

Methadone concentrations in blood, plasma, and oral fluid determined by isotope-dilution gas chromatography–mass spectrometry

Ya-Ching Hsu · Bud-Gen Chen · Shu-Ching Yang ·
Yu-Shan Wang · Shiao-Ping Huang · Mei-Han Huang ·
Tai-Jui Chen · Hsu-Chun Liu · Dong-Liang Lin ·
Ray H. Liu · A. Wayne Jones

Received: 7 July 2012 / Revised: 29 August 2012 / Accepted: 26 September 2012 / Published online: 23 October 2012
© Springer-Verlag Berlin Heidelberg 2012

Abstract Methadone (MTD) is widely used for detoxification of heroin addicts and also in pain management programs. Information about the distribution of methadone between blood, plasma, and alternative specimens, such as oral fluid (OF), is needed in clinical, forensic, and traffic medicine when analytical results are interpreted. We determined MTD and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in blood, plasma, blood cells, and OF by gas chromatography–mass spectrometry (GC-MS) after adding deuterium-labeled internal standards. The analytical limits of quantitation for MTD and EDDP by

this method were 20 and 3 ng/mL, respectively. The amounts of MTD and EDDP were higher in plasma (80.4 % and 76.5 %) compared with blood cells (19.6 % and 23.5 %) and we found that repeated washing of blood cells with phosphate-buffered saline increased the amounts in plasma (93.6 % and 88.6 %). Mean plasma/blood concentration ratios of MTD and EDDP in spiked samples ($N=5$) were 1.27 and 1.21, respectively. In clinical samples from patients ($N=46$), the concentrations of MTD in plasma and whole blood were highly correlated ($r=0.92$, $p<0.001$) and mean (median) plasma/blood distribution ratios were 1.43

Published in the special paper collection *Forensic Toxicology* with guest editors Kazuhito Watanabe and Satoshi Chinaka.

Ya-Ching Hsu, Bud-Gen Chen, and Shu-Ching Yang contributed equally to this work.

Parts of this work were presented at the 50th Conference of TIAFT 2012 in Hamamatsu.

Y.-C. Hsu · B.-G. Chen · S.-P. Huang · M.-H. Huang ·
R. H. Liu (✉)
Department of Medical Laboratory Science and Biotechnology,
Fooyin University,
151 Ching-Hsueh Road, Ta-Liao,
Kaohsiung 83102, Taiwan
e-mail: rayliu@uab.edu

S.-C. Yang
Department of Pathology, College of Medicine, National Cheng
Kung University,
No. 138, Sheng Li Road,
Tainan City 704, Taiwan

Y.-S. Wang · H.-C. Liu · D.-L. Lin
Department of Forensic Toxicology, Institute of Forensic
Medicine, Ministry of Justice,
New Taipei City 235, Taiwan

T.-J. Chen
Department of Psychiatry, E-Da Hospital,
No. 1, Yida Road, Jiaosu Village, Yanchao District,
Kaohsiung City 82445, Taiwan

A. W. Jones
Department of Forensic Genetics and Forensic Toxicology,
National Board of Forensic Medicine,
Artillerigatan 12,
587 58 Linköping, Sweden

(1.41). The correlations between MTD in OF and plasma ($r=0.46$) and OF and blood ($r=0.52$) were also statistically significant ($p<0.001$) and the mean OF/plasma and OF/blood distribution ratios were 0.55 and 0.77, respectively. The MTD concentration in OF decreased as salivary pH increased (more basic). These results will prove useful in clinical and forensic medicine when MTD concentrations in alternative specimens are compared and contrasted.

Keywords Blood · Plasma · Oral fluid · Methadone · EDDP · GC-MS

Introduction

First synthesized in Germany in the early 1940s for use as an analgesic and spasmolytic drug, methadone (MTD) was approved by the US Food and Drug administration in 1965 to treat heroin addiction [1, 2]. Methadone maintenance treatment (MMT) subsequently became accepted worldwide for rehabilitation of heroin addicts, although this approach was not implemented in Taiwan until 2006. Those involved with the MMT program are well aware that methadone might be diverted from the licit to the illicit drug market, thus increasing the risk for methadone-related overdose deaths [3–10]. For safety and to ensure an effective treatment, the concentrations of MTD in blood, plasma, and alternative specimens must be carefully controlled by highly reliable analytical methods.

A 2004 publication by Jones and Larsson [11] emphasized the lack of published data on the distribution of drugs and poisons between plasma and whole blood. This information is needed when therapeutic concentrations are compared and contrasted with the concentrations determined in whole blood, such as in forensic toxicology and legal medicine. The uneven distribution of drugs between plasma and blood cell is also important in clinical pharmacology when pharmacokinetic parameters are calculated from concentration–time (C-T) profiles [11]. The parameters will differ depending on whether C-T profiles are constructed from the analysis of drugs in plasma or whole blood [12]. Present knowledge about the distribution of MTD between plasma, whole blood, blood cell, and oral fluid (OF) is rather limited despite a growing interest in the use of alternative specimens, such as OF, in clinical, forensic, and especially traffic medicine when impaired drivers are apprehended and prosecuted [13–15].

