International Journal of Mass Spectrometry 299 (2011) 145–150

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Evaluation of extractive electrospray ionization and atmospheric pressure chemical ionization for the detection of narcotics in breath

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ARTICLE INFO

Article history:
Received 28 August 2010
Received in revised form 8 October 2010
Accepted 8 October 2010
Available online 15 October 2010

Keywords:
Extractive electrospray ionization
Atmospheric pressure chemical ionization
Breath analysis
Narcotic

ABSTRACT

Diagnosis by online breath analysis using mass spectrometry is challenging because of the low concentrations of pertinent compounds in breath. Here we investigate extractive electrospray ionization and atmospheric pressure chemical ionization for the detection of narcotics in breath. The limit of detection was evaluated for morphine, fentanyl, norfentanyl, naloxone, cocaine, γ-hydroxybutyrolactone (GBL), and nicotine. They were found to be in the low fmol/s range, depending on the ionization system used. Data was obtained on four different mass spectrometers: A quadrupole time-of-fight instrument, a 3D ion trap, a linear ion trap, and a portable ion trap. A system was developed to mix reference compounds with breath for the investigation of the ionization sources as well as for standardization and online quantification of semi-volatile compounds in breath.

1. Introduction

Human breath is composed of many compounds that can potentially provide information about a person's health status. It is known that diseases like diabetes (via acetone [1]) or cancer [2] can be diagnosed just by the smell of breath. In addition to compounds that originate from basic human metabolism, artificial compounds such as pharmaceuticals and their metabolites, even some with quite low volatility, are exhaled [1,3]. The detection of specific pharmaceutical compounds in breath could provide important information on the health status and serve as a new tool for the investigation of drug metabolism and pharmacokinetics. One can even envision the analysis of breath as a fast and comfortable feedback tool to monitor and control the needed dose of a particular drug. In the special case of anesthesiology, it would be really helpful to monitor breath of patients in order to deliver an optimized drug level during surgery. To establish an online detection method for narcotics used in anesthesia, a sensitive, accurate, and fast detection method is needed. Mass spectrometry is the most promising detection technology in this field. It provides high sensitivity (amol) as well as high accuracy (by MS^n) and even enables online detection.

The idea of breath analysis by mass spectrometry, at least in offline detection mode, is not new. Offline sampling techniques, e.g., sampling bags [4] or solid phase micro extraction (SPME) [5] are well established for the detection of volatile organic compounds (VOCs) in combination with gas chromatography–mass spectrometry (GC–MS) [6,7]. Most of the compounds that were detected in breath this way had molecular masses below 200 g/mol [8] and have a vapor pressure lower than water. Compounds used in anesthesia usually have higher masses and lower volatility than the VOCs typically detected. Analyses have also been made of exhaled breath condensate (EBC) [3], which even showed the presence of completely nonvolatile compounds such as proteins. Thus, nonvolatile species are apparently exhaled via breath, but they are very low in concentration [7,9,10]. To detect nonvolatiles such as narcotics, especially with an online method, a very sensitive detection system is needed.

Ionization methods that permit online detection by mass spectrometry have also been developed, in particular two subambient ionization techniques, i.e., selected ion flow tube mass spectrometry (SIFT-MS) [2,11] and proton transfer reaction mass spectrometry (PTR-MS) [12,13]. SIFT-MS reaches sensitivities in the range of high ppb range for volatile compounds, whereas PTR-MS has even sub-ppb sensitivity for some compounds. Both methods operate at pressure of a few mbar, and need infusion of the analyte in gaseous form, which is problematic for nonvolatiles. In parallel, ambient ionization methods such as ambient pressure chemical ionization (APCI) [14–16], glow discharge [17,18], fused droplet electrospray ionization (FD-ESI) [19,20] and extractive electron spray ionization (EESI) were developed [21–23]. The figures of merit of these ambient ion sources have recently been reviewed in detail by Chen et al. [24]; most of them show LODs in the low ppb range. In contrast to the methods operating at reduced pressure, the ambient methods are able to handle nonvolatile compounds.

