

Sensitive detection of drug vapors using an ion funnel interface for secondary electrospray ionization mass spectrometry

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In this study, we use an ion funnel (IF) at ambient pressure to enhance the sensitivity of secondary electrospray ionization (SESI). Atenolol, salbutamol and cocaine as test compounds are delivered to the SESI interface in the gas phase and are charged with three nano electrosprays. In our experiments, we show that the compounds can be detected at concentrations in the low pptv range, which is an increase of two orders of magnitude compared with the results without the IF. With a standard SESI interface, the compounds could not be detected at all. With the use of the SESI IF interface for the headspace analysis of bananas and limes, we can detect many more compounds and at higher intensities than with a standard SESI interface. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: secondary electrospray ionization; ion funnel; biological samples; surface analysis; online mass spectrometry

INTRODUCTION

Mass spectrometry has become a very powerful analytical tool over the last few years. Instruments have become smaller and easier to handle, detection limits have steadily improved, and sample introduction has been simplified. However, better sensitivity often comes at the cost of a more complex sample introduction. The larger the share of ions generated that reach the detector, the better the sensitivity. Although guiding ions towards the detector can be easily achieved with electric or magnetic fields and at low pressure (to maximize the mean free path of ions and therefore minimize the number of ions being lost due to collisions with neutral molecules), sample introduction is simplest and most convenient at ambient pressure. Unfortunately, the transfer from ambient to sub-ambient pressure results in great ion loss. In the case of electrospray ionization (ESI), both measurements and calculations show that only a small fraction of typically 1%^[1] of the ions generated can be focused into the mass spectrometer.^[2]

Knowing the detailed chemical composition of gasses is important and may be vital in many situations in life: airport security screens passengers and baggage for drugs and explosives,^[3,4] surgeons monitor oxygen and carbon dioxide levels in the breath of patients,^[5] chemical sensors in mines alert miners in case of elevated carbon monoxide levels.^[6] All these examples have some common characteristics: results have to be available immediately, only a very small number of substances need to be monitored simultaneously, and detection is limited to compounds of low molecular weight and usually, high vapor pressure. Building a device that can both handle online analysis and simultaneously detect a variety of compounds with high sensitivity is difficult. Besides ion mobility spectrometry,^[7] which is mainly used for airport security and military purposes, there are two other mass spectrometric techniques that meet the

requirements mentioned earlier, proton transfer reaction mass spectrometry (PTR-MS) developed by Hansel *et al.*^[8] and selected ion flow tube mass spectrometry (SIFT-MS) developed by Smith and Španěl.^[9,10] Both techniques are capable of analyzing ambient gasses (e.g. air, breath) online. The sensitivities obtained depend on the compounds investigated and may, under optimal conditions, be in the sub-pptv range (e.g. in the case of PTR-MS of Her Majesty's explosive (HMX)^[11]) or in the sub-ppbv range (e.g. SIFT-MS analysis of aromatic hydrocarbons.^[12]) Except for a handful of recently investigated compounds of heavier molecular mass, such as sesquiterpenes^[13,14] HMX^[15] and trinitrotoluene (TNT),^[11,15] both techniques focus on volatile organic species with molecular weights below 200 Da.

In the case of secondary ESI (SESI)^[16–19] and desorption ESI (DESI),^[20,21] detecting compounds of higher m/z is possible. Both ionization techniques originate from ESI,^[22] where the analyte molecules are contained within the charging spray. In the case of SESI, the gaseous analyte is intercepted by an electrospray to obtain analyte ions. For all techniques mentioned, the available mass range is restricted only by the mass spectrometer, typically 50–20 000 m/z . However, the sensitivities obtained when measuring gaseous samples are considerably lower compared with PTR-MS and SIFT-MS. This can be explained by the fact that for both SESI and DESI, analyte ions are not produced directly by the high voltage spray source. It is therefore expected that the ion transfer is similar if not worse than for ESI. Furthermore, a

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large fraction of the analyte molecules are never ionized because of the geometry of SESI experiments and the short time during which charge transfer has a chance to occur. It is thus not surprising that SESI is less sensitive than ESI. If this transmission problem could be overcome, SESI might allow for the online detection of gas-phase compounds that cannot be detected with current techniques so far. Possible areas of application are breath monitoring of anesthetics during surgery, of substances of abuse at parties, or of doping compounds during sport events.

