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Development and validation of a highly sensitive gas chromatographic–mass spectrometric screening method for the simultaneous determination of nanogram levels of fentanyl, sufentanil and alfentanil in air and surface contamination wipes

Nadine F.J. Van Nimmen*, Hendrik A.F. Veulemans

Laboratory for Occupational Hygiene and Toxicology, Department of Occupational, Environmental and Insurance Medicine, Katholieke Universiteit Leuven, Kapucijnenvoer 35, 6th Floor, B-3000 Leuven, Belgium

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Abstract

A highly sensitive gas chromatographic–mass spectrometric (GC–MS) analytical method for the determination of the opioid narcotics fentanyl, alfentanil, and sufentanil in industrial hygiene personal air samples and surface contamination wipes was developed and comprehensively validated. Sample preparation involved a single step extraction of the samples with methanol, fortified with a fixed amount of the penta-deuterated analogues of the opioid narcotics as internal standard. The GC–MS analytical procedure using selected ion monitoring (SIM) was shown to be highly selective. Linearity was shown for levels of extracted wipe and air samples corresponding to at least 0.1–2 times their surface contamination limit (SCL) and accordingly to 0.1–2 times their time weighted average occupational exposure limit (OEL-TWA) based on a full shift 960 l air sample. Extraction recoveries were determined for spiked air samples and surface wipes and were found to be quantitative for both sampling media in the entire range studied. The air sampling method's limit of detection (LOD) was determined to be 0.4 ng per sample for fentanyl and sufentanil and 1.6 ng per sample for alfentanil, corresponding to less than 1% of their individual OEL for a full shift air sample (960 l). The limit of quantification (LOQ) was found to be 1.4, 1.2, and 5.0 ng per filter for fentanyl, sufentanil, and alfentanil, respectively. The wipe sampling method had LODs of 4 ng per wipe for fentanyl and sufentanil and 16 ng per wipe for alfentanil and LOQs of respectively, 14, 12, and 50 ng per wipe. The analytical intra-assay precision of the air sampling and wipe sampling method, defined as the coefficient of variation on the analytical result of six replicate spiked media was below 10 and 5%, respectively, for all opioids at all spike levels. Accuracy expressed as relative error was determined to be below 10%, except for alfentanil at the lowest spike level (−13.1%). The stability of the opioids during simulated air sampling was investigated. For fentanyl and sufentanil a quantitative recovery was observed at all spike levels, while for alfentanil recoveries ranged from 60.3 to 85.4%. When spiked air samples were stored at ambient temperature and at −15 °C quantitative recovery was found for fentanyl and sufentanil after 7 and 14 days. For alfentanil a slight loss seemed to occur upon storage during 7 days, being more explicit after 14 days. Ambient storage of spiked wipes seemed to lead to significant losses of all opioids studied, yielding recoveries of 37.7–88.3%. Upon storage of similar wipes at −15 °C a significantly higher recovery was found ranging from 77.3 to 88.3%. The developed analytical and sampling procedures have been recently applied in an explorative field study of which the results of surface contamination wipe sampling are presented in this paper. To our knowledge, this is the first study addressing the development and validation of analytical procedures for the assessment of external occupational exposure to potent opioid narcotics.

Keywords: Validation; Air analysis; Surface contamination; Fentanyl; Sufentanil; Alfentanil; Opioids

1. Introduction

The opioid compounds fentanyl, sufentanil, and alfentanil are members of a group of synthetic narcotic analgesics that are structurally different from opium derived substances but have comparable pharmacological properties. Their main therapeutic effects are analgesia, sedation, and attenuation of responses to potent sympathetic stimuli. The opioid compounds have a wide range of side effects including respiratory depression, nausea, miosis, bradycardia, and induction of dependence. Fentanyl, the first of the 4-anilinopiperidine series of opioid mu agonists, is chemically related to

* Corresponding author. Tel.: +32-16-33-6995; fax: +32-16-33-6997.
E-mail address: nadine.vannimmen@med.kuleuven.ac.be (N.F.J. Van Nimmen).

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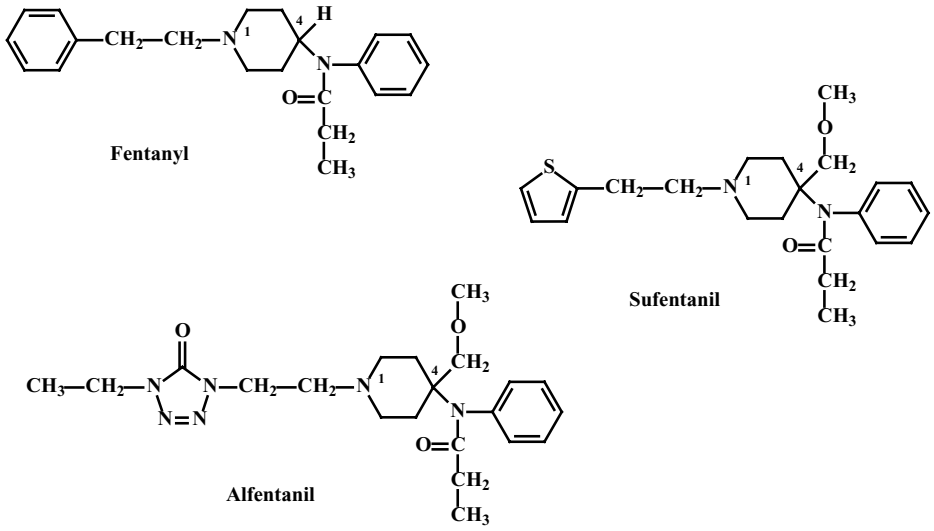

Fig. 1. Structure of the opioid narcotics fentanyl, sufentanil, and alfentanil.

meperidine and has been reported to be 50–100 times more potent than morphine [1]. Fentanyl was introduced into clinical practice in the early 1960s and it remains the most widely used anaesthetic in the world [2]. Sufentanil and alfentanil are also widely used to provide potent analgesia, as primary anaesthetic agents in very high doses during cardiac surgery, and in intensive care medicine [3]. The structural difference in the nature of the 1-substituent and the degree of substitution at the 4-position (Fig. 1) affect their relative potency and duration of action. Sufentanil is the most potent of the series and is about 2000 times as potent as the opioid prototype morphine, yet it has a shorter duration of action than fentanyl [4]. Alfentanil has the most rapid analgesic onset and exhibits about 30 times the clinical potency of morphine [3,5].