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doi:10.1016/j.ijms.2010.10.011

EESI (and the related technique FD-ESI) as well as APCI [25] are the most promising techniques for the online detection of non-volatiles in aerosols like breath: EESI showed very high sensitivity and has been claimed to easily detect compounds such as caffeine in breath [23]. APCI is expected to have very high ionization efficiency for polar and non-polar compounds. Both EESI and APCI may profit from the humidity of breath, which could have an enhancing influence since more protons are available for protonation of analyte molecules. In contrast to EESI, APCI can be performed at higher temperatures which allows both the transport into gas phase and the efficient ionization of non-volatiles [26]. For EESI, a stable ESI plume is necessary and at high temperatures (>150 °C) this is not the case anymore.

In this work, the ionization efficiency for a range of narcotics using both APCI and EESI were compared, and limits of detection (LODs) were determined. The LODs were evaluated with four different mass analysers. The performance was compared with direct infusion ESI [27] and APCI [10]. In a second step, we investigated whether narcotics could be detected in breath. Experiments with humans and animals are very complicated, time-consuming, and expensive. Before tests with patients will be performed, all aspects of the ionization system as well as the mass analyzer have to be optimized. To develop and validate a system for accurate and quantitative detection of target compounds in breath, a reference system is needed as well. Such a tool was also developed, and will be presented below. It enables mixing breath with well-defined amounts of reference compounds and directs the obtained gas or aerosol mixture into the ionization source. In a proof of principle study, nicotine was quantified after smoking, and an online profile of nicotine in breath was recorded.

2. Experimental

2.1. Systems and chemicals

In this study, four different mass spectrometers were used: a quadrupole time-of-fight instrument, a 3D ion trap, a linear ion trap, and a portable ion trap. The quadrupole time-of-flight mass spectrometer (Q-TOF Ultima, Waters, Milford, USA) was equipped with a Z-Spray source in the standard configuration. Standard ESI measurements were performed with this system. The inlet is a cone with an opening of about 0.5 mm I.D. A 3D ion trap (LCQ-Deca, Thermo Finnigan, San Jose, USA) and a linear ion trap (LTQ-Deca XP Plus Thermo Finnigan, San Jose, USA) as well as a portable rectilinear ion trap (Mini 10.5, Aston Labs, Purdue University, USA) were used.

Solvents were purchased from Sigma–Aldrich (Buchs, SG, Switzerland) and narcotic standards from Lipomed AG (Arlesheim, Switzerland). The water used was purified by ion exchange at ETH Zürich and then filtered and deionized a second time by a Millipore® system. As test compounds, nicotine, cocaine, morphine, fentanyl, sulfentanyl, norfentanyl, naloxone caffeine and gamma-hydroxy butyrolacton (GBL) were used (Lipomed AG).

2.2. Limits of detection

The limit of detection is defined by a ratio of 3:1 between the signal of the compound of interest and the noise/background signal at the same mass. To determine this value, different strategies are known. A very common but not always accurate method to estimate LODs is to generate a high signal and calculate the amount of compound that would be needed for obtaining a signal-to-noise ratio of 3. This method has the obvious drawback that near to the LOD, one cannot expect the same linear or proportional response than at high concentrations. This occurs due to the unknown contribution of background to the signal as well as the non-linear behavior of mass analyzers over the whole signal range (e.g., saturation effects of multi-channel plate detectors, or space charge effects in the source). Another, more precise method is to dilute the analyte in a stepwise fashion until a signal-to-noise ratio of 3:1 is reached. This method is very time-consuming but more accurate. In this work we used a combined approach to reach quite accurate LODs. The analyte was diluted stepwise until a signal-to-noise ratio close to 3:1 was reached (less than 10:1). The LOD was then calculated proportionally. This leads to a good estimate of the real LOD and has the advantage of being not too time-consuming. LODs in mass spectrometry are always a question of optimization, maintenance, and age of the equipment, as well as the level of background noise and pollution. Since these values depend on so many factors, the LODs will vary over time and from laboratory to laboratory.

In this work, both direct infusion ESI and APCI, as well as gas-phase methods, including neutral desorption EESI and APCI, are utilized. The concentrations of the compounds delivered as vapors and in solution, however, cannot be directly compared. In order to be able to circumvent this problem, the rate of molecules entering the ionization device was calculated. For direct infusion ESI and direct infusion APCI, the infusion rate of the solution was multiplied by the concentration of the solution. For direct infusion experiments, a syringe pumpe was used, and flow rates were optimized to be about 1–20 μL/min. Gas delivery was made by a heated APCI Probe (Waters Waters, Milford, USA), which was attached to the home-built ion sources.