This article reports use of a GC-MS method to determine the distribution of MTD and its metabolite EDDP between blood, plasma, and blood cell and OF. Experiments were done *in vitro* by spiking blood with known amounts of MTD and EDDP and also by analysis of clinical specimens obtained from patients undergoing MMT.

This current study contributes to better understanding the distribution of methadone and its metabolite between plasma, whole blood, and oral fluid. Data made available through this study will be helpful to the interpretation of analytical findings derived from (a) compliance testing in patients enrolled in MMT or pain management programs, (b) therapeutic concentration determination, and (c) forensic and traffic screening of motorists suspected of driving under the influence of drugs.

Materials and methods

Chemicals, calibration standards, and reagents

Authentic standard substances and internal standards (ISs), MTD, EDDP, MTD- d_9 , and EDDP- d_3 (in 0.1 mg/mL methanol solution) were purchased from Cerilliant (Round Rock, TX, USA). All solvents and reagents were of analytical or HPLC grade including ethyl acetate (Ferk, Berlin, Germany); hexane, isopropanol, and acetonitrile (Merck, Darmstadt, Germany); sodium carbonate (Na_2CO_3) and sodium hydrogen carbonate (NaHCO_3 ; Mallinckrodt, Phillipsburg, NJ, USA); sodium chloride (NaCl), sodium hydrogen phosphate (Na_2HPO_4), and potassium dihydrogen phosphate (KH_2PO_4 ; Merck, Darmstadt, Germany); and potassium chloride (KCl; Panreac, Barcelona, Spain).

Carbonate buffer (pH 10.2) was prepared from 4.2 g NaHCO_3 (2 M) and 10.6 g Na_2CO_3 (1 M) in 100 mL aqueous solution. Phosphate buffer saline (PBS, pH 7.4) was prepared from 1,428 mM NaCl, 101 mM Na_2HPO_4 , 26.8 mM KCl, and 17.6 mM KH_2PO_4 , and then diluted ten times.

Calibration curves included seven concentration data points for MTD (0–300 ng/mL) and EDDP (0–50 ng/mL) showed a linear response with coefficients of determination (r^2) of 0.9959 and 0.9980, respectively. Calibration standards were prepared by adding appropriate volumes of 1.0 or 10 $\mu\text{g/mL}$ MTD (in methanol) and 0.1 or 1.0 $\mu\text{g/mL}$ EDDP (in methanol) to 1 mL of the sample blank. Mean analytical recoveries of MTD and EDDP from plasma were 84.1 % ($N=3$) and 93.9 % ($N=3$), respectively.

In vitro experiments (five replicates) with plasma, blood cell, and whole blood

The specimens of whole blood used for *in vitro* distribution studies were obtained from a local hospital blood bank. Aliquots (3 mL) were spiked with 60 μL of methanol solution containing 10 $\mu\text{g/mL}$ of MTD or EDDP to give 200 ng/mL of the analytes in the whole blood. Plasma and blood cells were obtained by centrifugation at 2,500 rpm for 10 min. The supernatant plasma fraction and bottom layer

(blood cell) were carefully separated and transferred to clean dry tubes. Whole blood, plasma, and blood cell samples thereby prepared were then analyzed for their contents of the analytes (described later). (For samples from clinical sources, they were mostly stored at $-20\text{ }^{\circ}\text{C}$ until analysis, typically within 1 month after preparation.)

A set of “washing experiments” were conducted to understand whether the drugs found in the blood cell sample can be removed. Specifically, the blood cell sample was washed with phosphate buffer saline (PBS) as follows: 1.5 mL PBS was added to the blood cell portion (derived from 3 mL whole blood), followed by centrifugation at 2,500 rpm for 10 min. The supernatant (wash solution) was added to the plasma portion prior to extraction and analysis of MTD and EDDP. This washing was done 0, 2, 3, and 4 times before the bottom layer (blood cell) was transferred to a clean glass tube for analysis. Wash solutions were combined with the plasma portion for analysis to minimize the number of experiments and to assure the analytes’ contents are higher than the analytical method’s limits of quantitation.