In this study, we combine SESI with an ion funnel (IF) interface that works at ambient pressure to investigate whether and to what extent the sensitivity of SESI experiments can be improved. Our IF design is based on publications of Smith and co-workers^[23]. Technical details can be obtained from Meier et al.^[24] The compounds tested here (atenolol, cocaine and salbutamol, which were chosen because they all are on the world anti-doping agency's list of prohibited compounds) could not be detected with standard SESI experiments. In contrast, our new interface allows for detection limits as low as 13 fmol/s (34 pptv) in the case of atenolol. Furthermore, when measuring the headspace of banana skin and slices of limes, we could detect many compounds with molecular weights up to 500 Th that have so far been below the limit of detection.

EXPERIMENTAL SECTION

All experiments in this publication were conducted on a commercial 3D ion trap mass spectrometer (LCQ-Deca, Thermo Finnigan, San Jose, USA). Details on the self-made charging spray interface used for the SESI experiments as well as the IF used for enhancing the sensitivity have been described before.^[24] Standard SESI experiments were conducted using the neutral sample introduction system shown in Figure 1 (left) without the IF. The neutral gaseous sample molecules introduced via this system were ionized with three charging sprays as described before.^[25]

The SESI setup used in this study is shown in detail in Figure 1. The neutral sample introduction consists of a 1/16" Swagelok Tee (# 3; Swagelok Corp., Solon, OH, USA), a polyimide-coated fused silica capillary (# 4; id 250 μm , od 350 μm , BGB Analytik, Boeckten, Switzerland) to help nebulize the analyte molecules with nitrogen as nebulizing gas (# 2, 500 ml/min, controlled by a mass flow controller, F 201-CV, Bronkhorst High-Tech B.V., Ruurlo, Netherlands) and a polyimide-coated fused silica capillary (# 1; id 75 μm , od 150 μm , BGB Analytik, Boeckten, Switzerland) for sample introduction (flow rate 5 $\mu\text{l}/\text{min}$, controlled by the

LCQ syringe pump). The sample spray (# 4) was mounted airtight and on-axis into a heating coil (# 5) that was maintained at $\sim 150^\circ\text{C}$ during the whole experiments to prevent sample molecules from condensing onto the polyether ether ketone surface of the transfer tube inside the heating coil. The evaporated sample molecules were then introduced into the charging spray unit (# 9) via a 1/4" Teflon Union (# 6) and a 1/16" Swagelok cap (# 7) holding a stainless steel capillary (# 10; id 500 μm , od 700 μm). The charging spray unit was heated to 75°C with three heating cartridges (# 8; Probag Wärmetechnik AG, Niederbuchsiten, Switzerland) to prevent sample molecules from condensing; 75° is still below the boiling point of the solvent mixture, which is used for the charging sprays. The distance between the end of the stainless steel capillary delivering the sample molecules and the three charging sprays (# 11) was optimized to ≈ 10 mm to yield highest signal intensity.

The charging sprays were infused at 0.05 ml/min and consisted of MeOH: H₂O in a 1:1 ratio and acidified with 1% acetic acid. A voltage of 3.5 kV was applied. The inlet capillary of the mass spectrometer was held at 250°C and at a voltage of +10 V. Other LCQ parameters were optimized for maximum ion yield. Data acquisition was controlled by the Xcalibur 2.0 software (Thermo Fisher Scientific, Waltham, MA, USA). Mass spectra were collected in scanning mode for 120 s in the m/z range of 100–500 Th.

