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Trace detection of hormones and sulfonamides in viscous cosmetic products by neutral desorption extractive electrospray ionization tandem mass spectrometry

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A sensitive method based on a geometry-independent neutral desorption (GIND) in combination with extractive electrospray ionization mass spectrometry (EESI-MS) has been developed for fast detection of illicit additives such as sulfonamides and hormones in highly viscous cosmetic products. The method gave a low limit of detection (LOD) (in the range of 0.001–1 ng/g), acceptable relative standard deviation (RSD = 6.8–11.4%) and reasonable recovery (87–116%) for direct measuring of nine types of hormones and sulfonamides in the cosmetic products. The average measurement time for two types of samples was less than 1 min. Trace amounts of analytes in commercial cosmetic products have been quantitatively detected, without any sample pretreatment. The experimental results showed that non-volatile illicit additives such as sulfonamides and hormones could be sensitively liberated using the GIND device for quantitative detection from the highly viscous cosmetic products, demonstrating that GIND-EESI-MS is a promising tool for high throughput, sensitive and quantitative analysis of highly complex viscous samples.

Keywords: ND; EESI; viscous samples; cosmetic products; hormones; sulfonamides; quantitative detection; high throughput analysis

INTRODUCTION

Cosmetic products have become more and more important in our daily life for enhancing the appearance, skin care, odor of the human body and so on. Cosmetic products are not only the daily necessities for urban women, but are also becoming more and more popular for men in many countries. This leads to a huge market for cosmetic products. According to the euromonitor data report released in 2008, the world cosmetics market growth increased from 170 billion Euros in 2001 to more than 220 billion Euros in 2007. Moreover, this market is expected to grow at an average of 3% per annum and in 2011 will reach 250 billion Euros.[1] Thus, safety and quality control of cosmetic products is urgently required.

Cosmetics are complex mixtures composed of oils, fats, waxes, surfactants, moisturizing agents, emulsifiers, preservatives, pigments, flavors and so on. In order to achieve 'magic effects' functions such as whitening, eliminating freckles, increasing skin elasticity, removing acne and so on, illicit additives such as hormones and antibiotics were usually added to the cosmetics. These illicit additives bring serious risk to human health.[2] For instance, the long-term exposure to hormones in cosmetics could result in metabolic disorders and/or occurrence of cancer.[3,4] Overdosage of antibiotics can weaken the immunity system and even lead to death due to its hypersusceptibility.[5] Thus, addition of either hormones or sulfanilamides to the cosmetics has been banned by both the European Union Directive 76/768/EEC and Hygienic Standard for Cosmetics of China. However, cosmetic products containing illicit additives are still in the market.

Until now, liquid chromatography (LC)[6–11] or its combination with mass spectrometry (MS) (e.g. LC/MS)[12–14] is the most common method for detecting additives in cosmetics. However, the sample pretreatment required by these techniques makes the whole analysis process laborious and time consuming (>40 min), having difficulty in fast screening inferior commercial products in the market. Besides, there are large numbers of samples in the market for quality control, and thus an appropriate analytical technique for practical sample analysis must meet the demanding requirements for high throughout, high sensitivity and high specificity.

MS is an analytical tool with high sensitivity and high specificity. The throughput of MS has been dramatically improved by Cooks *et al*. by introducing desorption electrospray ionization (DESI).[15] DESI could tolerate complex matrices and form analyte

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Journal of MASS SPECTROMETRY

ions on sample's surfaces without separating the matrix. Later on, other ambient ionization techniques[16,17] such as direct-analysis-in-real-time (DART),[18] desorption atmospheric pressure chemical ionization (DAPCI),[19–21] atmospheric-pressure solids analysis probe (ASAP),[22] electrospray laser desorption/ionization (ELDI),[23] plasma-assisted desorption/ionization (PADI),[24] low temperature plasma (LTP),[25,26] dielectric barrier discharge ionization (DBDI),[27,28] probe electrospray ionization (PESI),[29,30] extractive electrospray ionization (EESI)[31–33] and so on were developed for direct analysis of complex samples without sample pretreatment. However, rapid and quantitative analysis of viscous samples has been still challenging in MS, because the analytes are usually the heterogeneous liquid phase in the high viscous samples which led to the difficulty in liberation of analytes for sensitive MS analysis.

We have demonstrated that by directing a nitrogen gas beam into the bulk liquid, analytes in the highly viscous liquids such as ionic liquids[34] and edible oil samples[35] or on the surfaces of greasy cheese products[36] and toothpaste products[37] can be sampled for EESI-MS analysis.[38,39] This strategy separates the extractive ionization process from the sampling process, improving the toleration of the complex matrix for sensitive mass spectrometric analysis. With a sealable ND device,[40] the analytes liberated from the sample surface were efficiently transferred to the EESI source, preventing the analyte loss at the maximal degree; therefore rapid identification and quantification of picogram of non-volatile compounds (e.g. explosives) was achieved from the human skin surface. Recently, a geometry-independent neutral desorption (GIND) device[33] was reported by us for sensitive sampling of explosives on various surfaces. In comparison with previously reported ND devices, the GIND device requires no optimization of the parameters (e.g., the angles or distances among the gas beam, the surface and the collecting tube), allowing easy operation and high throughput analysis without significant material loss after the ND process. In this study, some illicit additives including sulfonamide and hormones in viscous cosmetics were rapidly detected and identified by GIND-EESI-MS using a sealable GIND device.

EXPERIMENTAL

Chemicals and reagents

Testosterone, progesterone (98%), estrone (99%) and 4-aminobenzoic acid were bought from Acros organics (New Jersey, USA). Methyltestosterone (98.5%), estradial (99.0%), diethylstilbestrol (99.0%), sulfanilamide, sulfapyridine, sulfadiazine, sulfadimethoxine, sulfamethizole, sulfachloropyridazine, sulfathiazole, sulfisoxazole, sulfamethazine and sulfamethoxazole (99%) were bought from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Dexanethasone, betamethasone (98%) and beclomethasone dipropionate (99%) were bought from Sigma-Aldrich (St. Louis, MO, USA). Individual stock standard solutions (1000 mg/l) were prepared in methanol and stored at −20 °C. The cosmetic products including night cream, day cream and anti-sunlight oil were purchased from a local supermarket. No further treatment was performed before the samples were analyzed by ND-EESI-MS.

