Rapid characterization of complex viscous samples at molecular levels by neutral desorption extractive electrospray ionization mass spectrometry

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In this protocol, the sample (which could be a bulk or heterogeneous fluid, or a greasy surface) is treated with a neutral desorption (ND) sampling gas beam, and the resulting analyte mixtures are directly characterized by extractive electrospray ionization mass spectrometry (EESI-MS). The ND device can be specifically constructed such that the sampling gas beam is bubbled through the liquid sample (microjet sampling) or directed to impact the sample surface (e.g., for the analysis of a material like cheese). The ND-EESI-MS analysis process requires no sample pretreatment because it can tolerate an extremely complex matrix. ND-EESI-MS allows real-time, online chemical profiling of highly viscous samples under ambient conditions. Both volatile and nonvolatile analytes from viscous samples can easily be detected and quantified by ND-EESI-MS, thereby providing an MS-based analytical platform for multiple disciplines (e.g., for the food industry, for drug discovery, and for the biological and life sciences). Here we describe the ND-EESI-MS protocol for viscous sample analysis, including the experimental design, equipment setup, reagent preparation, data acquisition and analysis steps. The data collection process takes <1 min per sample, although the time required for the whole procedure, which largely depends on the experimental preparation processes, might be considerably longer.

INTRODUCTION

Development of the protocol

Mass spectrometry (MS) can provide unparalleled sensitivity and specificity for the detection and identification of trace amounts of chemical compounds in a mixture, which is mostly attributed to its unique capability to measure the mass-to-charge ratio (m/z) of the charged particles[1]. The generation of charged molecules or molecular fragments is a prerequisite for MS analysis. In the early days, analyte ions were traditionally created under vacuum conditions using the classical ionization techniques such as electron impact[2] or chemical ionization (CI)[3]. The vacuum system involved in these ionization techniques has been designed to be compatible with the vacuum environment of the mass spectrometer, even though vacuum conditions are not absolutely necessary for ion creation. Later, the introduction of atmospheric pressure ionization (API) techniques such as electrospray ionization (ESI)[4,5] and atmospheric pressure CI (APCI)[6] successfully allowed the production of analyte ions outside the vacuum chamber. This greatly eased the application of MS, especially for sample manipulation. However, for either the ionization techniques requiring a vacuum environment (e.g., electron impact and CI) or the API techniques used at atmospheric pressure (e.g., ESI and APCI), samples with complex matrix still have to be pretreated (e.g., extraction, purification and/or chromatography separation) before MS analysis[7–11], making the analytical work laborious and time consuming.

In the summer of 2004, Cooks and co-workers[12] showed that desorption electrospray ionization (DESI) could produce ions of analytes on solid surfaces with high efficiency under ambient conditions. With DESI, specific analytes (including small organic molecules[13], such as propranolol, testosterone, dobutamine, verapamil, chloramphenicol, ibuprofen, diazepam, roxithromycin, carbamazepine, acetylcholine and angiotensin; and large biopolymers[14], such as cytochrome *c* (12.3 kDa), lysozyme (14.3 kDa), apomyoglobin (16.9 kDa), β-lactoglobulin B (18.2 kDa), chymotrypsinogen A (25.6 kDa), ovalbumin (44.4 kDa) and BSA (66.4 kDa)) in complex matrices on solid surfaces have been directly desorbed/ionized, thereby dramatically simplifying the analysis process and improving the analytical speed for MS analysis of complex samples. Following DESI, new ionization techniques such as direct analysis in real time[15,16], laser ablation with electrospray ionization[17–19], dielectric barrier discharge ionization[20], desorption atmospheric pressure CI[21,22], atmospheric solids analysis probe[23], low-temperature plasma probe[24,25], electrospray-assisted laser desorption/ionization[26–28], atmospheric pressure glow discharge ionization[29], desorption atmospheric pressure photoionization[30] and so on have been developed for fast and direct ambient analysis of complex samples on solid surfaces with minimal sample pretreatment. In addition, by drying liquid samples (e.g., urine[31]) on a paper surface, they can be embedded on solid surfaces for MS analysis using the above-mentioned ionization methods. This has further extended these ambient ionization techniques for the fast analysis of liquid samples while minimal sample pretreatment is needed. A tutorial review is recently available to summarize the fundamental principles, physical processes and typical applications of ambient ionization techniques[32].

Early researchers reported that electrospray plumes could effectively ionize either particles or vapor molecules[33–35]. Motivated by the need for direct analysis of liquid samples, especially the nonvolatile compounds therein, Cooks and co-workers[36] further developed the extractive electrospray ionization (EESI) technique in 2006. In EESI, liquid samples are neutrally sprayed to intersect an electrospray plume and then ionized by the charged droplets created by the ESI[37]. In comparison with the desorption/ionization processes occurring on a 2D surface[38], in EESI, analytes in the

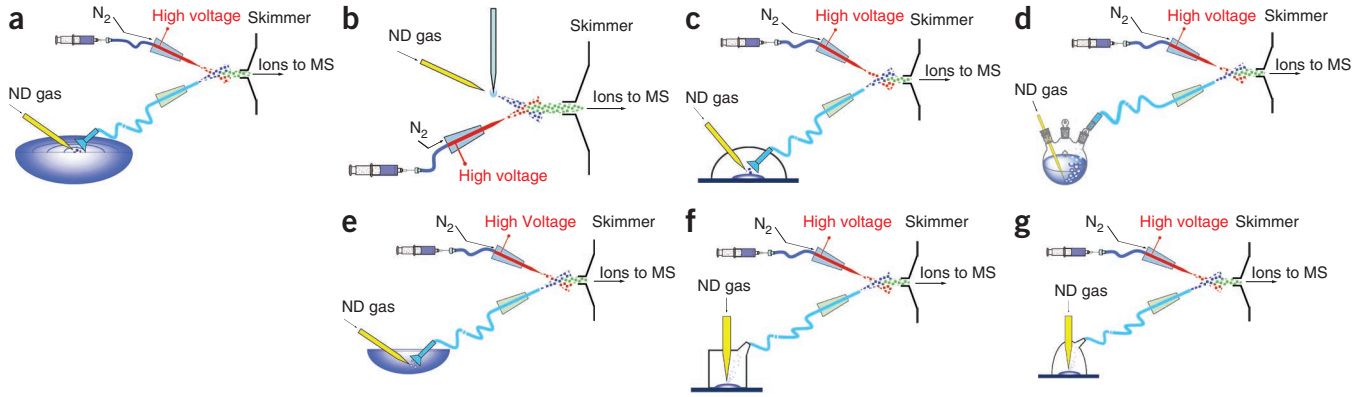


Figure 1 | Schematic diagrams of seven types of ND sampling devices. (**a**–**g**) A typical open-air ND sampling device (**a**); a simplified open-air ND sampling device (**b**); a sealable ND sampling device(**c**); a microjet ND sampling device (**d**); a simplified microjet ND sampling device (**e**); a typical GIND sampling device(**f**); and an improved GIND sampling device (**g**), which is easier for cleaning.

matrix are subjected to the extractive ionization in a 3D space, in which the matrix is dispersed over a relatively large volume[36,39,40]. Consequently, ion suppression effects are reduced, enabling continuous analysis of complex samples by EESI-MS without significant loss of sensitivity. Additionally, with EESI, the bulk liquids are isolated from the high electric field and are also not in contact with the electrospray solutions (e.g., an acidic methanol/water solution), resulting in improved safety and lower sample contamination. Actually, besides liquid samples, aerosols, such as those in the exhaled breath[41,42], can be conveniently introduced into the EESI source for direct ionization and subsequently analyzed by mass spectrometers. As a result, EESI-MS has been demonstrated to be a promising tool for the direct analysis of liquids and aerosols without sample pretreatment.

Viscous samples are common in daily life and also have important roles in research laboratories, industries, life sciences and many other aspects of modern science and technology. For example, blood is a typical heterogeneous liquid of high viscosity, and blood analysis is important for human health evaluation. Generally, a specific analyte in viscous samples can be analyzed by techniques such as HPLC/GC (gas chromatography), GC/LC-MS and spectroscopy methods. Before analysis of an actual sample, tedious sample pretreatments including liquid-liquid extraction, solid-phase extraction, matrix cleanup and preconcentration are usually required. The sample pretreatment steps prevent high-throughput analysis and likely produce biased recovery for the analytes. Therefore, the sensitive, selective and fast analysis of these viscous samples at molecular levels remained a challenge for the analytical science. With this regard, the analytical method of EESI-MS coupled with neutral desorption (ND) sampling has been investigated for the ambient analysis of various viscous samples (e.g., viscous liquids and pastes)[43–49].

For rapid characterization of viscous samples by EESI-MS, a neutral gas beam is directed to impact the samples for ND (schematically shown in **Fig. 1a**) so that the analytes liberated from the bulk samples can be transferred to the EESI source for direct ionization[50]. As shown in **Figure 1a**, the ND sampling process is separated from the extractive ionization process, enabling the ionization to occur in a 3D space without contacting with the bulk sample. This makes ND-EESI-MS tolerate extremely complex matrices. As the analytes can be continuously liberated from the bulk samples without substantial sensitivity loss, ND-EESI-MS realizes real-time, online chemical profiling of highly viscous samples under ambient conditions. Moreover, by using biological/chemical compatible reagents (e.g., nitrogen gas or air for most cases) for the ND process, the potential for undesired biological/chemical reactions of the analytes can be minimized, and thus the physiological/pathological status of the analytes can be maximally preserved (especially important for biosamples[51–54], such as skin and tissues). It is noteworthy that, in recent studies[55,56], native proteins (e.g., myoglobin and lysozyme) in aqueous solutions have been successfully detected with EESI-MS, which not only opens the possibility for rapid detection of both small and large molecules in biological sample by using EESI-MS but also leads EESI-MS to the next stage, in which the rapid detection of proteins in complex viscous samples becomes possible.