Atenolol was obtained from Sigma-Fine Chemicals (St. Louis, MO, USA). Cocaine (methyl (1*R*,2*R*,3*S*,5*S*)-3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1] octane-2-carboxylate) was obtained from Lipomed AG (Arlesheim, Switzerland). Ventolin[®] containing salbutamol ((*RS*)-4-[2-(*tert*-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)phenol, 5 mg/ml) sold by GlaxoSmithKline (Brentford, UK) was obtained from a local pharmacy. Acetic acid and methanol were obtained from Acros Organics (Geel, Belgium). Nanopure water with a resistivity of > 18.1 M Ω cm was obtained from a NANOpure water purification system (Barnstead, IA, USA). The charging spray solutions were prepared weekly.

The limits of detection (LODs) were determined as follows: A dilution series of the compounds was prepared. The samples were measured in triplicate, with increasing concentrations for each compound. A blank was run between any two measurements until the signal intensity of the compound being measured returned to the level of the preceding blank (usually 30–60 s). Because there is no window integrated into the IF to monitor the electrosprays, single measurements lasted at least 4 min to ensure that the sprays worked steadily. Intervals of roughly 200 scans (30 s) were averaged and normalized by dividing by the

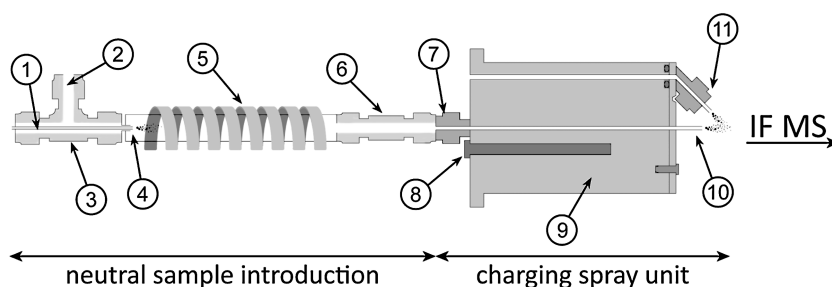
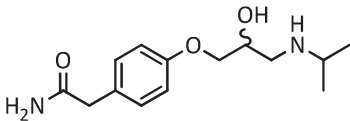
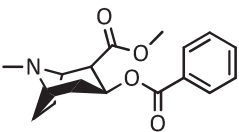
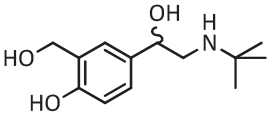


Figure 1. The SESI interface: (1) polyimide-coated fused silica capillary (id 75 μm , od 150 μm) for sample introduction; (2) polyimide-coated fused silica capillary (id 250 μm , od 350 μm) to help nebulize the analyte molecules with nitrogen as nebulizing gas (500 ml/min); (3) Swagelok Tee to mount (1) and (2); (4) secondary electrospray ionization spray mounted on-axis into heating coil (5) that prevents sample molecules from condensing on surface; (6) Teflon straight fitting, (7) Swagelok cap to mount (10); (8) heating cartridge; (9) heated charging spray unit; (10) stainless steel capillary; (11) charging sprays.

total ion current to allow for a direct comparison between measurements. The limits of detection (LOD) was defined to be three times the normalized signal of the noise present in the blank and was calculated from the lowest concentration with a normalized signal that had an $S/N > 3$.

RESULTS AND DISCUSSION

In the first set of experiments, and to prove that both our SESI spray interface as well as the SESI IF spray interfaces are working, we used atenolol, which is a selective β_1 receptor antagonist (β -blocker) used by patients suffering from cardiovascular diseases; salbutamol, which is a short-acting β_2 -adrenergic receptor agonist used by patients suffering from asthma or chronic obstructive pulmonary disease for the relief of bronchospasm and cocaine

Table 1. The chemicals used in this study shown with their molecular weight and their most abundant signal			
Compounds used in this study			
Compound	Structure	Molecular weight	m/z
Atenolol		266.34	267
Cocaine		303.15	304
Salbutamol		239.15	240

