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Two techniques to sample non-volatiles in breath—exemplified by methadone

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Keywords: exhaled breath, endogenous particles, particle collection, exogenous compounds

Abstract

The particles in exhaled breath provide a promising matrix for the monitoring of pathological processes in the airways, and also allow exposure to exogenous compounds to be assessed. The collection is easy to perform and is non-invasive. The aim of the present study is to assess if an exogenous compound—methadone—is distributed in the lining fluid of small airways, and to compare two methods for collecting methadone in particles in exhaled breath. Exhaled particles were collected from 13 subjects receiving methadone maintenance treatment. Two different sampling methods were applied: one based on electret filtration, potentially collecting exhaled particles of all sizes, and one based on impaction, collecting particles in the size range of 0.5–7 μm, known to reflect the respiratory tract lining fluid from the small airways. The collected samples were analyzed by liquid chromatography mass spectrometry, and the impact of different breathing patterns was also investigated. The potential contribution from the oral cavity was investigated by rinsing the mouth with a codeine solution, followed by codeine analysis of the collected exhaled particles by both sampling methods. The results showed that methadone was present in all samples using both methods, but when using the method based on impaction, the concentration of methadone in exhaled breath was less than 1% of the concentration collected by the method based on filtration. Optimizing the breathing pattern to retrieve particles from small airways did not increase the amount of exhaled methadone collected by the filtration method. The contamination from codeine present in the oral cavity was only detected in samples collected by the impaction method. We conclude that methadone is distributed in the respiratory tract lining fluid of small airways. The samples collected by the filtration method most likely contained a contribution from the upper airways/oral fluid in contrast to the impaction method.

1. Introduction

Exhaled breath contains particles carrying non-volatile substances [1–3]. The main components, lipids and proteins, are derived from the respiratory tract lining fluid (RTLF) [4–8]. The collection procedure is non-invasive, can be repeated within a short time span and is convenient compared to bronchoalveolar lavage (BAL), for example. The small mass sampled is, however, challenging to analyze. Nevertheless, exhaled particles provide a new and promising matrix for the analysis of biomarkers.
Collection of exhaled particles can be performed using various collection principles. Exhaled breath condensate (EBC), obtained by cooling exhaled breath, was the first method used [9, 10]. Reproducibility, however, has been found to be a problem [9]. A novel method of collecting exhaled particles by impaction was presented in 2009 by Almstrand et al [3], and has since then been further optimized to specifically sample lining fluid from the small airways [11], now termed the PExA® method. Finally, Beck et al presented a collection device based on an electret filter, the SensAbues® method, as an easy-to-use method for the testing of abused drugs [12].

The interest in the non-volatiles in exhaled particles has focused on endogenous compounds, such as the biomarkers of inflammation (see, for example, [13–17]). Exogenous compounds as the biomarkers of exposure, such as metals in exhaled particles as an organ-specific marker of exposure to welding fumes [18, 19], have recently been investigated. Exogenous compounds with alternative routes of entry, such as drugs, may be distributed to the RTLF via the circulation. Recently, a non-volatile drug, namely methadone, was detected in exhaled breath by the SensAbues® method [12, 20]. It is, however, unclear if the origin is the RTLF from the peripheral airways, from the upper airways or oral fluid. Particles exhaled and collected by the PExA® method have been shown to consist of the RTLF of peripheral airways generated during inspiration from a residual volume by the airway opening of closed small peripheral airways [11, 21]. We therefore considered it of interest to explore whether the PExA® method was able to detect and quantify methadone in exhaled particles and to compare it with the SensAbues® method.

Thus, the present study aims at testing the hypothesis stating that exhaled methadone originates in the RTLF of peripheral airways. The following specific questions were addressed:

- Is methadone in exhaled particles detectable and quantifiable by the PExA® method?
- If so, are the amounts of methadone collected by the SensAbues® and PExA® methods similar?
- If they are not, how can this be explained?

### 2. Materials and methods

#### 2.1. Subjects

Fourteen subjects (ten males, four females, age range 26–60 years) undergoing methadone maintenance treatment were recruited for a clinical study at two treatment units at Sahlgrenska University Hospital, Gothenburg. The subjects met the DSM-IV criteria for opiate dependence and fulfilled the Swedish legal criteria for methadone maintenance treatment. The subjects were in a steady state and received the dose (solution, p.o.) supervised between 8 and 9 o’clock in the morning. The sampling of the exhaled breath was performed before noon, but the time between dosing and sample collection was not noted. The subjects were asked about their health status and medication and their daily dose of methadone was noted. In addition to particle collection, the examinations included samples of the oral fluid and spirometry. One subject (subject 2) did not complete the sampling procedure because of difficulties following the given instructions. Subject 14 did not succeed in performing the spirometry test correctly.