Application of the method

Until now, ND-EESI-MS has been successfully applied for rapid characterization of samples with a wide range of viscosities, including perfume[43], beer[47], ionic liquid[45], edible oil[48], honey[45], cheese[49] and toothpaste[46], with their viscosities ranging from a few centipoise (cP) to 300,000 cP (**Figs. 1** and **2**). The analytes tested by ND-EESI-MS are summarized in **Table 1**. For all the application examples, no sample pretreatment was performed. Analytes, including both volatile and nonvolatile compounds (**Table 1**) in the viscous samples with different viscosities, can be directly detected using ND-EESI-MS. This makes ND-EESI-MS an attractive tool to characterize highly viscous samples involved in multiple disciplines, such as food industry, chemical industry and drug discovery. With the high content information provided by the EESI-MS spectra, samples can be readily classified by the ND-EESI-MS method. For example, four types of commercial cheese products have been successfully classified by using ND-EESI-MS, and both volatile (e.g., butyric acid and valeric acid) and nonvolatile compounds (e.g., decanoic acid) in cheese samples have been detected[49]. In addition, ND-EESI-MS is also a sensitive method for quantitative determination. For example, the limit of detection (LOD) of diethyl phthalate (DEP) in ethanol (the major matrix of perfume products) is <100 p.p.b. (wt/wt), with a dynamic response range of four orders of magnitude[43]. The LOD of diethylene glycol (DEG) in toothpaste (viscosity > 300,000 cP; **Fig. 2**) is ~200 p.p.b. (wt/wt), with a signal response range of five orders of magnitude[46]. Usually, the targeted analytes in viscous

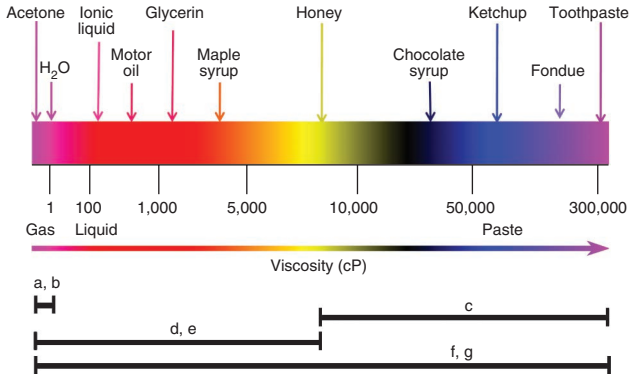


Figure 2 | The viscosity spectrum, in which the viscosities of various viscous samples (20 °C, atmospheric pressure) are presented. Centipoise (cP) is the centimeter-gram-second unit of dynamic viscosity, defined as 'shear stress required to move one layer of fluid along another over a total layer thickness of 1 cm at a shear rate of 1 cm s^{-1}'. Letters above the scale bars correspond to the individual ND device (shown in **Fig. 1**) that could be used to analyze the material. (Copyright: Wiley-VCH Verlag. Reproduced with permission from ref. 45.)

samples can be quantified using a standard addition method[43,46], which minimizes the matrix effects caused by the complex matrices. These experimental results have confirmed that the targeted analytes even in high-viscosity samples (e.g., toothpaste) can be rapidly and directly detected by ND-EESI-MS, with both qualitative and quantitative information. Note that even though ND-EESI-MS can be theoretically used for detection of many kinds of molecules, it is particularly good for molecules with high polarity and/or high proton affinity, whereas it works less well for nonpolar molecules.

Compared with the separation technique-based methods for the analysis of viscous samples, the key merits of ND-EESI-MS include the following: (i) viscous samples can be directly analyzed without tedious pretreatment; (ii) both volatile and nonvolatile molecules can be detected; (iii) there is good toleration of matrices; (iv) there is minimal chemical contamination and better biological/chemical compatibility; and (v) remote analysis of ambient samples by MS becomes possible. This is particularly useful for monitoring samples (e.g., explosives) under unusual conditions (e.g., explosions, biohazards and so on). The high-throughput, real-time and online analysis of various viscous samples can be realized by ND-EESI-MS, thus providing a powerful MS-based platform for multiple disciplines.

Experimental design

As shown in previous studies[45–49,51–54], ND-EESI can be easily implemented in different instruments without resorting to sophisticated instrumentation. Once a mass spectrometer with an API interface is available, ND-EESI-MS can be constructed using commonly available materials and parts in a research lab, and the most important steps include (i) fabricating an ND device, (ii) coupling an ND device to the EESI source and (iii) assembling the ND-EESI source to an API mass spectrometer. In addition, the design and implementation of ND-ESSI-MS can be tailored according to the specific conditions of a laboratory to meet particular research purposes.

To date, ND devices have evolved from the open-air ND devices[43] to sealable ND devices[44–48], and then to geometry-independent ND (GIND) devices with improved sampling performance[49,57]. At the very beginning, the open-air ND device (**Fig. 1a**) was used for ND sampling and could be easily constructed by using a gas emitter,

TABLE 1 | Analysis of various samples with different viscosities by ND-EESI-MS.

Sample types	Ion detection mode	Appropriate standard	Results[a]	Reference
Perfumes	Positive	Diethyl phthalate	LOD <100 p.p.b. (wt/wt)	43
Beer	Positive and negative	Amino acids	Simultaneous identification of trace analytes in beer (low p.p.m. or sub-p.p.m.), including volatile esters (e.g., ethyl acetate and isoamyl acetate), free fatty acids (e.g., caproic acid, caprylic acid and capric acid), semi/nonvolatile organic or inorganic acids (e.g., lactic acid) and various amino acids	47
Ionic liquid	Positive	Fructose, 1-ethyl-3-methylimidazolium chloride (EMIMCl)	Identification of fructose, HMF and EMIMCl; the reaction kinetics can be monitored in real time	45
Edible oil	Positive	*E*-2-hexenal	Simultaneous identification of volatile compounds (e.g., *E*-2-hexenal) and nonvolatile phenolic compounds (e.g., sinapic acid, coumaric acid and tyrosol) with different polarities	48
Honey	Positive	5-OH-HMF	Rapid fingerprinting and classification of different grades of honey	45
Cheese	Negative	Butyric acid	Simultaneous identification of volatile analytes (e.g., butyric acid and valeric acid) and non-volatile analytes (e.g., decanoic acid)	49
Toothpaste	Positive	Diethylene glycol (DEG)	LOD <200 p.p.b. (wt/wt)	46

[a]Analytes are qualified and quantified by characteristic fragment ions in MS^2 spectra.

a sample collector and two gas delivery tubes. During the ND sampling process, neutral gas introduced through a gas delivery tube (i.d., 3 mm) is ejected from the gas emitter (i.d., 80–800 μm); the gas emitter can be easily fabricated using a glass tube (i.d., 3 mm) to impact the liquid sample surface. However, experimental results have indicated that chemicals desorbed from the sample were lost in the open air, causing a decrease in detection sensitivity[57]. Moreover, even though slightly viscous liquids (e.g., perfume[43]) and some biological samples (e.g., human skin[52]) can be effectively analyzed by the open-air ND device, the sampling performances are unsatisfactory for highly viscous samples or greasy surfaces. As a result, we suggest that the open-air ND device is more suitable for liquid samples with relatively low viscosities (e.g., acetone, water and perfume) rather than for those with medium or high viscosities. The liquid surfaces to be sampled by the ND device shown in **Figure 1a** can be from a bulk solution in a container (e.g., basin or beaker) or from other sources of interest (e.g., lake or river).

It is noteworthy that, when necessary, the device in **Figure 1a** can be further simplified such that the sample collector is not applied (**Fig. 1b**). In this case, the droplet of the liquid sample hangs on the tip of a pipette tube or a glass rod (o.d., 0.1–5 mm), and fine aerosols are formed under a gentle stream of neutral gas; these aerosols are subsequently transported to the EESI source for ionization. This simplified ND device is particularly suitable for the rapid screening of samples with low viscosity. Additionally, the cross-contamination and/or carryover effect can be minimized by using a disposable pipette or a glass rod as the sample holder. Similar to the device in **Figure 1a**, this simplified device is also not suitable for medium- and high-viscosity samples because the small-size aerosols required for ionization are hard to obtain. It is also possible for the device shown in **Figure 1b** to get poor signals from highly volatile analytes because the volatile compounds may be mostly evaporated before they can be sampled by the gas beam.

To improve the sampling effectiveness of the open-air ND sampling devices, the sealable ND sampling strategy has been introduced as schematically illustrated in **Figure 1c**, in which an airtight C-shaped glass cell is added to cover the open-air ND sampling device. Therefore, the ND is performed in an enclosed chamber and, thus, more desorbed chemicals can be collected and transported to the EESI source. In this way, analytes can be detected with improved sensitivity. In theory, the sealable ND sampling device shown in **Figure 1c** can be used for all kinds of viscous samples. However, because of the small size of the sealable device (e.g., i.d., 20 mm), the device would be insufficient to cover a large liquid container; on the contrary, it is also inconvenient to apply the device to the surface of liquid samples. Therefore, we recommend using the device in **Figure 1c** to analyze paste samples or greasy surfaces.

Furthermore, this strategy has been extended to sample viscous liquids using a microjet ND sampling device (**Fig. 1d**). In the sealable ND device that involves the microjet sampling mechanism, a sharp stream of neutral gas is introduced into the bulk solution to form bubbles. The bubbles move upward to the liquid surface, burst and generate microdroplets at the gas-liquid interface. Afterward, the neutral microdroplets formed inside the enclosed chamber are carried by the gas flow, passing through the air stream outlet to the EESI source. More analytes, including volatile, semivolatile and even nonvolatile compounds, can be liberated from the viscous liquid samples with the microjet ND sampling device[47]. Thus, the microjet ND sampling device would be recommended for the analysis of viscous liquid samples with relatively high viscosity (e.g., ionic liquid[45], beer[47], edible oils[48] and honey[45]). As a small amount of sample (e.g., 2 ml) can be effectively analyzed by this device coupled to EESI-MS, this method would also be of interest for the analysis of plasma for which the analytical procedure would be similar to that for the analysis of edible oils[48].

