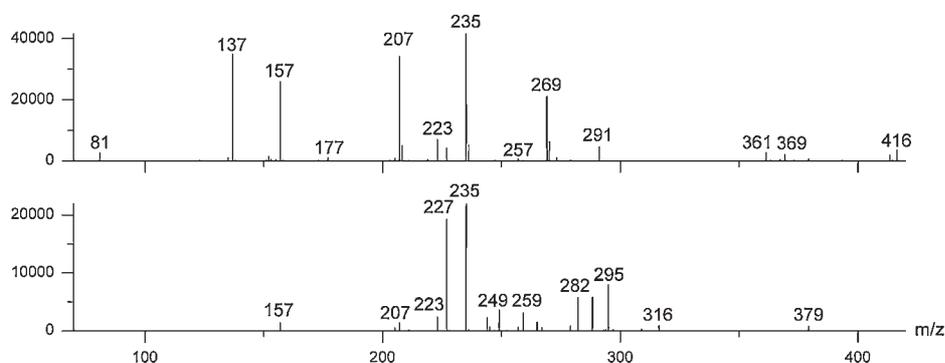
Figure 3. Chemical fingerprints of 'ETH Zurich 150' perfume obtained by EESI-MS (top) and direct infusion ESI (bottom). The peaks at m/z 282 and 295 in the bottom spectrum are chemical noise.

Table 1. Peak lists of the EESI-MS and the direct infusion ESI-MS fingerprints of the 'ETH Zurich 150' perfume (+++ high intensity signal; + observed; − not observed)

m/z	EESI-MS	ESI-MS	m/z	EESI-MS	ESI-MS	m/z	EESI-MS	ESI-MS
59	+	−	229	+	+	363	+	−
81	+	−	235	+++	+++	367	+	−
83	+	−	241	+	+	369	+	−
95	+	−	244	+	+	373	+	−
109	+	−	245	+	+	379	+	+
117	+	−	247	+	+	383	+	+
123	+	−	249	+	+	393	+	−
127	+	−	252	+	+	413	+	−
137	+++	+	257	+	+	416	+	+
152	+	+	259	+	+	425	+	−
155	+	−	265	−	+	431	+	−
157	+++	+	269	+++	−	438	+	+
173	+	−	273	+	−	441	+	−
177	+	+	288	−	+++	453	+	+
193	+	−	291	+	+	467	−	+
197	+	+	293	+	+	471	−	+
203	+	+	316	−	+	476	−	+
205	+	+	323	+	+	478	+	−
207	+++	+	331	+	+	488	+	−
209	+	+	341	+	+	508	−	+
211	+	+	345	+	−	517	+	+
219	+	+	351	+	−	523	−	+
223	+	+	357	+	−	526	−	+
227	+	+++	361	+	−	541	−	+

before infusion as described by Marques *et al.*[6] MS settings were the same in both experiments. One can see that the spectra obtained look quite different. It is worth noting that most of peaks are present in both fingerprints although some low intensity signals cannot be seen in the overview spectrum. Complete peak lists are presented in Table 1. Notably, the direct infusion ESI-MS fingerprint is richer in signals in the high *m/z* range compared to that obtained by EESI-MS. This is likely due to the generally lower volatility and thus less efficient extraction of heavier compounds by EESI-MS. A number of peaks were detected in the EESI mass spectrum only. This discrepancy is most pronounced for *m/z* values below 200, but also obvious in the 300–400 Da range (Table 1). Our interpretation is that these signals are due to minor components of the perfume that are ionized by EESI (but not by ESI) due to the slightly different charging mechanism. Both methods thus appear to complement each other, and if used together give more comprehensive mass spectrometric information of perfumes.