Prior to formulation in various dosage devices, fentanyl, sufentanil, and alfentanil are synthesized as neat powdery chemicals. To limit and control the potential exposure and the health risk of workers engaged in the synthesis and formulation of these potent narcotics, monitoring programs are required. For the most part, occupational exposure is assumed to occur in the handling of the opioids in the form of finely divided solids. Hence, personal air sampling is considered as a standard tool for quantitative respiratory exposure risk assessment [6]. However, before any monitoring of exposure can be sensibly carried out, criteria have to be established specifying what level of exposure will be acceptable [7]. Few therapeutic substances, however, have occupational exposure limits (OELs) set by regulatory bodies. In view of respiratory exposure, Sargent and Kirk [8] proposed a pharmacology rather than toxicity based model for establishing in-house OELs for pharmaceutical products. For fentanyl, alfentanil, and sufentanil a quantitative time weighted average occupational exposure limit (OEL-TWA) is derived using the general equation employed by Sargent, complemented by a traditional ‘safety factor’ approach induced by uncertainties in quantitative assessments. This corporate internal OEL-TWA for fentanyl has been set at 0.0001 mg/m^3 (0.1 μg/m^3). Reflecting their relative potency, the in-house OEL-TWA of alfentanil and sufentanil has been set at 0.001 mg/m^3 (1 μg/m^3) and 0.000032 mg/m^3 (0.032 μg/m^3), respectively. In recognizing the acute effects of the compounds, also a short-term exposure limit (OEL-STEL) has been defined at three times the individual OEL-TWA. Hence, the OEL-STEL for fentanyl, alfentanil, and sufentanil were set at 0.0003 mg/m^3 (0.3 μg/m^3), 0.003 mg/m^3 (3 μg/m^3), and 0.0001 mg/m^3 (0.1 μg/m^3), respectively.

Surface contamination surveys for pharmaceutical active material in industrial hygiene settings are also not uncommon [6]. Surface contamination assessment is assumed to be an indirect measure for potential dermal exposure. Wipe sampling is an important worksite analysis tool for identifying hazardous conditions, and for evaluating the effectiveness of personal protective equipment, housekeeping, and decontamination programs, even though there are few specific criteria for acceptable surface contamination amounts [9]. In order to allow for relevant quantitative surface monitoring programs, maximum allowable surface contamination levels must be defined. The in-house values for maximum surface contamination levels are based on the assumption that a worker is allowed to receive an equivalent ‘dose’ through respiratory exposure as via dermal exposure during a single working day. This maximum allowable dose is calculated by multiplying the OEL-TWA (mg/m^3) by 10 m^3, the average worker’s breathing volume during an 8 h-working day. Assuming a skin exposure through an anatomical area equivalent to a single hand press (100 cm^2), a contaminant transfer rate of 100% and a subsequent dermal absorption of 100%, this dose would be the maximum amount allowed on a working surface of 100 cm^2. Based on the outlined rationale (personal communication), the tentative surface contamination limit (SCL) for fentanyl,

alfentanil, and sufentanil was set at a rather stringent level of 1, 10, and 0.32 μg/100 cm^2, respectively. However, some controversy still exists regarding the magnitude of factors that could be applied for surfaces that are unlikely to come into contact with the unprotected skin of the worker, like floors and walls. For this type of surfaces, setting a more tolerable surface contamination limit would seem acceptable.

Wipe sampling techniques are also used to assess surface contamination on the skin in order to get information about actual dermal exposure. However, there are concerns related to direct wipe sampling of the skin, including the possibility of promoting skin absorption with the use of certain solvents [9]. In addition, up till now no quantitative dermal occupational exposure limits (DOELs) exist to protect workers against adverse effects from uptake through skin absorption [10].

Prior to setting up industrial hygiene programs, focussing on the assessment of occupational respiratory exposure and surface contamination wiping, analytical methods had to be developed. Due to the high potency of the opioid narcotics, reflected by their low OEL and SCL-values, considerable efforts were taken by the pharmaceutical industry to reduce occupational exposure and to protect workers, particularly those involved in the opioid synthesis. Hence, environmental concentrations are expected to be very low and special emphasis was to be placed on the sensitivity of the analytical methods. In this study, we have developed and comprehensively validated a highly sensitive gas chromatographic–mass spectrometric (GC–MS) analytical method to monitor airborne concentrations of fentanyl and related compounds as low as <1 to 2 ng/m^3 based on a full shift air sample of 960 l. Moreover, the method's excellent sensitivity allows peak air sampling to be performed at which opioid levels equal to or less than 10% of their OEL-STEL values can be detected. Finally, the same analytical method was also applied to wipe samples and allows detecting opioid surface contamination levels less than 5 ng/100 cm^2.

The analytical work described in this study was complemented with research on the development and validation of sensitive analytical procedures in the scope of a biological monitoring approach of opioid exposed workers. This research is presented in detail elsewhere [11].

2. Experimental

2.1. Chemicals and materials

Fentanyl citrate, alfentanil hydrochloride, sufentanil citrate and the internal standard analogues [$^2H_5$]fentanyl citrate, [$^2H_5$]alfentanil hydrochloride and [$^2H_5$]sufentanil citrate were kindly provided by Janssen Pharmaceutica (Beerse, Belgium). Methanol (HPLC grade) was obtained from Fisher Chemicals (Leicester, UK). Dimethyldichlorosilane was supplied by Supelco (Bellefonte, USA). Extraction vials (4 ml) and autosampler vials (1 ml) were obtained from Machery-Nagel (Düren, Germany). VWR (Leuven, Belgium) supplied the 30 ml extraction vials, the TNT® Blue nitrile disposable gloves (Ansell Protective Products) and a Heidolph Reax 2 auto shaker. IOM personal inhalable samplers and IOM filter cassettes (25 mm) were supplied by JS Holdings (Hertfordshire, UK). Glass fiber filters (binder-free, 0.5 μm, 25 mm i.d.) were from Pall Corp. (MI, US). Regal surface wipes (5 cm × 5 cm, 8 ply) were from Johnson & Johnson Medical Ltd. (Skipton, UK). Polyethylene glycol (average MN 200), toluene (spectrophotometric grade) and acetone (HPLC grade) were obtained from Sigma–Aldrich (Steinheim, Germany). A Gilibrator-2 primary flow calibrator and the Gilair 3 and Gilair 5 air sampling pumps were supplied by Gilian, Sensidyne (FL, US).