The microjet ND sampling device presented in **Figure 1d** can be further simplified as shown in **Figure 1e**. Clearly, the ND device shown in **Figure 1d** differs from the one shown in **Figure 1e** in the following aspects: (i) no container (e.g., a two-neck round-bottom flask) is required by the device shown in **Figure 1e**; (ii) bulk sample of large volume (e.g., lake water, river water and so on) can be directly sampled by the device shown in **Figure 1e**; and (iii) the ND ejector and the sample collector are immersed in the liquid (**Fig. 1e**); thus, the sampling process is carried out in a liquid-sealed system. The simplified microjet ND sampling device (**Fig. 1e**) enables the analysis of liquid samples in large-size containers (in which the inner diameter of the container is larger than the outer diameter of the microjet ND sampling device, e.g., a basin) or even *in situ* analysis of ambient water samples (e.g., lake water).

For the construction of the devices shown in **Figure 1d** and **e**, the experimenter should properly cover the tip of the ND gas transfer line to ensure that the samples are effectively collected. In addition, inert reagents or gas are preferably used for the ND sampling in order to minimize potential changes to the liquid-sealed system, particularly for the ongoing chemical or biological process. An unexpected decrease or elimination of the signal suggests that the properties of the ongoing chemical or biological system have been affected by the microjet sampling process. In such a case, changing the ND gas is usually recommended. This ensures that the properties of the analytes are not influenced by the analytical procedure.

With the application of geometry-dependent ND devices (**Fig. 1a–e**), the parameters (e.g., angles and distances) generally need to be optimized before analysis to ensure a high quality of sampling performance. However, the time spent on optimization would heavily depend on the understanding of the principles of the ND sampling process and the experiences of the operator; thus, it may take just 2 min for a skilled operator, whereas it may require 30 min or even longer for a beginner.

In contrast, operation would be more simplified by using a GIND device (**Fig. 1f,g**), for which no device optimization is required as the ND parameters have been fixed during the fabrication. The GIND device is airtight and can directly cover the samples (e.g., paste) or the surfaces of various samples, including liquid, human skin, clothes and so on. Therefore, the GIND device can be applied to both low- and high-viscosity samples. Note that the GIND devices presented in **Figure 1f,g** are theoretically the same; however, the one in **Figure 1g** would be somewhat superior to that in **Figure 1f**, as the former is more convenient for cleaning and has a smaller dead volume. The GIND device is, therefore, more sophisticated to construct and more difficult to fabricate but has the advantages of improved performance and easier operation. The simple versions of a GIND device can, however, be homemade using commercially available parts with minimal instrumentation (e.g., modifying a top part of a gas washing bottle)[49].

In summary, on the basis of the characteristics of individual type of ND sampling device specified above, the ND sampling devices are able to sample all types of viscous samples of various viscosities as shown in **Figure 2**. Open-air ND sampling devices (**Fig. 1a,b**) are

mostly suitable for liquid samples with low viscosity; microjet ND sampling devices (**Fig. 1d**,**e**) can be used for liquid samples with both low and relatively high viscosity; sealable ND device (**Fig. 1c**) is preferable for highly viscous samples (e.g., pastes); and finally, GIND devices (**Fig. 1f**,**g**) can be applied to all kinds of viscous samples with a wide variety of viscosities.

The application of ND sampling devices is rather flexible and can be adjusted according to the conditions of an individual laboratory. For example, low-viscosity liquid samples (e.g., perfume) can be deposited on a surface (e.g., paper or skin) and analyzed using a sealable ND device (**Fig. 1c**) or a GIND device (**Fig. 1f**,**g**); alternatively, the high-viscosity samples can be diluted using a proper solvent (e.g., methanol, water and so on) and sampled using the open-air ND sampling devices (**Fig. 1a**,**b**).

Theoretically, any type of neutral gas can be used for ND, but nitrogen gas or air is favored, as less chemical contamination is introduced in the sampling process. By using moist air (relative humidity ≥ 65%) rather than nitrogen gas, desorption performance can be further improved; this may be attributed to the micro water droplets, which extract analytes on the sample surface[58]. Moist air can be easily prepared in the lab by introducing ambient air through water contained in a two-neck glass flask. Moreover, selective desorption can be achieved with the application of specific chemicals (e.g., acetone). To improve the specificity and selectivity of the method, other reactive reagents can also be added into the electrospray solution. For example, the selective detection of DEG in toothpaste has been completed by using 10 μmol l^{-1} ammonium acetate in methanol solution[46], and nonpolar sulfur-containing species have been sensitively detected by using an $AgNO_3$/water solution[41]. It is important to note, though, that the native conditions of the samples may be affected (e.g., proteins), especially when a biologically incompatible chemical reagent is used. In this regard, the neutral nitrogen gas beam is preferably used in this protocol.

In theory, an EESI source can be coupled to any type of MS instrument with an API interface; however, the structure of the EESI source and the type of the MS instrument will influence the method sensitivity. To the best of our knowledge, the highest sensitivity has been obtained by using an ion-trap mass spectrometer (Finnigan LTQ-XL), and the result was comparable with that achieved by ESI-MS[52]. Another merit of an ion-trap mass spectrometer is that the multistage tandem mass spectrometric analysis (MS^n) can be performed with a single mass analyzer. MS^n analysis is generally required for the identification of a specific chemical when the sample is directly analyzed without matrix cleanup. This is because false signals may be produced by the matrix compounds in the MS^1 spectrum; however, the fragment ions of the false signals in MS^n spectrum ($n \geq 2$) are rarely the same as those of the targeted analytes. This means that false signals can be excluded by MS^n ($n \geq 2$, the higher the value of n, the better confidence, depending on the fragments observed in the n stage of a MS^n experiment) analysis. In principle, ND-ESSI can also be implemented in a miniature mass spectrometer (especially those using ion traps as the mass analyzers) for *in situ* analysis, but it has not been reported yet.

On the basis of the ND-EESI-MS spectra, fingerprints of samples can be obtained and the statistical analysis (e.g., principal component analysis (PCA) for sample classification) can be performed if necessary. Long-term stability of the ND-EESI-MS platform is required to ensure satisfactory results, even when statistical analysis is used to process the data. To evaluate the sustained performance of the ND-EESI method and the MS instrument, qualitative and quantitative analysis of standards are preferably performed before the analysis of actual samples, such that both the MS instrument and the ND-EESI method can be properly calibrated. Moreover, quantification of trace amounts of analytes can be achieved by using the characteristic fragments observed in the MS^n spectra. In this protocol, quantitative analysis has been tentatively performed using the standard addition method, and examples have been provided, including the quantification of DEP in perfume products[43] and DEG in toothpaste products[46]. For both qualitative and quantitative analysis, at least six replicates are required to get meaningful results. To obtain reproducible results, the experimental conditions should be strictly controlled and doubly checked before the sample analysis or after running the analysis for a relatively long duration (longer than 2 h), particularly when a homemade ND device and/or EESI source is used. Conventional analytical methods, such as GC/MS or liquid chromatography (LC)-MS combined with sample pretreatment (e.g., solid-phase extraction) can be used to confirm the results obtained using ND-EESI-MS.

Finally, it is noteworthy that when compared with the protocol reported previously[53], the protocol described here is typically designed for the analysis of viscous samples (a few cP to 300,000 cP), whereas the former one is more suitable for the analysis of biological surfaces (e.g., human skin) that are complex matrix samples but usually have low viscosities. In addition, versatile devices have been introduced in this paper, with regard to the analytical requirements of different types of viscous samples (e.g., liquid, gel or solid samples with viscosities from a few cP to 300,000 cP).

MATERIALS

REAGENTS

! CAUTION For your safety, please read the material safety data sheet for every chemical reagent before use. Store all reagents and solvents according to manufacturer's instructions.

! CAUTION All sample items (perfumes, foods and so on) should be stored according to manufacturer or supplier's instructions, as appropriate.

- *Perfume samples*: Eighteen fragrances of different brands were bought in local shops (i.e., 'Weekend' by Burberry; 'Relaxing Fragrance' by Shiseido; 'Be Delicious' by DKNY; 'Beautiful' by Estee Lauder; 'Hugo XY' by Hugo Boss; 'le Male' by Jean-Paul Gaultier; 'ETH Zurich 150' by Givadaun; 'Bright Crystal' by Versace; 'Option' by Nova; 'CK One' by Calvin Klein; 'Miss Dior' and 'Midnight Poison' by Christian Dior; 'Clinique Happy for Men' and 'Clinique Happy Heart' by Clinique; 'Opium', 'Opium Shanghai' and 'Opium Imperiale' by Yves Saint Laurent; and 'White Musk' eau de toilette by The Body Shop).
- *Edible oil samples*: Four types of edible oils (extra virgin olive oil, sunflower oil, rapeseed oil and sesame oil) were obtained from local grocery stores.
- *Beer samples*: Three types of beers (pale pilsener, white/wheat beer and lager beer) were purchased from local supermarkets.
- *Honey samples*: Two different grades of honey samples (grade A and substandard) were purchased from a local grocery store.
- *Cheese samples*: Four types of commercial cheese products were randomly selected from three well-known supermarkets (Denner, Coop and Migros) in Switzerland. Cheeses were produced in Switzerland by raw milk (Emmentaler and Gruyère) or pasteurized milk (Saint Paulin and Tilsiter). Sample qualities were guaranteed regarding their expiration dates.
- *Toothpaste samples*: Commercial toothpaste products were bought from local supermarkets.