(Table 1). The LOD were determined and compared with the LODs obtained using both a standard SESI as well as the SESI spray interface without the IF. Figure 2 shows two experiments using atenolol as analyte, for the SESI interface without IF (Figure 2, top) and for the SESI interface with IF (Figure 2, bottom). It can easily be seen that the IF helps to increase the sensitivity, in the case of atenolol by about two orders of magnitude. For the standard SESI setup, no signal could be observed up to a concentration of 1 nmol/s. Higher concentrations were not used to prevent memory effects. Identical to a previous publication of us^[24], the SESI IF setup was carefully investigated and compared with the SESI interface and the standard SESI interface. Table 2 shows the LODs obtained for the different experiments; values are expressed as fmol of analyte delivered in the gas-phase per second. The distance between the sprays and the inlet of the mass spectrometer was 12 cm for the experiments 'Standard SESI setup far' and 2 cm for the 'Standard SESI setup close', respectively. The experiments 'SESI interface w/o IF' and 'SESI interface IF on' correspond to the setups depicted in Figure 2. As expected, the best sensitivity is achieved in the 'SESI interface IF on' experiments. Comparable with the study on extractive ESI (EESI) using an IF^[24], the geometry of the IF by itself helps to improve the sensitivity of the experiments, as can be seen when the LODs obtained for 'IF off' are compared with these obtained with the other setups. However, it is very interesting to note that using the 'SESI interface w/o IF' geometry detection of the compounds was possible, whereas no signal could be obtained in the 'SESI interface close' setup. The distance between charging sprays and the inlet of the mass spectrometer in case of the 'SESI interface close' setup is obviously too short and does not allow for the gaseous analyte molecules to be efficiently charged.

In a second series of experiments, the SESI interface was modified as depicted in Figure 3. Two holes were cut into a standard 50 ml Falcon tube and were sealed air-tight with septa to mount stainless steel capillaries that allowed for a steady nitrogen flow through the tube. The flow rate was set to 500 ml/min and was controlled by a mass flow controller (F 201-CV, Bronkhorst High-Tech B.V., Ruurlo, Netherlands). By blowing nitrogen over freshly cut banana skin (5 cm long, 2 cm wide) or slices of limes (1/8th of a lime) that were placed into

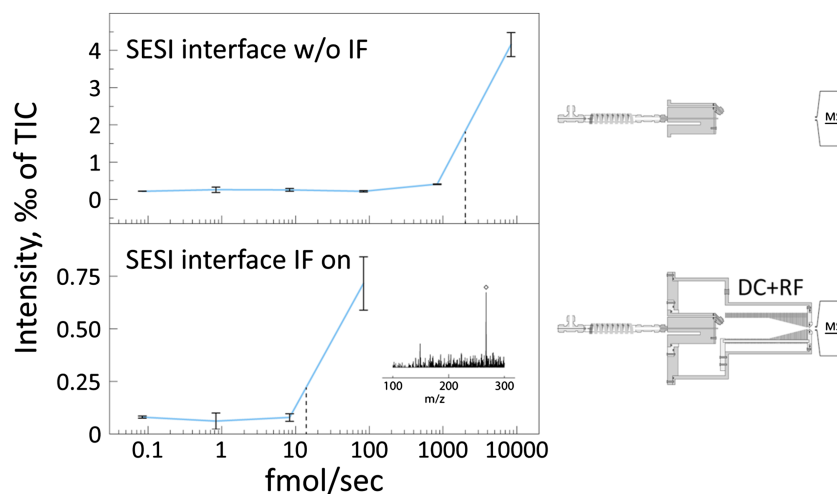


Figure 2. Determination of limits of detection using atenolol for secondary electrospray ionization experiments without (top) and with ion funnel (bottom). Note that the x -axis is logarithmic. The inset (spectrum) shows the signal of atenolol (marked with a diamond) at a concentration of 15 fmol/s.