Sample preparation

For analysis, to 1 mL of the biological sample (whole blood, plasma, blood cell, or oral fluid) was added 10 μL (10 $\mu\text{g}/\text{mL}$) MTD- d_9 and 30 μL (1 $\mu\text{g}/\text{mL}$) EDDP- d_3 as internal standards. The sample (whole blood and blood cell samples were first lysed by vortexing) was then deproteinized by adding 1-mL acetonitrile, followed by centrifugation, and then removal of the supernatant. This process was repeated and the combined supernatant was mixed with 1 mL carbonate buffer (pH 10.2). Both methadone and EDDP were extracted with 4.5 mL hexane/isopropanol (8:1) mixture by mechanical shaking. The extract was evaporated to dryness under a slow stream of nitrogen without applying heat and re-constituted with 50 μL ethyl acetate before analysis by GC-MS.

Clinical samples

Specimens of blood and oral fluid were collected from patients enrolled in a MMT program at E-Da Hospital (Kaohsiung, Taiwan), after informed consent was obtained and approval by the ethical review board at this institution. Dosage and typical patient information, such as gender, age, weight, etc., are maintained in the patients’ record files. These information were critically evaluated in our report [16] on the effects of genetic factors upon the drug concentrations in plasma, but not reported in this current article.

Specimens were collected upon patients’ arrival to take their daily dose. In general, their last dose was taken

approximately 23 h earlier. Blood specimens were taken by venipuncture into evacuated tubes with K_2EDTA as preservative. Following specimen collection, the hematocrit (HCT) of the blood samples was determined by Automatic Hematology Analyzer 1800i (SYSMEX, Kobe, Japan). Samples for analysis (whole blood, plasma, and blood cell) were prepared as described in the “*In vitro experiments (five replicates) with plasma, blood cell, and whole blood*” section without spiking the analytes, then frozen separately at $-20\text{ }^{\circ}\text{C}$ until analysis typically within 1 month.

Near simultaneous samples of oral fluid were collected without stimulation. Patients were asked to rinse with the water provided, make tongue and lip movements for about 30 s, then “spit” (through a large-diameter straw) into a clean centrifugation tube. Oral fluid pH was determined by METER SP701 (SUNTEX, Taipei, Taiwan). The oral fluid samples were stored at $-20\text{ }^{\circ}\text{C}$ until analysis typically within 1 month. For analysis, saliva specimens were allowed to thaw, centrifuged (3,000 rpm for 5 min), and a precise volume of clear supernatant phase was mixed with 1 mL carbonate buffer (pH 10.2) and extracted with hexane/isopropanol (8:1). The extract was evaporated to dryness under a slow stream of nitrogen and re-constituted with 50 μL ethyl acetate for GC-MS analysis.

No formal study was conducted to evaluate the stability of the analytes in the samples (blood and oral fluid) stored under $-20\text{ }^{\circ}\text{C}$. However, repeated analysis of samples stored for different times did not indicate any deterioration trend of the analytes included in this study.

GC-MS analysis

An Agilent 6890N gas chromatograph/5975 mass selective detector system operating at 70 eV with ion source temperature set at $230\text{ }^{\circ}\text{C}$ was used for this study. The gas chromatograph was equipped with a 30-m HP-5 (Wilmington, DE, USA) capillary column crosslinked 5 % phenyl methyl siloxane with 250- μm I.D. and 0.25- μm film thicknesses. The injector temperature and GC-MS interface temperature were maintained at $280\text{ }^{\circ}\text{C}$. The sample was introduced into the gas chromatograph in splitless mode and the helium carrier gas flow rate was set at 1.0 mL/min. The initial oven temperature was held at $160\text{ }^{\circ}\text{C}$ for 4 min, then raised to $250\text{ }^{\circ}\text{C}$ at $10\text{ }^{\circ}\text{C}/\text{min}$, and held for 1 min. The analytical limits of quantitation of MTD and EDDP by this method were 20 and 3 ng/mL, respectively.

Based on intensity and cross contribution parameters made available from our earlier study [17], selected ion monitoring was done at m/z 72, 223, 294/78, 226, 303 for MTD/MTD- d_9 , and m/z 262, 276, 277/265, 279, 280 for EDDP/EDDP- d_3 and the ions underlined were used for quantitation.

Results

Relative amounts of methadone in plasma, blood cell, and whole blood

Five replicates of in vitro experiments (starting from spiking the whole blood with the analytes) were conducted to study the distribution of MTD in plasma and blood cell portions. Whole blood, plasma, and blood cell samples were prepared and analyzed separately. The amounts of drugs found in the plasma and the blood cell portions were summed and found to be compatible to the amounts found in the whole sample (data not presented), indicating the validity of the experimental design.

When whole blood samples were spiked with known amounts of MTD and EDDP, we found that both drugs were predominantly contained in the plasma fraction, 80.4 % and 76.5 %, respectively, compared with blood cell, 19.6 % and 23.5 %, respectively (Table 1). This suggests that plasma/whole blood distribution ratios of MTD and EDDP will be greater than unity (1.0) and mean concentration ratios ($N=5$) of 1.27 and 1.21, respectively, were obtained by analysis of whole blood and plasma.