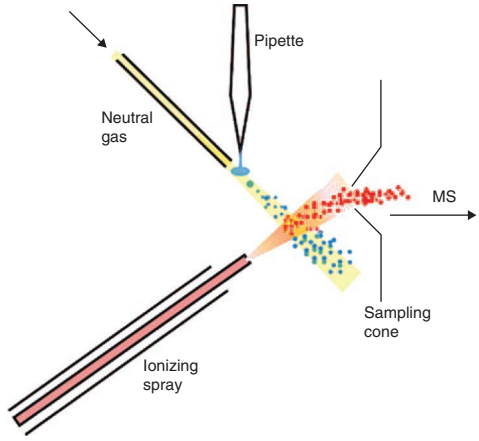
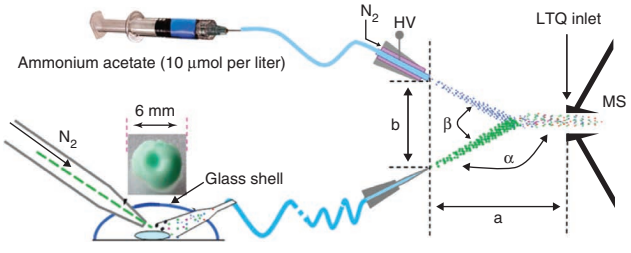


Figure 3 | Schematic diagram of EESI-MS combined with a simplified open-air ND sampling device for the analysis of perfume samples. About 1 µl of perfume sample filled in a 10-µl pipette tip is desorbed and successively transported from the pipette tip into the EESI source by a gentle stream of nitrogen gas with the flow rate from 100 to 400 liters per h. (Copyright: American Chemical Society. Reproduced with permission from ref. 43.)

- Nicotine (analytical reagent grade) **! CAUTION** It is poisonous, may be fatal if inhaled, swallowed or absorbed through the skin in large doses. It is readily absorbed through the skin. Typical permissible exposure limit is 0.08 p.p.m. Skin-Rabbit lethal dose (LD50) is 50 mg kg^{-1}.
- Caffeine (analytical reagent grade) **! CAUTION** It can be adsorbed into the body by inhalation and by ingestion. **! CAUTION** Evaporation at 20 °C is negligible; however, a harmful concentration of airborne particles can be reached quickly on dust forming. **! CAUTION** Short-term exposure may affect the central nervous system and cardiovascular system, resulting in insomnia, excitement, tachycardia and polyuria.
- Diethyl phthalate (DEP, >99.5% purity) was purchased from Fluka.
- Fructose (99.9%) was provided by Mallinckrodt.
- 1-Ethyl-3-methylimidazolium chloride (EMIMCl) was supplied by Solvent-Innovation.
- Chromium (II) chloride ($CrCl_2$) was bought from Sigma-Aldrich.
- Diethylene glycol (DEG, analytical reagent grade) was bought from Sinopharm Chemical Reagent.
- Ammonium acetate (analytical reagent grade) was purchased from Sinopharm Chemical Reagent.
- Deionized water was used in the experiments and provided by the Chemistry Department facility at East China Institute of Technology or an ultrapure water system (Barnstead Nanopure Diamond, Barnstead, analytic D11901).
- Methanol (HPLC grade) **! CAUTION** It is flammable, causes eye and skin irritation, and is harmful if swallowed; avoid contact with the eyes, skin and clothes.
- Ethanol (75% (vol/vol), medical grade) was purchased from a local hospital **! CAUTION** It is flammable and contact with eyes may cause irritation.
- Acetic acid (HPLC grade) **! CAUTION** It is poisonous and corrosive. Liquid and mist cause severe burns to all body tissues. It may be fatal if swallowed and harmful if inhaled. Inhalation may cause lung and tooth damage. It is a flammable liquid and vapor **! CAUTION** Contact with eyes may cause irritation.
- Cotton swabs (medical grade) were purchased from a local hospital.
- Compressed nitrogen (99.995%) **! CAUTION** May cause asphyxiation in high concentrations.

EQUIPMENT

- Refrigerator (laboratory equipment with its working range from −20 to 4 °C)
- Syringes (Hamilton, cat. nos. 81165 and 81265)
- Syringe pumps (Harvard), flow rate ranging from 1.4×10^{-9} liter per h (for the smallest syringe with the size of 0.5 µl) to 26 ml min^{-1} (for the largest syringe with the size of 60 ml)
- Water bath and controller (laboratory equipment). Temperature adjustable from room temperature (22 °C) to 90 °C
- Temperature meters (to ensure laboratory equipment is within its working range from −40 to 60 °C)
- Mass spectrometers: ESI-QToF Ultima (Waters Micromass) and Finnigan LTQ-XL (Thermo Scientific)
- Xcalibur software (version 2.0) (Thermo Fisher)



- MassLynx software (version 4.0) (Waters)
- ND devices (homemade devices, specially designed for different types of viscous samples; see EQUIPMENT SETUP)
- EESI source (homemade device, implemented on a commercial QToF or Finnigan LTQ-XL mass spectrometer; see EQUIPMENT SETUP)
- Plastic pipette (D10, Gilson)

REAGENT SETUP

Chemical reagents and solvents All the chemical reagents and solvents obtained should be of the highest purity available, and should be stored properly according to the manufacturer's instructions and used directly without any further purification.

EQUIPMENT SETUP

Open-air ND sampling device The simplified open-air ND sampling device, as presented in **Figure 3**, has been used in this protocol for the analysis of perfume, which is a liquid sample with relatively low viscosity. Even though the sampling device is recommended for the analysis of low-viscosity samples, highly viscous samples diluted with a proper solvent can also be sampled with this device. In this simplified open-air ND device, a plastic pipette (D10, Gilson) is directly adopted as the sample holder and a Teflon tube (inner diameter (i.d.), 7 mm) is used to introduce the ND gas to the sample. The pipette tip is 2 cm away from the sampling cone of the ESI source. The gas emitter can be simply fabricated using the commercially available pipette (1–5 µl); however, in this case, the background effects caused by the plastic pipette should be considered during the identification of some targeted compounds such as DEP. The sample collector is not needed in this simplified sampling protocol. This simplified ND device can benefit a high-throughput analysis, as the steps of sample deposition and evaporation are eliminated[40]; however, it can be challenging to use this device to reliably reproduce the results because setup parameters can be difficult to control.

Sealable ND sampling device In this protocol, the sealable ND device (**Fig. 4**) has been used for highly viscous toothpaste samples and the microjet ND device (**Fig. 5**) has been applied to the analysis of low- and medium-viscosity samples (e.g., beer, ionic liquid, edible oil and honey). In the sealable ND device, a C-shaped glass cell has been adopted to cover the sampling area airtightly; the cell inner diameter is 20 mm and the sealable sampling area is ~10 mm^2 (**Fig. 4**). With the application of a sealable ND sampling device, the sensitivity of the ND-EESI-MS method can be substantially improved because the neutral aerosols can be collected more effectively in an enclosed system. To achieve high sensitivity, make sure the sampling area has an airtight cover and that the analytes can be exclusively transferred to the EESI

Figure 4 | Schematic diagram of EESI-MS combined with a typical sealable ND sampling device for the analysis of toothpaste samples. The distance between the inlet of the LTQ instrument and the gas outlets (a) is 10 mm. The distance between the two spray tips (b) is 2 mm. The angle between the sample outlet and the heated capillary of the LTQ-MS instrument (α) is 150°. The angle between the sample spray beam and the electrospray beam (β) is 60°. A sealable C-shaped glass cell is added to an open-air ND device, resulting in significant sensitivity improvement of ND-EESI-MS. (Copyright: American Chemical Society. Reproduced with permission from ref. 46.)

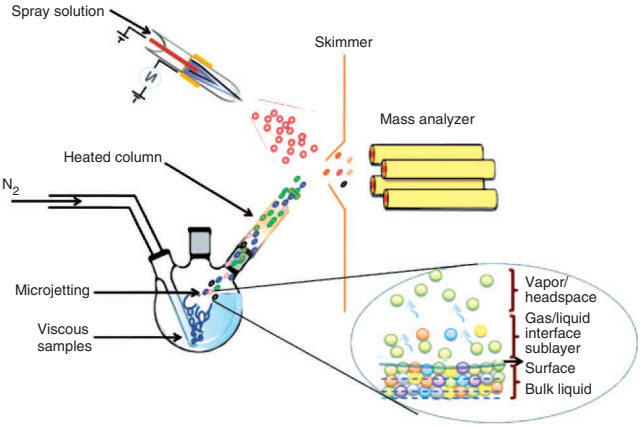


Figure 5 | Schematic diagram of EESI-MS combined with a microjet ND sampling device for the analysis of low- and medium-viscosity liquid samples. Samples include beer, ionic liquid, edible oil and honey. In a microjet ND sampling device, a sharp stream of nitrogen gas is directed into the viscous liquids, and fine droplets are produced by a mechanism known as microjet sampling; in this case, volatile, semivolatile and even nonvolatile compounds in viscous liquids can be liberated and transported into the EESI source. (Copyright: Wiley-VCH Verlag. Reproduced with permission from ref. 45.)

source without loss during transportation. In a sealable ND sampling device, samples are completely isolated from the high-voltage probe, thereby leading to a higher degree of safety. Using a sealable ND device, the desorption effectiveness can be enhanced by using a gas flow of higher velocity, which can be accomplished by using either a large flux or a small aperture. Analytes are significantly diluted when a large flux of gas is used, which can result in poor signals. Alternatively, a small aperture (preferably, i.d. ≤80 μm) is more practical for improving method sensitivity, although it might make the fabrication of the gas emitter more difficult, as such a tiny aperture is usually not readily available.