2.2. Instrumentation and chromatographic conditions

The analyses were carried out on a Hewlett-Packard 6890 series gas chromatograph equipped with an autosampler and a 5973 series mass selective detector (MSD) in electron impact (EI) mode (70 eV). A 3 μl aliquot of the sample was introduced in a splitless way onto a DB5-MS (J&W) column with a nominal length of 30 m, an internal diameter of 0.25 mm and a film thickness of 0.1 μm. A constant high purity Helium flow of 2.5 ml/min was applied through the column. The GC separation was obtained using a program with an initial oven temperature of 120 °C that was increased at a rate of 60 °C/min to a final temperature of 280 °C. The oven was held at the final temperature for an additional 3.0 min. The injector and MS source temperature were maintained at 230 °C. The MS quadrupole temperature was held at 150 °C. The mass selective detection system was operated in the selected ion monitoring (SIM) mode. Base ion fragments occurring at *m/z* 245 for fentanyl, and *m/z* 250 for [$^2H_5$]fentanyl, *m/z* 289 for sufentanil and alfentanil and *m/z* 294 for [$^2H_5$]sufentanil and [$^2H_5$]alfentanil were monitored and used for subsequent quantification. Individual ion dwell times were set at 75 ms for the opioid base ion fragments and at 25 ms for the base ion fragments of the penta-deuterated analogues.

2.3. Sampling procedures

Personal sampling pumps were calibrated using a soap bubble calibrator (Gilibrator-2) to deliver a constant flow of 2 l/min. Personal air sampling was performed by drawing a known quantity of air through a 25 mm glass fiber filter mounted in an IOM sampling head, clipped near the workers breathing zone. In this configuration, the IOM sampler effectively traps particles up to 100 μm in aerodynamic diameter and closely simulates the manner in which airborne workplace particles are inhaled through the nose and mouth [12]. Full shift as well as peak air samples were taken and had total air volumes of respectively, 960 and 30 l. After sampling, the filter cassette was placed in a sealing cap and shipped to the lab.

Prior to wipe sampling, the surface wipes were wetted with methanol. Any excess solvent was removed by squeezing the wipe. New sets of clean disposable nitrile gloves were used for each sample to avoid contamination of the wipe by previous samples and to prevent contact with the surface. Using clean tweezers, an area of 100 cm^2 was covered, rolling the wipe slightly back and forth over the sampling area, in a horizontal direction. The wipe was turned and the same sampling area was covered in a vertical direction. Firm pressure was applied when wiping. After sampling, each wipe was placed in an individual 30 ml vial. With each set of air samples and wipes, at least one field blank was included. All samples were stored at −30 °C until analysis.

2.4. Sample preparation

Extraction solvent was prepared by adding [$^2H_5$]fentanyl citrate, [$^2H_5$]alfentanil hydrochloride and [$^2H_5$]sufentanil as an internal standard in a final concentration of 50, 500, and 15 ng/ml of methanol, containing 0.01% polyethylene glycol as a competitive agent for active sites present in glass ware.

Using tweezers, the sampled glass fiber filters were removed from the IOM sampler and were placed into a 4 ml glass vial. Two milliliters of extraction solvent were pipetted into the vial. The inside wall of the sampler was rinsed if necessary. The wipe samples were transferred to individual 30 ml screw top vials and 20 ml of extraction solvent was added. The vials were shaken in a Heidolph Reax 2 autoshaker at 50 rpm for 30 min and subsequently sonicated for an additional 30 min. On each occasion, at least one laboratory blank and a set of duplicate spiked quality control (QC) air samples and wipes were processed in the same way as the filter and wipe samples. One milliliter of each sample was transferred into a 2 ml automatic sampler vial and analyzed.

2.5. Preparation of linear regression calibrators and QC air samples and wipes

It should be noted that all concentrations mentioned in this paper refer to the free base. Stock standard solutions of fentanyl citrate and sufentanil citrate (0.1 mg/ml) and of alfentanil hydrochloride (1 mg/ml) were prepared in methanol from the respective pure chemicals. A working spiking solution was prepared by appropriate dilution of the stock solution in methanol to yield concentrations of 20 μg fentanyl/ml, 200 μg alfentanil/ml, and 6.5 μg sufentanil/ml.

Using the working spiking solution, linear regression calibrators were prepared in the extraction solvent in a range corresponding to at least 0.1–2 times the individual analytes OEL for a full shift air sample and accordingly in a range of 0.1–2 times their SCL. A summary of the individual nominal concentrations of the opioid analytes applied in the regression calibrators is given in Table 1. QC calibrators were prepared in a similar way, corresponding to individual opioid levels of 0.1, 0.5, 1, and 2 times the OEL or SCL.

Table 1
Nominal concentrations for the opioid narcotics in the linear regression calibrators applied, corresponding with various airborne concentrations for a full shift 960 l air sample and equally corresponding to different surface contamination levels

Airborne concentration level at 960 l	Surface contamination level at 100 cm^2	Fentanyl (ng/ml)	Alfentanil (ng/ml)	Sufentanil (ng/ml)
0.1 OEL	0.1 SCL	5	50	1.5
0.25 OEL	0.25 SCL	13	125	3.9
0.5 OEL	0.5 SCL	25	250	7.5
1 OEL	1 SCL	50	500	15
1.5 OEL	1.5 SCL	75	750	23
2 OEL	2 SCL	100	1000	31

OEL, time weighted average occupational exposure limit; SCL, surface contamination limit.

QC air samples and wipes were prepared by applying microliter quantities of the working spiking solution to the sampling material to cover concentrations corresponding to 0.1, 0.5, 1, and 2 times the OEL or SCL for all analytes. QC air samples and wipes were allowed to dry and were processed as described in Section 2.4.

3. Results

3.1. Specificity

Under the chromatographic conditions described in Section 2, all analytes of interest were well separated on the GC–MS chromatogram. No significant interference was observed in extracted blank air samples or wipes at the retention time of the compounds. Representative chromatograms of a blank air sample and a QC sample spiked at a level corresponding to 1 OEL of the individual opioids are shown in Figs. 2 and 3. The retention times for fentanyl, sufentanil and alfentanil were 3.97, 4.17, and 4.72 min, respectively. It was observed that in practice, extraction of particularly surface wipes resulted in less clean extracts, as compared to air sample extracts. Nevertheless, the GC–MS analytical method operated in the SIM mode is quite insensitive to the presence of interfering compounds and offers specificity, which would probably not be reached by scanning analytical procedures.