To investigate how these narcotics behave and how to detect them in breath, a mixing system for breath and narcotics was designed (Fig. 1). It was constructed based on a commercial APCI spray source (for a Q-TOF Ultima Instrument from Micromass). For delivery of reference compounds, a fused silica capillary with a inner diameter of 150 μm was used. The nebulization gas flow in this reference channel was adjusted to a rate of 50–150 L/h, which is in the same range as the flow rate by breathing into the system. The reference solution was delivered with a 250 μL syringe pump (Hamilton, Bonaduz, Switzerland) with a flow rate between 1 and 20 μL/min

3. Results and discussion

To enable breath-by-breath analysis, a high scan rate is needed. For one breath lasting five seconds, a rate of 0.1–0.01 s per scan is needed. Additionally, a sensitivity in the range of pg/mL breath is needed to detect even fairly concentrated compounds [28,29]. Moreover, the possibility to carry out multiple MS experiments such as MS/MS is needed to gain an acceptable selectivity and to identify unknown compounds. Furthermore, it would be desirable to have a mobile instrument to place it, e.g., in a hospital. Table 1 shows the mass spectrometers we considered in this study and tested for breath analysis.

Although the LODs for these mass analyzers are not directly comparable since many factors influence the signal, we evaluated the LODs for a few compounds of interest, to learn which system (mass analyzer, ionization technology) is the most promising for breath analysis, especially for the detection of narcotics (Table 2).

From the data presented in Table 1, it can be seen that the Mini 10.5 is not acceptable for online detection due to its low scan speed, even though it may still be possible to improve this a bit. Moreover, as shown in Table 2, the LOD even in direct ESI mode is already too high for this instrument. One could think of using this instrument to analyze multiple breath strokes to obtain online information, but even in this case, the sensitivity is too low; for achieving a good signal-to-noise ratio, more than 100 scans would be needed, which is far too slow. Neither the LOD nor the scan speed for the Mini

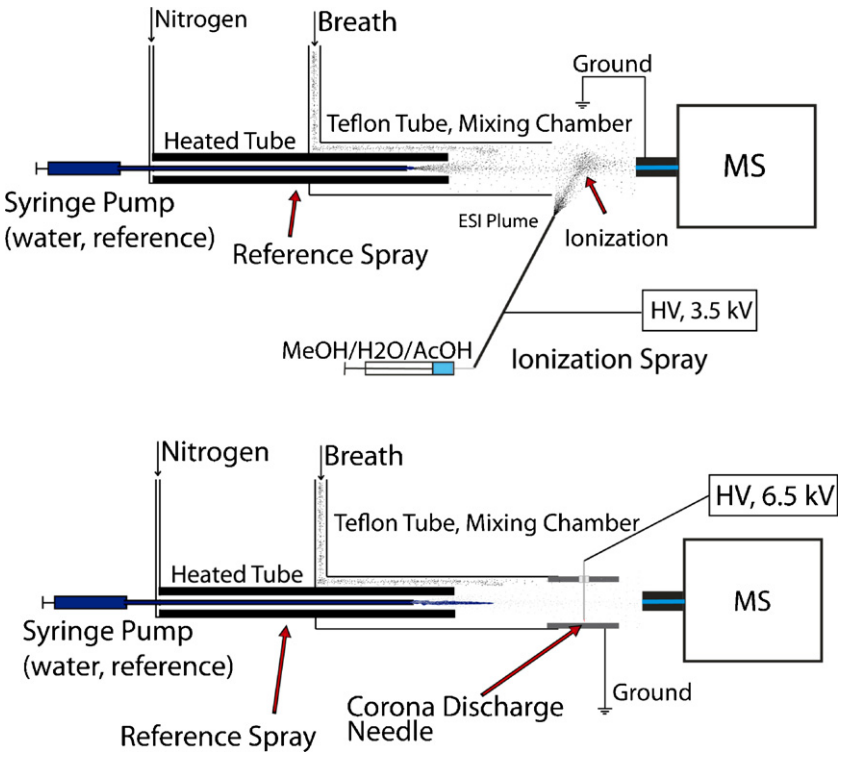

Fig. 1. Reference system for breath analysis. The reference system for mixing breath and standard consists of a heated tube (T_{max} = 350 °C), which evaporates the standard dissolved in water. Heated breath (by flowing around the heated tube) is mixed with a reference spray (70–120 °C). The mixture is delivered to the point of ionization. The top schematic shows the EESI system where an ESI spray plume intercepts the outlet of the mixing device. The ESI spray was operated at 3.5 kV without sheath gas with a 1:1 methanol:water solution containing 0.1% acetic acid. APCI was performed with a self-made system that consists of a grounded metal tube and a needle that is placed 3 mm from the surface. The voltage applied was 6.5 kV and the current 5 mA to sustain a stable glow discharge.