In the microjet ND sampling device, the sample holder is a two-neck round-bottom glass flask (or a three-neck round-bottom flask with the middle neck capped). The gas emitter is a Teflon tube connected with a cone-shaped tip (e.g., pipette tip and stainless steel needle). The sample collector/transfer line is a Teflon tube with a large diameter (e.g., i.d. 5 mm). The length of the tube used in the experiment can vary over a large range, for example, a 120-cm-long Teflon tube has been used for edible oil samples[45], whereas a 30-cm-long one has been used for beer samples[47]. The outlet of the tube is 2 cm away from the ESI tip. Under the microjet ND sampling strategy, the ND performance can be significantly improved, especially for semivolatile and nonvolatile compounds. However, the overall performance largely depends on the working parameters, the chemicophysical properties of the analyte and the viscosity of the sample. Online, real-time monitoring of chemical reactions can also be achieved by applying a microjet sampling strategy. The flow rate of the desorption gas introduced into the liquid is a critical parameter because a low flow rate would result in weak signals, whereas a high flow rate would shorten the residence time of aerosol droplets inside ionization region and reduce the ionization efficiency. The optimum flow rate depends on the sample viscosity and the tube inner diameter. For example, the flow rates selected for beer (low-viscosity) and edible oil (medium-viscosity) samples are 50 (ref. 47) and 100 liters per h[48], respectively, with a tube inner diameter of 5 mm in both cases. The tube exporting the analytes from the sample collector can be heated when necessary (e.g., 80 °C), but the heating temperature should be appropriately chosen when heat-sensitive compounds are analyzed.

GIND sampling device The GIND sampling device shown in **Figure 6** has been used to sample analytes from cheese products by introducing a bell-shaped glass envelope (inset in **Fig. 6**; i.d. 30 mm, length 8 cm)[49]. With regard to its construction, a GIND device would be more sophisticated and difficult to create than a geometry-dependent ND device; however, a simple version of a GIND device can be easily modified from the top part of a gas washing bottle[49] (**Fig. 6**). This might be the easiest way to construct a GIND device. For the GIND device shown in **Figure 1f** (ref. 57), the gas emitter is fabricated with a tube (stainless steel, i.d. 5.36 mm, length 5 cm) and a cone-shaped tip (i.d. 0.1 mm, outer diameter (o.d.) 1 mm). The gas emitter is installed perpendicularly to the sample surface to make fabrication easier. The sample collector can be made with stainless steel (i.d. 10 mm, o.d. 12 mm, length 12.36 cm). Additionally, we recommend installing a layer of elastic inert rubber (thickness, 2 mm) along the bottom edge of the collector for better airtightness. Finally, the collected sample aerosols can be carried out to the EESI source through a sample transfer line (Teflon tube, i.d. 3 mm, o.d. 5 mm).

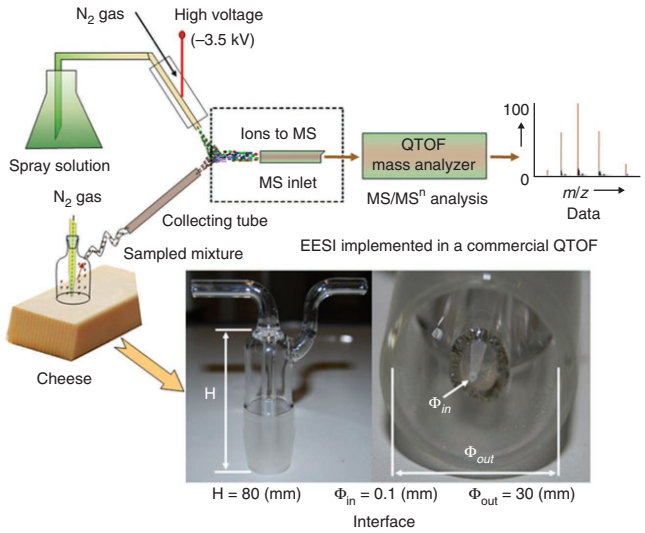


Figure 6 | Schematic diagram of EESI-MS combined with a GIND sampling device. This setup is used for the analysis of greasy surfaces of cheese samples. In this GIND sampling device, a geometry-independent bell-shaped glass envelope is used to cover the sampling area airtightly, which contributes to the enhancement of sampling effectiveness and efficiency. (Copyright: Springer. Reproduced with permission from ref. 49.)

ND interface for sampling ND interface for sampling involves the application of the gas emitter and the sample collector in the ND device. The distance between the tip of the gas emitter and the sample surface can vary from 1.5 to 30 mm, depending on the velocity of the gas stream. The typical linear velocity of a ND gas beam is estimated as 10 m s^{-1} and can be created by using a gas flow with the flow rate of 1–3 liters per min[53]. The angles formed between the sample surface and axes of gas beam/sample collection tube can vary in the ranges of 20–60° and 60–20°, respectively, and the recommended combination is 30° and 45°. ▲ **CRITICAL** We recommend using a standard compound (e.g., caffeine, nicotine or arginine) of relatively high concentrations (e.g., 50 p.p.b.) first to make the optimization process easier. ! **CAUTION** The interface parameters should be optimized to obtain a stable and sensitive signal; however, the optimizing time can vary from minutes to hours, largely depending on the experience of the experimenter.

Implementing ND-EESI on a commercial ESI interface In this protocol, the homemade EESI can be implemented on a commercial Quadrupole time-of-flight MS (QTOF-MS) or linear trap quadrupole MS (LTQ-MS) without any hardware modification. ▲ **CRITICAL** The glass shell of the QTOF-MS instrument should be properly installed to prevent any gas leakage in the source region, and the differential pumping system of the MS instrument should be properly maintained; this is particularly important for using open-air ND devices because the vacuum created by the instrument can facilitate analyte transportation. Virtually any type of MS instrument with an ESI/APCI source (or similar instruments with an API interface) can be coupled to an EESI source. To date, besides the QTOF-MS and LTQ-MS used in this protocol, LCQ-MS and Orbitrap-MS instruments have also been successfully combined with a homemade EESI source. ! **CAUTION** Some source modification may be required on the basis of the original configuration of the interface.

The outlet of the sample transfer line is carefully positioned to form an angle, typically ranging from 60° to 90° against the electrospray beam.

Generally, a mixture of methanol/water/acetic acid (45/45/10 (vol/vol/vol)) can be used as the electrospray solution. The typical infusion rate of the solution is 2–5 μl min^{-1}. If necessary, reactive chemicals can be added into the electrospray solution to improve method selectivity. The ion detection mode is selected on the basis of the targeted analytes; for example, the positive ion mode is used to detect DEG in toothpaste[46] and the negative ion mode is used to detect fatty acids in cheese[49]. The EESI source temperature is usually kept at 150 °C for most compounds, but it can be optimized for better signal response by changing values from 50 to 450 °C. The typical ESI voltage is ~3 kV for the QTOF-MS instrument and 4 kV for the LTQ-XL mass spectrometer. With a sophisticated EESI source, the best sensitivity can be easily achieved by precisely adjusting the working parameters of the EESI source.

MS analysis. MassLynx software (version 4.0) is used for the QTOF-MS (QTOF Ultima, Waters Micromass) and Xcalibur software (version 2.0) is used to control the Thermo Scientific LTQ-XL-MS. As mentioned above, other MS instruments can also be used in conjunction with the proper software. MS^n ($n \geq 2$) analysis can be performed when collision-induced dissociation is used. In this protocol, for the LTQ-MS instrument, the collision energy is in the range of 10–35% units (arbitrary units defined by the manufacturer) and for the QTOF-MS instrument, the collision energy is from 5 to 25 eV. ▲ **CRITICAL** Only the major fragments in the MS^n ($n \geq 2$) spectra of individual targeted analytes are used for qualitative and quantitative analysis. In this case, false signals caused by the complex matrix can be excluded.

PROCEDURE

Prepare samples

1| Samples of interest (perfume/oil/honey/beer/cheese/toothpaste; option A) are used for sample fingerprinting and/or the detection of targeted analytes. Fructose dehydration reaction solution (option B) is used for the online monitoring of the reaction. Stock standard solutions of DEP and DEG (option C) are used to prepare a series of standard solutions.

(A) Prepare perfume/oil/honey/beer/cheese/toothpaste samples

(i) Take any refrigerated samples out of refrigerator (stored at 3–4 °C); note that nonperishable items such as toothpaste do not require refrigeration.

(ii) Place the sample under ambient conditions (i.e., at room temperature, 22 °C).

(iii) Wait until all samples have equilibrated to room temperature.

(iv) Record the time spent for the whole equilibration process.

! CAUTION All samples should be kept in their original packages; care should be taken to avoid any potential contamination during this process.

(B) Prepare fructose dehydration reaction solution

(i) Dissolve fructose and $CrCl_2$ (catalyst) in EMIMCl (an ionic liquid) contained in a two-neck round-bottom glass flask[45,59].

(ii) Heat the reaction solution to the temperature of 80 °C.

(C) Prepare stock standard solutions of DEP and DEG

(i) Dissolve DEP in ethanol and dissolve DEG in water.

! CAUTION The concentration of the stock solution depends on the solubility of the chemical in the selected solvent.

(ii) Mix the solution using a desktop vortex (or something similar) to make the solution homogeneous.

(iii) Store the stock solution in the refrigerator at 3–4 °C if it is not used immediately.

! CAUTION Standard chemicals used to prepare stock solutions are generally stored in the refrigerator at 3–4 °C. Hence, before preparing a stock solution, these chemicals should be taken out of the refrigerator and kept under ambient conditions until they reach room temperature. Similarly, a refrigerated stock solution should be allowed to equilibrate to room temperature before use.

! CAUTION Potential evaporation of solvent should be avoided, especially when a relatively volatile solvent (e.g., methanol) is used; the concentration of the stock solution would be increased by the loss of solvent.

Prepare a series of standard solutions

2| Take out the stock solution and keep at room temperature for a while. *Note*: Standard solutions are prepared for qualification and/or quantification of targeted analytes.

3| Add a known amount of a stock solution into a volumetric flask and dilute it with the appropriate solvent.

! CAUTION Potential evaporation of solvent should be avoided, especially when a relatively volatile solvent (e.g., methanol) is used; the concentration of the stock solution would be increased by the loss of solvent.

Prepare spiked samples

4| Spike native samples with a series (at least three) of known amounts of the standard chemical. *Note*: Samples spiked with a standard chemical (spiked samples) are prepared for quantification analysis and recovery determination.

5| Mix the sample thoroughly to make it homogeneous.