ND sampling

Similar to the previous report,[37] a home-made glass shell (i.d., 20 mm) was utilized to air-tightly cover the sampling area

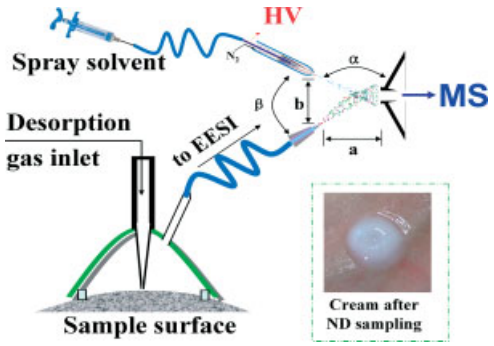


Figure 1. The scheme of the GIND-EESI with a sealable GIND device. The inset shows the picture of the cream on a skin surface sampled by the GIND device.

(∼50 mm^2) and the GIND device (Fig. 1). The surface of the viscous cosmetics (∼0.1 g) was impacted by a pure nitrogen gas beam (room temperature, velocity ∼3.4 m/s, flow 2.7 ml/s) ejected from an aperture (i.d. 100 μm) for desorption sampling. The distance between the GIND gas emitter and the gel surface was 1.5 mm. Under the experimental conditions, the viscous cosmetic samples formed no visible mist inside the GIND device, probably because the desorbed analytes were sampled in a relatively gentle way to form a light aerosol, which was carried as a flow (velocity 3.4 m/s, flux 2.7 ml/s) into the EESI source using the sample transfer line (Teflon tube, i.d. 3 mm).

A fraction of a commercial cosmetic sample (∼100 mg) was deposited on a paper surface or a skin surface, and then was subjected to GIND sampling without further treatment. The method was extended for sampling trace amounts of cosmetic ingredients of the residues coated on a skin surface, from which the analytes were also detected from the contaminated skin surfaces.

EESI-MS analysis

A home-made EESI source was coupled to an linear trap quadrupole (LTQ-XL) mass spectrometer (Fig. 1) (San Jose, CA, USA) for the direct analysis of sampled illicit additives and contaminants in cosmetics. The angle (β) between the sample outlet and the electrospray beam was 60°. The angle (α) between the electrospray beam and the heated capillary of the LTQ instrument was 150°, which was equivalent to the one formed by the sample outlet and the heated capillary of the LTQ instrument. The distance between the inlet of the LTQ instrument and the gas outlets was 15 mm and the distance between the two spray tips was 6 mm.

A methanol/acetic acid (100 : 0.1, v : v) solution infused at 5 μl/min was electrosprayed as the extraction solvent to generate primary ions. The sample plume was directed to intersect the ESI plume for extractive ionization of the analytes. The analyte ions were then guided through the heated capillary of the LTQ mass spectrometer for mass analysis. The temperature of the heated capillary was maintained at 300 °C. A positive ion detection mode was used for most experiments, with an ESI voltage of +4.0 kV. A mass range between 50 and 600 Da was scanned for each measurement. The pressure of nitrogen gas was 2.0 MPa. The default values of voltages for the heated capillary, ion optics and the detectors were used without further optimization. Collision-induced dissociation (CID) experiments were performed by applying 10–35% (arbitrary units defined by the LTQ instrument) of the collision energy to the precursor ions isolated with a window width of 1.6 mass/charge (m/z) units.

For CID experiments, the pressure was 8×10^{-5} Pa inside the ion trap, where the water content was estimated as 1×10^{-5} Pa, which was high enough to promote water clustering reaction inside the ion trap.[41]

Preparation of spiked standard samples

An analyte-free cosmetic sample (2.0 g each) was put into seven beakers, respectively (10 ml, Tianjin Tianbo Glass Instrument Co., Ltd., Tianjin, China), then an analyte methanol solution (400 µl each) was added to a beaker. Finally, a series of seven different concentrations of standard analyte cosmetic samples was prepared. These samples were stirred for 30 min to achieve the homogeneous distribution of analytes inside cosmetics. These mixtures were partially dried in a vacuum (10 mTorr, 298 K), until the final mass loss was 400 mg for each beaker. Because of the volatility of methanol, the solvent evaporated easily within minutes while other ingredients were unchanged. By this means, the viscosity of the sample should not be significantly changed by doping the analytes.

RESULTS AND DISCUSSION

Optimization of the GIND-EESI conditions

GIND device simplifies the operation and optimization of the ND device, and only the pressure of the desorption nitrogen gas is the key parameters of GIND sampling. If the nitrogen gas flow for the GIND process is lower than 0.1 ml/s, no analyte can be liberated from the highly viscous cosmetics such as creams and cleansing agents. However, if the gas flow is too high, deducted signals are observed. In our experiment, the pressure of nitrogen gas for analysis of sulfanilamide was taken as the standard, which was optimized in the range of 1.0–2.4 MPa (gas flow rate 1.7–4.1 m/s) (Fig. 2(a)). The signal intensity of *m/z* 173 $(M+H)^+$ was increasing with elevated pressure of the nitrogen gas for ND from 1.0 to 2.0 MPa (gas flow rate 1.7–3.4 m/s), the maximum signal was reached at 2.0 MPa (gas flow rate 3.4 m/s). Further increase in the pressure led to severely deducted signals and serious sample sparkling, which caused contamination of the GIND device with visible sample spots, and part of the sample transfer line as well, by large droplets of the cosmetic sample. In such a case, in order to avoid carry over effect of the sample, it is highly recommended to replace the GIND device and the sample transfer line before the next sample is loaded for analysis. Besides the contamination, a strong nitrogen flow resulted in an analyte aerosol flux with much higher speed, which probably blew the analytes away from the EESI source without producing detectable signals. Thus, the pressure of nitrogen gas was chosen as 2.0 MPa (gas flow rate 3.4 m/s).

Similarly, the working conditions for the EESI source including the voltage for electrospray, the temperature of the ion transfer capillary, the composition of the electrospray solvent, the flow rate of the spray solvent and extraction solvent were optimized. Generally, acidic solvent (methanol/water/acetic acid = 50 : 48 : 2, v : v : v) can be used for analysis of most compounds, especially for those with strong polarities. In this study, it was found that the slightly acidified methanol (methanol/acetic acid = 100 : 0.1, v : v) produced the best signal for most analytes, probably because a better electrospray will be produced with acidic methanol solution and the analytes can be extracted better in methanol than water. This is also in agreement with the extraction mechanism of EESI.[42] Thus the methanol/acetic acid (100 : 0.1, v : v) solvent was infused at 5 µl/min as the electrospray solvent for all the experiments. The effect of the electrospray voltage on the signal levels is shown in Fig. 2(b). The signal levels of the analytes were also increased when the electrospray voltage was increased from +1 to +4 kV, because the primary droplets were charged better when a higher voltage was used, which produce more primary ions. No significant increase in the signal level was observed when the voltage was higher than 4 kV, probably because the efficiency of primary ion generation was not accordingly enhanced. Therefore, the electrospray voltage was kept as 4.0 kV for all measurements. The solvated ions generated by the extractive ionization process undergo desolvation during the ion transfer. Certainly, the temperature of the heated capillary influences the desolvation