the Falcon tube, volatile compounds released by the fruits were detected in the headspace. Figure 4 shows the background-subtracted spectra obtained for the 'SESI interface w/o IF' (top) and the 'SESI interface IF on' setup (bottom) of bananas (left) and limes (right). As expected from the results obtained in the first set of experiments described above, the 'SESI interface IF on' setup allowed for the detection of many more compounds and at higher intensities. Previous gas chromatography mass spectrometry studies on bananas^[26] and limes^[27] could identify a variety of volatile compounds in the mass range covered by

our instrument (50–500 Th). The signals with m/z that match compounds identified in these published studies are marked with lower-case letters. Even without confirming the signals with MS/MS data in these proof-of-principle experiments, we can show that this newly built SESI IF interface is a powerful technique to detect volatile compounds on-line in the gas phase. In addition, it is interesting to note that our interface is capable of detecting compounds up to 500 Th. To our knowledge, no other online mass spectrometric technique has detected components in the headspace with such high m/z so far.

Table 2. The limits of detection for atenolol, salbutamol and cocaine using the ion funnel (left) and the control experiments (right). Without the ion funnel, the compounds could not be detected at all

Limits of detection [fmol/s] for SESI experiments						
Compound	Ion funnel		Control experiments			
	IF on	IF off	SESI interface w/o IF	SESI interface close	Standard SESI setup far	Standard SESI setup close
Atenolol	13 ± 2	83 ± 12	1390 ± 108	> 1E+06	> 1E+06	> 1E+06
Salbutamol	18 ± 4	125 ± 18	2416 ± 241	> 1E+06	> 1E+06	> 1E+06
Cocaine	26 ± 2	50 ± 14	323 ± 52	> 1E+06	> 1E+06	> 1E+06

SESI, secondary electrospray ionization, IF, ion funnel.

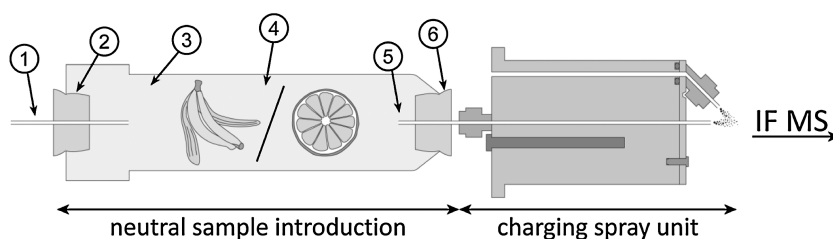


Figure 3. The headspace interface: (1) needle introducing nitrogen at 500 ml/min; (2) septum to hold (1); (3) 50 ml Falcon tube; (4) banana or lime sample placed in (3); (5) stainless steel capillary transferring neutral sample molecules towards charging sprays and IF-MS; (6) septum to hold (5) in place.