Effect of washing blood cell with buffer on plasma concentrations

During in vitro experiments, we studied the effect of washing blood cell with buffer on the amounts of MTD and EDDP in the plasma fraction. Table 2 shows that amount of drug and percentage in plasma increased as the number of washings increased with a corresponding decrease in amount and percentage of drug in blood cell. Note also that the total amount of drug (plasma + blood cell) remained approximately the same regardless of number of washes, confirming the reliability of the analytical data.

Methadone in blood, plasma, and oral fluid from patients

Table 3 presents summary statistics for the concentrations of MTD determined in blood, plasma, and OF in clinical samples ($N=46$) from patients enrolled in a methadone

Table 2 Effect of number of washings of separated blood cell with phosphate buffer saline on the amounts of methadone (MTD) and EDDP in plasma and blood cell fractions

Analyte	Number of washings	Amount (ng) and (%) in plasma ^a	Amount (ng) and (%) in blood cells ^b	Total amount of drug, ng ^c
MTD	0	508 (80.3 %)	124 (19.6 %)	632
	2	524 (89.4 %)	62 (10.6 %)	586
	3	582 (92.3 %)	48 (7.7 %)	630
	4	557 (93.6 %)	38 (6.4 %)	595
EDDP	0	458 (76.5 %)	141 (23.5 %)	599
	2	480 (84.9 %)	85 (15.0 %)	565
	3	506 (87.8 %)	70 (12.2 %)	576
	4	492 (88.6 %)	63 (11.4 %)	555

Data shown are mean values for ($N=5$) spiked samples

^a Significant increase as number of washes increased by one-way ANOVA ($p<0.05$)

^b Significant decrease as number of washes increased by one-way ANOVA ($p<0.05$)

^c No significant differences in total drug amount by one-way ANOVA ($p>0.05$)

maintenance program. The corresponding descriptive statistics for pH of oral fluid and HCT of blood samples are also shown because these might be important variables in the distribution of drugs.

The scatter plot in Fig. 1 shows that the concentrations of MTD in plasma and whole blood are highly correlated with Pearson's $r=0.92$ ($p<0.001$). Furthermore, the plasma/blood distribution ratio of MTD was positively correlated ($r=0.64$, $p<0.001$) with HCT of blood samples (Fig. 2). The higher the percentage of blood cell in the blood sample (increasing HCT), the larger was the plasma/blood distribution ratio of MTD.

A much weaker correlation and larger scatter of the data points were observed for MTD in plasma compared with oral fluid ($r=0.46$) and also for oral fluid and blood ($r=0.51$) as depicted in Figs. 3 and 4.

The OF/plasma concentration ratio of MTD depended to some extent on the salivary pH (Fig. 5), decreasing as pH increased ($r=-0.51$), suggesting lower concentration of MTD in OF under more basic physiological conditions. A similar negative correlation ($r=-0.46$) was observed for the

Table 1 Summary statistics of amounts of methadone (MTD) and EDDP in plasma, blood cell, and whole blood (HCT=36.8 %) and the corresponding percentages of drug in plasma and blood cell fractions

Analyte	Amount in plasma, ng	Amount in blood cell, ng	Amount in whole blood, ng	% drug in blood cell	% drug in plasma	Plasma/blood concentration ratio ^a
MTD	170±12.2	41±6.9	211±10.0	19.6	80.4	1.27
EDDP	153±4.2	47±5.7	200±2.8	23.5	76.5	1.21

Data represent mean±SD for $N=5$ spiked replicates

^a Ratio of concentrations of drug by direct analysis of plasma and whole blood

Table 3 Descriptive statistics showing mean (\pm standard deviation) concentrations (ng/mL) of methadone in whole blood (B), plasma (P), and oral fluid (OF) from MMT patients

Variable	Mean \pm SD	Median	Min–Max	
Blood HCT	42.7 \pm 2.71 %	42.8 %	38–49 %	
Salivary pH	6.93 \pm 0.64	7.01	5.5–8.0	
Blood (B) conc.	161 \pm 66 ng/mL	152 ng/mL	47–307 ng/mL	
Plasma (P) conc.	231 \pm 107 g/mL	213 ng/mL	63–512 ng/mL	
Oral Fluid (OF) conc.	124 \pm 98 ng/mL	105 ng/mL	25–401 ng/mL	
P/B ratio	1.43 \pm 0.22	1.41	0.94–1.89	
Corresponding values for blood HCT (%) and pH of oral fluid (OF) are also shown	OF/B ratio	0.77 \pm 0.46	0.76	0.13–1.97
	OF/P ratio	0.55 \pm 0.35	0.75	0.08–1.42

OF/blood distribution ratio of MTD and salivary pH (data not shown).

