Biochemical Pharmacology 98 (2015) 502–510

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

First report on the pharmacokinetics of tramadol and *O*-desmethyltramadol in exhaled breath compared to plasma and oral fluid after a single oral dose

Markus R. Meyer[a,*], Staffan Rosenborg[a,b], Marta Stenberg[b], Olof Beck[a,b]

[a] Karolinska Institutet, Department of Laboratory Medicine, Division of Clinical Pharmacology, Stockholm, Sweden
[b] Karolinska University Laboratory, Department of Clinical Pharmacology, Stockholm, Sweden

ARTICLE INFO

Article history:
Received 11 August 2015
Accepted 9 September 2015
Available online 18 September 2015

Keywords:
Tramadol
Exhaled breath
Oral fluid
Blood plasma
LC–MS/MS
Pharmacokinetics

ABSTRACT

Exhaled breath (EB) is a promising matrix for bioanalysis of non-volatiles and has been routinely implemented for drugs of abuse analysis. Nothing is known regarding the pharmacokinetics of therapeutics and their metabolites in EB. Therefore, we used tramadol as a model drug. Twelve volunteers received a single oral dose of tramadol and repeated sampling of EB, plasma, and oral fluid (OF) was done for 48 h using a particle filter device for EB and the Quantisal-device for OF. Samples were analyzed with LC–MS/MS and the pharmacokinetic correlations between matrices were investigated. The initial tramadol half-life in EB was shorter than in plasma but it reappeared in EB after 8–24 h. The ratio of *O*-desmethyltramadol to tramadol was considerably lower in EB and OF compared to plasma. This pilot study compared for the first time the pharmacokinetics of a therapeutic drug and active metabolite in different biomatrices including EB and demonstrated its potential for bioanalysis.

1. Introduction

In the fields of pharmacology and toxicology, there is presently a search and interest in biological matrices alternative to venous blood and urine. Two promising alternatives are dried blood spots (DBS) and oral fluid (OF) [1,2]. These are presently undergoing development aiming to improve and standardize the sampling procedure. The interest in these alternatives is driven by the advancement in analytical methodology, especially the sensitivity and selectivity of LC–MS, which makes less sample material required for the analytical investigation [1,2].

Yet another matrix that has attracted attention recently is exhaled breath (EB). This matrix has the feature of being readily and non-invasively available. Determination of therapeutic drugs in EB has found interest in connection with anesthetic agents, e.g., propofol and fentanyl, but also other drugs [3]. For toxicology applications, a number of abused drugs has been detected in EB shortly after intake [4]. Patients on methadone maintenance treatment receiving daily doses always have methadone present in EB before daily dose intake [4,5]. Also tramadol was reported to be detectable in EB but not *O*-desmethyltramadol (ODMT) [6].

Two studies with controlled intake of THC and cocaine has been performed and confirmed that these drugs are detected in EB after intake [7,8]. Both these studies have indicated a very rapid elimination of the substances from EB and the parent drugs were only detected for a few hours.

Interest in EB has mainly been focused on volatile components. From studies on EB condensate it has become evident that EB also contains non-volatile both low and high molecular weight components [9–11]. It is now established that these non-volatiles components may exit lung as part of microparticles formed during normal breathing and carry material originating from distal parts of the airway system [9].

Tramadol (T) is widely used in the treatment and prophylaxis of moderate to moderately severe chronic pain such as postoperative, dental, cancer, and acute musculoskeletal pain [12]. T pharmacologically acts via two ways. It activates μ-opioid receptors mainly through its active metabolite ODMT. T itself acts via norepinephrine and serotonin reuptake inhibition in the CNS, which inhibits pain transmission in the spinal cord [13,14]. The metabolism to ODMT is mainly catalyzed by cytochrome P450 2D6 (CYP2D6) [14]. Besides its therapeutic use, T was also found to be abused and reports of its abuse have increased [15–18].

Individualized patient treatment needs reliable but also easy-to-handle tools to monitor the dose and effects of administrated drugs. Hence, EB testing has become an emerging alternative to

* Corresponding author.
E-mail address: markusr.meyer@gmx.de (M.R. Meyer).

http://dx.doi.org/10.1016/j.bcp.2015.09.008
0006-2952/

common drug testing matrices such as urine, hair, or OF [3]. Different drugs of abuse have been shown to be detected in EB based on the formation of aerosol particles from the airway lining fluid by the breathing process [7,8,19–24]. However, nothing is known so far regarding the pharmacokinetics of drugs and their metabolites in EB particularly compared to blood plasma (BP) and OF. Such data are of importance to further characterize EB as tool for drug testing and therapeutic drug monitoring. Moreover, such data will help to better understand how drugs are incorporated into EB. Therefore, the aim of the presented study was to use T as a model drug and to analyze the pharmacokinetic correlations of T and its active metabolite ODMT between different matrices after controlled single administration.

2. Materials and methods

2.1. Chemicals and reagents

T, ODMT, T-d3C13, and ODMT-d6 were obtained as ampoulled stock solutions from LGC Standards AB (Borås, Sweden). Methanol and acetonitrile of LC–MS grade were from Fisher Scientific AB (Gothenburg, Sweden). All other chemicals and reagents from Merck KGaA (Darmstadt, Germany). The Milli-Q water was of ultrapure quality and prepared in-house.

2.2. Clinical study

The clinical study was performed at the Clinical Pharmacology Trial Unit at the Karolinska University Hospital, Huddinge, Sweden. As the aim was not to study the effect or safety of tramadol, it was not classified as a clinical trial according to the European guidelines [25]. However, the study was conducted according to the Declaration of Helsinki (Fortaleza, 2013) and, as applicable, to International Conference on Harmonization guidelines for Good Clinical Practice (ICH-GCP). The study was approved by the Regional Ethics Committee in Stockholm (No. 2015/103-31/2).

Subjects were recruited via advertising at the University Campus and the CPTU website (www.karolinska.se/kfp). After written and oral information they gave informed consent. Thereafter they were interviewed and physically examined. Subjects were eligible if they were 18–65 years of age, willing to follow the restrictions of the study and gave informed consent. They were excluded if they had a current and clinically significant medical, surgical or psychiatric illness, current abuse of alcohol or illicit drugs, seropositivity for hepatitis or HIV, known hypersensitivity to tramadol or any excipient. Females were not eligible if they were pregnant or breastfeeding. Subjects were also excluded if they had participated in another clinical trial during the last three months or if the investigator considered them unsuitable for the study.

2.3. Tramadol administration and sampling

Eligible subjects received a single oral dose of tramadol (50 mg, Tramadol Actavis® capsules). Sampling of EB, OF, and BP was done predose and at 0.5, 1, 1.5, 2, 3, 4, 8, 11, 24, 32 and 48 h after dosing.

The sampling of breath was done as published previously [26]. Briefly, a commercial sampling device was used (PSM Sweden, Uppsala, Sweden) and micro-particles present in the EB were selectively collected by letting the EB during normal breathing pass through a mouth-piece constructed to only allow micro-particles to pass through. The micro-particles passing the mouth-piece were collected on a polymer filter inside the device [4]. The sampling procedure was standardized by filling of a plastic bag and collecting about 30 L of EB. Following sampling, the device was sealed with plugs and stored at −20 °C.