3.2. Linearity

Six level calibration curves for the opioid compounds were obtained by plotting the peak area ratio of the quantification ion of the analyte and its respective deuterated internal standard against the corresponding concentrations of the analyte in the calibrators. At each calibration level three replicate samples were analyzed. Linear regression analysis of the calibration plots resulted in the equations and correlation coefficients listed in Table 2. For fentanyl and sufentanil a negative intercept was observed, being not significant at

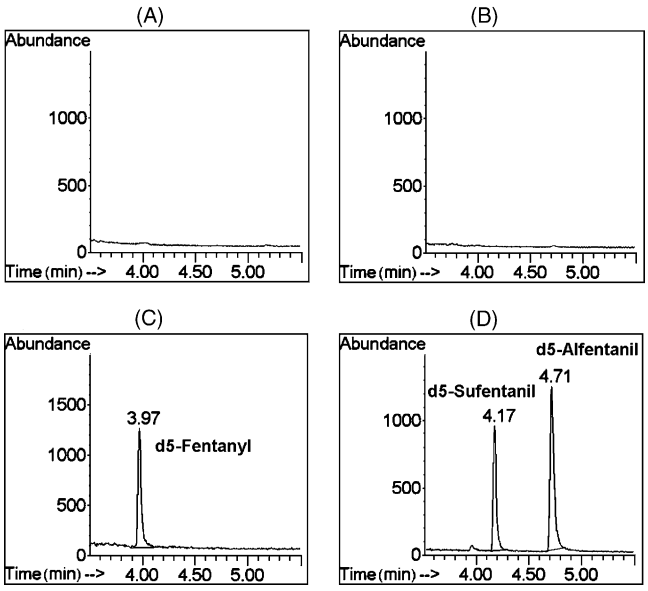

Fig. 2. Example chromatogram of an extracted blank air sample showing peaks at *m/z* 250 (C) and *m/z* 294 (D) from the internal standards [$^2H_5$]fentanyl (d_5-fentanyl) and [$^2H_5$]alfentanil (d_5-alfentanil) and [$^2H_5$]sufentanil (d_5-sufentanil), respectively. At their respective retention times, no interference is observed at the ion fragments *m/z* 245 (A) and *m/z* 289 (B) monitored for the opioid narcotics.

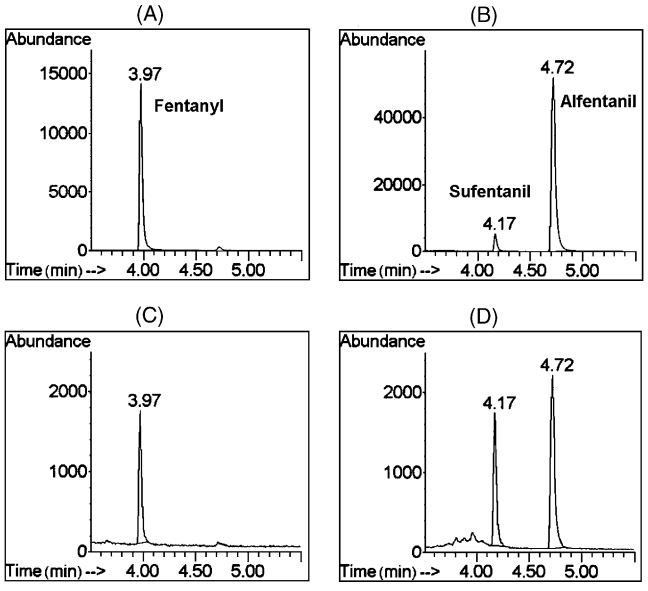

Fig. 3. Example chromatogram of a QC air sample spiked with the opioid narcotics at a level corresponding with 1 OEL for a full shift air sample (960 l). Ion fragments monitored for the opioid narcotics (*m/z* 245 (A) and *m/z* 289 (B)) as well as those monitored for the deuterated analogues (*m/z* 250 (C) and *m/z* 294 (D)) are shown.

the $\alpha = 0.05$ level. For alfentanil a significant ($P = 0.037$) but analytically irrelevant negative intercept was observed, probably caused by a minor shift in the slope of the regression curve due to slightly raised data points at the end of the curve. The method showed good linearity over the entire ranges studied corresponding to 0.1–2 times the OEL of the individual compounds for a 960 l air sample and equally for the corresponding range of the SCL.

3.3. Limits of detection (LODs) and limits of quantification (LOQs)

In order to achieve and maintain the desired sensitivity, several factors needed to be addressed. The use of high purity solvents and disposable extraction vials and a dedicated cleaning procedure for other materials aided in avoiding contamination. Other precautions are discussed in length elsewhere [11]. Briefly, they included the addition of a small percentage of polyethylene glycol (0.01% (v/v)) to the extraction solvent and the use of freshly silanized GC inlet liners to prevent the adsorption of the analytes of interest to active sites present in different glass materials.

The primary target limit of detection (LOD) was defined as the equivalent amount of material that would be collected on a filter when sampling at atmospheric concentrations of the test substance at 0.1× the OEL-TWA for a 960 l air sample and equally the quantity of the opioid narcotic that would be sampled on a 100 cm^2 wipe at 0.1 × SCL. This corresponds to a sample containing 5 ng/ml fentanyl, 50 ng/ml alfentanil and 1.5 ng/ml sufentanil. The second target limit of detection was to be able to sample for 10% of the OEL-STEL for a 30 l air sample, resulting in extracted concentration levels of 0.50, 5.5, and 0.15 ng/ml for fentanyl, alfentanil, and sufentanil, respectively. The target LODs were determined by injecting standards at these levels a minimum of 10 times. Acceptance criteria for LOD injections, i.e. a signal-to-noise ratio (S/N) of at least 3 and a coefficient of variation (CV) for relative peak areas of repetitive injections no greater than 10%, were met for all opioid narcotics (Table 3). The actual calculated detection limits and quantification limits are presented in Table 4. All calculations for these limits were

Table 2
Linear regression models ($Y = ax + b$) for the opioid narcotics for concentrations ranging from 0.1 to 2 times their individual occupational exposure limit (OEL-TWA for a 960 l air sample) and surface contamination limits (SCL)

	Slope (95% C.I.)	Intercept (95% C.I.)	Intercept *P*-value	R^2
Fentanyl	31.59 (29.33–33.85)	−0.023 (−0.150 to 0.104)	0.64	0.997
Alfentanil	3.49 (3.46–3.52)	−0.045 (−0.086 to −0.0046)	0.037	0.999
Sufentanil	124.71 (118.05–131.37)	−0.016 (−0.13 to 0.099)	0.72	0.999

95% C.I. = 95% confidence interval; *Y*, peak area ratio *m/z* 245/*m/z* 250 (fentanyl) and peak area ratio *m/z* 289/*m/z* 294 (alfentanil and sufentanil).