A multiple regression analysis with OF/plasma or OF/blood ratios of MTD as dependent y variable and salivary pH and blood HCT as two independent x variables showed that only salivary pH was statistically significant as an influencing factor on the drug distribution ratios.

Discussion

Collecting oral fluid samples for accurate quantitation of drugs is not a trivial matter. Interested readers are referred to a review [18] on potential variables. We have conducted a government supported study on this subject matter and developed a collection device that was granted a patent by the Taiwanese government [19]. Many commercially available collection devices are much more convenient for oral fluid drug screening purpose; however, the oral fluid collection method adopted in this study can provide the best quantitative data.

The GC-MS method used for analysis of MTD and EDDP in biological fluids was highly reliable and was fully validated in an article published by us in 1991 [20]. Oral fluid data obtained by this method were found compatible to those derived from a LC-MS-MS method in another

laboratory (not reported). Furthermore, the sum of analytes found in plasma and blood cell samples was statistically identical to the amount found in the whole blood sample from the same source.

The concentration distribution of drugs and their metabolites between different biological specimens is gaining in importance considering the increasing use of alternative specimens in clinical, forensic, and traffic medicine [21, 22]. In clinical therapeutics, concentrations of drugs are traditionally determined in samples of plasma or serum, whereas in forensic toxicology drugs and metabolites are determined in whole blood, which is often hemolyzed [23]. This complicates direct comparisons between therapeutic drug concentrations and the equivalent toxic or lethal concentrations, such as in medical examiner cases and in blood samples from drug-impaired drivers [24]. In order to compare and contrast results from clinical and forensic samples, it is necessary to know how drugs distribute between the plasma and erythrocyte fractions of whole blood [25].

Many drugs are tightly bound to plasma proteins, which means that the concentrations in plasma or serum are higher than in blood cell and also higher than in the whole blood, depending in part on HCT [11]. This was found for diazepam [11], which had a plasma/blood distribution ratio of 1.8:1 and also tetrahydrocannabinol (THC), which had a plasma/blood ratio closer to 2:1 [26]. For water-soluble

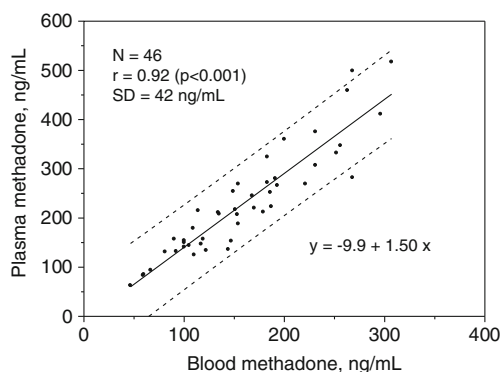


Fig. 1 Correlation (Pearson) between concentrations of MTD in plasma and whole blood for $N=46$ clinical samples. SD=residual standard deviation and *dashed lines* show 95 % prediction intervals

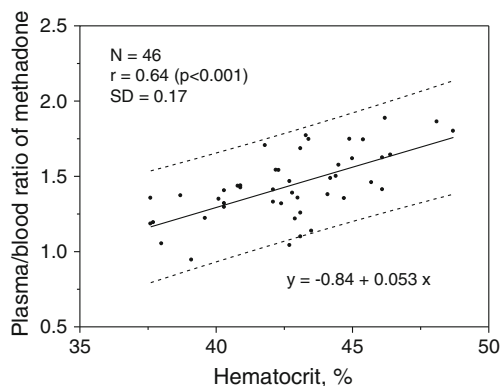


Fig. 2 Correlation (Pearson) between plasma/blood concentration ratio of MTD and blood HCT for $N=46$ clinical samples. SD=residual standard deviation and the *dashed lines* show 95 % prediction intervals

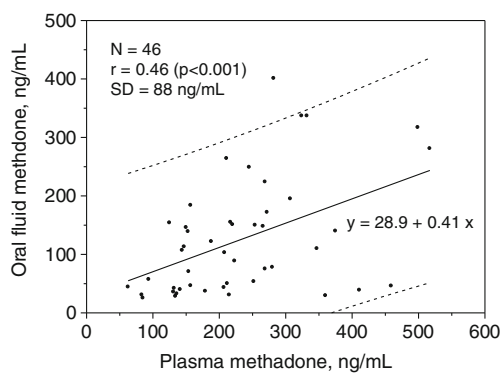


Fig. 3 Correlation (Pearson) between OF and plasma concentrations of MTD for $N=46$ clinical samples. SD=residual standard deviation and the *dashed lines* show 95 % prediction intervals

non-protein bound drugs, such as ethanol, methanol, and isopropanol, the plasma/whole blood distribution ratios are in good agreement with the distribution of water in the specimens, about 1.15:1 [27, 28]. The plasma to blood ratio defines the drug concentration in plasma compared to whole blood and provides an indication of the extent of drug binding to plasma proteins compared with the drug's affinity for blood cell. The plasma/blood ratio is an important parameter in forensic toxicology and also in conjunction with clinical pharmacokinetics, when volumes of distribution and elimination half-lives are calculated [29].