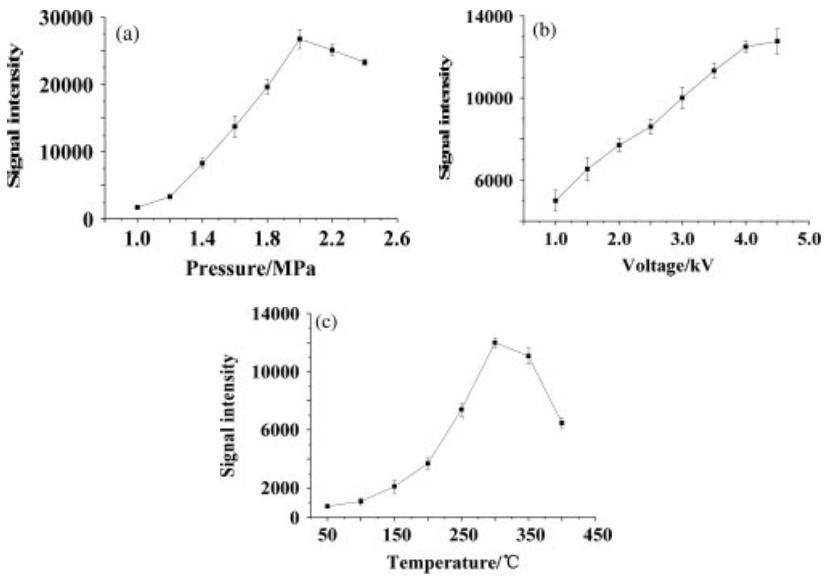


Figure 2. Optimization of GIND-EESI working conditions. (a) Effects of nitrogen gas pressure for ND on the EESI-MS signal intensity. (b) Effects of electrospray voltage on the EESI-MS signal intensity. (c) Effects of temperature of the ion transfer capillary on the EESI-MS signal intensity.

Journal of MASS SPECTROMETRY

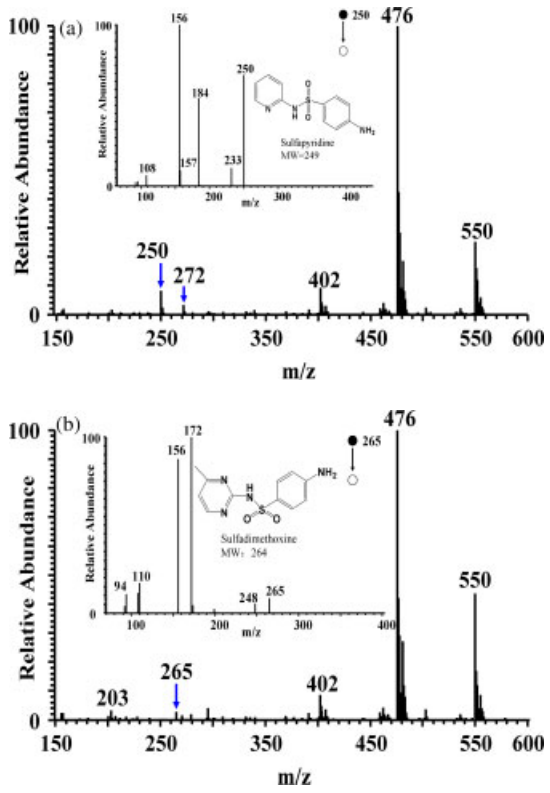


Figure 3. Typical GIND-EESI mass spectra recorded from commercial cosmetic products spiked with antibiotics (100 ng/g). (a) Sulfapyridine, the inset shows the major fragments obtained in the CID experiments. (b) Sulfadimethoxine, the inset shows the MS/MS spectrum of the protonated sulfadimethoxine.

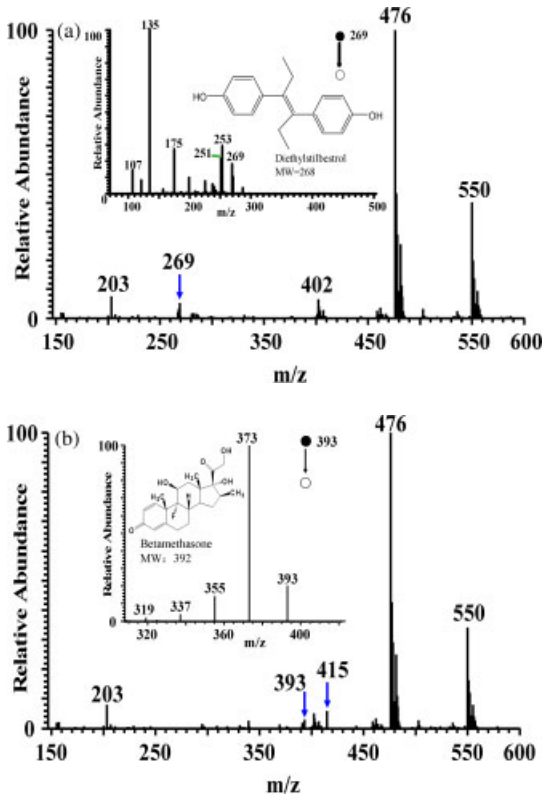


Figure 4. Typical GIND-EESI mass spectra recorded from commercial cosmetic products spiked with hormones (50 ng/g). (a) Diethylstilbestrol, the inset shows the major fragments obtained in the CID experiments. (b) Betamethasone, the inset shows the MS/MS spectrum of the protonated betamethasone.

process. Figure 2(c) shows the effect of the temperature on the signal intensities of sulfanilamide (*m/z* 173). When the capillary was heated to more than 300 °C, the thermo-induced dissociation of analyte could occur, resulting in the reduced signal level.

GIND-EESI-MS analysis of cosmetics

Sulfonamides

Sulfonamides are a kind of antibiotics with low cost and are often added illegally to the cosmetics to strengthen its ability to cure acne. However, the long-term use of sulfonamides cannot only lead to bacteria and/or virus drug resistance, but also result in decreased immunity and other serious health consequences.[43,44] Therefore, the detection of sulfonamide in the cosmetic products is quite demanding.