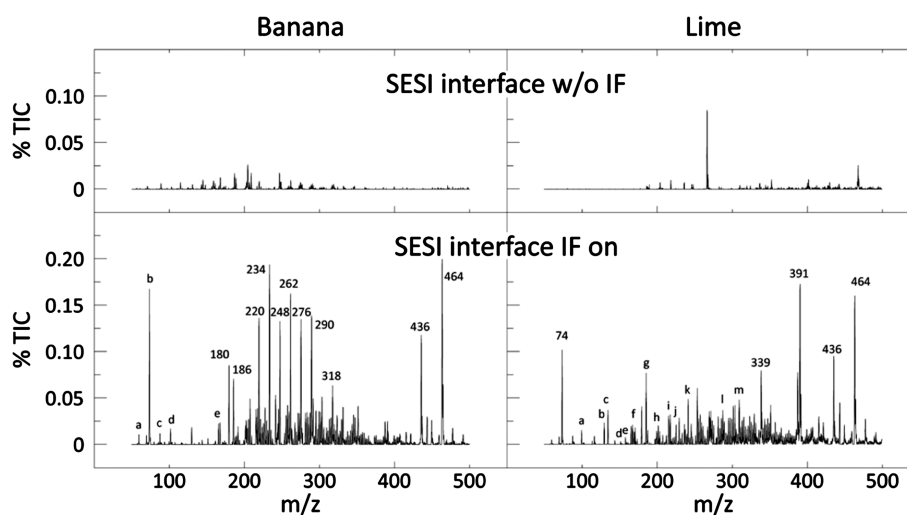


Figure 4. Left: headspace mass spectra of banana skin for experiments without (top) and with ion funnel interface (bottom). The spectra are background subtracted. Putative assignments of compounds found in previous studies^[26]: (a) 1-Propanol, (b) 1-Butanol, (c) 2-Pentanol, (d) 1-Hexanol, (e) Eugenol. Right: headspace mass spectra of limes for experiments without (top) and with ion funnel interface (bottom). The spectra are background subtracted. Putative assignments of compounds found in previous studies^[27]: (a) Hexanal, (b) Octanal, (c) *p*-Methylacetophenone, (d) Geraniol, (e) Citronellol, (f) Decanal, (g) Dodecanal, (h) Citronellyl acetate, (i) Tetradecanal, (j) Pentadecanal, (k) Hexadecanal, (l) α -Bisabolol, (m) Ethyl linoleate.

CONCLUSIONS

A heated SESI interface has been built and coupled to an IF that works at ambient pressure. With the use of atenolol, cocaine and salbutamol as test compounds, we could show that the SESI interface alone has sensitivity in the low ppbV range. The use of the IF further increased the sensitivity by another two orders of magnitude, into the low pptV range. Headspace analyses of banana skin and slices of limes showed that with the new IF interface, the detection of volatile compounds up to 500 *m/z* is possible, compounds that have so far been below the detection limit.