The characteristics of the subjects are shown in table 1. The spirometry results were normal for all subjects, but one (subject 8). One subject (subject 14) did not succeed in performing the test correctly.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>FVC (% pred)</th>
<th>FEV1 (% pred)</th>
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<td>F</td>
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</table>

Subject 2 did not complete the sampling procedure because of difficulties following the given instructions. Subject 14 did not succeed in performing the spirometry test correctly.

### 2.2. Collection of exhaled breath

#### 2.2.1. **SensAbues®**

The SensAbues® collection device (SensAbues AB, Sollentuna, Sweden) has been described elsewhere [12, 23]. A schematic presentation of the device is shown in figure 1. In short, particles in the exhaled breath are collected by passing it through a mouth-piece, separating any fluid and larger particular matter from the exhaled breath. Inside the device, a porous polymer filter made of electret material is mounted to collect the particles. As the filter is porous, it has no defined pore size. The filtration mechanism is based on electrostatic forces, diffusion, impaction and

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**Table 1. Characteristics of the study subjects.**

<table>
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<th>Subject</th>
<th>Sex</th>
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<th>Height (cm)</th>
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</tbody>
</table>

Subject 2 did not complete the sampling procedure because of difficulties following the given instructions. Subject 14 did not succeed in performing the spirometry test correctly.
interception. For particles smaller than 1 μm, electrostatic forces dominate [24]. The collection efficiency is 99% in the particle diameter range of 0.5–20 μm [25].

Normally, a small part of the airstream (approximately 3%) is diverted from the mouthpiece to a plastic bag for the standardization of the exhaled volume. When the bag is full, it indicates that about 30 l have passed the sampling device. However, during most of the experiments, a flow meter (OEM Flow Sensor Spiroson-AS, Medical Technologies, Zürich, Switzerland) was connected to the device to obtain a more precise measurement of the breathing volumes. In the clinical study, the subjects were asked to breathe tidally during the collection, wearing a nose-clip, until a total air volume of 20 l had been sampled and measured by the Spiroson flow meter. In some of the additional sub-studies, other sampling volumes were used (see below).

After the sampling, the mouthpiece was removed and the device was sealed with plugs and stored at −20 °C until analysis.

2.2.2. PExA®

The PExA® instrument (PExA AB, Göteborg, Sweden) has been described previously [3]. A schematic presentation of the instrument is shown in figure 2. Briefly, the subject exhales through a double valve system. The first valve is a two-way non-rebreathing valve (Hans Rudolph, 112630-1420A, Hans Rudolph, Shawnee, KS, US), which allows the subject to inhale filtered room air (Whatman HEPA-CAP®, GE Healthcare, Little Chalfont, UK) and exhale either to waste or into the instrument by activating the second valve. The airway reopening maneuver includes exhalation to a residual volume, breath holding for five seconds, inhalation to total lung capacity immediately followed by slow exhalation. Only the final exhalation, from the total lung volume, is sampled by the PExA® instrument. A reservoir facilitates the counting and particle collection of the whole volume that is exhaled into the instrument. Between the sampling maneuvers, the subject breathes tidally to waste.

The particles are collected by a modified multi-stage inertial impactor (PM10 Impactor, Dekati Ltd, Tampere, Finland) on a hydrophilic polytetrafluoroethylene filter (Millipore FHLC 02500, Merck, Darmstadt, Germany). The filter has a pore size of 0.45 μm, but just acts as a membrane to collect the impacted particles and no air passes it. Particles between 0.5 and 7 μm are collected on the membrane, due to the cut-off limits of the impactor. The particles are counted by an optical particle counter with a sizing capability in eight size intervals from 0.41 to 4.55 μm (Grimm 1.108, Grimm Aerosol Technik GmbH, Airnring, Germany). The mass of the particles sampled is calculated using the information from the particle counter, assuming a spherical particle shape and a density close to water. In practice, about 80% of the particle mass is in particles with a diameter of less than 2 μm [21, 26]. Particles with a diameter less than 0.5 μm will pass the impactor. To determine the potential fraction of methadone in this particle fraction, an elctret filter of the same type used in SensAbues® was placed after the impactor and analyzed for methadone. The instrument box and the tubing to the two-way valve have a thermostat set at 36 °C, although the two-way valve is not fully controlled by it. Finally, the flow through the instrument is measured by a flow meter (OEM Flow Sensor Spiroson-AS, Medical Technologies, Zürich, Switzerland), which monitors the flow velocities and exhaled volume.