The GIND-EESI mass spectra recorded from commercial cosmetic products spiked with sulfapyridine (100 ng/g) are shown in Fig. 3(a). The predominant peaks at *m/z* 476 and 550 were likely due to the major components in the cosmetic samples, because these two peaks were commonly detected as the major signals in all the cosmetic samples tested (Figs 3(b), 4(a) and (b)). Theoretically, these components can be easily identified using tandem MS experiments. However, since we have focused on the detection of hormones and sulfonamides in this study, these peaks (i.e., at *m/z* 476 and 550) have not been particularly identified by performing CID experiments. Besides the common base peaks in the full scan mass spectra, the peaks at *m/z* 250 and 272 observed in Fig. 3(a) are corresponding to the protonated sulfapyridine and its sodium complex $[249+Na]^+$, respectively. This can be demonstrated by the further fragmentation of protonated sulfapyridine (*m/z* 250) in MS^2, from which the peaks *m/z* 233, 184, 156 and 108 are ascribed to the loss of NH_3, H_2SO_2, $C_5H_6N_2$ and $C_5H_6ON_2S$, respectively. The relatively low abundances of the peaks at *m/z* 233 and 108 suggest that the cleavage of NH_3 or $C_5H_6ON_2S$ was not favored under these experimental conditions. Similarly, the sodiated sulfapyridine (*m/z* 272) yielded major fragments of *m/z* 178, 130 by the loss of $C_5H_6N_2$ and $C_5H_6ON_2S$, respectively. No product ion of *m/z* 206 was detected, showing that the cleavage of H_2SO_2 was not preferable for the sodiated molecules. These data indicated that the sodium ion was bound to the O═S═O group rather than the benzene ring.

Figure 3(b) shows the GIND-EESI-MS spectrum recorded from a face care cream spiked with sulfadimethoxine (100 ng/g). The sulfadimethoxine (MW 264) was detected as protonated molecules at *m/z* 265. During the CID process, if the positive charge was localized in the benzene ring, the precursor ions (*m/z* 265) could break the N–C bond or the N–S bond to produce major fragments of *m/z* 172 and 156, respectively. If the positive charge was localized on the pyrimidine ring, the peaks at *m/z* 94 and 110 were observed for the same breakages (inset of Fig. 3(b)). The low abundances of the peaks at *m/z* 94 and 110 suggested that the proton was most likely stabilized by the benzene ring. The sulfanilamide (MW 172) was detected as protonated molecules at *m/z* 173 and sodiated molecules at *m/z* 195. Upon CID, the protonated sulfanilamide (*m/z* 173) preferably loses NH_3 to give a major peak at *m/z* 156. If the collision energy was further increased (≥30%), small fragments

of *m/z* 92 were also detected, with low abundance, due to the loss of SO_2 from the precursor ions (*m/z* 156). Similarly, other sulfonamides were detected from the spiked cosmetics products, at trace levels, and were identified by the MS/MS data (Table 1). As summarized in Table 1, the characteristic fragment of *m/z* 156 was abundantly detected from all the sulfonamides. Thus, the signal intensity of the ionic fragment (*m/z* 156) was chosen for quantification of sulfonamides in the cosmetic products.

Hormones

Sex hormones and glucocorticoid hormones are two types of typical hormones added illegally into cosmetics to eliminate freckles and increase skin elasticity. Sex hormones including male and female hormones are steroids that control sexual maturity and reproduction. Apparently, hormone abuse may result in metabolic disorders, even production failure. Ambient desorption ionization MS was used to rapidly determine hormones and their metabolites. For example, the detection limit under 1 ng of anabolic steroids in the raw urine was achieved by combining reactive desorption electrospray ionization (reactive DESI) and tandem MS.[45] But for the highly viscous sample, the overall efficiency of the surface desorption/ionization is unsatisfactory. Alternatively, ND successfully samples the analytes from the complex heterogeneous mixture with high efficiency using positive extractive ionization. In this study, this method was applied to cosmetics analysis.

In the GIND-EESI mass spectrum, trace amounts of diethylstilbestrol (MW 268, 50 ng/g) spiked into a night cream were detected as protonated molecules at *m/z* 269 (Fig. 4(a)). Upon CID, the precursor ions (*m/z* 269) generated fragments of *m/z* 253, 251, 175 and 135 by the loss of CH_4, water, C_6H_6O, $C_9H_{10}O$, respectively. The fragmentation pattern of the protonated diethylstilbestrol was consistent with that obtained using authentic diethylstilbestrol. These data confirm with the successful detection of diethylstilbestrol from the viscous cosmetic samples.

Figure 4(b) shows the GIND-EESI-MS spectrum of betamethasone (50 ng/g) in a cream. The peak at *m/z* 393 is corresponding to the protonated molecule of betamethasone (MW 392). It should be noted that the sodiated molecule *m/z* 415 $[M+Na]^+$ was also detected from the sample, probably because the sodium content was not negligible in the tested sample. During the CID process, the precursor ions (*m/z* 393) generated major peaks at *m/z* 373, 355, 337 and 319 by the loss of HF, and three water successively (inset of Fig. 4(b)). The abundance of its fragment at *m/z* 373 was much higher than other peaks, showing that the HF loss is relatively preferable during the CID process. The interesting fragmentation pathways increased the confidence for specific detection of betamethasone in the complex matrix. Similarly, other hormones were sensitively detected by the method reported here. Table 2 summarizes the MS/MS data for the cosmetic products spiked with trace amounts of hormones. It should be noted that the molecular structures for the hormones examined were quite different from each other. Consequently, unlike the sulfonamides, there was no common fragment observed in the MS/MS data. The quantification of the specific hormone should be carried out based on the abundance using different fragments.

Sensitivity and reproducibility

The low limit of detection (LOD) values (in the range of 0.001–1 ng/g) for most illicit additives were obtained in the MS/MS experiments (Table 3). The LOD values were measured using blank samples spiked with diluted authentic analyte solutions when the signal intensities reached three times the noise level. The low LOD values were favored by the low noise levels detected in the MS/MS spectrum. These data showed that the method established here is sensitive for rapid screening of cosmetics contaminated by the illicit additives.

Under the experimental conditions, the relative standard deviation (RSD) was obtained by measuring the abundances of characteristic fragment (MS/MS) of the illicit compounds spiked in the cream samples for multiple times. For example, the RSD ($n = 10$) for estrone, diethylstilbestrol, testosterone and betamethasone were 10.5, 11.4, 6.8 and 8.0%, respectively. Note that the concentrations of the analytes spiked in the cream samples were equal to 50 times the LOD values for the ten measurements. The RSD results showed that this method provided a reasonable deviation for trace detection of the analytes.