Figure 4 shows EESI-MS fingerprints of three authentic samples of the 'Opium' brand obtained from three different stores. All the spectra exhibit the same set of characteristic compounds but also reveal relative intensities that are almost identical (the intensity ratio fluctuation for two major peaks, *m/z* 137 and 219, is below 10%). This was also found to be valid for EESI-MS fingerprints of the other brands. Table 2
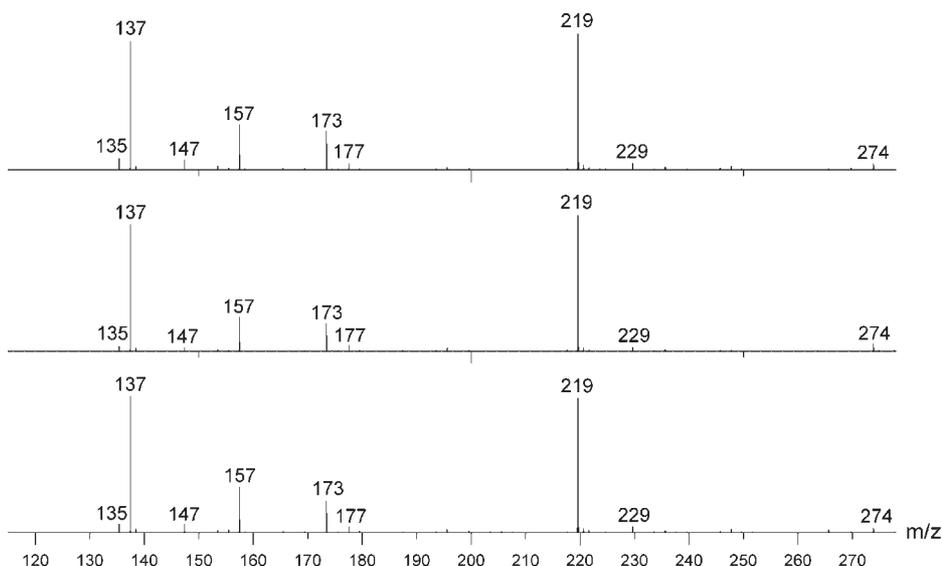
Figure 4. Positive mode EESI-MS fingerprints of three authentic 'Opium' perfumes obtained at different locations.

Table 2. Intensities of the 15 most abundant peaks (relative to the major peak at m/z 235) in the EESI-MS fingerprint of the 'ETH Zurich 150' perfume. Ten fingerprints of the same sample were obtained to give a RSD for the intensity value of each peak

m/z	137	207	157	269	223	291	227	416
Relative intensity, %	88.2 ± 7.5	85.3 ± 3.7	66.7 ± 5.7	57.1 ± 4.8	17.8 ± 3.0	12.4 ± 3.1	9.3 ± 2.1	9.0 ± 1.1
RSD, %	8.5	4.3	8.5	8.4	16.9	25.0	22.6	12.2

m/z		369	361	81	209	413	257	219
Relative intensity, %		6.9 ± 1.8	6.8 ± 1.3	6.1 ± 1.0	4.3 ± 0.7	4.3 ± 1.0	1.6 ± 0.2	1.2 ± 0.2
RSD, %		26.1	19.1	16.4	16.3	23.3	12.5	16.7

demonstrates intensity ratio fluctuations for 15 major peaks in the EESI-MS fingerprint of the 'ETH Zurich 150' brand. Ten measurements on the same sample were done to calculate relative standard deviations (RSDs) for each value. One can see that for the five most abundant compounds (m/z 137, 157, 207, 235, 269) the intensity ratio fluctuation is below 10%. Lower intensity peaks show reproducibility within 30%. Therefore, even if two samples exhibit the same set of characteristic compounds, they can be discriminated based on larger differences in relative intensities of these compounds. The following example shows how this feature can significantly facilitate differentiation in some cases.