6| Allow the spiked samples to stabilize; this generally takes ≥2 h for liquid samples and 24 h for solid samples (e.g., soil, sediment, powder).
▲ **CRITICAL STEP** We recommend that the amount of the standard chemical added be 0.5–2 times the amount of the analyte in native samples.
! **CAUTION** Samples that have been spiked with solutions should be dried at room temperature in the dark to remove the solvent completely.
▲ **CRITICAL STEP** The response of the analyte in a spiked sample should be within the linear dynamic range of the response curve or the analyte cannot be appropriately quantified. It is also important to ensure that the spiked standard analyte is evenly distributed in the sample.
! **CAUTION** Spiked samples are prepared by adding chemicals that are unstable in ambient conditions; they should be analyzed as soon as possible to minimize analyte decomposition.

Calibrate MS instrument
7| Set ESI source parameters as follows: ESI voltage, +3 kV; infusion rate of electrospray solution, 2 μl min^{-1}; source temperature, 80 °C; cone voltage, +50 V; first ion tunnel radio frequency (RF1) voltage, 45 V; and the voltage of the multichannel plate detector, 2,300 V.
▲ **CRITICAL STEP** For the proper performance of ND-EESI-MS, we strongly recommend calibrating the MS instrument before analysis according to the instrument manufacturer's instructions.

8| Record multiple (more than four) full-scan spectra of the standard solution in the absence of the collision gas.
! **CAUTION** A typical standard solution used for low-mass-range calibration is 10 mg per liter of sodium formate solution in 2-propanol/H_2O (90/10 (vol/vol))[53].
! **CAUTION** A mass accuracy of 10 p.p.m. should be achieved after calibration.
? TROUBLESHOOTING

Optimize EESI source
9| Optimization of the EESI source as performed in this protocol is adapted from the procedure reported previously[53]. First, pour the standard solution, such as 0.001 mol per liter of nicotine in methanol/water (1/1, (vol/vol)), into a sealable flask. Nicotine is used as a standard for source optimization.

10| Introduce the neutral nitrogen gas through a gas supply tube; the outlet of the tube is immersed in the bulk solution.

11| The bubbles generated by the gas carry analyte molecules into another gas tube connected to the flask. As a result, analyte molecules are transported into the EESI source for ionization.

12| Optimize source position, source voltage, source temperature, electrospray solution flow rate and other source parameters at constant gas flow rate, until a stable and intense signal (e.g., protonated nicotine, *m/z* 163) is obtained.

Optimize individual ND devices
13| With EESI source and MS instrument parameters optimized, ND device parameters (such as the gas emitter incident angle, the sample collecting angle, the tip-to-sample surface distance and gas flow rate) can be further optimized when necessary. A standard solution, for example, 0.001 mol per liter of nicotine, caffeine or arginine in methanol/water (1/1, (vol/vol)), can be used for ND device optimization, and the signal (e.g., protonated nicotine, *m/z* 163; protonated caffeine, *m/z* 195; and protonated arginine, *m/z* 175) should be obtained at the highest level after ND device optimization. Use option A to optimize the device for the detection of DEP in perfume samples (**Fig. 3**). Use option B to optimize the device for the analysis of edible oil, honey and beer samples, and for online monitoring of the fructose dehydration reaction (**Fig. 5**). Use option C to optimize a sealable ND device (**Fig. 4**) or a GIND device (**Fig. 6**) for the analysis of toothpaste and cheese samples.
(A) Optimize a simplified open-air ND sampling device
(i) Fill 1 μl of standard solution in a pipette tip.
(ii) Carry out the ND sampling process as described in the EQUIPMENT SETUP (Open-air sampling device) section.
(B) Optimize a sealable ND device used for viscous liquid samples
(i) Add an amount of the standard solution into a two-neck round-bottom flask.
(ii) Carry out the ND desorption sampling process as described in the EQUIPMENT SETUP (Sealable ND sampling device) section.

(C) Optimize a sealable ND device (for viscous solid samples) or a GIND device

(i) Deposit an amount of the standard solution on a surface (e.g., paper).

(ii) Dry the solution under ambient conditions.

(iii) Carry out the ND desorption sampling process as described in EQUIPMENT SETUP (the Sealable ND sampling device and GIND sampling device sections, respectively).

! CAUTION The EESI source region should be properly sealed before carrying out ND to take the advantage of vacuum-assisted analyte transportation; otherwise, the analytes may not be able to reach the EESI source effectively. For the same reason, when a commercial ESI interface is involved, the glass cover of the source region should be tightly fixed to the MS instrument and the differential pumping system should work appropriately.

14| Carry out sampling process for practical samples, standard solutions and spiked samples by following the steps described in options A–E.

! CAUTION For the more accurate and precise quantification analysis, a sealable ND/GIND sampling device is recommended, especially when volatile compounds are targeted. Use option A for sampling perfume. Use option B for sampling edible oil, honey and fructose dehydration reaction solution. Use option C for sampling toothpaste and cheese. Use option D for sampling standard solutions. Use option E for sampling spiked samples.

! CAUTION We suggest measuring each sample independently at least four times.

! CAUTION After each measurement, the entrance of the mass spectrometer should be carefully and thoroughly cleaned with cotton swabs and a methanol and water solution (1/1, (vol/vol)).

(A) Sample low-viscosity liquid samples (e.g., perfume) with the simplified open-air ND device

(i) Fill 1 μl of liquid sample in a pipette tip.

(ii) Put the pipette tip in a proper position, as specified in the EQUIPMENT SETUP (Open-air sampling device) section.

(iii) Carry out the ND sampling process under the optimized experimental conditions.

(B) Sample low- and medium-viscosity liquid samples (e.g., edible oil, honey, ionic liquid) by a microjet ND sampling device

(i) Add an appropriate amount of liquid sample into a two-neck round-bottom flask.

(ii) Carry out the microjet ND sampling process as described in the EQUIPMENT SETUP (Sealable ND sampling device) section under the optimized experimental conditions.

! CAUTION For the real-time online monitoring of reactions, the microjet ND sampling process can be performed at desired time points during the reaction. For example, we selected ten specific time points for the online monitoring of the fructose dehydration reaction[45].

! CAUTION Any effervescent vicious liquid samples (e.g., beer) should be carefully added into the flask to prevent overflowing.

! CAUTION Even though a high desorption gas flow rate can improve ND sampling performance, it can also agitate the sample violently and cause negative effects; for example, during the microjet ND sampling process of beer samples, the transport line could be filled with foam at a gas flow rate of 80 liters per h[47].

(C) Sample high-viscosity samples (e.g., toothpaste, cheese) by a sealable ND device or a GIND device

(i) Cover the sample airtightly with a sealable device; for example, a C-shaped glass shell has been used for toothpaste samples (**Fig. 4**)[46] and a geometry-independent glass envelope has been used for cheese samples (**Fig. 6**)[49].

! CAUTION When an appropriate amount of sample is used (e.g., 0.2 g of toothpaste sample), the whole sample can be covered under a sealable device.

(ii) Carry out the ND sampling process as specified in EQUIPMENT SETUP (in the Sealable ND sampling device and GIND sampling device sections, respectively).

(D) Sample the standard solutions by individual ND devices

(i) Sample the standard solutions by individual ND devices as described in options A–C.

! CAUTION For a sealable ND sampling device used for high-viscosity samples (e.g., **Fig. 2f,g**), standard solutions are firstly deposited on a solid surface (e.g., paper) and dried before ND sampling process.

(E) Sample the spiked samples by individual ND device

(i) Sample the spiked samples by individual ND devices exactly in the same way as their native counterparts.

15| Acquire data with the assistance of appropriate MS software. For example, mass spectra can be acquired and processed by MassLynx software (version 4.0) when a QTOF-MS instrument is used or by Xcalibur software (version 2.0) when a LTQ-MS instrument is used.

! CAUTION During data acquisition, the mass spectra are obtained strictly following the steps and standard procedures specified in the instrument manuals.

16| Carry out background subtraction in the same way as that reported previously[53]. In short, the background subtraction should be performed strictly based on the standard commands implemented in the commercial software.
▲ **CRITICAL STEP** Background subtraction is necessary to reduce background effects resulting from instrument contamination and/or complex matrix.

Quantitative analysis

17| Obtain the standard curve on the basis of the signal intensity and the concentration of the targeted analyte in a series of standard solutions.

18| Estimate the instrumental detection limit according to the linear response curve; the instrumental detection limit in this protocol is determined as the analyte amount when the signal-to-noise ratio is equal to 3.

19| Obtain the working curve by plotting the signal intensity against the concentration of spiked analyte in the samples.

20| Determine the concentration of the targeted analyte in the samples by extrapolating the working curve to the *x* axis. The absolute value of the *x* intercept will be the concentration of the analyte in the sample.
▲ **CRITICAL STEP** Quantitative analysis using spiked samples is known as the standard addition method through which matrix effects can be reduced.

21| Evaluate analyte recovery (*R*) by comparing the concentration detected in the spiked sample with the corresponding amounts added in the sample, as shown in the following equation:

$$R = \frac{m_1 - m_0}{m} \times 100\%$$

where *R* is the recovery of the targeted analyte, m_1 is the total amount of the analyte found in the spiked sample, m_0 is the analyte amount measured in the native sample and *m* is the analyte amount added into the native sample.
▲ **CRITICAL STEP** For recovery determination, matrix effects on detection sensitivity can be evaluated by using the spiked samples.

? TROUBLESHOOTING

No signal is obtained from the EESI source using a standard solution

- Double-check the ESI source to ensure that it works properly.
- Make sure that a proper gas stream is supplied for the ESI spray and/or for the sample spray.
- Ensure that the standard compounds can be ionized in the ESI source.
- Make sure that the standard compounds are delivered to the EESI source through the sample transfer line or the desolvation gas line of the QTOF-MS instrument. Cluster formation can sometimes be observed when the sample of heavy humidity is delivered through the desolvation gas line of the QTOF-MS instrument; this can be avoided by properly heating the gas line.