During the clinical study, particle collection was done using the airway reopening maneuver and the total exhaled volume was 60 l, corresponding to roughly a 100–150 ng particle sample. In the additional studies, other sampling volumes were used (see below).

2.2.3. Sampling of oral fluid

The sampling of oral fluid was done using the Quantisa™ collection device containing a stabilizing buffer solution and an absorbing pad (Immunalysis, Pomona, CA, US). According to the manufacturer, the device collects a volume of 1 ± 0.1 ml.

2.3. Procedures and subjects in additional sub-studies

2.3.1. Oral fluid contribution

Five healthy subjects (two males, three females, age 23–64 years) rinsed their mouth cavity with a 30 mg l⁻¹ codeine solution for 5 s and subsequently breathed 20 l tidally through a SensAbues® device. Collection was done before and after the treatment.

Another ten healthy subjects (nine males, one female, age 23–37 years) similarly rinsed their mouths and in another experiment gurgled their throats with codeine solution for 5 s and subsequently about 120 ng exhaled particles were collected by the PExA® method, using the airway reopening maneuver. Collections were done before any treatment and after each of the two treatments. The three parts of the experiment were performed on different days.
2.3.2. Impact of breathing pattern on the collection of methadone in SensAbues®

Eight healthy subjects (six males, two females, age range 34–59 years), undergoing methadone maintenance treatment, first breathed tidally through the SensAbues® device. The procedure was performed until the plastic bag indicated that a total volume of about 30 l had passed the sampling device. Then the subjects were asked to mimic the airway reopening maneuver normally used during a PExA® measurement. After two tidal breathings they exhaled to a residual volume, held their breath for 5 s, made an inhalation to total lung capacity and finally exhaled through the sampling device.

2.3.3. Particle losses in the PExA® instrument and in SensAbues®

Seven healthy subjects (three males, four females, age 35–65 years) were asked to breathe into a modified PExA® instrument, where the valves were stripped off and the tubing between the mouthpiece and the particle counter was reduced to a minimum. The subjects breathed through a simple mouthpiece directed towards the sampling point of the particle counter. These measurements, where particle loss was minimized, were then compared to measurements where a two-way non-rebreathing valve was inserted between the mouthpiece and the sampling point of the particle counter. The subjects breathed a total volume of 201 tidally in triplicate. The experiments were performed at room temperature and the valve did not have a thermostat.

Four subjects undergoing methadone maintenance treatment were asked to breathe 30 l tidally through the SensAbues® device. The devices were then carefully dismantled and the mouthpiece, front filter holder, filter and rear filter holder were each separately rinsed with methanol. The analysis then followed the standard procedure for methadone.

2.4. Chemical analysis

Extraction of the collected sample in the SensAbues® devices was performed with 7 ml of methanol. The device was put on top of a disposable glass tube and methanol was sequentially added in three portions to a total volume of 7 ml directly on the filter. No deliberate washing of the front filter holder, filter and rear filter holder was done, but methadone from the rear filter holder was able to contribute to the total amount analyzed. The resulting eluate (approximately 5 ml) was evaporated to dryness using a heated vacuum centrifuge. The residue was redissolved in 1% NH₄ aqueous solution containing...
Table 2. Methadone in exhaled particles, as collected by the PExA® and SensAbues® devices respectively, expressed as pg per liter of exhaled breath and in oral fluid expressed as μg L⁻¹ liquid from 13 subjects undergoing methadone maintenance treatment. Their daily doses of methadone are also noted.

<table>
<thead>
<tr>
<th>id</th>
<th>PExA® method</th>
<th>SensAbues® method</th>
<th>Oral fluid</th>
<th>Dose</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Methadone pg</td>
<td>Volume of air l</td>
<td>Methadone pg</td>
<td>Volume of air l</td>
</tr>
<tr>
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<td>0.18</td>
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</table>

* Outlier, excluded from the calculations of the median.