Blood was collected after venous puncture into EDTA vacutainer tubes. Samples were centrifugated and the plasma transferred into Nunc cryotube vials, which were stored at −20 °C. An assumed volume of 1 mL of OF was collected using a commercial sampling device (Quantisal, Immunalysis, Pomona, CA, USA). Following sampling, the device was sealed with plugs, left at room temperature for at least six hours and then stored at −20 °C.

2.4. Sample preparation

Following storage, the breath collection devices were analyzed based on a previously published method with modification [26,27]. Briefly, the breath testing devices were put on top of glass test-tubes and analytes were eluted from a prewetted filter with methanol avoiding any contact with the inner walls of the device. Following evaporation to dryness, the extract was reconstituted in MeOH/water (1:1, v/v) and 2 μL injected onto the LC–MS/MS system.

A volume of 100 μL of the BP samples was diluted with 50 μL of internal standard solution in water and 150 μL of acetonitrile. After shaking and centrifugation the supernatant was transferred into glass vials and 2 μL injected onto the LC–MS/MS system.

A volume of 100 μL of OF was diluted with 50 μL of internal standard solution in water and 50 μL of acetonitrile. After shaking and centrifugation the supernatant was transferred into glass vials and 2 μL injected onto the LC–MS/MS system.

2.5. Liquid chromatography–triple quadrupole mass spectrometry (LC–MS/MS)

The LC–MS/MS system consisted of a Thermo Fisher Scientific TSQ Quantiva triple quadropole mass spectrometer connected to a Dionex Ultima 3000 UHPLC. The liquid chromatography system was composed of an Ultimate 2000 SRD degasser, Ultimate 3000 RS binary solvent pump system, column oven and Ultimate 3000 RS autosampler. The softwares used were Chromeleon Xpress v. 3, Xcalibur 3.0.63 and Thermo TSQ Tune Master v. 2.3.0.

The chromatographic system further included an ACQUITY UPLC BEH Phenyl column (100 mm × 2.1 mm, particle size 1.7 μm) with a gradient system consisting of A = water + 0.2% aqueous ammonia (25%) and 0.4% aqueous ammonium formate (1 M) and B = methanol + 0.2% aqueous ammonia (25%) and 0.4% aqueous ammonium formate (1 M).

The gradient using a flow rate of 500 μL/min was programmed as follows. From 0.0 to 0.4 min 30% B, 0.4 to 2.5 min 90% B, 2.51 to 3.0 min 99% B, and 3.05 to 3.85 min 30% B. The column was maintained at 60 °C.

The global MS settings for analyzing T, ODMT, T-d3C13, and ODMT-d6 were as follows. Positive spray voltage, 2500 V; sheath gas, 10 arbitrary units (Arb); aux gas, 15 Arb; sweep gas, 0 Arb; ion transfer tube, 240 °C; vaporizer, 450 °C, cycle time, 0.1 s; Q1 and Q3 resolution, 0.7 FWHM; CID gas, 1.5 mTorr; source fragmentation, 10 V. The individual SRM settings per compound are summarized in Table 2a.

2.6. Method validation

The used method for quantification of T and ODMT in BP, OF, and breath was validated in accordance to the "Guideline on bioanalytical method validation" published by the European Medicines Agency [28]. Briefly, the method was tested for selectivity using ten blank samples each, carry-over (using a blank sample following the high quality control, QC), lower limit of quantification (LLOQ) defined as lowest calibration standard, within-run and between-run accuracy using five samples per level at four (six for EB) concentration levels (LLOQ, low QC, medium QC

and high QC) with an additional dilution QC for BP and OF, within-run and between-run precision, matrix effect using six blank samples each, and stability (freeze–thaw stability, short term stability, sample processing temperature stability). The calibration consisted of six (eight for EB) concentration points equally distributed over the whole range. The individual calibrator and QC concentrations are summarized in Table 2b. For quantification, the ratios of the analyte vs internal standard (T vs T-d3C13 and ODMT vs ODMT-d6) were used. The analytical runs consisted of a blank sample, a zero sample, calibration standards, three levels of QC samples (low, medium and high) in duplicate and the study samples. All calculations were done using GraphPad Prism 5.00 (GraphPad Software, San Diego, CA, USA).

2.7. CYP2D6 genotyping

Subjects were genotyped for CYP2D6 genotype according to the accredited method used at the Clinical Pharmacology Laboratory at the Karolinska University Hospital. Commercially available TaqMan® reagents for *3, *4, *10, *17 and *41 alleles and copy number analysis were used on a StepOne Plus Real-Time PCR System (Applied Biosystems, Inc., Foster City, CA, USA).

2.8. Pharmacokinetic calculations

Non-compartmental pharmacokinetic analysis was performed using PKSolver [29] for Microsoft Excel. GraphPad Prism 6.05 (GraphPad Software, Inc., San Diego, CA, USA) was used for correlation analyses and drawing figures.

3. Results

3.1. Subjects and safety

Twelve healthy volunteers (six men and six women) were recruited to take a 50 mg oral dose of T. Their median age was 25.5 years (range 19–42). All but one subject (92%) reported at least one adverse event (AE). In total, 24 AEs were reported, 16 of these were

Table 1
Genotype and selected individual and mean/median values of pharmacokinetic parameters of all twelve subjects.