Table 3
Signal-to-noise ratio (S/N) and R.S.D. of 10 replicate injections of a standard solution of the opioid narcotics at concentrations corresponding with 0.1 times the surface contamination limit (SCL) and equally 0.1 times the time-weighted average occupational exposure limit (OEL-TWA) for a 960 l air sample and at 0.1 times the short term exposure limit (OEL-STEL) for a 30 l air sample

	0.1 SCL and 0.1 OEL-TWA		0.1 OEL-STEL	
	S/N	R.S.D. (%, $n = 10$)	S/N	R.S.D. (%, $n = 10$)
Fentanyl	55:1	4.4	9:1	1.5
Alfentanil	130:1	0.9	22:1	3.3
Sufentanil	22:1	4.1	3:1	6.3

based on signal-to-noise ratios of at least 3 for the LODs and at least 10 for the LOQs. From the data in Table 4 it is concluded that the developed analytical method shows excellent sensitivity with LODs of 0.2% (alfentanil) to 1.0% (sufentanil) of their individual SCL and OEL (960 l air sample) and LOQs of 0.5% (alfentanil) to 4.0% (sufentanil) of their individual SCL and OEL at the same conditions. Moreover, the method's excellent sensitivity allows peak air sampling and partial shift sampling to be performed, which in some cases may be desirable or necessary. A sample chromatogram of the primary target LOD standard is presented in Fig. 4.

3.4. Extraction recovery

The extraction recovery of the opioids from air samples and wipes was determined by processing and analyzing a set of six replicate glass fiber filters and wipes spiked with various amounts of the compounds at the QC levels. Recovery was expressed as the percentage of the compound found on the spiked samples to those found in spiked aliquots of extraction solvent. Validation criteria for extraction efficiency were average recoveries of at least 90% and a R.S.D. of less than 10%. Extraction was found to be quantitative for all opioids at each spike level for both spiked glass fiber filters and wipes, as shown in Tables 5 and 6.

3.5. Intra-assay precision and accuracy using spiked samples

The analytical intra-assay precision of the air sampling method was defined as the coefficient of variation resulting from the analysis of a set of six replicate filters spiked with various concentrations of the opioid narcotics corresponding to the QC calibrators. The accuracy was determined by comparing the means of measured concentrations with the nominal concentration for the same QC calibrators. Intra-assay precision data and accuracy of the analytical air sampling method are presented in Table 7. R.S.D.s were below 2% at

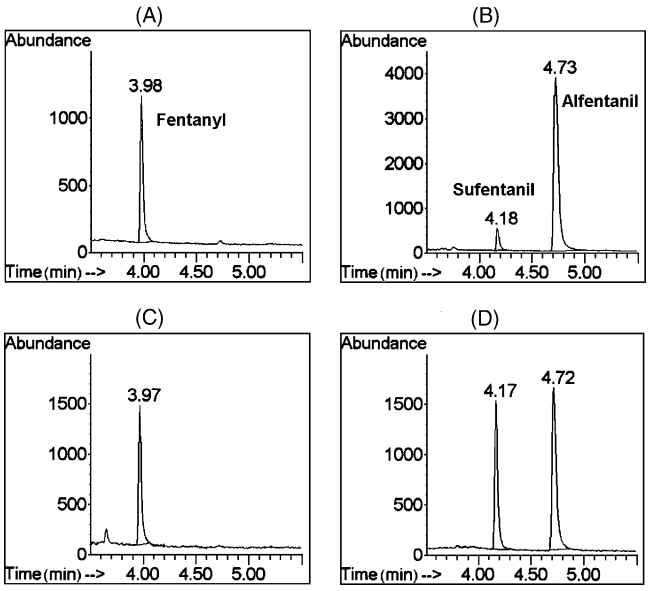

Fig. 4. Example chromatogram of a QC air sample spiked with the opioid narcotics at the primary target LOD level corresponding with 0.1 OEL for a full shift air sample (960 l). Ion fragments monitored for the opioid narcotics (*m*/*z* 245 (A) and *m*/*z* 289 (B)) as well as those monitored for the deuterated analogues (*m*/*z* 250 (C) and *m*/*z* 294 (D)) are shown.

Table 5
Extraction recoveries of the opioid narcotics determined on a set of six replicate filters spiked with various amounts of opioid narcotics, corresponding with different airborne concentrations for a full shift 960 l air sample

	Extraction recovery (%) from spiked filters ($n = 6$)			
	0.1 OEL	0.5 OEL	1 OEL	2 OEL
Fentanyl	98.2 ± 3.7	100.1 ± 5.7	98.0 ± 4.2	102.9 ± 3.9
Alfentanil	100.5 ± 1.2	100.7 ± 0.8	99.9 ± 1.4	98.7 ± 1.4
Sufentanil	101.2 ± 1.8	100.4 ± 1.3	100.1 ± 1.5	99.2 ± 1.9

OEL, time weighted average occupational exposure limit.

Table 4
LODs and LOQs of the opioid narcotics in air samples and wipes, calculated at a signal-to-noise ratio of respectively, S/N 3 and S/N 10, and expressed in ng/ml extract, ng per sampled filter, ng per wipe and as a percentage of their individual time weighted average occupational exposure limit (OEL) for a full shift 960 l air sample and accordingly their individual surface contamination limit (SCL)

	LOD				LOQ			
	ng/ml	ng per filter	ng per wipe	%OEL, SCL	ng/ml	ng per filter	ng per wipe	%OEL, SCL
Fentanyl	0.2	0.4	4	0.4	0.7	1.4	4	1.4
Alfentanil	0.8	1.6	16	0.2	2.5	5.0	16	0.5
Sufentanil	0.2	0.4	4	1.0	0.6	1.2	4	4.0

Table 6
Extraction recoveries of the opioid narcotics determined on a set of six replicate wipes spiked with various amounts of opioid narcotics, corresponding with different levels of surface contamination

	Extraction recovery (%) from spiked wipes ($n = 6$)			
	0.1 SCL	0.5 SCL	1 SCL	2 SCL
Fentanyl	102.8 ± 2.8	101.2 ± 1.8	99.5 ± 1.7	103.7 ± 2.6
Alfentanil	99.2 ± 2.3	100.7 ± 0.9	98.3 ± 2.3	101.7 ± 1.2
Sufentanil	96.4 ± 2.6	103.5 ± 1.6	98.9 ± 4.2	101.2 ± 4.3

SCL, surface contamination limit.

all spike levels of alfentanil and sufentanil and ranged from 3.6 to 6.9% for various fentanyl levels. Accuracy, expressed as relative error, was determined to be below 10% for all opioids at all spike levels, except for alfentanil at the lowest spike level (−13.1%).