50% methanol. The filter-membranes from the PExA® measurements were extracted with 7 ml of methanol by putting the membrane in a disposable glass tube containing the solvent and sonicated for 10 min. The methanol extract was evaporated and re-dissolved as described above. The recovery from the SensAbues® filters was more than 90% [27]. No specific extraction experiments were performed on the membranes used in the PExA® method, but the recovery was estimated to be the same as for SensAbues®.

Methadone and codeine were analyzed in the final extracts using a liquid chromatography and mass spectrometry method operating in selected reaction monitoring mode. The method for analyzing methadone has been described elsewhere [20]. The limit of detection for methadone and codeine was 1 pg/sample and the limit of quantification 5 pg/sample.

3. Results

Methadone was detected and quantified in all samples collected by the two methods. The results are given in table 2. The median concentration of methadone sampled by the PExA® method was 0.28 pg l⁻¹ exhaled breath (range 0.18–1.03) and sampled by SensAbues® 9.5 pg l⁻¹ exhaled breath (range 4.0–77.6), after exclusion of a probable outlier (subject seven; 847 pg l⁻¹). This subject had problems following the breathing instructions and a massive contamination of oral fluid was suspected. A pairwise comparison showed that the concentration was on average 51 times higher per liter of exhaled breath in the samples collected by SensAbues® than the samples collected by the PExA® method. There was no significant correlation between the concentration of methadone in the samples from PExA® and SensAbues® (rₛ = 0.43, p = 0.17). No methadone was detected in the electret filter placed after the impactor in the PExA® instrument.

The median methadone concentration in the oral fluid was 112 μg L⁻¹ (range 21.9–322). The concentration of methadone was more than three orders of magnitude higher in the sampled particles as compared to the oral fluid, based on PExA® samples with a mass of 120–150 ng, or a volume of 0.12–0.15 ml. There was no significant correlation between the concentration of methadone in the oral fluid and the concentration in the samples collected by the PExA® method (rₛ = 0.45, p = 0.128), but there was a trend towards a correlation with samples collected by SensAbues® (rₛ = 0.57, p = 0.055).

There was no correlation between lung function and the total amount collected or the concentration of methadone per liter of exhaled breath for any of the methods.

Applying the airway reopening maneuver during the collection of methadone with SensAbues® resulted in a lower concentration (median 79.5 pg/sample, range 23–998) as compared to normally practised tidal breathing (median 426 pg/sample, range 38–1890).

Codeine concentrations in samples collected by SensAbues® ranged from <5 to 85 000 pg/sample, after rinsing the mouth with a codeine solution. When sampling was done from nasally expired air with a closed mouth (n = 5), no codeine could be detected in any sample (data not shown). For the PExA® method, the codeine concentrations were below the limit of detection (1 pg/sample) in all samples, i.e. both from the mouth rinsing and throat gurgling experiment.

The PExA® instrument includes a two-way non-rebreathing valve that significantly traps exhaled particles as compared to the experimental setting without the valve (paired t-test p = 0.024). The mean particle number passing the valve was 82% (range 59%–120%).
There was a loss of particles in all size fractions in most subjects, but most notably in the largest fraction between 2.98 and 4.55 μm in diameter, where 53% of the particles passed the valve.

Methadone was distributed in different parts of SensAbues® after sampling the subjects undergoing methadone maintenance treatment as shown in table 3. Only 12% of the methadone collected in the device (mouthpiece not included) was found in the filter.

**4. Discussion**

The present study shows that a non-volatile exogenous compound, i.e. methadone, is detectable and quantifiable in exhaled breath by two quite different collecting principles. The equipment based on impaction collected less than one percent of the amount deposited in the filtration device, considering the total amount deposited in the latter device (table 3). These results raise the question of why the impaction device collects such a small fraction of the total amount of exhaled methadone as well as more general questions on particle formation and on the impact of the design of sampling devices too.

The impactor of the PExA® method collects particles between 0.5 and 7 μm in diameter. Particles larger than 7 μm are trapped by the initial stage of the impactor or even earlier in the sampling system and are not collected. The optical particle counter registers particles 0.41–4.55 μm in diameter. The number concentration in the eight size intervals of the counter enables the calculation of the volume and mass of particles between 0.41 and 4.55 μm, assumed to be an estimate of the mass collected by the impactor. The particle counter, however, rarely detects particles larger than 2 μm and the mass median diameter is 0.7–1.0 μm. In practice, particles of 2 μm or less in diameter constitute about 80% of the particle mass [21, 26].