According to primary literature and Goodman & Gillman's reference book [30, 31], methadone is 89 ± 1.4 % bound to plasma proteins and the mean plasma/blood concentration ratio is 1.33:1 as determined by radioimmunoassay [30]. This result is in good agreement with the present work using spiked blood samples (mean 1.27:1, Table 1) and also in specimens from methadone maintenance patients (mean 1.41:1, Table 3).

The discrepancy between the P/B ratio of MTD for spiked female blood (HCT=36.8 %) and the clinical samples (mean HCT=42.6 %), mainly from male MMT patients (male=36, female=10), is attributed to the difference in HCT (see Fig. 2). According to Malarkey and McMorow

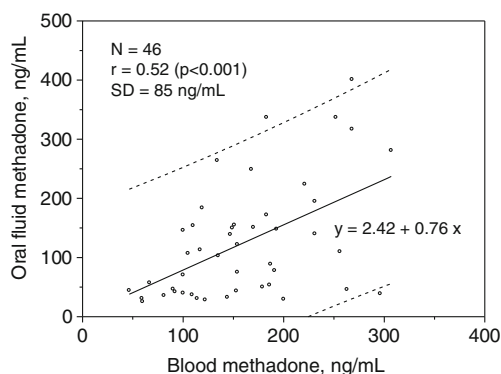


Fig. 4 Correlation (Pearson) between OF and blood concentrations of MTD for $N=46$ clinical samples. SD=residual standard deviation and the *dashed lines* show 95 % prediction intervals

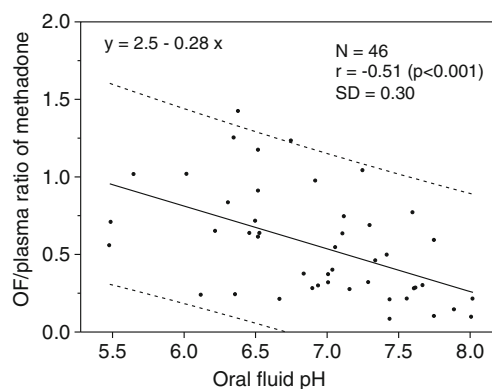


Fig. 5 Correlation (Pearson) between OF/plasma concentration ratio and pH of oral fluid for $N=46$ clinical samples. SD=residual standard deviation and the *dashed lines* show 95 % prediction intervals

[32], normal HCT values for females and males are 35.9–44.6 % and 41.5–50.4 %, respectively. The lower the hematocrit of the blood specimen the less blood cells are available to dilute the MTD concentration in plasma, which means the concentration in whole blood is higher and the calculated plasma/blood ratio is lower [33].

The amounts of MTD and EDDP in the plasma fraction of blood increased after blood cell were repeatedly washed with phosphate buffer (Table 2), which might have implications for the choice of analytical specimen in therapeutic drug monitoring programs. After centrifugation to obtain plasma or serum, unless the blood cell is carefully washed to remove any residual drug, the concentration is underestimated. This problem could be avoided if methadone was routinely determined in whole blood instead of plasma, although if this was done, therapeutic concentrations of this drug would need to be adjusted downwards because the plasma/blood ratio is greater than unity (1.0). Moreover, the concentrations of methadone in whole blood will depend to some extent on the HCT of specimen (Fig. 2) because a large volume of blood cell, containing little drug, dilutes the concentration in plasma and in whole blood [33]. Bloods with an unusually low HCT, such as from patients with anemia or other blood disorder, would be expected to have higher concentration of methadone compared with normal bloods or those with elevated HCT.

Methadone is a basic drug ($pK_a=8.6$), which has consequences for its passage across biological membranes in relation to the pH-partition hypothesis and plasma pH, which is close to 7.35–7.45 [29]. Moreover, only the protein-free fraction of drug is capable of transferring from plasma into saliva and OF [29]. The closer the pH of saliva to the pK_a of methadone, the more free-drug passes by diffusion from plasma to saliva, which supports the negative correlation between OF/plasma ratio and salivary pH shown in Fig. 5 [34]. Normalization of the OF concentration of MTD to a constant salivary pH was found (as would be

expected) to improve the correlation with plasma concentrations (data not shown).

This study verifies that oral fluid is a viable biological specimen for analysis of basic drugs, such as methadone [35, 36], but it would not be correct to predict the concentration in plasma from the concentration measured in saliva. Although the OF and plasma concentrations were significantly correlated ($r=0.46$), the individual data points were widely scattered as evidenced by the large residual standard deviations (SD) of ± 88 ng/mL (Fig. 3). The residual SD was about half (± 42 ng/mL) for the scatter plot depicting MTD concentrations in plasma and whole blood (Fig. 1).