Similar tests were run to determine the analytical intra-assay precision and accuracy of the wipe sampling method using a set of six replicate wipes spiked with the opioid narcotics at QC calibrator levels. The data presented in Table 8 show coefficients of variations below 5% and accuracy, expressed as relative error, below 10% for each opioid at all spike levels. Again, only alfentanil at the lowest spike level shows a relative error of −13.1%. The previous data indicate that the developed method shows an excellent inter-assay precision and accuracy even at ultra-low opioid spike levels.

3.6. Inter-assay precision and accuracy using QC level calibrators

The inter-assay precision of the analytical method was defined as the coefficient of variation resulting from the analysis of the QC level calibrators at five different time intervals over a period of approximately five months. The accuracy was determined by comparing the means of measured concentrations with the nominal concentration for the same QC calibrators. The data presented in Table 9 show R.S.D.s below 5% for all sufentanil and alfentanil spike levels. For fentanyl R.S.D.s range from 1.5 to 11.3% for decreasing spike levels. Accuracy, expressed as relative error, was below 6% for each opioid at all spike levels, indicating an excellent accuracy and reproducibility of the analytical method over time.

3.7. Stability of the opioid narcotics on glass fiber filters during simulated air sampling

To estimate the stability of the opioids during actual air sampling, a simulation test was performed in which the compounds were spiked individually ($n = 3$) at each of their QC levels on blank glass fiber filters. Using calibrated personal sampling pumps, air at a flow rate of 2 l/min was drawn through the spiked filters for 240 min to simulate half shift air sampling. After simulated air sampling, the filters were analyzed and spike recoveries were compared to non-sampled filters. The results are presented in Table 10. For fentanyl and sufentanil a quantitative recovery is observed for all spike levels at the sampling conditions described above. In contrast, air sampling through alfentanil-spiked filters seemed to lead to loss of the active compound, with recoveries ranging from 60.3 to 85.4% as compared to non-sampled spiked filters. This apparent loss could be caused by irreversible adsorption on the active (silanol-) sites present in the glass fiber filters or could be an indication of partial breakdown of the compound. However, no additional compounds were observed in the SIM chromatograms of the sampled filters. Moreover, when glass fiber filters were used that

Table 7
The air sampling method's intra-assay precision expressed as the R.S.D. ($n = 6$) and the methods accuracy expressed as relative error (R.E.) ($n = 6$) for the opioid narcotics spiked on glass fiber filters at the QC calibrator levels

	Intra-assay precision (R.S.D.) and accuracy (R.E.) ($n = 6$)			
	0.1 OEL	0.5 OEL	1 OEL	2 OEL
Fentanyl				
Added (ng)	10	50	100	200
Mean ± S.D. (ng)	9.0 ± 0.33	49 ± 3.4	93 ± 3.6	201 ± 7.6
R.S.D. (%)	3.6	6.9	3.8	3.8
R.E. (%)	−6.1	−2.5	−7.5	0.74
Alfentanil				
Added (ng)	110	540	1100	2180
Mean ± S.D. (ng)	96 ± 1.1	517 ± 3.9	1062 ± 15	2360 ± 33
R.S.D. (%)	1.1	0.8	1.4	1.4
R.E. (%)	−13.1	−4.3	−3.4	8.3
Sufentanil				
Added (ng)	3.1	15	31	62
Mean ± S.D. (ng)	2.9 ± 0.05	15 ± 0.2	30 ± 0.5	60 ± 1.1
R.S.D. (%)	1.8	1.3	1.5	1.9
R.E. (%)	−7.1	−2.5	−3.0	−8.6

Table 8
The wipe sampling method's intra-assay precision expressed as the R.S.D. ($n = 6$) and the methods accuracy expressed as R.E. ($n = 6$) for the opioid narcotics spiked on wipes at the QC calibrator levels

	Intra-assay precision (R.S.D.) and accuracy (R.E.) ($n = 6$)			
	0.1 SCL	0.5 SCL	1 SCL	2 SCL
Fentanyl				
Added (μg)	0.10	0.50	1.0	2.0
Mean ± S.D. (μg)	0.09 ± 0.003	0.47 ± 0.008	0.98 ± 0.02	2.0 ± 0.05
R.S.D. (%)	2.7	1.8	1.8	2.5
R.E. (%)	−9.1	−5.6	−1.9	0.86
Alfentanil				
Added (μg)	1.0	5.0	10.0	20.0
Mean ± S.D. (μg)	0.87 ± 0.02	4.6 ± 0.04	9.7 ± 0.22	20.2 ± 0.24
Precision (%)	2.3	0.93	2.3	1.2
Accuracy (%)	−13.1	−8.4	−2.7	1.2
Sufentanil				
Added (μg)	0.03	0.16	0.33	0.65
Mean ± S.D. (μg)	0.03 ± 0.0008	0.15 ± 0.002	0.32 ± 0.014	0.66 ± 0.028
R.S.D. (%)	2.7	1.5	4.3	4.2
R.E. (%)	1.5	−4.5	−1.9	0.77

Table 9
The analytical method's inter-assay precision expressed as the R.S.D. ($n = 5$) and the methods accuracy expressed as R.E. ($n = 5$) for the opioid narcotics at the QC calibrator levels

	Inter-assay precision (R.S.D.) and accuracy (R.E.) ($n = 5$)			
	0.1 OEL, SCL	0.5 OEL, SCL	1 OEL, SCL	2 OEL, SCL
Fentanyl				
Added (ng/ml)	5.2	26.0	52.0	103.0
Mean ± S.D. (ng/ml)	5.0 ± 0.57	25.2 ± 1.4	51.8 ± 2.3	103.0 ± 1.5
R.S.D. (%)	11.3	5.5	4.4	1.5
R.E. (%)	−3.2	−2.9	−0.30	−0.041
Alfentanil				
Added (ng/ml)	51.5	260.0	515.0	1030.0
Mean ± S.D. (ng/ml)	48.5 ± 1.3	247.8 ± 2.6	510.7 ± 9.4	1033.6 ± 3.2
Precision (%)	2.6	1.0	1.8	0.3
Accuracy (%)	−5.8	−4.7	−0.83	0.35
Sufentanil				
Added (ng/ml)	2.0	10.0	20.0	40.0
Mean ± S.D. (ng/ml)	1.9 ± 0.07	9.9 ± 0.22	19.9 ± 0.13	40.1 ± 0.07
R.S.D. (%)	3.8	2.3	0.63	0.18
R.E. (%)	−3.4	−0.76	−0.58	0.22