No signal is obtained from the ND-EESI source using a standard solution

- Double-check the EESI source, as described above, to ensure that the EESI source works properly.
- Check whether the glass shell of the TOF-MS instrument has tightly covered the MS interface and is able to seal the source region properly without any gas leakage.
- Check the sample collecting tube to ensure that the transfer line is not blocked.
- Check the differential pumping system to ensure that all the pumps are working properly to provide a good vacuum for the instrument.
- Check whether there are contaminants or carryovers on the MS interface that may have been created

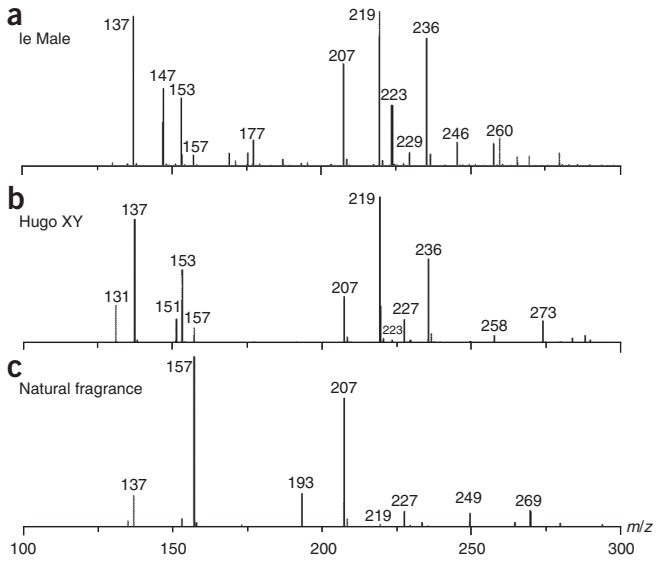


Figure 7 | Mass spectra of three different perfume products recorded by EESI-MS combined with a simplified ND sampling device. (**a**–**c**) 'le Male' by Jean Paul Gaultier (**a**), 'Hugo XY' by Hugo Boss (**b**) and 'Natural fragrance' by Shiseido (**c**). (Copyright: American Chemical Society. Reproduced with permission from ref. 43.)

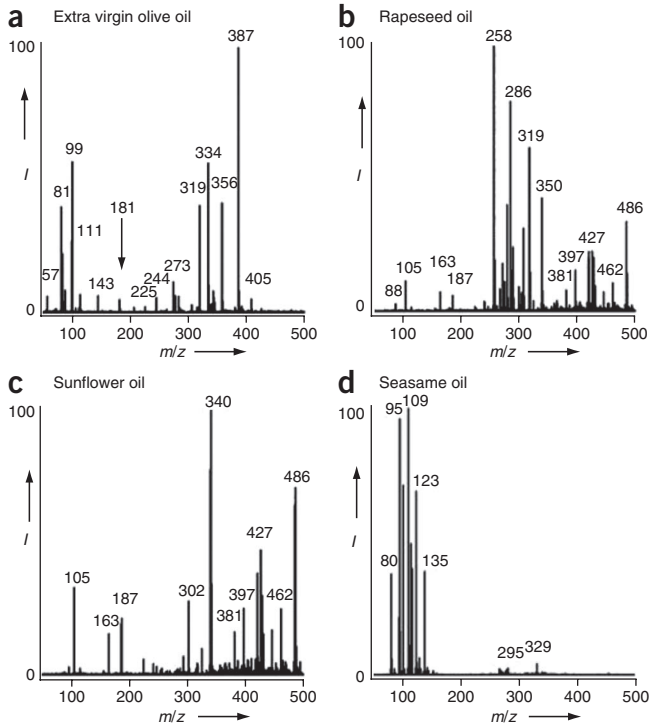


Figure 8 | Mass spectra of four types of edible oils recorded by EESI-MS combined with a microjet ND sampling device. (**a**–**d**) Extra virgin olive oil (**a**), rapeseed oil (**b**), sunflower oil (**c**) and sesame oil (**d**). (Copyright: Royal Society of Chemistry. Reproduced with permission from ref. 48.)

by the measurements performed before the ND-EESI experiments.

- Check that the gas emitter and the sample collector are properly positioned.

● TIMING

Step 1A: 1–2 h
Step 1B: 30–60 min
Step 1C: 2–3 h
Steps 2 and 3: 2–3 h
Steps 4–6: 1–2 h
Steps 7 and 8: 30–60 min
Steps 9–12: 5–30 min; the time for optimizing the EESI source is heavily dependent on the experience of the operator
Step 13: 5–30 min; however, the time for ND device optimization is heavily dependent on the experience of the operator
Steps 14 and 15: ≤5 min
Step 16: 1–3 min
Steps 17–21: 30–60 min

ANTICIPATED RESULTS

In this protocol, samples of various viscosities (ranging from a few cP to 300,000 cP), such as perfume, beer, ionic liquid, edible oil, honey, cheese and toothpaste, are rapidly analyzed and characterized by ND sampling coupled to EESI-MS method.

With the application of a proper ND sampling device, chemical fingerprints of samples can be effectively obtained by EESI-MS analysis. For example, in the case of low-viscosity samples (i.e., commercial perfume products), EESI-MS fingerprints of three different samples have been obtained (**Fig. 7**) with a simplified ND sampling device. Obviously, the origins of the perfume products can be easily identified based on the mass spectra. Even though some peaks are observed in all three mass spectra, their relative intensities are very different, indicating different concentrations of these ingredients. Similarly, the medium-viscosity (e.g., edible oils and honeys) and high-viscosity samples (e.g., cheese) can also be easily classified by EESI-MS spectra (**Figs. 8**–**10**) when a sealable ND sampling or a GIND sampling device is used. In addition, the chemical reaction of fructose conversion to 5-hydroxymethylfurfural (a promising surrogate for petroleum-based chemicals)[45] has been successfully monitored in real time (**Fig. 11**) on the basis of the chemical fingerprints obtained using microjet ND sampling coupled to EESI-MS.

Specific analytes in the samples we investigated have been confirmed using the present analytical protocol with available standard chemicals. As shown in **Figures 12** and **13**, DEP in perfume samples and DEG in toothpaste

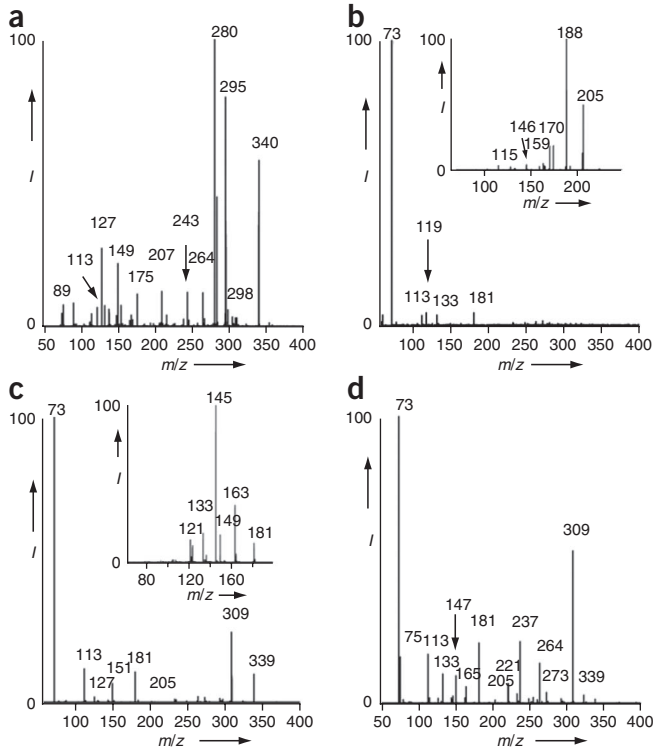


Figure 9 | Mass spectra of two different grades of honey recorded by EESI-MS combined with a microjet ND sampling device. (**a**–**d**) A substandard honey (**a**); grade A honey (**b**,**c**); and grade A honey (bulk liquid) (**d**). (Copyright Wiley-VCH Verlag. Reproduced with permission from ref. 44.)