The present study gives a detailed appraisal of the distribution of methadone between plasma, whole blood, and oral fluid in patients enrolled in a MMT program. MTD concentrations were higher in plasma than in whole blood as expected from knowledge of the high protein binding of this opioid drug. The concentrations of MTD in OF were lower than in plasma and whole blood with mean OF/plasma and OF/blood distribution ratios of 0.55 and 0.77, respectively (Table 3). These results do not agree with two previous studies in which concentrations of MTD were higher in OF compared with plasma [37, 38]. Whether this discrepancy depends on the way saliva was collected, e.g., by spitting or use of specially designed OF collecting devices, remains to be investigated. Noteworthy however is that the mean (median) OF/plasma distribution ratio of MTD reported by us in Table 3 of 0.55 was in good agreement with the theoretically predicted value of 0.58 (range 0.45–0.70) determined elsewhere [39].

In conclusion, OF is a suitable non-invasive and alternative matrix for analysis of MTD in connection with screening of motorists suspected of driving under the influence of drugs [40, 41] or when testing compliance in patients enrolled in MMT or pain management programs [42]. The plasma to blood ratios of drugs are important parameters to consider when therapeutic concentrations are compared and contrasted with those determined in whole blood in forensic and traffic medicine.

Acknowledgments This study was partially supported by funding provided by the National Science Council (NSC 99-2113-M-242-003 and NSC 100-2113-M-242-001).

References

- Kreek MJ, Vocci FJ (2002) History and current status of opioid maintenance treatments: blending conference session. *J Subst Abuse Treat* 23:93–105
- Dole VP, Nyswander M (1965) A medical treatment for diacetylmorphine (heroin) addiction—a clinical trial with methadone hydrochloride. *JAMA* 193:646–650
- Drummer OH, Opeskin K, Syrjanen M, Corder SM (1992) Methadone toxicity causing death in ten subjects starting on a methadone maintenance program. *Am J Forensic Med Pathol* 13:346–350
- Milroy CM, Forrest AR (2000) Methadone deaths: a toxicological analysis. *J Clin Pathol* 53:277–281
- Seymour A, Black M, Jay J, Cooper G, Weir C, Oliver J (2003) The role of methadone in drug-related deaths in the west of Scotland. *Addiction* 98:995–1002
- Shields LB, Hunsaker JC III, Corey TS, Ward MK, Stewart D (2007) Methadone toxicity fatalities: a review of medical examiner cases in a large metropolitan area. *J Forensic Sci* 52:1389–1395
- Laberke PJ, Bartsch C (2010) Trends in methadone-related deaths in Zurich. *Int J Legal Med* 124:381–385
- Okie S (2010) A flood of opioids, a rising tide of deaths. *N Engl J Med* 363:1981–1985
- Albion C, Shkrum M, Cairns J (2010) Contributing factors to methadone-related deaths in Ontario. *Am J Forensic Med Pathol* 31:313–319
- Madden ME, Shapiro SL (2011) The methadone epidemic: methadone-related deaths on the rise in Vermont. *Am J Forensic Med Pathol* 32:131–135
- Jones AW, Larsson H (2004) Distribution of diazepam and nordiazepam between plasma and whole blood and the influence of hematocrit. *Ther Drug Monit* 26:380–385
- Rowland M, Tozer TN (1995) Clinical pharmacokinetics—concepts and applications. Williams & Wilkins, Baltimore
- Toennes SW, Steinmeyer S, Maurer H-J, Moeller MR, Hauert GF (2005) Screening for drugs of abuse in oral fluid—correlation of analysis results with serum in forensic cases. *J Anal Toxicol* 29:22–27
- Vindenes V, Lund HME, Andresen W, Gjerde H, Ikdahl SE, Christophersen AS, Ølestad EL (2012) Detection of drugs of abuse in simultaneously collected oral fluid and blood from Norwegian drug drivers. *Forensic Sci Int* 219:165–171
- Wiese Simonsen K, Steentoft A, Hels T, Bernhoft IM, Rasmussen BS, Linnet K (2012) Presence of psychoactive substances in oral fluid from randomly selected drivers in Denmark. *Forensic Sci Int* 221:33–38
- Yang S-C, Huang S-P, Yang M-Y, Liu RH, Wang S-J, Wu H-C, Huang Y-J, Li J-H (2011) Can pharmacogenetic studies improve the effectiveness of methadone maintenance programs? 2011 Annual Meeting of American Academy of Forensic Sciences; Chicago, IL, US
- Liu RH, Wang S-M, Canfield DV (2010) Quantitation and mass spectrometric data of drugs and isotopically labeled analogs. CRC Press, Boca Raton
- Crouch DJ (2005) Oral fluid collection: the neglected variable in oral fluid testing. *Forensic Sci Int* 150:165–173
- Wang CT, Lin H-C, Liao LF, Liu RH (2011) Taiwan Patent No. M412772
- Baugh LD, Liu RH, Walia AS (1991) Simultaneous gas chromatography/mass spectrometry assay of methadone and 2-ethyl-1,5-dimethyl-3,3-diphenyl-pyrrolidine (EDDP) in urine. *J Forensic Sci* 36:548–555
- Bosker WM, Huestis MA (2009) Oral fluid testing for drugs of abuse. *Clin Chem* 55:1910–1931
- Gallardo E, Barroso M, Queiroz JA (2009) Current technologies and considerations for drug bioanalysis in oral fluids. *Bioanalysis* 1:637–667
- Jones AW, Holmgren A, Kugelberg FC (2007) Concentrations of scheduled prescription drugs in blood of impaired drivers. Considerations for interpreting the results. *Ther Drug Monit* 29:248–260
- Levine B (ed) (2006) Principles of forensic toxicology, 2nd edn. CRC Press, Boca Raton
- Drummer OH (2006) Drug testing in oral fluid. *Clin Biochem Rev* 27:147–159