Quantitative analysis

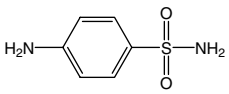
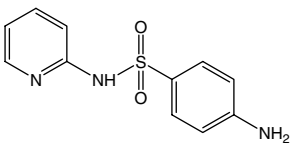
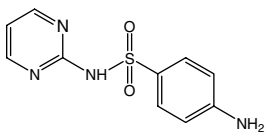
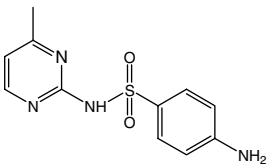
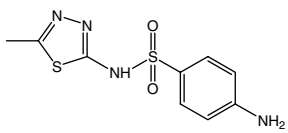
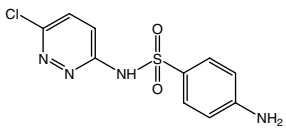
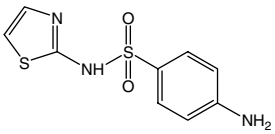
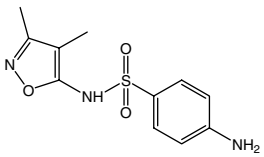
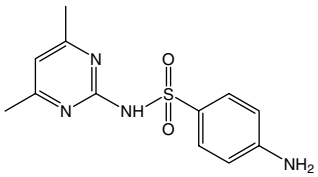
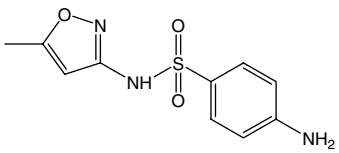
By using the characteristic fragments obtained in the MS/MS experiments, the signal levels (intensity) linearly responded to the concentrations of the illicit additives spiked into the cosmetic products. For example, the linear dynamic responses were obtained for the trace amounts of testosterone (Fig. 5(a)) and sulfanilamide (Fig. 5(b)), showing a linear equation of $y = 84.4x + 26.4$ ($R^2 = 0.978$) and $y = 112.6x$ ($R^2 = 0.991$), respectively. The slope for the sulfanilamide (112.6) was slightly higher than that for the testosterone (84.4), suggesting that the sensitivity for sulfanilamide detection was relatively higher than that for testosterone detection. This is consistent with the LOD data (Table 3). The intercepts for the calibration curves were close to zero, showing that the analytes were not found in the original samples.

Sometimes a cosmetic sample may contain more than one illicit additive (e.g. hormones and sulfonamides together). To test if any signal suppression is caused by the other analyte in the same sample, testosterone (100 ppb) and sulfanilamide (100 ppb) were simultaneously added into a day cream sample and were then homogeneously mixed before the sample was subjected to GIND-EESI-MS analysis. As a result, the signal of testosterone and sulfanilamide was detected as 8350 and 12 200 cps, respectively, in the MS/MS experiment. These values were close to those shown in Fig. 5(a) and (b), respectively, which were obtained using the cosmetic sample that contained only one analyte, either testosterone (100 ppb) or sulfanilamide (100 ppb). These data showed that every analyte could be individually detected using GIND-EESI-MS, although the analytes were present in a single sample with complex matrix. This merit could be ascribed to the advanced feature of GIND-EESI-MS, which tolerates extremely complex matrices by separating the GIND sample process from the EESI process in both space and time, resulting in reduced ionization suppression effects.

Once the sample was contaminated by the illicit additives, the intercept could not be close to the zero point. For example, the sulfapyridine showed linear responses to concentration in the spiked samples, with a linear equation of $y = 34.9x + 197$ ($R^2 = 0.993$) (Fig. 5(c)). However, the intercepts of the calibration curves were significantly higher than zero, because the analytes existed in the cream samples with considerable amounts. As shown in Fig. 5(c), the original concentrations of the analytes were deduced as 5.6 ng/g. The data show that the method can be used

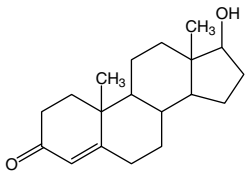
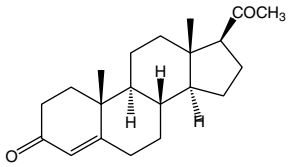
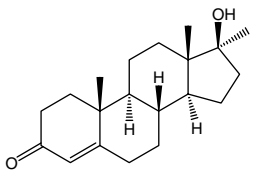
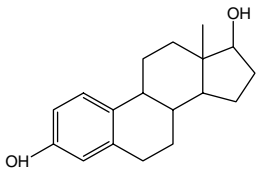
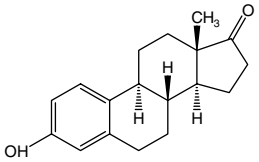
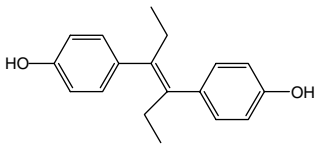
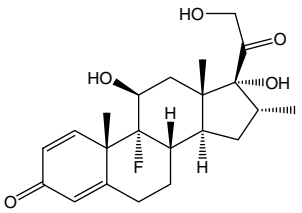
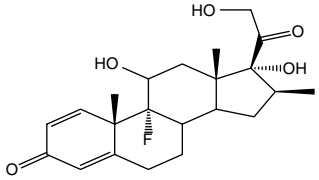
Journal of MASS SPECTROMETRY

Table 1. Mass spectral data for GIND-EESI-MS analysis of sulfonamides

Analytes	Molecular structures	Analyte ions observed (*m/z*)		Product ions MS/MS (*m/z*)
		$M+H^{+}$ [a]	$M+Na^{+}$	
Sulfanilamide		173	195	156
Sulfapyridine		250	272	184, 156, 94
Sulfadiazine		251	273	156, 94
Sulfadimethoxine		265	287	172, 156, 108, 92
Sulfamethizole		271	293	156, 115, 92
Sulfachloropyridazine		286	–	156, 92
Sulfathiazole		256	–	156, 92
Sulfisoxazole		268	–	156, 113, 92
Sulfamethazine		279	301	186, 156, 124, 92
Sulfamethoxazole		254	276	236, 188, 156

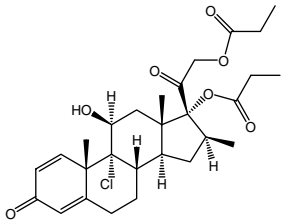
[a] Ions used to generate the fragments observed in this table.