		$t_{1/2}$ (h)		T_{max} (h)		C_{max} (μg/L)		AUC 0-inf$_{obs}$ (μg*h/L)		Cl/F$_{obs}$	
Subject	Genotype	T	ODMT	T	ODMT	T	ODMT	T	ODMT	T	ODMT
Blood plasma											
1	*1*1	4.5	4.8	1.0	1.5	124	29	945	388	0.05	0.13
2	*1*1	4.7	5.2	1.5	2.0	130	36	983	405	0.05	0.12
3	*1*1	4.0	4.3	2.0	2.0	100	34	628	275	0.08	0.18
5	*41*5	5.1	6.3	1.0	1.5	91	33	695	439	0.07	0.11
6	*1*1	4.9	6.6	1.5	2.0	95	40	719	454	0.07	0.11
7	*10*41	4.6	4.9	2.0	2.0	73	38	465	313	0.11	0.16
8	*1*4	5.6	6.1	1.5	1.5	275	23	1890	203	0.03	0.25
9	*1*4	4.9	5.3	1.5	1.5	228	44	1640	399	0.03	0.13
10	*1*41	6.4	6.8	4.0	4.0	188	27	1977	305	0.03	0.16
11	*4*4	9.0	10.8	4.0	4.0	268	5.0	3679	94	0.01	0.53
12	*1*1	5.2	5.2	1.0	1.0	166	59	855	419	0.06	0.12
13	*1*1	5.1	3.3	1.5	2.0	81	37	429	205	0.12	0.24
Mean	–	5.4	5.8	1.9	2.1	152	34	1242	325	0.06	0.19
Median	–	5.0	5.3	1.5	2.0	127	35	900	351	0.06	0.15
Exhaled breath											
1	*1*1	4.7		2.0	3.0	740	30	6439		0.01	
2	*1*1	8.0		8.0	8.0	364	44	1879		0.03	
3	*1*1	6.3		1.9	3.9	67	11	606		0.08	
5	*41*5	6.3	5.9	1.9	8.0	696	10	7054	129	0.01	0.39
6	*1*1	8.3	6.8	1.0	11.0	695	41	5746	683	0.01	0.07
7	*10*41	19	1.2	2.0	2.0	429	9.1	2166	23	0.02	2.16
8	*1*4	6.6		1.5	1.5	87	1.5	740		0.07	
9	*1*4	4.2	4.5	8.0	8.0	1090	97	7510	766	0.01	0.07
10	*1*41	8.4		8.0	8.0	254	6.5	1820		0.03	
11	*4*4	12		8.0	8.0	576	2.8	4521		0.01	
12	*1*1	7.7	14	1.0	1.0	455	25	3386	231	0.01	0.22
13	*1*1	28		3.0	11.0	58	4.0	677		0.07	
Mean	–	10	6.5	3.9	6.1	459	23	3545	366	0.03	0.58
Median	–	7.9	5.9	2.0	8.0	442	11	2776	231	0.02	0.22
Oral fluid											
1	*1*1	4.7	4.8	1.0	4.0	1384	52	7574	443	0.007	0.11
2	*1*1	3.8	5.0	3.0	3.0	959	80	5248	430	0.01	0.12
3	*1*1	3.6	4.3	1.5	4.0	459	20	2769	174	0.02	0.29
5	*41*5	8.1	5.1	3.0	11	580	2.4	5184	30	0.01	1.66
6	*1*1	4.5	3.5	1.5	1.5	3905	158	13133	870	0.004	0.06
7	*10*41	6.7	7.8	1.0	1.0	2777	21	16603	236	0.003	0.21
8	*1*4	5.5	5.1	2.0	2.0	1895	48	7046	233	0.007	0.22
9	*1*4	4.4	4.0	1.5	2.0	784	38	4604	287	0.01	0.17
10	*1*41	6.6	5.2	3.0	3.0	1010	27	6581	211	0.008	0.24
11	*4*4	11	7.4	8.0	8.0	1351	8.0	12805	72	0.004	0.69
12	*1*1	4.4	4.5	1.0	1.0	1513	58	5053	264	0.01	0.19
13	*1*1	3.8	3.6	2.0	2.0	913	65	3146	260	0.02	0.19
Mean	–	5.6	5.0	2.4	3.5	1461	48.12	7479	293	0.01	0.35
Median	–	4.6	4.9	1.8	2.5	1181	43.00	5915	248	0.01	0.20

Cl/F$_{obs}$: oral clearance, $t_{1/2}$: half life; T_{max}: time to reach maximal plasma concentration; C_{max}: maximal plasma concentration; AUC: area under the curve. EB C_{max} is in pg/filter and AUC in pg*h/filter.

Table 2a
Selected reaction monitoring (SRM) parameter for analyzing tramadol (T), *O*-desmethyltramadol (ODMT), and the stable isotope labeled internal standards. The quantifier fragment ions are highlighted in bold. CE, collision energy; RF, radio frequency.

Analyte	Precursor (*m*/*z*)	Fragment ions (*m*/*z*)	CE (V)	RF lens (V)
T	264.2	264.2	5	42
		58.2	17	42
T-d3C13	268.2	**58.2**	16	43
ODMT	250.2	250.2	5	39
		58.2	17	39
ODMTd-6	256.2	**64.2**	17	39

considered at least possibly related to the study drug and all AEs were mild. Seven subjects (58%) reported mild euphoria and four subjects (33%) reported drowsiness/tiredness associated with expected peak concentrations of tramadol. Two subjects (17%) reported headache and one subject (8%) each reported nausea, hoarseness and increased tactile sensibility.

3.2. CYP2D6 subject genotyping

The results of the CYP2D6 genotyping are summarized in Table 1. Ten out of twelve subjects were classified – based on the codeine/morphine scheme – as extensive metabolizers (genotypes *1*1, *1*4, *1*41, *10*41, activity score 1.0–2.0), one as intermediate metabolizer (*41*5, activity score 0.5) and one as poor metabolizer (*4*4, activity score 0) [30,31]. This also reflects the expected phenotype distribution among Caucasians [30,31].

3.3. Method validation

The analytical work was based on using liquid chromatography–tandem mass spectrometry LC–MS/MS in selected reaction monitoring (SRM) mode and the method was successfully validated in accordance to the criteria of the European Medicines Agency [28]. Using the quantifier fragment ion of each analyte, the method was selective enough down to the LLOQ. No carry-over problems could be observed and the LLOQ could be defined to be 1 μg/L for BP and OF and 10 pg/filter for EB. Example chromatograms of BP, EB, and OF samples can be found in Fig. 1. Matrix effects in form of ion suppression with means ranging from 11 to 16% for BP, from 40 to 52% for EB, and from 12 to 14% for OF were observed but could be compensated by the individual internal standards. Hence, the CV of the IS-normalized matrix factor was not greater than 15% for the low and at a high QC levels. All analytes showed sufficient stability during the tested conditions. The mean within-run and between-run accuracies in BP ranged from 0.2 to 19% but were within 20% of the nominal values for the LLOQ QC and within 15% for the low, medium,

Table 2b
Calibrator (CA) and quality control (QC) concentrations used for quantification of tramadol (T) and *O*-desmethyltramadol (ODMT) in blood plasma (BP), exhaled breath (EB), oral fluid (OF).

Analyte	CA BP (μg/L)	CA EB (pg/filter)	CA OF (μg/L)	QC BP (μg/L)	QC EB (pg/filter)	QC OF (μg/L)
T and ODMT	1	10	1	1	10	1
	40	40	40	3	30	3
	80	80	80	100	100	100
	120	120	120	180	180	180
	160	160	160	1800	400	1800
	200	200	200		800	
		600				
		1000				