26. Schwilke EW, Karschner EL, Lowe RH, Gordon AM et al (2009) Intra- and inter-subject whole blood/plasma cannabinoid ratios determined by 2-dimensional electron impact GC-MS with cryo-focusing. *Clin Chem* 55:1188–1195
27. Iffland R, West A, Bilzer N, Schuff A (1999) Zur Zuverlässigkeit der Blutalkohol-bestimmung. Das Verteilungsverhältnis des Wassers zwischen Serum und Vollblut. *Rechtsmedizin* 9:123–130
28. Charlebois RC, Corbett MR, Wigmore JG (1996) Comparison of ethanol concentrations in blood, serum, and blood cells for forensic applications. *J Anal Toxicol* 20:171–178
29. Hinderling PH (1997) Red blood cells: a neglected compartment in pharmacokinetics and pharmacodynamics. *Pharmacol Rev* 49:279–295
30. Inturrisi CE, Colburn WA, Kaiko RF, Houde RW, Foley KM (1987) Pharmacokinetics and pharmacodynamics of methadone in patients with chronic pain. *Clin Pharmacol Ther* 41:392–401
31. Brunton LL, Lazo JS, Parker KL (eds) (2006) *Goodman & Gilman's the pharmacological basis of therapeutics*, 11th edn. McGraw-Hill, New York
32. Malarkey LM, McMorrow ME (eds) (2000) *Nurse's manual of laboratory test and diagnostic procedure*, 2nd edn. W.B. Saunders Co., Philadelphia
33. Rowland M, Emmons GT (2010) Use of dried blood spots in drug development: pharmacokinetic considerations. *AAPS J* 12:290–293
34. Aps JKM, Martens LC (2005) The physiology of saliva and transfer of drugs into saliva. *Forensic Sci Int* 150:119–131
35. Kintz P, Samyn N (2002) Use of alternative specimens: drugs of abuse in saliva and doping agents in hair. *Ther Drug Monit* 24:239–246
36. Schramm W, Smith RH, Craig PA, Kidwell DA (1992) Drugs of abuse in saliva: a review. *J Anal Toxicol* 16:1–9
37. Bermejo AM, Lucas ACS, Tabernero MJ (2000) Saliva/plasma ratio of methadone and EDDP. *J Anal Toxicol* 24:70–72
38. Wolff K, Hay A (1991) Methadone in saliva. *Clin Chem* 37:1297–1298
39. Shiran MR, Hassanzadeh-Khayyat M, Iqbal MZ, Lagundoye O, Seivewright N, Lennrd MS, Tucker GT, Tostami-Hodjegan A (2005) Can saliva replace plasma for the monitoring of methadone. *Ther Drug Monit* 27:580–586
40. Wille SMR, Raes E, Lillsunde P, Gunnar T, Laloup M, Samyn N, Christophersen AS, Moeller MR, Hammer KP, Verstraete AG (2009) Relationship between oral fluid and blood concentrations of drugs of abuse in drivers suspected of driving under the influence of drugs. *Ther Drug Monit* 31:511–519
41. Engblom C, Gunnar T, Rantanen A, Lillsunde P (2007) Driving under the influence of drugs—amphetamine concentrations in oral fluid and whole blood samples. *J Anal Toxicol* 11:176–280
42. Pfaffe T, Cooper-White J, Beyerlein P, Kostner K, Punyadeera C (2011) Diagnostic potential of saliva: current state and future applications. *Clin Chem* 57:675–687