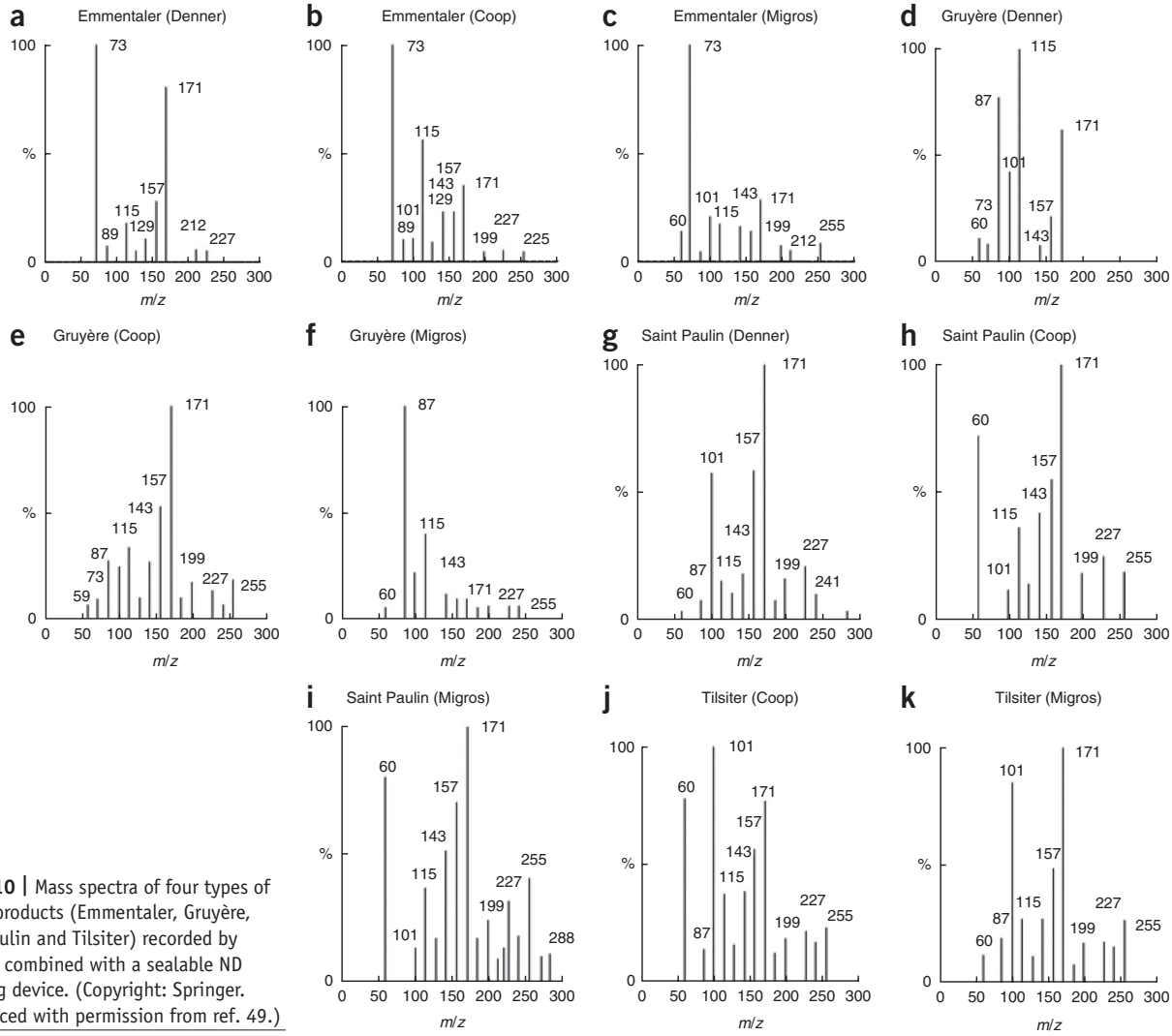


Figure 10 | Mass spectra of four types of cheese products (Emmentaler, Gruyère, Saint Paulin and Tilsiter) recorded by EESI-MS combined with a sealable ND sampling device. (Copyright: Springer. Reproduced with permission from ref. 49.)

samples are qualified by comparing the MS/MS or MS/MS/MS spectra with the reference spectra of authentic chemicals. Furthermore, DEP in perfume samples (**Fig. 14**) and DEG in toothpaste samples (**Fig. 15**) are quantitatively determined using the major characteristic fragments of individual targeted analyte in the MS^n spectra. The LODs are far below 100 p.p.b. (wt/wt) for DEP in ethanol[43] and ~200 p.p.b. (wt/wt) for direct detection of DEG in toothpaste[46].

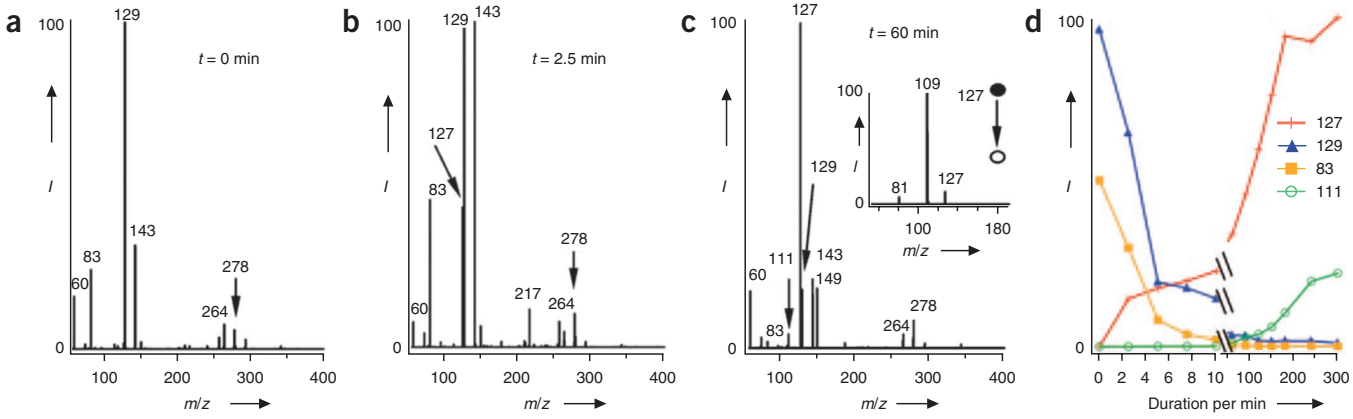


Figure 11 | Mass spectra for fructose dehydration reaction. (**a**–**c**) Spectra recorded at 0 min (**a**), 2.5 min (**b**) and 60 min (**c**)(*Inset*: MS/MS spectrum of HMF). (**d**) Intensities of protonated HMF (*m/z* 127), 1-methylimidazolium (*m/z* 83), 1-ethyl-3-methylimidazolium (*m/z* 111) and hydrated 1-ethyl-3-methylimidazolium (*m/z* 129) by EESI-MS combined with microjet ND sampling. In addition, the reaction has occurred in the viscous ionic liquid of EMIMCI. (Copyright: Wiley-VCH Verlag. Reproduced with permission from ref. 45.)

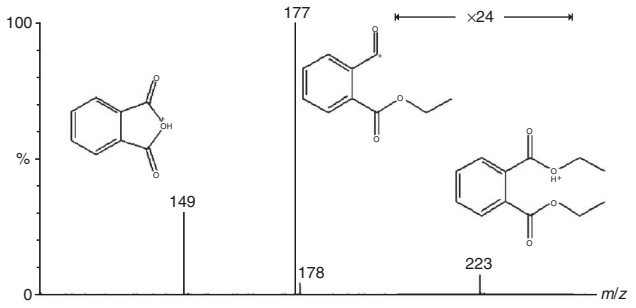


Figure 12 | MS/MS spectrum of DEP in perfume samples analyzed by ND-EESI-MS. (Copyright: American Chemical Society. Reproduced with permission from ref. 43.)

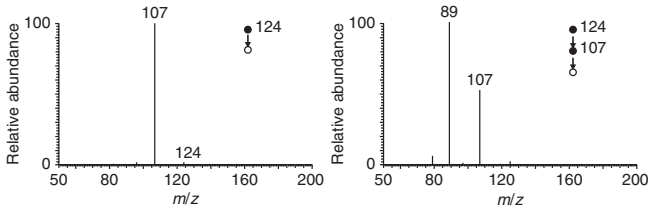


Figure 13 | MS/MS and MS/MS/MS spectra of DEG in toothpaste samples analyzed by ND-EESI-MS. (Copyright: American Chemical Society. Reproduced with permission from ref. 46.)

The recovery of DEG in the toothpaste sample is in the range of 97.6–102.4% (ref. 46). With the application of a sealable ND/GIND sampling device, volatile and semivolatile compounds can also be quantitatively detected; for example, the LOD for nicotine in aerosol standard samples has been determined to be 0.05 fg ml^{-1} (ref. 42). Although the ionization mechanisms of secondary ESI and EESI have an impact on when gas-phase (SESI) or condensed-phase (EESI) analytes are probed, we recommend that the sample plume interact with charged droplets (rather than gas-phase ions) to implement EESI.

In conclusion, with the proper application of individual ND devices, trace analytes present in either low- or high-viscosity samples (e.g., ionic liquids, oil, wine, beer, cheese, toothpaste and so on, with their viscosities ranging from a few cP to 300,000 cP) can be sensitively and directly detected with no sample pretreatment. Real-time, online monitoring of chemical reactions occurring in complex viscous systems (e.g., ionic liquids) has also been successfully achieved without interfering with the ongoing process or contaminating the sample. Specific analytes in highly viscous samples (e.g., DEG in toothpaste) have been quantified using ND-EESI-MS, and satisfactory recoveries (e.g., 97.6–102.4%) have been obtained. Experimental data have shown that ND-EESI-MS can substantially simplify the procedure for the rapid characterization of viscous samples at the molecular level, thus providing a powerful MS-based analysis platform for multiple disciplines.

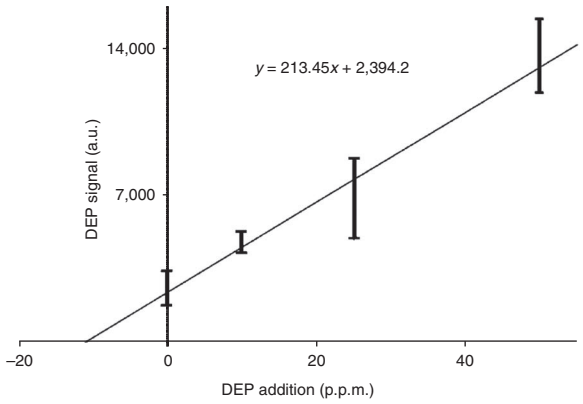


Figure 14 | Quantification of DEP in 'le Male' perfume using the standard addition method. (Copyright: American Chemical Society. Reproduced with permission from ref. 43.)

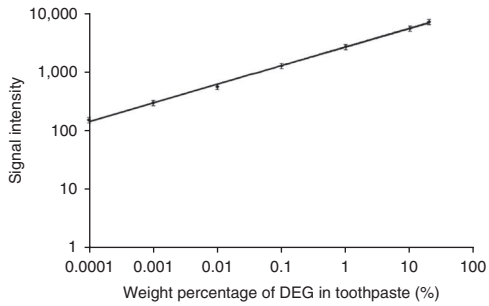


Figure 15 | Quantification of DEG in toothpaste using the standard addition method. (Copyright: American Chemical Society. Reproduced with permission from ref. 46.)

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AUTHOR CONTRIBUTIONS X.L. prepared the manuscript, discussed its implications and commented on the manuscript at all stages; B.H. was involved in most of the experiments; J.D. performed the experiment of selective detection of diethylene glycol in toothpaste products; H.C. conceived the concept, designed all the experiments, outlined the protocol and revised the manuscript.

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