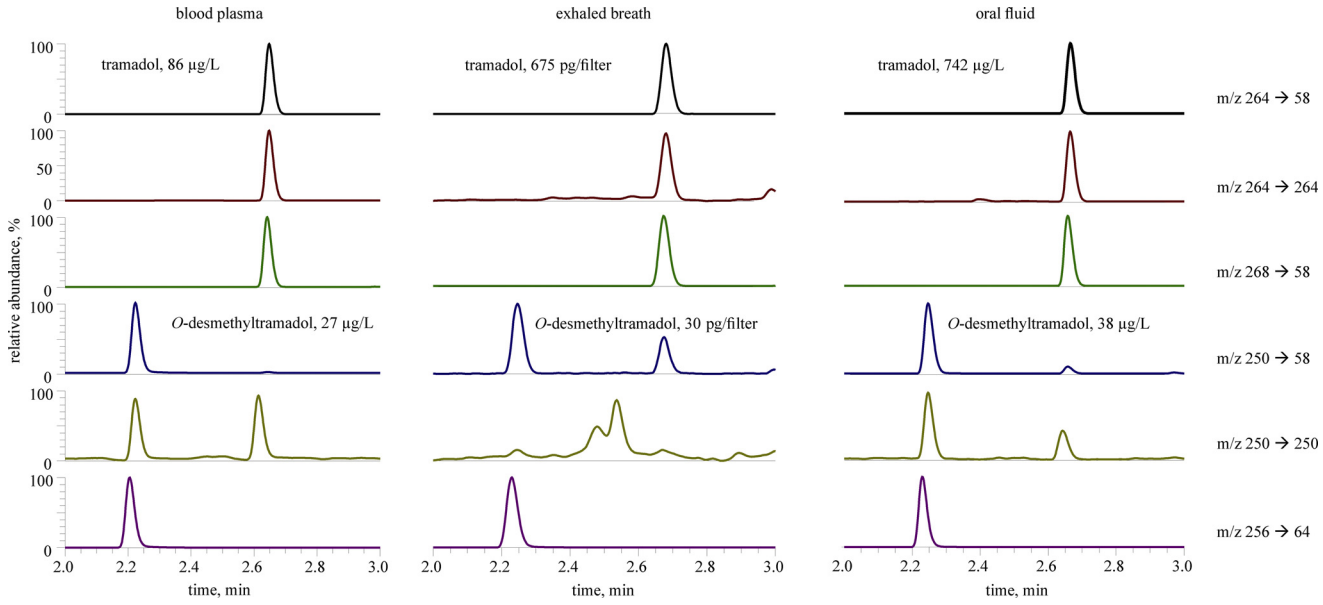

Fig. 1. Example mass chromatograms of tramadol and *O*-desmethyltramadol after analysis of BP, exhaled breath, and oral fluid from one subject sampled after 6 h post ingesting a single oral dose of 50 mg tramadol.

high QC and dilution QC. For EB values ranged from 0.2 to 15% and for OF from 0.06 to 14%. The mean within-run and between-run precisions in BP ranged from 0.6 to 15%, for EB from 0.8 to 17% and for OF from 0.75 to 12%. Precisions were within 20% for the LLOQ QC and within 15% for the low, medium, high QC, and dilution QC. Analytes were stable at room temperature for 6 h, for at least four weeks at −20 °C and after three freeze–thaw cycles. Processed samples were stable in the autosampler for at least 96 h.

3.4. Sample analysis

T and ODMT were not detected in any of the predose samples but were detectable in BP until 48 h with the exception of two subjects, in EB until 48 h with the exception of one subject. ODMT was only detectable in EB in about 38% (55 of 144) of the samples and no longer than 24 h with the exception of two subjects. In OF, T was detectable up to 48 h with the exception of two subjects and ODMT up to 32 h with the exception of two samples. The following eight samples were determined above the calibration range and were considered as estimated values: EB, T in subject 9 at 8 h; OF, T in subject 6 at 1, 1.5, 2 h; subject 7 at 1, 1.5, 2 h; subject 8 at 2 h. The concentrations vs time profiles of T and ODMT in BP, EB, and OF and the mean ODMT/T ratios vs sampling time can be found in Figs. 2 and 3, respectively. In most subjects, tramadol rapidly appeared in EB and then decreased again. A secondary peak was noted 8–24 h after dosing in eight out of the twelve subjects (Figs. 2 and 4).

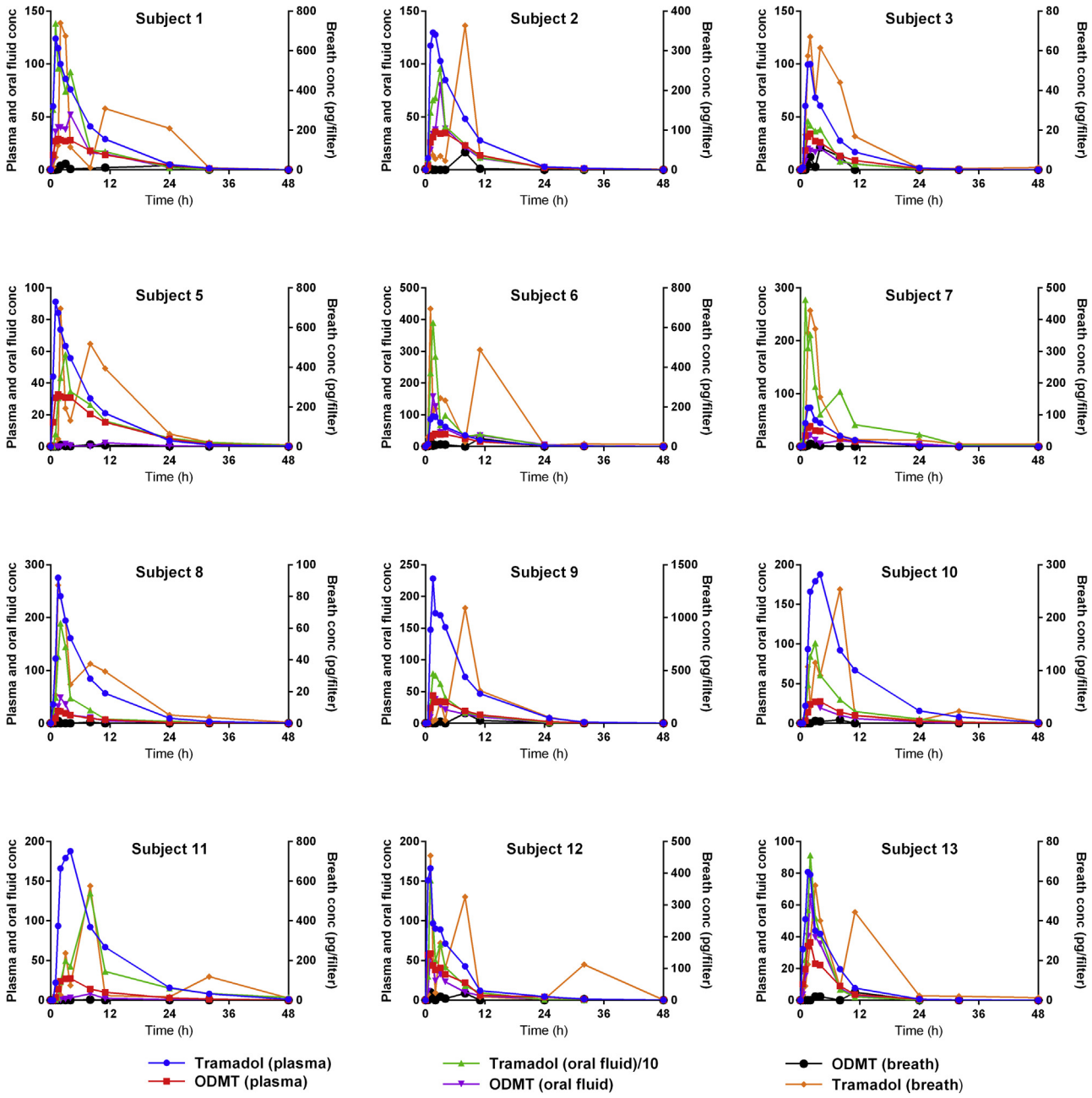

Fig. 2. Individual concentration–time profiles of tramadol and *O*-desmethyltramadol (ODMT) in blood plasma (μg/L), exhaled breath (pg/filter), and oral fluid (μg/L) including the intermediate metabolizer (subject 5) and the poor metabolizer genotype (subject 11). For increased visibility, the tramadol concentrations in oral fluid have been divided by 10.