Table 1
Mass analyzers considered in this study [30,31].

Mass spectrometer	Scan speed	Mass range	Accuracy	MS^n	Portability
Q-TOF (Ultima, Waters)	<0.1 s/scan	50–10,000 *m/z*	10 ppm	2	No
3D Trap (LCQ Deca XP, Thermo Scientific)	0.1–0.5 s/scan	50–4000 *m/z*	100 ppm	10	Bench top
Linear Trap (LTQ, Deca XP plus, Thermo Scientific)	0.1–0.5 s/scan	50–4000 *m/z*	100 ppm	10	Bench top
Portable Ion Trap (Mini 10.5, Aston Labs Purdue)	1 s/scan	50–600 *m/z*	600 ppm	10 (Theoretically no limitation)	Handheld

10.5 are thus competitive and we conclude that this system is not capable of online breath detection. Furthermore, it turned out to be neither very stable nor useful for applications in a hospital environment. On the other hand, for the Q-TOF as well as for the ion traps, the LODs were not strongly dependent on the mass analyzer used for most compounds. Since the Q-TOF is fairly big and not mobile, it seems that an ion trap is the only reasonable choice for on-site breath analysis. Since ion traps are bench-top instruments, it is possible with few compromises to use them as quasi mobile devices. Moreover, they offer the ability to enhance the sensitivity by ion accumulation, and to perform optimization of background by MS/MS experiments.

Table 2
Comparison of LODs (in fmol/s) for different mass analyzers employed. The LOD was calculated in fmol of compound per second delivered to the ionization device. This enabled us to compare methods where the sample was delivered in solution with techniques where the sample is delivered in the gas phase only. For samples in the gas phase, it had to be taken into account that the final concentration at the ionization device depends on the gas flow. The gas flow was adjusted to 150 L/h (nitrogen), and is within the same range as that of breath.

MS instrumentation and ionization system	Liquid delivery					Gas delivery	
	ESI Q-TOF (Q-TOF Ultima)	ESI linear trap (LTQ)	ESI 3D-trap (LCQ)	ESI portable ion trap (Mini 10.5)	APCI 3D trap (LCQ)	APCI Q-TOF (Q-TOF Ultima)	EESI Q-TOF (Q-TOF Ultima)
Morphine	0.7	1.2	2.5	583	12.5	1090	136
Fentanyl	0.7	2.5	n/d	124	n/d	248	248
Sulfentanyl	8.3	535	n/d	151	n/d	1100	1462
Naloxone	7.6	10	n/d	25	n/d	510	1019
Norfentanyl	2.2	72	n/d	36	n/d	751	751
Cocaine	0.8	0.2	0.2	137	0.3	176	28
Nicotine	0.3	n/d	n/d	n/d	n/d	12	206
GBL	5.8	n/d	n/d	n/d	n/d	388	775

n/d: not determined.

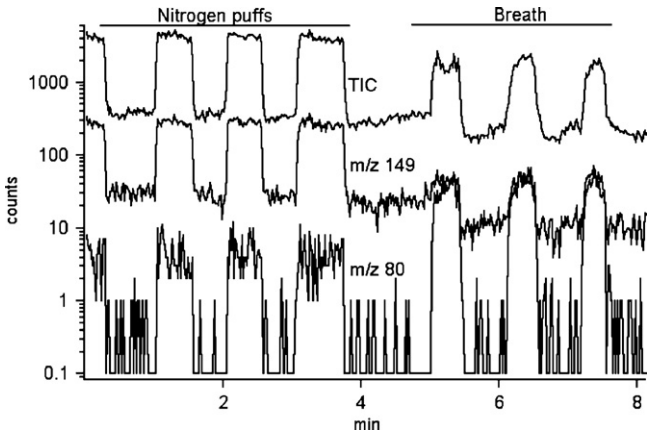

Fig. 2. Online breath analysis by APCI. The total ion current (TIC) as well as the phthalate ion m/z 149 (MH^+) and pyridine m/z 80 (MH^+) are monitored. The flow rate of the nitrogen puffs as well as the breath was approximately 5 mL/min.