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REFERENCES

- [1] A. El-Faramawy, K. W. M. Siu, B. A. Thomson. Efficiency of nano-electrospray ionization. *J Am Soc Mass Spectrom* **2005**, *16*, 1702.
- [2] R. D. Smith, J. A. Loo, C. G. Edmonds, C. J. Barinaga, H. R. Udseth. New developments in biochemical mass spectrometry: electrospray ionization. *Anal Chem* **1990**, *62*, 882.
- [3] Ionoscan Sentinel II, Smiths Detection, Smiths Group, London, UK.
- [4] GE Entry Scan. General Electric Company, Fairfield, CT, USA.
- [5] A. O. Soubani, Noninvasive monitoring of oxygen and carbon dioxide. *Am J Emerg Med* **2001**, *19*, 141.
- [6] Carbon Monoxide Sensor, Futurlec, London, UK.
- [7] A. B. Kanu, P. Dwivedi, M. Tam, L. Matz, H. H. Hill. Ion mobility – mass spectrometry. *J Mass Spectrom* **2008**, *43*, 1.
- [8] A. Hansel, A. Jordan, R. Holzinger, P. Prazeller, W. Vogel, W. Lindinger. Proton transfer reaction mass spectrometry: on-line trace gas analysis at the ppb level. *Int. J. Mass Spectrom. Ion Processes* **1995**, *149*, 609.
- [9] N. G. Adams, D. Smith. The Selected ion flow tube (SIFT): a technique for studying ion-neutral reactions. *Int. J. Mass Spectrom. Ion Phys.* **1976**, *21*, 349.
- [10] P. Španěl, D. Smith. Progress in SIFT-MS: breath analysis and other applications. *Mass Spectrom Rev* **2011**, *30*, 236.
- [11] C. A. Mayhew, P. Sulzer, F. Petersson, S. Haidacher, A. Jordan, L. Märk, P. Watts, T. D. Märk. Applications of proton transfer reaction time-of-flight mass spectrometry for the sensitive and rapid real-time detection of solid high explosives. *Int. J. Mass Spectrom.* **2010**, *289*, 58.
- [12] B. M. Ross. Sub-parts per billion detection of trace volatile chemicals in human breath using selected ion flow tube mass spectrometry. *BMC Research Notes* **2008**, *1*, 41.
- [13] F. Dhoooghe, C. Amelynck, N. Schoon, E. Debie, P. Bultinck, F. Vanhaecke. A selected ion flow tube study of the reactions of H₃O⁺, NO⁺ and O²⁺ with a series of sesquiterpenes. *Int. J. Mass Spectrom.* **2008**, *272*, 137.
- [14] T. S. Wang, D. Smith, P. Španěl. Selected ion flow tube, SIFT, studies of the reactions of H₃O⁺, NO⁺ and O²⁺ with eleven C₁₀H₁₆ monoterpenes. *Int. J. Mass Spectrom.* **2003**, *228*, 117.
- [15] S. Jürschik, P. Sulzer, F. Petersson, C. A. Mayhew, A. Jordan, B. Agarwal, S. Haidacher, H. Seehauser, K. Becker, T. D. Märk. Proton transfer reaction mass spectrometry for the sensitive and rapid real-time detection of solid high explosives in air and water. *Anal Bioanal Chem* **2010**, *398*, 2813.
- [16] M. Tam, H. H. Hill. Secondary electrospray ionization-ion mobility spectrometry for explosive vapor detection. *Anal Chem* **2004**, *76*, 2741.
- [17] W. E. Steiner, B. H. Clowers, P. E. Haigh, H. H. Hill. Secondary ionization of chemical warfare agent stimulants: atmospheric pressure ion mobility time-of-flight mass spectrometry. *Anal Chem* **2003**, *75*, 6068.
- [18] C. Wu, W. F. Siems, H. Hill. Secondary electrospray ionization ion mobility spectrometry/mass spectrometry of illicit drugs. *Anal Chem* **2000**, *72*, 396.
- [19] P. Martínez-Lozano, J. Rus, de la G. F. Mora, M. Hernández, de la J. F. Mora. Secondary electrospray ionization (SESI) of ambient vapors for explosive detection at concentrations below parts per trillion. *J Am Soc Mass Spectrom* **2008**, *20*, 287.
- [20] Z. Takats, J. M. Wiseman, B. Gologan, R. G. Cooks. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science* **2004**, *306*, 471.
- [21] Z. Takats, J. M. Wiseman, R. G. Cooks. Ambient mass spectrometry using desorption electrospray ionization (DESI): instrumentation, mechanisms, and applications in forensics, chemistry, and biology. *J Mass Spectrom* **2005**, *40*, 1261.
- [22] M. Yamashita, J. B. Fenn. Electrospray Ionization source. Another variation on the free-jet theme. *J Phys Chem* **1984**, *88*, 4451.
- [23] R. T. Kelly, A. V. Tolmachev, J. S. Page, K. Tang, R. D. Smith. The ion funnel: theory, implementations and applications. *Mass Spectrom Rev* **2010**, *29*, 294.
- [24] L. Meier, C. Berchtold, S. Schmid, R. Zenobi. Extractive electrospray ionization mass spectrometry – enhanced sensitivity using an ion funnel. *Anal Chem* **2012**, *84*, 2076.
- [25] L. Meier, S. Schmid, C. Berchtold, R. Zenobi. Contribution of liquid-phase and gas-phase ionization in extractive electrospray ionization mass spectrometry of primary amines. *Eur. J. Mass Spectrom.* **2011**, *17*, 345.
- [26] J. M. F. Nogueira, P. J. P. Fernandes, A. M. D. Nascimento. Composition of volatiles of banana cultivars from Madeira island. *Phytochem Anal* **2003**, *14*, 87.
- [27] M. G. Chisholm, M. A. Wilson, G. M. Gaskey. Characterization of aroma volatiles in key lime essential oils (*Citrus aurantifolia* Swingle). *Flavour Fragrance J.* **2003**, *18*, 106.