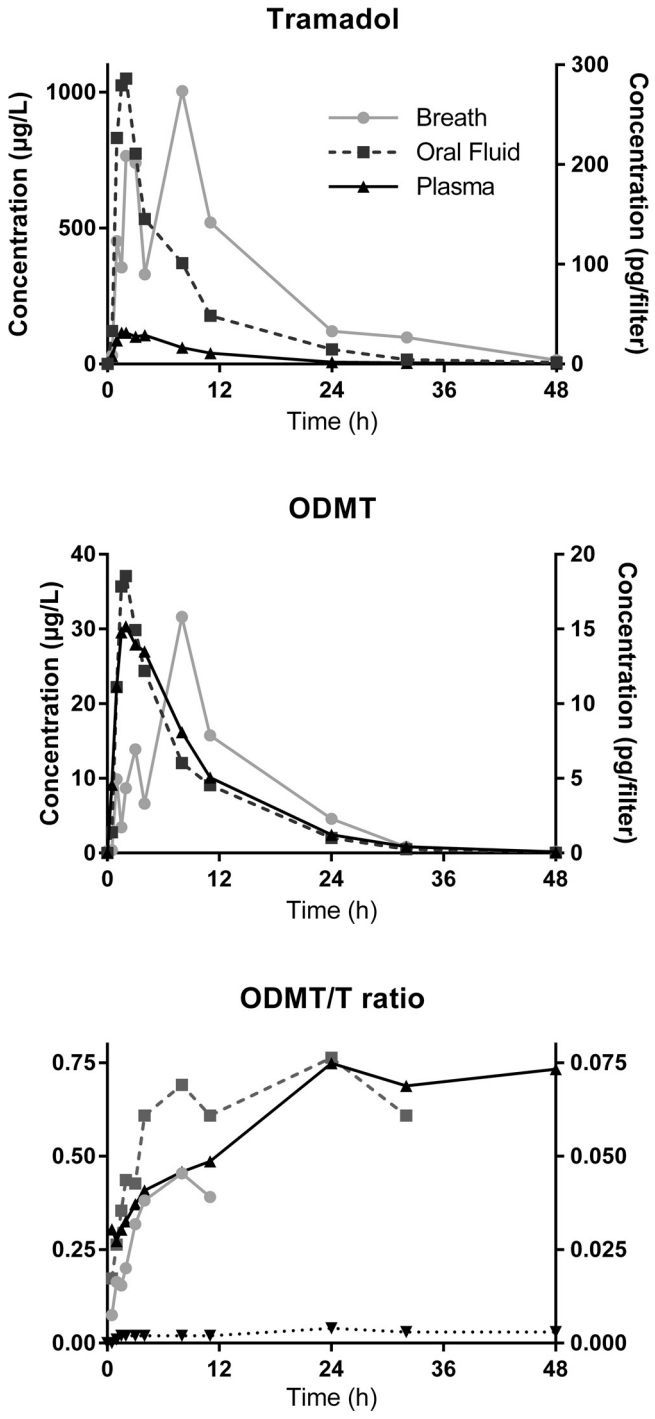

Fig. 3. Mean *O*-desmethyltramadol (ODMT)/tramadol (T) ratios vs sampling time in blood plasma (black), exhaled breath (grey), and oral fluid (dashed). The dotted line represents the ODMT/T ratio in plasma for the poor metabolizer (subject 11) and was excluded from the mean concentration of the remaining subjects (solid black line, triangles).

3.5. Pharmacokinetic calculations

The main pharmacokinetic parameters for the noncompartmental analyses are given in Table 1.

4. Discussion

The breath sampling and Quantisal devices used for sampling the EB and OF respectively, were used for the first time within this study to analyze pharmacokinetics of T and ODMT after controlled single administrations. Previous investigators have never analyzed EB and used other sampling strategies for OF. The analytical method was shown to be sufficient and reliable for analysis of T and ODMT in the studied biosamples. For BP and OF, dilution studies were added to meet the unexpectedly high concentrations in several study subjects. For EB, no dilution studies were possible with regards to the sample devices. Therefore, the calibration range and the QCs were extended to also meet the unexpectedly high concentrations. Nevertheless, one sample was still above the calibrated range and has to be considered as estimated. T and ODMT were detectable in BP until 48 h after administration with the exception of two subjects. T was detectable in EB until 48 h after dose intake with the exception of one subject, whereas ODMT was only detectable in about 38% (55 of 144) of the samples and no longer than 24 h with the exception of two subjects. In OF, T was detectable up to 48 h with the exception of two subjects and ODMT up to 32 h again with the exception of two samples. These data show that EB should be suitable to allow reliable monitoring of a T intake at least until 32 h after a single oral dose. In the case of chronic use or higher single doses, detection times should increase. In conclusion, the use of particularly EB but also OF for qualitative screening purposes on drugs or drugs of abuse was clearly demonstrated and detection windows were quite similar to those in BP.

Only one study with no detailed kinetic data on T and ODMT in OF was published so far [32] and no data are available in literature on the pharmacokinetics in EB. Hence, pharmacokinetic data for T and ODMT in EB and OF after controlled administration were presented for the first time in this study. Plasma kinetics after controlled oral administration of 100 mg to multiple volunteers were published in the past [33], in part even after chiral separation [34–36] and additional ultracentrifugation for measuring only the free fraction [37]. With regard to the fact that in the current study the administred dose was 50 mg and the total plasma concentrations were measured, pharmacokinetics in BP were comparable to published data. Mean T_{max} values for BP in the present study were 1.8 and 2.0 h for T and ODMT, compared to values ranging from 1.5 to 2.1 for T and from 1.5 to 3.4 ODMT in previous studies [33–35,37,38]. Values for $t_{1/2}$ in BP within the present study were 5.3 and 5.8 h for T and ODMT compared to values ranging from 4.6 to 7.6 and from 6.4 to 9.5 for T and ODMT, respectively. BP C_{max} values after 100 mg were ranging from 314 to 337 µg/L for T and were 88 µg/L for ODMT [33]. After administration of 50 mg in the presented study, mean BP concentrations were 152 and 34, respectively. No gender differences in the pharmacokinetics of T and ODMT were observed, which is in line with previous studies using BP only [33].