Table 2. Mass spectral data for GIND-EESI-MS analysis of hormones

Analytes	Molecular structures	Analyte ions observed (*m/z*)		Product ions MS/MS (*m/z*)
		$M+H^{+}$ [a]	$M+Na^{+}$	
Testosterone		289	311	271, 253, 243, 213, 109, 97
Progesterone		315	337	297, 279, 255, 239, 215, 109, 97
Methyltestosterone		303	325	285, 267, 227, 189, 109, 97
Estradial		273	295	255, 243, 173, 159, 135
Estrone		271	293	253, 227, 189, 157, 133, 85
Diethylstilbestrol		269	291	253, 225, 199, 175, 135, 107
Dexanethasone		393	415	373, 355, 337, 319, 279
Betamethasone		393	415	373, 355, 337, 319, 279, 237, 197, 177

Journal of MASS SPECTROMETRY

Table 2. (*Continued*)

Analytes	Molecular structure	Analyte ions observed (*m/z*) M+H^{+a}	Analyte ions observed (*m/z*) M+Na^{+}	Product ions MS/MS (*m/z*)
Beclomethasone dipropionate		522	544	503, 485, 429, 411, 393, 337, 319

[a] Ions used to generate the fragments observed in this table.

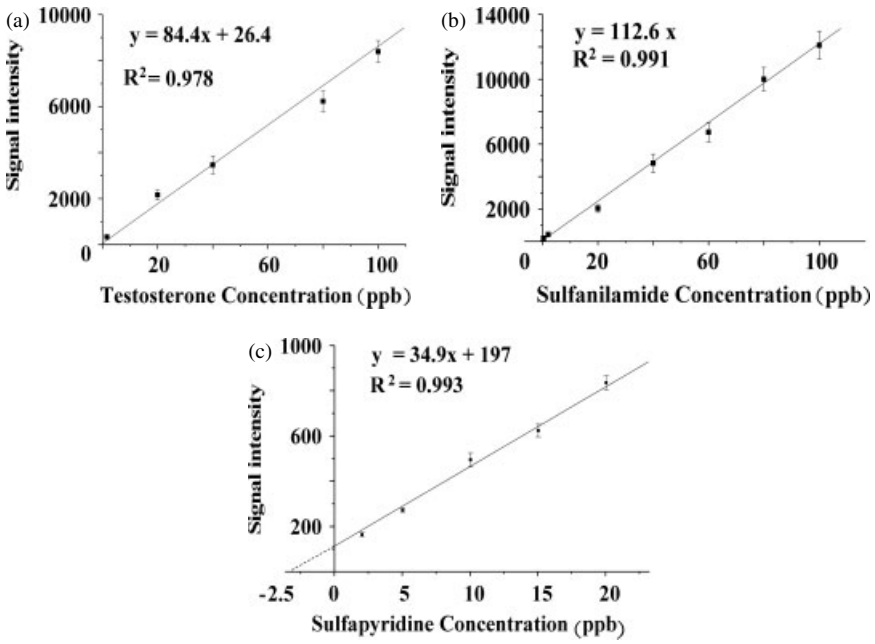


Figure 5. Quantitative detection of illicit additives in commercial cosmetic products. (a) Calibration curve for testosterone; (b) calibration curve for sulfanilamide and (c) calibration curve for sulfapyridine.

for rapid analysis of illicit additives in cosmetic products, with semi-quantitative information.

Following a procedure similar to that described in the experimental section for preparing and analyzing the spiked sample, extra experiments were performed to obtain the recovery of typical illicit additives added into the viscous cosmetic samples. As a result, the recovery of sulfapyridine (added 10 ng/g), sulfanilamide (added 20 ng/g), testosterone (added 10 ng/g) in the highly viscous cream was 108.1, 116.4 and 91.4%, respectively. The recovery was calculated using the following equation: Recovery = Mean value found/Amounts of analyte added, based on measurements of a series of illicit additives in cosmetics. The averaged recovery of most compounds (five measurements for each sample) added to relatively high concentration levels such as 50–200 ng/g were in the range of 90–110%. The relative narrow range of the recovery for measuring various concentration levels of the additives confirms that the GIND-EESI-MS has the capability to fastly detect trace illicit additives in actual samples of high viscosities.

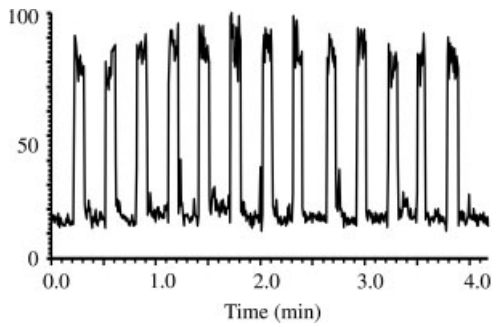


Figure 6. High throughput analysis of betamethasone in cosmetic products. The signal-to-noise ratio for the sodiated molecule was no less than 3.

High throughput analysis

For commercial product analysis, the analysis speed is also a crucial factor. Taking the advantages of direct GIND sampling and rapid extractive ionization, the method established here was

Table 3. Limits of detection of GIND-EESI-MS for illicit additives in cosmetics

Type of illicit additives	Analytes	Limit of detection (ng/g, $S/N = 3$)
Hormone	Testosterone	0.100
	Progesterone	0.100
	Methyltestosterone	0.100
	Estradial	0.100
	Estrone	0.100
	Diethylstilbestrol	1.000
	Dexanethasone	0.001
	Betamethasone	0.01
	Beclomethasone dipropionate	0.5
Antibiotics	Sulfanilamide	0.10
	Sulfapyridine	0.10
	Sulfadiazine	1.000
	Sulfadimethoxine	0.100
	Sulfamethizole	0.010
	Sulfathiazole	0.010
	Sulfisoxazole	0.010
	Sulfamethazine	0.010
	Sulfamethoxazole	0.010

LOD was calculated using the following equation: LOD $= 3\sigma c/S$, where σ is the standard deviation of the ten measurements performed on the blank sample, c the minimal concentration of a standard solution used to obtain the calibration curve and S the mean value of the signal of analytes at the concentration c.

able to analyze bulk sets of samples with improved throughput. As demonstrated, Fig. 6 shows the high throughput analysis of 13 cream samples spiked with betamethasone (10 ng/g) within 4 min. The extracted ion current of the protonated molecules (m/z 393) (up level) and the extracted ion current of the sodiated analytes (m/z 415, bottom level) responded rapidly to the samples. The response time (90% of the maximum) was no more than three scans. According to Fig. 6, it is possible to further improve the analysis speed since the intervals between each sample are much longer than the response time. Currently, the bottle neck of the throughput is the sample loading step, which takes about 1 min for each loading. However, in comparison with methods requiring separation steps, the method reported here is capable of semi-quantitatively analyzing highly viscous cosmetic products with significantly improved throughput.