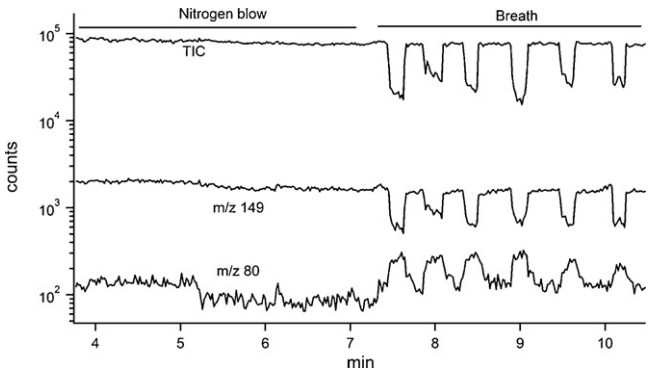

Fig. 3. Online breath analysis using EESI. We repeated the experiment shown in Fig. 2, but with EESI as an ionization source. In contrast to the observations in Fig. 2, the signal of the phthalate ion ($M+H^+$ at m/z = 149) decreases while an increase was found for pyridine ($M+H^+$ at m/z = 80) during breathing. The total ion current is not influenced by nitrogen, which also proves that the ionization plume is not blown away from the inlet of the mass spectrometer.

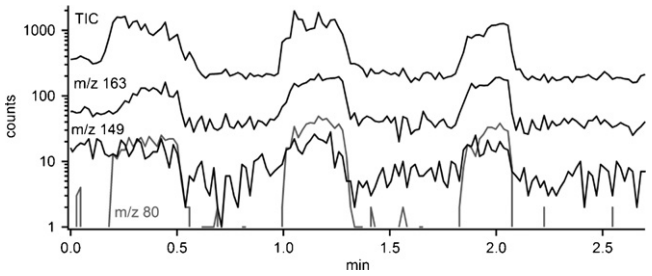

Fig. 4. Total ion current and selected ion traces when analyzing breath using APCI. As in the experiments with nitrogen, the pyridine ion signal (m/z = 80) moves with the total ion current (TIC). The same is true for the nicotine signal at m/z 163.

After studying the LOD for standard compounds, the next question was how the different ion sources and compounds behave if they are used to analyze breath. To investigate this, the device to mix breath with standards was used (Fig. 1). This system was validated by experiments with nicotine.

A series of background experiments were performed. Clean water (Mili-Q) was sprayed as a blank into the breath mixing device and ionized by EESI and APCI. For APCI we typically observed a fairly low background. The highest signal observed was at m/z 149 with an intensity of about 70 counts per second; this m/z is a well known precursor for phthalates and frequently detected as background molecule [32]. With EESI, a variety of signals were observed. The highest had a m/z of 100 and an intensity of about 420 counts/s. Phthalate at m/z 149 was also observed, with an intensity of about 250 counts/s. With EESI, more background is expected due to infusion of the solvent. For impurities in the ionization solvent (methanol, water, acetic acid) the ESI mechanism applies directly, which means that all the impurities are ionized well. In contrast, with APCI there are simply less compounds (no additional solvent) that could lead to background signals. Such background signals are usually subtracted from the spectra, but we observed a strong change for the background molecules while breathing into the system. To clearly differentiate pure background signals from breath signals, three puffs of nitrogen were delivered to investigate the behavior of these background molecules as well as the influence of the gas flow. Fig. 2 reports the signal intensities of m/z = 80 (pyridine) and m/z = 149 (phthalate) as well the total ion current (TIC) for the APCI source. The ion at m/z 149 was taken as the dominant background signal.