Data were obtained from the analysis of the QC calibrators at five different time intervals over a period of approximately five months.

were spiked with the internal standard [$^2H_5$]alfentanil prior to sampling, the observed recovery inconsistency seemed to be compensated and a quantitative recovery was calculated, as shown in Table 10. The results indicate that air sampling for alfentanil would be preferably performed on [$^2H_5$]alfentanil pre-spiked filters, requiring some discipline of the industrial hygienist and possibly constituting a small but potential risk on (cross-) contamination of sampling equipment and samples. Also the use of pre-silanised glass fiber filters to prevent irreversible alfentanil adsorption might serve to overcome the apparent compound loss during sampling.

Table 10
Recovery of the opioid narcotics individually spiked on blank glass fiber filters at their QC levels after simulated half shift air sampling (480 l)

	Recovery (%) after simulated air sampling ($n = 3$)			
	0.1 OEL	0.5 OEL	1 OEL	2 OEL
Fentanyl	98.5 ± 2.3	101.4 ± 4.5	98.0 ± 1.2	104.3 ± 2.8
Alfentanil	77.2 ± 6.7	85.4 ± 2.7	78.6 ± 6.6	60.3 ± 2.0
Sufentanil	99.8 ± 3.5	99.5 ± 2.3	98.5 ± 2.3	98.5 ± 2.3
Alfentanil[a]	100.7 ± 2.5	100.1 ± 0.35	100.3 ± 0.78	97.9 ± 4.4

[a] Recovery of alfentanil individually spiked on [$^2H_5$]alfentanil pre-spiked glass fiber filters at similar conditions.

Table 11
Overall stability of the opioid narcotics spiked on blank glass fiber filters and stored during 7 and 14 days at ambient conditions and at −15 °C

	Recovery from stored glass fiber filters (%) ($n = 12$)			
	7 days ambient	14 days ambient	7 days at −15 °C	14 days at −15 °C
Fentanyl	97.6 ± 2.8	99.8 ± 2.6	99.5 ± 4.0	99.5 ± 4.3
Alfentanil	92.1 ± 2.5	73.7 ± 5.3	91.0 ± 4.1	72.5 ± 4.8
Sufentanil	100.1 ± 4.1	100.5 ± 2.6	100.4 ± 1.9	97.8 ± 2.5
Alfentanil[a]	96.4 ± 1.1	96.4 ± 7.9	96.6 ± 1.7	96.2 ± 8.6

For each storage condition, recoveries were averaged over the individual opioid QC levels ($n = 12$).
[a] Recovery of alfentanil individually spiked on [$^2H_5$]alfentanil pre-spiked glass fiber filters at similar conditions.

3.8. Stability of air samples and wipes during storage under different conditions

To evaluate the stability of air samples and surface contamination wipes during storage, various experiments were set up with glass fiber filters and wipe samples spiked with the opioid narcotics at their QC calibrator levels. At each QC level, two sets of six spiked samples were prepared. One set tested the storage stability at ambient conditions and the other set at −15 °C. Upon storage during 7 days, three replicates at each storage condition were analyzed and recoveries of the opioid narcotics were compared to freshly spiked samples. After storage during 14 days, the remaining samples were analyzed and again recoveries were determined.

Table 11 shows the recoveries of the opioid narcotics spiked on blank glass fiber filters and stored under the various conditions mentioned above. For each storage condition, recoveries were averaged over the individual opioid QC levels ($n = 12$). For fentanyl and sufentanil quantitative averaged recoveries are observed at any storage condition and standard deviations are well below 5%. For alfentanil a slight loss seemed to occur when spiked glass fiber filters are stored during 7 days, yielding recoveries of 91.0–92.1%. Upon storage during 14 days, the alfentanil recoveries tend to decrease to 72.5–73.7%. A significant difference (t-test, $P < 0.00001$) between alfentanil recoveries determined upon storage during 7 and 14 days was observed while within the same storage period no significant difference (t-test, $\alpha = 0.05$) between storage conditions (ambient, −15 °C) was found.

Again, when glass fiber filters were used that were spiked with the internal standard [$^2H_5$]alfentanil prior to storage, the observed recovery inconsistency seems to be largely compensated and a quantitative recovery was found. This observation would again favor the use of [$^2H_5$]alfentanil pre-spiked glass fiber filters for air sampling for alfentanil, as was already indicated in the previous section.

Table 12 shows the recoveries of the opioid narcotics spiked on wipes and stored under the same various conditions. Ambient storage of surface contamination wipes seemed to lead to significant losses of all opioids studied, yielding recoveries of 37.8–60.9% after 7 days and 37.9–58.4% after 14 days of storage. Upon storage at −15 °C significant higher opioid recoveries were found ranging from 77.2 to 88.3% at 7 days and 79.6 to 86.7% at 14 days. It was observed that within a single storage condition, no significant difference was found in recoveries after 7 and 14 days (t-test, $\alpha = 0.05$). Pre-spiking of wiping samples with the deuterated analogues of the opioid narcotics would probably also compensate for the observed discrepancy in recovery. However, in view of the potential risk of contaminating the working environment with the deuterated opioid narcotics and hence increasing the risk of exposure of the workers, this would not be acceptable.

Taken into account the potential loss during storage of alfentanil containing air samples and opioid surface contamination wipes in general, it was recommended that all samples were stored at −15 °C prior to analysis. Whenever feasible, air samples containing alfentanil should be analyzed within one week.

3.9. Application

The procedures described have been applied in an explorative field study involving personal air sampling and surface contamination monitoring during a three weeks

Table 12
Overall stability of the opioid narcotics spiked on wipes and stored during 7 and 14 days at ambient conditions and at −15 °C

	Recovery from stored wipe samples (%) ($n = 12$)			
	7 days ambient	14 days ambient	7 days at −15 °C	14 days at −15 °C
Fentanyl	37.8 ± 4.6	37.9 ± 8.6	77.2 ± 6.6	79.6 ± 6.5
Alfentanil	60.9 ± 5.5	58.4 ± 8.4	88.3 ± 3.8	86.7 ± 5.6
Sufentanil	53.7 ± 9.3	57.6 ± 9.1	86.2 ± 3.5	83.5 ± 8.0

For each storage condition, recoveries were averaged over the individual opioid QC levels ($n = 12$).