The genotypes of the subjects were ranked according to the codeine/morphine scheme [30,31]. This uses the percentage of codeine converted into morphine by CYP2D6 to define the enzymatic status of the subjects. Extensive metabolizers show an increased conversion of codeine to morphine as compared with poor metabolizers. It is obvious that the determined genotype not necessarily reflects the actual phenotype but the pharmacokinetic data in general fitted very well to the expectations [39,40]. As ODMT is mainly formed enzymatically via CYP2D6 [41], significantly higher BP AUCs for T and significant lower AUCs for ODMT were found for subject 11 (CYP2D6 poor metabolizer, activity score 0), which is in line with the literature [30]. Subject 11 also showed the highest half-life values (all >9 h) for T in all BP and OF. In EB, values did not correspond very well with the expected phenotype. In a study on the influence of the CYP2D6 phenotype and genotype on T biotransformation, authors reported that ODMT was formed to a lesser extent in individuals with one functional allele [42]. Despite markedly lower ODMT concentrations, average peak T

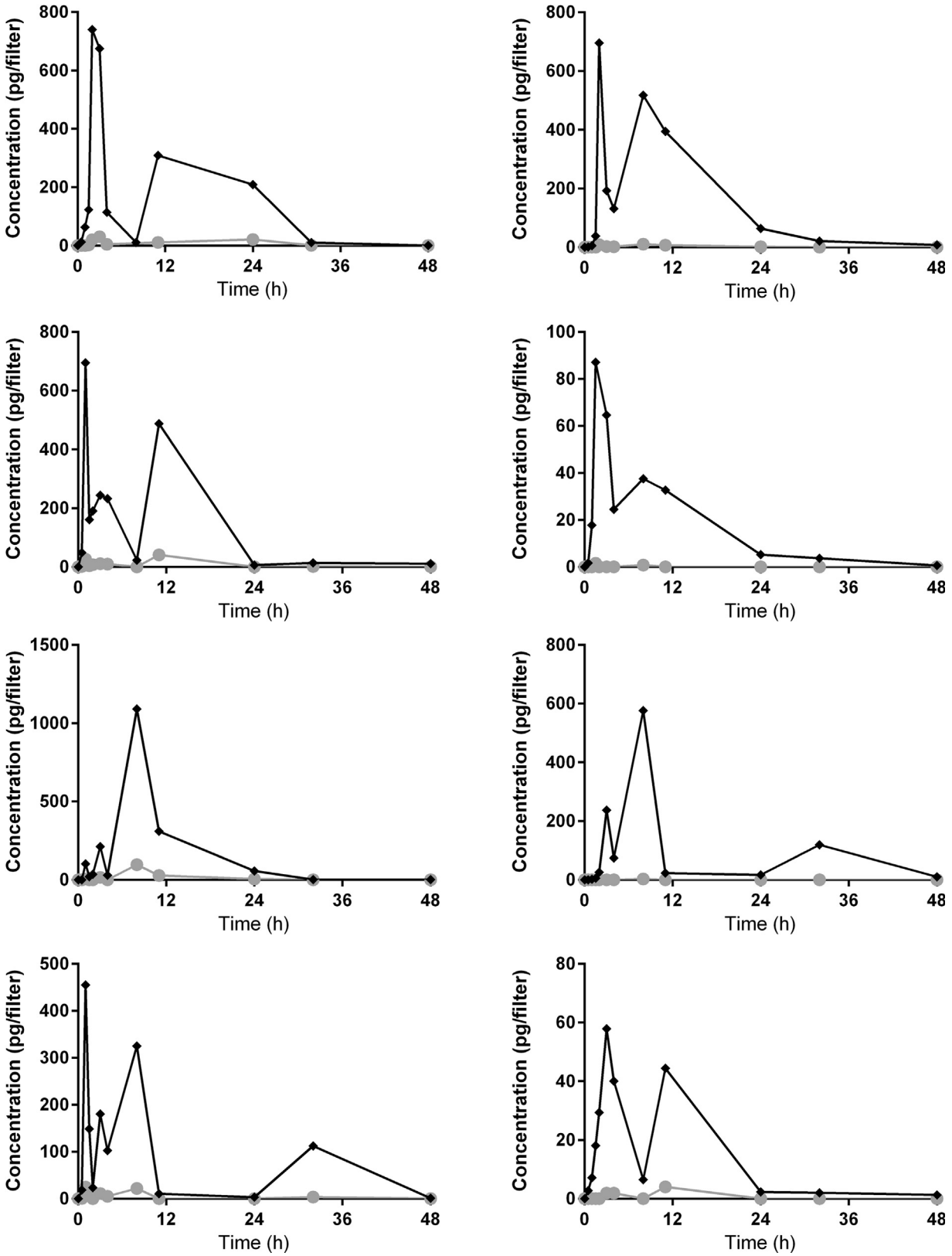

Fig. 4. Tramadol (black) and *O*-desmethyltramadol (grey) concentration vs sampling time profiles illustrating the biphasic kinetic and reappearance of tramadol in eight of the twelve subjects after about 8–24 h post dose.

concentrations did not appear to significantly differ between subjects with one versus two functional alleles. Similar results were observed in the study by Garcia-Quetglas et al. where the C_{max} of T did not differ significantly between poor and extensive metabolizers but the AUC did [36]. In the present study, the single poor metabolizer showed the highest AUC for T and the lowest AUC and C_{max} for ODMT but not the highest C_{max} for T. However, the ODMT/T ratio was significantly different from the extensive and

intermediate metabolizers. The ratios are shown in Fig. 3 for BP, EB, and OF. No significant differences of the pharmacokinetic data could be observed between the intermediate and extensive metabolizers. Genotype differences may be more pronounced at higher single or repeated doses of T. Stamer et al. and Gan et al. reported lower AUC of ODMT and higher clearance of T, respectively, in intermediate metabolizers compared to extensive metabolizers after intravenous administration of single doses up to 3 mg/kg of T [43,44].

AUC of T in EB neither correlated with concentrations in BP nor with concentrations in OF. This may be explained by fluctuations in the amount of EB particles collected within the used device. Previous studies have shown that there was indeed a high variability of mean particle concentrations among subjects [45]. However, such a limitation might be overcome by adding an endogenous marker to the analytical procedure compensating for these fluctuations [10]. Phospholipids may serve as endogenous standards as they are contained in particles originating from the respiratory tract lining fluid [45,46]. To demonstrate this, we analyzed all samples of subject 1 for palmitoyl palmitoleoyl PC (PC 16:0/16:1), which is an important surfactant component in the lung alveoli [47], and were able to detect it in every single sample [48]. However, further studies on this are encouraged for the future but we expect that using this endogenous standard, correlation of T concentrations in EB with T concentrations in BP or OF might improve. Another finding was the very low concentrations of ODMT in EB and the different time profiles of the ODMT/T ratios in the three matrices (Fig. 3). This indicates that EB neither reflects the situation in BP nor in OF but should be considered as an individual/separate compartment. This may limit the future use of EB as a matrix for therapeutic drug monitoring. In addition, the comparison of the AUC values (Table 1) did not reveal any correlation between T and ODMT within BP, EB or OF. However, the individual concentrations of T correlated significantly between BP and OF in all cases (mean $R^2 = 0.77$, SD = 0.17) but only in three cases between BP and EB (mean $R^2 = 0.31$, SD = 0.29). ODMT concentrations also correlated significantly between BP and OF with the exception of one subject (mean $R^2 = 0.71$, SD = 0.24). All in all, this leads to the conclusion that currently only OF could serve as alternative matrix for quantitative drug monitoring of T but not EB. Since significant correlations between OF and EB (mean $R^2 = 0.36$, SD = 0.30) concentration were only observed in four cases, a general contamination of EB by OF can be excluded. Hence, the value of EB and OF for quantitative therapeutic drug monitoring could not be shown. This is in contrast to the conclusions by Moore et al. who stated, after analyzing two paired blood-oral fluid specimens after unknown dose and time after ingestions, that OF could potentially be used for TDM of T. A further remarkable observation was the reappearance of T and ODMT after about 8–24 h in the EB samples in eight of the twelve subjects (see Fig. 4). This can also be observed in the mean T and ODMT concentration vs sampling time profiles shown in Fig. 3. The reason for this is currently unclear and remains to be explored in more detail in the future. One explanation might be redistribution after the 4-h sample as the subjects mainly remained supine during the more intensive sampling and then were more mobile until the 8- and 11-h samplings. Another possible explanation might be the turnover time of surfactant lipids, which is in steady state approximately 4–10 h [46,49]. Yet another possibility is that the observed biphasic pharmacokinetics of tramadol in EB may be explained by an accumulation of tramadol in lung tissue as has been noted in a case of fatal tramadol overdose and for other alkaline drugs [50,51].