The PExA® equipment thermostat is set to 36 °C to minimize the risk of particle loss due to condensation and changes in the particle size distribution. The two-way non-rebreathing valve is the only part of the instrument not fully thermostatically controlled. About 20% of the particles were lost over the entire measured size range in this valve and in the interval between 2.98 and 4.55 μm, it was almost 50%. The mechanism probably involves the rapid growth of particles by condensation, followed by sedimentation and impaction on the surfaces of the valve. These losses occur, notably, before the optical particle counter registers the particles. Losses could theoretically also be due to particles smaller than 0.5 μm, as they pass the impactor and are not collected. We did not, however, detect any methadone in the electret filters mounted after collecting the impactor stage in PExA®. The low mass of particles smaller than 0.5 μm and the circumstance in which the exhaled particle concentration in this size range has been shown to be lower in exhaled breath [26] probably explains the absence of methadone in particles smaller than 0.5 μm. Thus, the magnitude of the losses of particles in a size range less than 4.55 μm in diameter does not explain why the impaction equipment collects such a small fraction of exhaled methadone.

Particles collected by the PExA® method have been shown to originate from the RTLF of small airways [11, 21]. These particles are caused by the airway reopening following the airway closure of small peripheral airways [11, 28]. A plug of RTLF is formed when airways close during expiration. During the following inspiration, closed airways open, the plug ruptures and particles are formed. Thus, these particles are first inhaled and then exhaled, counted and collected. Analyses show a lipid [6] and protein [4] composition similar to that observed in bronchoalveolar lavage (BAL). The absence of any mucins also indicates a peripheral origin [29]. The same study did not detect any amylase in large pooled PExA® samples, indicating the absence of oral contamination. This was furthermore supported by the codeine experiment, which showed no detectable amounts of codeine and consequently the contribution from the oral fluid is negligible. These findings may be explained if the particles originating from the oropharyngeal tract are larger than 7 μm and are therefore not collected by the PExA® method. Furthermore, as the concentration of methadone in particles collected by PExA® is three orders of magnitude higher than in oral fluid, it is unlikely that contamination by the oral fluid explains the presence of methadone in the PExA® samples.

The PExA® method convincingly collects particles from the small airways. As methadone is present and can be quantified in samples collected by the PExA® method, this study shows that methadone is distributed to the RTLF in the small airways from the blood and thereby may represent a distribution compartment. This important finding facilitates the collection and analysis of endogenous exhaled particles for the testing and monitoring of methadone and probably other exogenous substances, e.g. drugs. It would, for example, be of high interest to compare the concentration of steroids in the lining fluid of the small airways, by inhaled versus oral treatment regimes.

**Table 3. Methadone distribution (pg/part) in different parts of the SensAbues® device.**

<table>
<thead>
<tr>
<th></th>
<th>Mouthpiece (pg)</th>
<th>Front filter holder (pg)</th>
<th>Filter (pg)</th>
<th>Rear filter holder (pg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>88</td>
<td>64</td>
<td>23</td>
<td>246</td>
</tr>
<tr>
<td>2</td>
<td>312</td>
<td>541</td>
<td>48</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>281</td>
<td>587</td>
<td>82</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>381</td>
<td>211</td>
<td>85</td>
<td>161</td>
</tr>
<tr>
<td>Median</td>
<td>297</td>
<td>376</td>
<td>65</td>
<td>84</td>
</tr>
</tbody>
</table>
The design of the filtration device theoretically facilitates the collection of particles of all sizes. In contrast with the impaction method, there is no intentional discrimination of particles, except for the baffles in the mouthpiece that are intended to prevent contamination from the mouth cavity. Electret filters effectively capture larger particles as well as those below 0.5 μm [30]. Consequently, the very high amount of exhaled methadone collected by this device, as compared to the PExA® method, is assumed to depend on the contribution from other sources than particles formed in the small airways, presumably from the oropharyngeal tract.