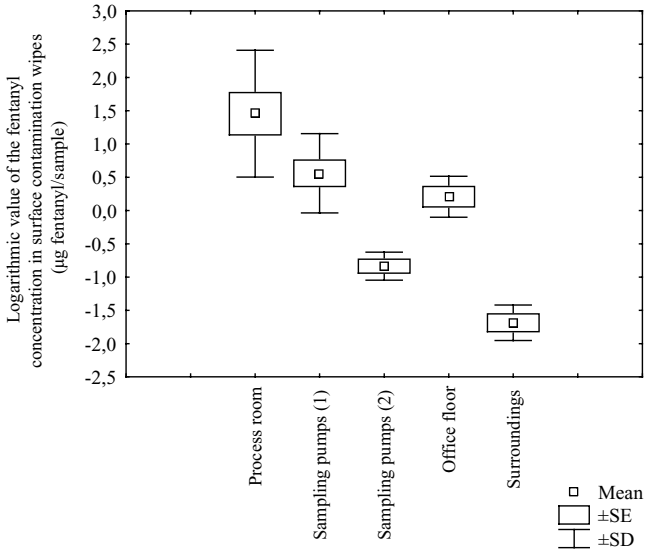

Fig. 5. Box and Whisker plot of the logarithmic values of the concentration of fentanyl in surface contamination wipes (μg fentanyl per sample).

fentanyl production campaign. The respiratory exposure data that were obtained from personal air sampling will be linked to other (biological) parameters monitored for opioid exposure and will be communicated elsewhere. Surface contamination monitoring was performed to evaluate the degree of contamination in the production facility but also focussed on the potential risk of secondary contamination of surfaces outside the actual process rooms. It was hypothesized that this contamination could result from the coming and going of production workers and the transfer of objects from the production facility to the surrounding office rooms. Nine wipe samples were taken on different surfaces at the process room, including centrifuges, steam taps, etc. (Fig. 5) ('Process room'). Fentanyl concentrations ranged from 0.7 to 371 μg per wipe; the median concentration was found to be 76 μg per sample. Also nine wipes were obtained from the surface of air sampling pumps used for personal monitoring of workers involved in the fentanyl production ['Sampling pumps (1)']. Four similar wipe samples were taken from air sampling pumps applied for personal air sampling of production workers, generally present in the same working area, but not directly involved in the fentanyl production ['Sampling pumps (2)']. Fentanyl concentrations measured in the former pump wipes ranged from 0.2 to 18 μg per sample, being significantly lower in the latter type of wipes, in which fentanyl concentrations were found in a range of 0.1 to 0.3 μg per wipe. Fentanyl concentrations were also determined in floor wipes ($n = 4$) at different locations at the office rooms ('Office floor'), and were demonstrated to range from 0.6 to 2.6 μg per sample. Finally, four wipes were taken from different surrounding surfaces ('Surroundings'), including doorknobs and handrails of stairs. The lowest concentrations of fentanyl were found in these samples, ranging from 0.01 to 0.05 μg per wipe. These results indicate that the developed analytical and sampling procedure is easily applicable to monitor fentanyl surface contamination. Although for each sampling condition, the number of wipe samples were small, it was shown that fentanyl concentrations were found to be the most elevated at surfaces in the production facility, as was expected. Measurements of surface contamination outside the production rooms seemed to confirm the hypothesis that secondary contamination could occur as a result from transfer processes. Fentanyl surface contamination levels found in the areas outside the actual process rooms, however, were found to be below the tentative surface contamination limit of 1 μg per wipe, except for three out of four wipes taken at the office floors. This may again touch upon the discussion of the acceptability of setting a more tolerable surface contamination limit for this type of surfaces.

4. Discussion

The present study describes the development and validation procedure of a highly sensitive GC–MS analytical and sampling procedure for the simultaneous determination of the opioid narcotics fentanyl, alfentanil, and sufentanil in air samples and surface contamination wipes. To our knowledge, this is the first study dealing with method development for the assessment of respiratory exposure of opioid production workers and the evaluation of surface contamination in industrial hygiene settings. Recently a study was published by Lambropoulos et al. [13] describing the development and validation of an HPLC assay for fentanyl, alfentanil, and sufentanil in wipe samples in order to control a cleaning procedure. The reported LODs and LOQs for the opioid studied, ranged from 20 to 100 ng per wipe and from 100 to 200 ng per wipe, respectively, being at least a 10-fold higher than the LODs and LOQs determined in our study. Precision, expressed as coefficient of variation on six replicate injections of the same sample is reported to be similar to our precision data, resulting although from the analyses of six individual spiked wipes. Lambropoulos et al. [13] also investigated the stability upon storage of opioids in wipe samples. Samples spiked with alfentanil in concentrations comparable with the QC calibrator level of 0.1 SCL in our study were found to yield recoveries of 92.0–100.1% upon storage at ambient temperature during 6 days or less. Accordingly, also quantitative recoveries were found for sufentanil spiked on wipes at concentrations equaling one SCL in our study and stored during 3 and 6 days at room temperature. In the same study, recoveries of 105.5 and 105.7% were reported when fentanyl spiked wipes were stored during 4 and 7 days at 5 °C, respectively. However, similar fentanyl spiked wipes yielded somewhat lower recoveries of 93.8–95.5% when stored at ambient conditions, which was also observed, in a much greater extent though, in our study. The type of wipes used by Lambropoulos et al. (Super POLX 1200 wipers, VWR Scientific products) possibly offers better stability for the opioids under various storage

conditions. Also the potential advantageous effect of storing opioid wipe samples in disposable polypropylene centrifuge tubes, as described by Lambropoulos et al., instead of the 30 ml glass extraction vials used in our study, will be taken into consideration.

In summary, a very sensitive GC–MS analytical method was developed and validated for the determination of fentanyl, sufentanil, and alfentanil in air samples and surface contamination wipes. The developed methods were evaluated in a small field study of which the wipe sampling results were presented. Future research will focus on overcoming the phenomena of potential loss of alfentanil during air sampling and subsequent sample storage exceeding 7 days, and the significant decrease in recovery of the opioids at ambient storage of wipe samples. The procedures will be subsequently applied in a large industrial hygiene survey to evaluate occupational exposure of opioid production workers to particularly fentanyl and sufentanil. The analytical and sampling procedures will be further evaluated and optimized for applicability within the scope of rapid screening of environmental exposure to potent opioid narcotics.

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