In conclusion, the present study reported for the first time the pharmacokinetics of a therapeutic drug and its active metabolite in exhaled breath in comparison to blood plasma and oral fluid to gain knowledge about drug vs metabolites ratios, pharmacokinetic parameters, and the possible use of EB as option in drug analysis. This study helps to understand the differences in pharmacokinetics between BP, EB, and OF. It further helps to better understand how drugs are excreted and eliminated via EB. The study also highlighted current limitations of EB as an alternative matrix particularly for therapeutic drug monitoring. However, it will serve as starting point to foster further research for optimizing sampling and analysis and the use of EB in drug research on lung disease.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgements

The work was funded by the Stockholm County Council ALF. We also thank RNs Peter Johansson and Karin Nordin and MDs Georgios Panagiotidis and Anders Helldén for excellent assistance during the study. We also thank Mrs. Inga Lill Reinholdsson for thorough monitoring of the study conduct and data collection as well as Ph.D. Shahid Ullah for analyzing the PCs.

References

[1] P.M. De Kesel, N. Sadones, S. Capiau, W.E. Lambert, C.P. Stove, Hemato-critical issues in quantitative analysis of dried blood spots: challenges and solutions, Bioanalysis 5 (2013) 2023–2041.
[2] W.M. Bosker, M.A. Huestis, Oral fluid testing for drugs of abuse, Clin. Chem. 55 (2009) 1910–1931.
[3] C. Berchtold, M. Bosilkovska, Y. Daali, B. Walder, R. Zenobi, Real-time monitoring of exhaled drugs by mass spectrometry, Mass Spectrom. Rev. 33 (2014) 394–413.
[4] O. Beck, N. Stephanson, S. Sandqvist, J. Franck, Detection of drugs of abuse in exhaled breath using a device for rapid collection: comparison with plasma, urine and self-reporting in 47 drug users, J. Breath Res. 7 (2013) 26006.
[5] O. Beck, S. Sandqvist, P. Eriksen, J. Franck, G. Palmskog, Method for determination of methadone in exhaled breath collected from subjects undergoing methadone maintenance treatment, J. Chromatogr. B 878 (2010) 2255–2259.
[6] O. Beck, Exhaled breath for drugs of abuse testing—evaluation in criminal justice settings, Sci. Justice 54 (2014) 57–60.
[7] K.N. Ellefsen, M. Concheiro, O. Beck, D.A. Gorelick, S. Pirard, M.A. Huestis, Quantification of cocaine and metabolites in exhaled breath by liquid chromatography-high-resolution mass spectrometry following controlled administration of intravenous cocaine, Anal. Bioanal. Chem. 406 (2014) 6213–6223.
[8] S.K. Himes, K.B. Scheidweiler, O. Beck, D.A. Gorelick, N.A. Desrosiers, M.A. Huestis, Cannabinoids in exhaled breath following controlled administration of smoked cannabis, Clin. Chem. 59 (2013) 1780–1789.
[9] P. Kuban, F. Foret, Exhaled breath condensate: determination of non-volatile compounds and their potential for clinical diagnosis and monitoring. A review, Anal. Chim. Acta 805 (2013) 1–18.
[10] K. Schwarz, H. Biller, H. Windt, W. Koch, J.M. Hohlfeld, Characterization of exhaled particles from the healthy human lung—a systematic analysis in relation to pulmonary function variables, J. Aerosol Med. Pulm. Drug Deliv. 23 (2010) 371–379.
[11] A. Bredberg, J. Gobom, A.C. Almstrand, P. Larsson, K. Blennow, A.C. Olin, et al., Exhaled endogenous particles contain lung proteins, Clin. Chem. 58 (2012) 431–440.
[12] S. Grond, A. Sablotzki, Clinical pharmacology of tramadol, Clin. Pharmacokinet. 43 (2004) 879–923.
[13] D. Lassen, P. Damkier, K. Brosen, The pharmacogenetics of tramadol, Clin. Pharmacokinet. 54 (8) (2015) 825–836.
[14] L. Gong, U.M. Stamer, M.V. Tzvetkov, R.B. Altman, T.E. Klein, PharmGKB summary: tramadol pathway, Pharmacogenet. Genom. 24 (2014) 374–380.
[15] R.C. Dart, N. Dasgupta, J.E. Bailey, H.A. Spiller, Interpreting poison center call volume associated with tramadol, Ann. Pharmacother. 45 (2011) 424.
[16] M.M. Bassiony, G.M. Salah El-Deen, U. Yousef, Y. Raya, M.M. Abdel-Ghani, H. El-Gohari, et al., Adolescent tramadol use and abuse in Egypt, Am. J. Drug Alcohol Abuse 41 (2015) 206–211.
[17] S. Babalonis, M.R. Lofwall, P.A. Nuzzo, A.J. Siegel, S.L. Walsh, Abuse liability and reinforcing efficacy of oral tramadol in humans, Drug Alcohol Depend. 129 (2013) 116–124.
[18] H. Zhang, Z. Liu, The investigation of tramadol dependence with no history of substance abuse: a cross-sectional survey of spontaneously reported cases in Guangzhou City, China, Biomed. Res. Int. 2013 (2013) 283425.
[19] A.C. Almstrand, B. Bake, E. Ljungstrom, P. Larsson, A. Bredberg, E. Mirgorodskaya, et al., Effect of airway opening on production of exhaled particles, J. Appl. Physiol. (1985) 108 (2010) 584–588.