The transfer of liquid oral fluid to the filter is an obvious explanation. One limitation of the present study is that we did not quantify the sampled material using the filtration device, which hypothetically may have revealed a larger sampled mass. There was a close to significant correlation between the concentration of methadone in the exhaled breath using the filter device and in the oral fluid (p = 0.057). The median concentration of methadone in the oral fluid was 112 μg l⁻¹. The mean amount of methadone sampled was 198 pg/sample, corresponding to the content in less than two microliters of oral fluid. The codeine experiments, using the filtration method, showed that the contribution from the mouth cavity varied from low to very high, which is consistent with earlier experiments with food dye solutions [31]. The oral fluid/saliva contamination of exhaled breath samples, especially of exhaled breath condensate, has been profoundly discussed [10, 31–36]. Most equipment described for the collection of EBC does not intentionally discriminate the particle sizes collected, just like the filtration method. Although the results of these studies are contradictory, they show that there is a considerable risk of contamination from oral fluid when the concentration of the compound of interest is high in oral fluid as compared to the concentration in particles from the airways. This is despite the fact that all equipment for collecting EBC has saliva traps.

The contribution of methadone from liquid oral fluid is probably random, just as the codeine experiment showed. However, except for the rejected outlier, where a massive contamination of oral fluid was suspected, the variation in the collected mass of methadone was of the same magnitude as the masses collected by the impaction method. Therefore, a possible contribution from particles/droplets larger than 7 μm must also be considered. Particle size distribution in the range of 0.7–1000 μm has been studied by Johnson et al during vocalization maneuvers, speech and coughing and they identified several modes in the particle size distribution [31]. Furthermore, Marteus et al studied the origin of nitrite and nitrate in EBC and suggested that a significant portion of respiratory droplets are formed in very proximal airways, including the oropharyngeal tract [34].

A possible mechanism for particles/droplets is a secondary formation from oral fluid or condensed water from the exhaled breath in the mouthpiece of SensAbues®. Indeed, a considerable part of the total amount of exhaled methadone was found in the mouthpiece and in the front filter holder. At an exhalation flow of 1 l s⁻¹, the air velocity in the mouthpiece was about 25 m s⁻¹, which is a velocity where the so-called mist flow of particles and gas can be produced [37].

Large particles forming in the central airways and/or the oropharyngeal tract at exhalation are also a possible source. The standard breathing maneuver applied in the PExA® method aims at a maximal amount of airway closure and has been shown to increase the exhaled particle concentration by an order of magnitude compared to tidal breathing [11]. When this breathing pattern was adopted for the SensAbues® method, there was no increase of methadone compared to tidal breathing. Thus, methadone produced by airway closure and the reopening of small airways is an exceedingly small proportion of the total amount collected by the SensAbues® device. This is consistent with the notion that large particles from more central airways or the oropharyngeal tract constitute the body of the collected particle mass by the filtration method.

To summarize, the very high amount of methadone collected by the filtration device is probably due to the considerable contribution of methadone from the central airways or oropharyngeal tract, which is a contribution not present in the PExA® method. We have not, however, been able to elucidate the exact mechanisms behind this contribution within the present study. The origin, formation and composition of particles/droplets from the upper airways need to be further elucidated.

One limitation of the present study is that the time was not registered between methadone dose administration and the collection of breath samples. Unfortunately this time span varied by hours. In a steady state, the methadone concentration in blood will at least double within a couple of hours after the administration of an oral dose solution [38] and return to the baseline concentration after about 10 h. Thus, a lack of correlation between the administered dose and amount or the concentration of methadone in oral fluid or samples collected by the PExA® or SensAbues® devices is not surprising. A study on the relation between the dose and amount detected by both methods and in the blood/urine is indeed warranted.

Another limitation is that no recovery experiments were performed on the membranes used in the impactation method. However, the recovery from the electret filters used in the filtration method was satisfactory (>90%) and our own experience of extraction from the membranes indicates that methanol extraction is probably just as effective. A marginal difference in
recovery can therefore not explain the huge difference between the methods. We conclude that methadone is indeed detectable and quantifiable in exhaled particles collected by the PExA® method, showing that endogenous compounds are distributed from the blood to the RTLF in the small airways. However, the amount of methadone collected by the PExA® method was a small fraction of that collected by the SensAbuses® method. This is most likely due to the fact that the PExA® method collects very small particles only, whereas the SensAbuses® method also collects much larger particles presumably originating from the throat and mouth.

Moreover, we conclude that the design of the collecting device has a crucial effect on the collection of non-volatiles in breath. The characteristics of any collection device should be thoroughly investigated and described according to the intended use of the device.

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