The ion at m/z of 80 (protonated pyridine) was monitored as a typical breath marker. Pyridine has been found in breath by Martinez-Lozano and Fernandez de la Mora [33] and was always observed in our breath experiment as well. After three puffs of nitrogen (with the same flow rate as breath), three breath sample puffs were delivered. With the APCI source, both the phthalate, which originates from the nitrogen stream and the pyridine from breath were observed as expected. With the EESI source there was a different outcome (Fig. 3). The nitrogen puffs did not influence the signal, and the breath led to a reduction of the total ion current as well as of the phthalate ion signal. In contrast, the breath marker pyridine shows a signal increase, which proves that this compound gets ionized in breath as well. If we compare EESI and APCI based on the data in Figs. 2 and 3, we conclude that in EESI a competition for protons occurs. In APCI we expect protonated water as the main and abundant proton source. In EESI ionization seems to proceed somewhat differently. Charges and protons from ionized molecules in the ESI solvent, background compounds included, are probably transferred. This could take place in the gas phase or within droplets. Since the EESI mechanism is still under discussion, we cannot say for sure where this charge transfer occurs. Our experiments, including the ones with nicotine (Fig. 5), show that there is a competition for charge within the EESI plume.

Nicotine was then mixed into the reference spray at a concentration of 1 μg/mL. Mixed with breath at an infusion rate of 3 μL/min, a final concentration of about 0.7 pg/mL for a gas-phase sample (breath) was reached. For APCI (Fig. 4), an enhancement of the nicotine signal was observed. We expected this, due to the higher humidity, which produces more available protons. In the case of EESI (Fig. 5) we observed the same signal loss for nicotine (MH^+ m/z = 163) as observed in Fig. 3.

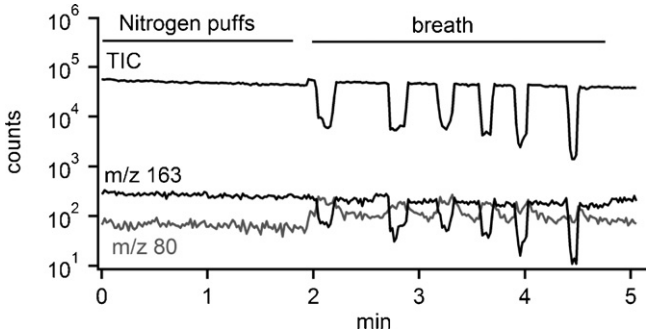

Fig. 5. Total ion current and selected ion traces when analyzing breath mixed with nicotine, using EESI. Pyridine ($[M+H]^+$ at m/z = 80) is still observed when nicotine is infused into the reference spray. As it was observed for the phthalate background, the nicotine signal decreases while breathing.

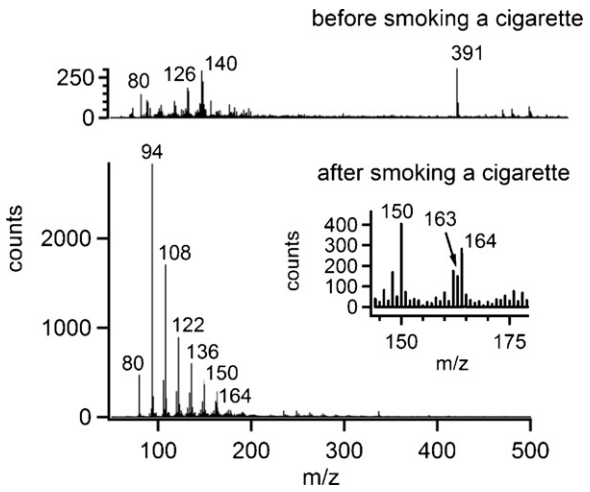

Fig. 6. Mass spectra of exhaled breath recorded using APCI. The spectra were collected over one long deep breath of about 30 s. The top spectrum refers to breath of a male human before smoking a cigarette. The signals observed were not all identified, although the signal for pyridine (m/z = 80) is obvious. After smoking a cigarette we observe many signals with high intensity. Also pyridine is increasing by a factor of at least 2. The intense pattern (m/z 94,108, 122, 136, 150, 164) seems to be alkyl homologs (Δm = 14). The signal of nicotine was clearly detected above the background.

Since nicotine turned out to be problematic in EESI for calibration, due to the signal loss in the reference spray, a calibration for nicotine was only made using the APCI source. This calibration was applied to quantify nicotine in breath after smoking a cigarette. Fig. 6 shows the large changes in breath signals before and after smoking a cigarette measured by the APCI source. Additionally, we observe many compounds that we have not identified yet. The signal of nicotine was detected very well just after smoking (inset, Fig. 6). Cotinine, which is the most important metabolite of nicotine, was not detected. A peak at m/z 177, which could refer to the cotinine molecular ion, was detected in the breath of nonsmokers as well as of smokers. Even after a nonsmoker smoked a cigarette, no change in the intensity of this signal was observed (data not shown).