[20] A. Helander, S. Ullah, O. Beck, Phosphatidylethanols in breath: a possible noninvasive screening test for heavy alcohol consumption, Clin. Chem. 61 (2015) 991–993.
[21] O. Beck, N. Stephanson, S. Sandqvist, J. Franck, Determination of amphetamine and methylphenidate in exhaled breath of patients undergoing attention-deficit/hyperactivity disorder treatment, Ther. Drug Monit. 36 (2014) 528–534.
[22] O. Beck, S. Sandqvist, J. Franck, Demonstration that methadone is being present in the exhaled breath aerosol fraction, J. Pharm. Biom. Anal. 56 (2011) 1024–1028.
[23] O. Beck, S. Sandqvist, P. Eriksen, J. Franck, G. Palmskog, Determination of methadone in exhaled breath condensate by liquid chromatography–tandem mass spectrometry, J. Anal. Toxicol. 35 (2011) 129–133.
[24] O. Beck, S. Sandqvist, I. Dubbelboer, J. Franck, Detection of delta9-tetrahydrocannabinol in exhaled breath collected from cannabis users, J. Anal. Toxicol. 35 (2011) 541–544.
[25] European Commission, Guidance Documents Applying to Clinical Trials in EudraLex—Volume 10.
[26] C. Skoglund, U. Hermansson, O. Beck, Clinical trial of a new technique for drugs of abuse testing: a new possible sampling technique, J. Subst. Abuse Treat. 48 (2015) 132–136.
[27] N. Stephanson, S. Sandqvist, M.S. Lambert, O. Beck, Method validation and application of a liquid chromatography–tandem mass spectrometry method for drugs of abuse testing in exhaled breath, J. Chromatogr. B 985 (2015) 189–196.
[28] EMA, Guideline on bioanalytical method validation, 2014.
[29] Y. Zhang, M. Huo, J. Zhou, S. Xie, P.K. Solver, An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel, Comput. Methods Programs Biomed. 99 (2010) 306–314.
[30] K.R. Crews, A. Gaedigk, H.M. Dunnenberger, T.E. Klein, D.D. Shen, J.T. Callaghan, et al., Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for codeine therapy in the context of cytochrome P450 2D6 (CYP2D6) genotype, Clin. Pharmacol. Ther. 91 (2012) 321–326.
[31] K.R. Crews, A. Gaedigk, H.M. Dunnenberger, J.S. Leeder, T.E. Klein, K.E. Caudle, et al., Clinical Pharmacogenetics Implementation Consortium guidelines for cytochrome P450 2D6 genotype and codeine therapy: 2014 update, Clin. Pharmacol. Ther. 95 (2014) 376–382.
[32] Y.H. Ardakani, M.R. Rouini, Improved liquid chromatographic method for the simultaneous determination of tramadol and its three main metabolites in human plasma, urine and saliva, J. Pharm. Biom. Anal. 44 (2007) 1168–1173.
[33] Y.H. Ardakani, M.R. Rouini, Pharmacokinetics of tramadol and its three main metabolites in healthy male and female volunteers, Biopharm. Drug Dispos. 28 (2007) 527–534.
[34] M.A. Campanero, E. Garcia-Quetglas, B. Sadaba, J.R. Azanza, Simultaneous stereoselective analysis of tramadol and its primary phase I metabolites in plasma by liquid chromatography. Application to a pharmacokinetic study in humans, J. Chromatogr. A 1031 (2004) 219–228.
[35] A. Ceccato, F. Vanderbist, J.Y. Pabst, B. Streel, Enantiomeric determination of tramadol and its main metabolite *O*-desmethyltramadol in human plasma by liquid chromatography–tandem mass spectrometry, J. Chromatogr. B 748 (2000) 65–76.
[36] E. Garcia-Quetglas, J.R. Azanza, B. Sadaba, M.J. Munoz, I. Gil, M.A. Campanero, Pharmacokinetics of tramadol enantiomers and their respective phase I metabolites in relation to CYP2D6 phenotype, Pharmacol. Res. 55 (2007) 122–130.
[37] N.V. de Moraes, G.R. Lauretti, M.N. Napolitano, N.R. Santos, A.L. Godoy, V.L. Lanchote, Enantioselective analysis of unbound tramadol, *O*-desmethyltramadol and *N*-desmethyltramadol in plasma by ultrafiltration and LC–MS/MS: application to clinical pharmacokinetics, J. Chromatogr. B 880 (2012) 140–147.
[38] M.R. Rouini, Y.H. Ardakani, F. Soltani, H.Y. Aboul-Enein, A. Foroumadi, Development and validation of a rapid HPLC method for simultaneous determination of tramadol, and its two main metabolites in human plasma, J. Chromatogr. B 830 (2006) 207–211.
[39] J.B. Dumond, M. Vourvahis, N.L. Rezk, K.B. Patterson, H.C. Tien, N. White, et al., A phenotype–genotype approach to predicting CYP450 and *P*-glycoprotein drug interactions with the mixed inhibitor/inducer tipranavir/ritonavir, Clin. Pharmacol. Ther. 87 (2010) 735–742.
[40] C. Deneux, V. Tardy, A. Dib, E. Mornet, L. Billaud, D. Charron, et al., Phenotype–genotype correlation in 56 women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency, J. Clin. Endocrinol. Metab. 86 (2001) 207–213.
[41] T. Lynch, A. Price, The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects, Am. Fam. Physician 76 (2007) 391–396.
[42] S.M. Abdel-Rahman, J.S. Leeder, J.T. Wilson, A. Gaedigk, R.R. Gotschall, R. Medve, et al., Concordance between tramadol and dextromethorphan parent/metabolite ratios: the influence of CYP2D6 and non-CYP2D6 pathways on biotransformation, J. Clin. Pharmacol. 42 (2002) 24–29.
[43] U.M. Stamer, F. Musshoff, M. Kobilay, B. Madea, A. Hoeft, F. Stuber, Concentrations of tramadol and *O*-desmethyltramadol enantiomers in different CYP2D6 genotypes, Clin. Pharmacol. Ther. 82 (2007) 41–47.
[44] S.H. Gan, R. Ismail, W.A. Wan Adnan, W. Zulmi, Impact of CYP2D6 genetic polymorphism on tramadol pharmacokinetics and pharmacodynamics, Mol. Diagn. Ther. 11 (2007) 171–181.
[45] A.C. Almstrand, E. Ljungstrom, J. Lausmaa, B. Bake, P. Sjovall, A.C. Olin, Airway monitoring by collection and mass spectrometric analysis of exhaled particles, Anal. Chem. 81 (2009) 662–668.
[46] J.J. Presneill, K. Nakata, Y. Inoue, J.F. Seymour, Pulmonary alveolar proteinosis, Clin. Chest Med. 25 (2004) 593–613 viii.
[47] W. Bernhard, S. Hoffmann, H. Dombrowsky, G.A. Rau, A. Kamlage, M. Kappler, et al., Phosphatidylcholine molecular species in lung surfactant: composition in relation to respiratory rate and lung development, Am. J. Respir. Cell Mol. Biol. 25 (2001) 725–731.
[48] S. Ullah, S. Sandqvist, O. Beck, Measurement of lung phosphatidylcholines in exhaled breath particles using a convenient collection procedure, Anal. Chem. (2015) (submitted for publication).
[49] A. Chander, A.B. Fisher, Regulation of lung surfactant secretion, Am. J. Physiol. 258 (1990) L241–53.
[50] T. Suhara, Y. Sudo, K. Yoshida, Y. Okubo, H. Fukuda, T. Obata, et al., Lung as reservoir for antidepressants in pharmacokinetic drug interactions, Lancet 351 (1998) 332–335.
[51] N.D. Bynum, J.L. Poklis, M. Gaffney-Kraft, D. Garside, J.D. Ropero-Miller, Postmortem distribution of tramadol, amitriptyline, and their metabolites in a suicidal overdose, J. Anal. Toxicol. 29 (2005) 401–406.