CONCLUSIONS

The method developed here is an attractive analytical tool for high throughput analysis of trace analytes in heterogeneous samples in highly viscous samples, with high sensitivity, high specificity and high throughput. Cosmetic products are types of heterogeneous liquid samples of high viscosities. Fast detection of trace compounds such as illicit additives in cosmetic products is highly desirable, but many challenges have to be faced due to the complexity of the samples. As demonstrated in this study, the analytes present in the viscous cosmetics products were sensitively sampled by using nitrogen gas beam, which was directed into the bulk liquids to liberate the analytes inside the matrix. Using a sealed geometry-independent ND device, the analytes were collected and transferred for post extractive ionization in a home-made EESI source, without significant material loss. The illicit additives including sulfonamides and hormones were thereby rapidly detected and identified by tandem MS, without any sample pretreatment. For most compounds tested, this method was in the range of 0.001–1 ng/g, and the reasonable RSD was in the range of about 6.8–11.4% for the ten measurements. The signals detected linearly responded to the concentrations of the illicit additives in the cosmetic product samples, showing a wide dynamic response range for most analytes and the capability for semi-quantitative analysis. Moreover, the analysis speed has improved and the analysis of three samples was completed within a few minutes.

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REFERENCES

[1] V. Dimitrova, M. Kaneva, T. Gallucci. Customer knowledge management in the natural cosmetics industry. *Ind. Manage. Data Syst.* **2009**, *109*, 1155.
[2] T. Kooyers, W. Westerhof. Toxicology and health risks of hydroquinone in skin lightening formulations. *J. Eur. Acad. Dermatol. Venereol.* **2006**, *20*, 777.
[3] M. P. Castanedo-Tardan, K. A. Zug. Patterns of Cosmetic Contact Allergy. *Dermatol. Clin.* **2009**, *27*, 265.
[4] P. D. Darbre. Environmental oestrogens, cosmetics and breast cancer. *Best Pract. Res. Clinc. Endocrinol.* **2005**, *20*, 121.
[5] C. Laguna, J. Cuadra, B. Martín-Gonzáleza, V. Zaragoza, L. Martínez-Casimiro, V. Alegre. Allergic Contact Dermatitis to Cosmetics. *Actas Dermosifiliogr.* **2009**, *100*, 53.
[6] W. Jin, Y. Yang, W. Wang, J. Ye. Simultaneous determination of antibiotics in anti-acne aosmetics by rapid LC with DAD. *Chromatographia* **2009**, *69*, 1.
[7] C. H. Lin, J. Y. Sheu, H. L. Wu, Y. L. Huang. Determination of hydroquinone in cosmetic emulsion using microdialysis sampling coupled with high-performance liquid chromatography. *J. Pharm. Biomed.* **2005**, *38*, 414.
[8] S. C. Rastogi, C. Zachariae, J. D. Johansen, C. Devantier, T. Menné. Determination of methyldibromoglutaronitrile in cosmetic products by high-performance liquid chromatography with electrochemical detection: Method validation. *J. Chromatogr.* **2004**, *1031*, 315.
[9] D. Takahiro, K. Keiji, T. Satoshi, T. Shuzo, I. Shozo. Simultaneous measurement of diazolidinyl urea, urea, and allantoin in cosmetic samples by hydrophilic interaction chromatography. *J. Chromatogr. B* **2009**, *877*, 1005.
[10] Y. Wen, Y. Wang, B. S. Zhou, Y. Xu, Y. Q. Feng. Determination of Sexual Hormones in Liquid Cosmetics by Polymer Monolith Microextraction Coupled with High Performance Liquid Chromatography. *Chin. J. Anal. Chem.* **2007**, *35*, 681.
[11] X.Y. Zhao, Y. F. Lin, X. Z. Hu, X. F. Fu, J. Li, P. Wang. Determination of 9 glucocorticoids in cosmetics by reversed 2 phase high performance liquid chromatography. *Chin. J. Anal. Lab.* **2009**, *28*, 111.
[12] D. T. Harwood, D. J. Handelsman. Development and validation of a sensitive liquid chromatography-tandem mass spectrometry assay to simultaneously measure androgens and estrogens in serum without derivatization. *Clin. Chim. Acta* **2009**, *409*, 78.
[13] P. Regal, B. I. Vázquez, C. M. Franco, A. Cepeda, C. Fente. Quantitative LC-MS/MS method for the sensitive and simultaneousdetermination of natural hormones in bovine serum. *J. Chromatogr. B* **2009**, *877*, 2457.
[14] T. J. Yang, F. J. Tsai, C. Y. Chen, T. C. C. Yang, M. R. Lee. Determination of additives in cosmetics by supercritical fluid extraction on-line headspace solid-phase microextraction combined with gas chromatography-mass spectrometry. *Anal. Chim. Acta* **2010**, *668*, 188.