The signal decrease for nicotine in breath was monitored for about 60 min as shown in Fig. 7. For validation, a calibration of nicotine by the reference spray as well as by the sample spray (nebulizing nicotine solution into the sample tube) was made. Although the variation was quite high and only the range between 0.6 and 13 pg/mL breath was calibrated, the curve showed a nice linearity

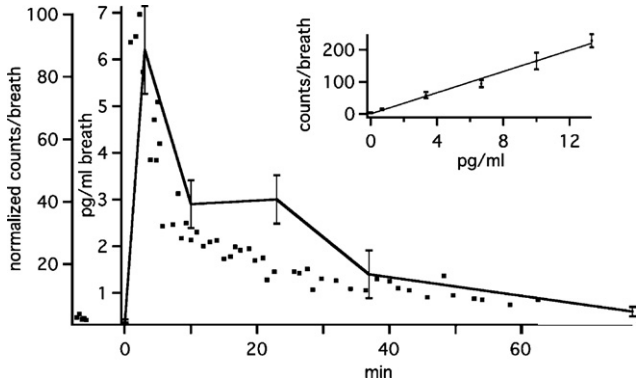

Fig. 7. Time evolution of the nicotine signal, using APCI. Every dot represents the accumulated counts for nicotine (m/z 163) of one deep breath of approximately 30 s. The dots before 0 min refer to the background level before smoking. The second graph shows a second series with quantification and six breath strokes averaged per point (the small inset shows the calibration).

of R = 0.995 (small box Fig. 7), apparently also unaffected by other signals close in mass, such as 162 and 164. Based on this calibration, a quantification of nicotine in breath after smoking was carried out and a maximum concentration of 6.2 (±0.9) pg/mL in breath was determined for a male smoker (line in Fig. 7). After approximately 70 min, the nicotine signal reached almost background level again. These results are in perfect agreement with literature [34]. Fig. 7 shows the kinetics of nicotine for a male nonsmoker after smoking a cigarette carried out in two different experiments.

Besides nicotine, other compounds that are used for pain treatment were tested. Morphine and fentanyl are the two important compounds for this purpose. We reached a limit of detection of 833 pg/s for morphine (10 pg/mL breath), which is comparable to the sensitivity without breath. Morphine behaved the same way as nicotine and the signal was independent of the way of delivery. For fentanyl, it turned out that it adsorbs to the cold PTFE surfaces. It seems that the non-polar fentanyl easily sticks to these surfaces, as opposed to the more polar nicotine and morphine, which seem to be transported well. On the other hand, we found that nicotine sticks to polar glass surfaces while fentanyl gets transported through glass tubes.

4. Conclusions

In this study we described a system for online breath analysis by EESI and APCI and a strategy for quantitation and calibration of narcotics in breath. The new experimental setup we developed enables mixing breath with volatile and nonvolatile compounds quantitatively. Nicotine is a fairly volatile compound and is easily ionized, i.e., APCI is suitable for efficient ionization. The quantification of nicotine shows that both the mixing process, as well as the delivery process are quantitative. Online breath analysis is challenging, but for the first time, a tool is available to directly compare mass spectrometers as well as ionization methods. The sensitivity for the detection of molecules in breath was found to be mostly dependent on the ionization method used; within a factor of 10 it was independent of the mass analyzer used. Only the portable ion trap clearly lacks sufficient sensitivity. Our new sample introduction system surely will have to be developed further, and be tested and validated for other compounds of interest before it can be applied broadly. For example, the surface properties of the delivery system were found to be critical for being able to work quantitatively. One of the next steps will be to compare the breath of patients treated with morphine or fentanyl, to validate our setup in a clinical environment.

Acknowledgments

We thank the Swiss National Science Foundation (grant no. K-23K1-122264) for financial support of this project. We thank the group of Prof. Leroux at ETH Zürich for making their LTQ-Deca-XP accessible to us. We also thank the University of Applied Sciences of Northwestern Switzerland for access to their LCQ-Deca.

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