In many countries a good part of the perfume market is occupied by fragrances 'inspired' by famous brands.[6] Producers of these inspired perfumes try to imitate the aroma of a famous brand. Commonly, an inspired perfume would come in a package resembling that of the famous brand, under a consonant name, e.g. 'Option' (inspired by 'Opium') or 'Bossage' (inspired by 'Hugo Boss'), but cost much less. To a certain extent it is possible to imitate famous fragrances since most of their ingredients are disclosed. It can be extremely difficult to recognize such a product by a simple smell test. Figure 5 shows EESI-MS fingerprints of 'Opium' by Yves Saint Laurent and its inspired analog 'Option' by a company called Nova. One can see that the characteristic compounds are very similar for these two samples. A straightforward way to differentiate these perfumes would be to perform a multivariate analysis (e.g. PCA – principal component analysis) of their peak lists. This would require some additional effort to separate MS peaks that are inherent to each sample (many of which have low intensities) from chemical and detector noise which are still present at low
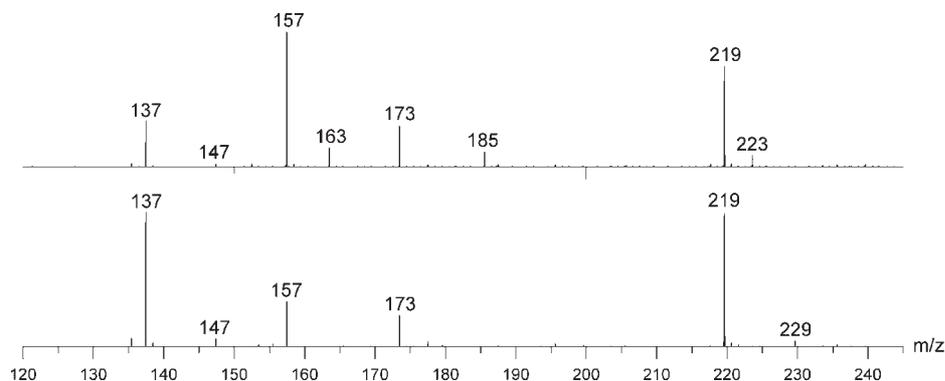
Figure 5. Positive mode EESI-MS fingerprints of inspired (top) and authentic (bottom) 'Opium' perfumes.
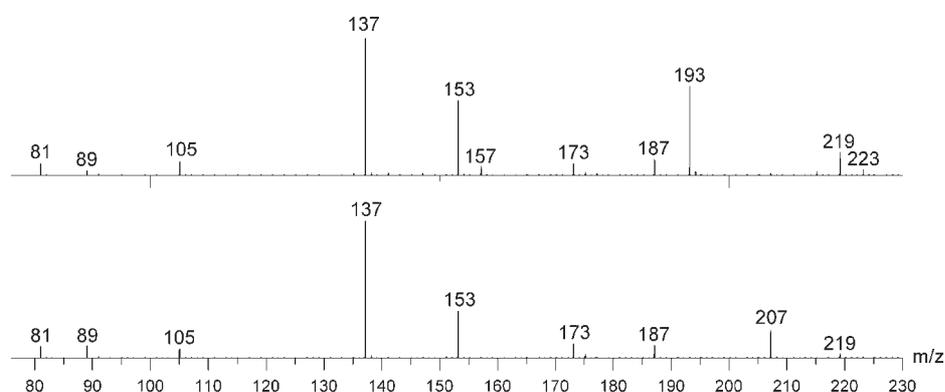
Figure 6. Positive mode EESI-MS fingerprints of counterfeit (top) and authentic (bottom) 'Miss Dior' perfumes. The peaks at m/z 105 and 187 in both spectra are chemical noise.

intensity even after the background subtraction procedure. Furthermore, PCA data treatment in itself is time-consuming. Fortunately, a rapid differentiation can be done by simply taking a closer look at the relative intensities of the major peaks from these two samples, e.g. *m/z* 137 and 157, that occur in the ingredients list for 'Opium'. One can also note that some peaks (e.g. *m/z* 163 and 185) only show up in the EESI-MS fingerprint of the inspired sample. This could alternatively be used for differentiation purposes.

The world of counterfeit perfumes is probably even larger than the world of inspired perfumes. Unlike inspired aromas which are prohibited to be sold in only a few countries, perfume forgery is subject to legal prosecution throughout the world. Figure 6 demonstrates EESI-MS fingerprints of 'Miss Dior' by Christian Dior and its counterfeit sample obtained on the black market. Here the difference is more pronounced than that between 'Opium' and 'Option' (Fig. 5) indicating different sets of characteristic compounds from the authentic and counterfeit samples (Fig. 6). There were many peaks (e.g. *m/z* 157, 193, 215, 223) detected with significantly higher signal levels from the counterfeit perfume, probably because low purity materials were used to make the counterfeit perfume. In some cases, impurities derived from natural products could be allergenic or toxic compounds, which in turn could be the molecular basis of known side effects resulting from counterfeit perfumes.[19,20] The degree of these effects would depend on the 'quality' of a particular counterfeit sample.

CONCLUSIONS

Rapid differentiation of perfume products, mixtures of complex chemical compositions, challenges many analytical tools since it requires high sensitivity, high throughput, and the feasibility for real-time, online analysis. Making use of the advantages of EESI, which tolerates complex matrices by separating the sampling process from the ionization process, we present a sample-preparation-free approach to differentiate perfume products online by extractive electrospray ionization mass spectrometry (EESI-MS). The experimental findings show that this method is applicable to rapid forgery detection. Due to its remarkably high throughput, this method can be particularly important for online product control in the perfume industry.

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