[15] Z. Takats, J. M. Wiseman, B. Gologan, R. G. Cooks. Mass Spectrometry Sampling under Ambient Conditions with Desorption Electrospray Ionization. *Science* **2004**, *306*, 471.
[16] A. Venter, M. Nefliu, R. G. Cooks. Ambient desorption ionization mass spectrometry. *Trend Anal. Chem.* **2008**, *27*, 284.
[17] H. W. Chen, G. Gamez, R. Zenobi. What Can We Learn from Ambient Ionization Techniques?. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 1947.
[18] R. B. Cody, J. A. Laramee, H. D. Durst. Versatile New Ion Source for the Analysis of Materials in Open Air under Ambient Conditions. *Anal. Chem.* **2005**, *77*, 2297.
[19] H. W. Chen, J. Zheng, X. Zhang, M. B. Luo, Z. C. Wang, X. L. Qiao. Surface desorption atmospheric pressure chemical ionization mass spectrometry for direct ambient sample analysis without toxic chemical contamination. *J. Mass Spectrom.* **2007**, *42*, 1045.
[20] X. L. Zhang, B. Jia, K. K. Huang, B. Hu, R. Chen, H. W. Chen. Tracing Origins of Complex Pharmaceutical Preparations Using Surface Desorption Atmospheric Pressure Chemical Ionization Mass Spectrometry. *Anal. Chem.* **2010**, *82*, 8060.
[21] S. P. Yang, J. H. Ding, J. Zheng, B. Hu, J. Q. Li, H. W. Chen, Z. Q. Zhou, X. L. Qiao. Detection of melamine in milk products by surface desorption atmospheric pressure chemical ionization mass spectrometry. *Anal. Chem.* **2009**, *81*, 2426.
[22] C. N. McEwen, R. G. McKay, B. S. Larsen. Analysis of solids, liquids, and biological tissues using solids probe introduction at atmospheric pressure on commercial LC/MS instruments. *Anal. Chem.* **2005**, *77*, 7826.
[23] J. Shiea, M. Z. Huang, H. J. Hsu, C. Y. Lee, C. H. Yuan, I. Beech, J. Sunner. Electrospray-assisted laser desorption/ionization mass spectrometry for direct ambient analysis of solids. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 3701.
[24] L. V. Ratcliffe, F. J. M. Rutten, D. A. Barrett, T. Whitemore, D. Seymour, C. Greenwood, Y. Aranda-Gonzalvo, S. Robinson, M. McCoustra. Surface analysis under ambient conditions using plasma-assisted desorption/ionization mass spectrometry. *Anal. Chem.* **2007**, *79*, 6094.
[25] Y. Y. Liu, X. X. Ma, Z. Q. Lin, M. J. He, G. J. Han, C. D. Yangœ, Z. Xing, S. C. Zhang, X. R. Zhang. Imaging Mass Spectrometry with a Low-Temperature Plasma Probe for the Analysis of Works of Art. *Angew. Chem. Int. Ed. Engl.* **2010**, *49*, 4435.
[26] N. Na, Y. Xia, Z. L. Zhu, X. R. Zhang, R. G. Cooks. Birch Reduction of Benzene in a Low-Temperature Plasma. *Angew. Chem. Int. Ed. Engl.* **2009**, *48*, 2017.
[27] L. C. Chen, Z. Yu, H. Furuya, Y. Hashimoto, K. Takekawa, H. Suzukiœ, O. Ariyada, K. Hiraoka. Development of ambient sampling chemi/chemical ion source with dielectric barrier discharge. *J. Mass Spectrom.* **2010**, *45*, 861.
[28] K. Hiraoka, S. Ninomiya, L. C. Chen, T. Iwama, M. K. Mandal, H. Suzuki, O. Ariyada, H. Furuyaœ, K. Takekawaœr. Development of double cylindrical dielectric barrier discharge ion source. *Analystœr* **2009**, *136*, 1210.
[29] L. C. Chen, K. Yoshimura, Z. Yu, R. Iwata, H. Ito, H. Suzuki, K, Mori, O. Ariyada, S. Takeda, T. Kubota, K. Hiraoka. Ambient Imaging Mass Spectrometry by Electrospray Ionization Using Solid needle as Sampling Probe. *J. Mass Spectrom.* **2009**, *44*, 1469.
[30] Z. Yu, L. C. Chen, H. Suzuki, O. Ariyada, R. Erra-Balsells, H. Nonami, K. Hiraoka. Direct profiling of phytochemicals in tulip tissues and in vivo monitoring of the change of carbohydrate content in tulip bulbs by probe electrospray ionization mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 2304.
[31] L. Zhu, G. Gamez, H. W. Chen, K. Chingina, R. Zenobi. Rapid detection of melamine in untreated milk and wheat gluten by ultrasound-assisted extractive electrospray ionization mass spectrometry (EESI-MS). *Chem. Commun.* **2009**, *5*, 559.
[32] H. W. Chen, S. P. Yang, M. Li, B. Hu, J. Q. Li, J. Wang. Sensitive detection of native proteins using extractive electrospray ionization mass spectrometry. *Angew. Chem. Int. Ed. Engl.* **2010**, *49*, 3053.
[33] H. W. Gu, S. P. Yang, J. Q. Li, B. Hu, H. W. Chen, L. L. Zhang, Q. Fei. Geometry-independent neutral desorption device for the sensitive EESI-MS detection of explosives on various surfaces. *Analyst* **2010**, *135*, 779.
[34] W. S. Law, H. W. Chen, J. H. Ding, S. P. Yang, L. Zhu, G. Gamez, K. Chingin, Y. L. Ren, R. Zenobi. Rapid characterization of complex viscous liquids at the molecular level. *Angew. Chem. Int. Ed. Engl.* **2009**, *48*, 8277.
[35] W. S. Law, H. W. Chen, R. Balabin, C. Berchtold, L. Meier, R. Zenobi. Rapid fingerprinting and classification of extra virgin olive oil by microjet sampling and extractive electrospray ionization mass spectrometry. *Analyst* **2010**, *135*, 773.
[36] Z. C. Wu, K. Chingin, H. W. Chen, L. Zhu, B. Jia, R. Zenobi. Sampling analytes from cheese products for fast detection using neutral desorption extractive electrospray ionization mass spectrometry. *Anal. Bioanal. Chem.* **2010**, *397*, 1549.
[37] J. H. Ding, H. W. Gu, S. P. Yang, M. Li, J. Q. Li, H. W. Chen. Selective Detection of Diethylene Glycol in Toothpaste Products Using Neutral Desorption Reactive Extractive Electrospray Ionization Tandem Mass Spectrometry. *Anal. Chem.* **2009**, *81*, 8632.
[38] H. W. Chen, A. Wortmann, R. Zenobi. Neutral desorption sampling coupled to extractive electrospray ionization mass spectrometry for rapid differentiation of biosamples by metabolomic fingerprinting. *J. Mass Spectrom.* **2007**, *42*, 1123.
[39] H. W. Chen, S. P. Yang, A. Wortmann, R. Zenobi. Neutral Desorption Sampling of Living Objects for Rapid Analysis by Extractive Electrospray Ionization Mass Spectrometry. *Angew. Chem. Int. Ed. Engl.* **2007**, *119*, 7735.
[40] H. W. Chen, B. Hu, Y. Hu, Y. F. Huan, Z. Q. Zhou, X. L. Qiao. Neutral Desorption Using a Sealed Enclosure to Sample Explosives on Human Skin for Rapid Detection by EESI-MS. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 719.
[41] C. S. Hoaglund-Hyzer, D. E. Clemmerœ. Ion Trap/Ion Mobility/Quadrupole/Time-of-Flight Mass Spectrometry for Peptide Mixture Analysis. *Anal. Chem.* **2001**, *73*, 177.
[42] W. S. Law, R. Wang, B. Hu, C. Berchtold, L. Meier, H. W. Chen, R. Zenobi. On the Mechanism of Extractive Electrospray Ionization. *Anal. Chem.* **2010**, *82*, 4494.
[43] G. F. Pang. *Modern Analytical Technology for Pesticide Residue and Veterinary Drug Residue*, Science Press: Beijing, **2007**.
[44] J. S. Li, Y. M. Qiu, C. Wang. *Residue Analysis for Veterinary Drugs*, Shanghai Science and Technology Press: Shanghai, **2002**.
[45] G. Huang, H. Chen, X. R. Zhang, R. G. Cooks, Z. Ouyang. Rapid Screening of Anabolic Steroids in Urine by Reactive Desorption Electrospray Ionization. *Anal. Chem.* **2007**, *79*, 8327.