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Time-Dependent Postmortem Redistribution of Opioids in Blood and Alternative Matrices

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Abstract

Forensic postmortem case interpretation can be challenging, in particular due to postmortem redistribution (PMR) phenomena. Recent studies have shown that computed tomography (CT)-guided collection of biopsy samples using a robotic arm (virtobot) provides a valuable tool for systematic studies on time-dependent PMR. Utilizing this strategy, several cases involving opioid use such as methadone, fentanyl, tramadol, codeine, oxycodone and hydrocodone were evaluated for time-dependent concentration changes and potential redistribution mechanisms. Upon admission to the institute (t1), blood (femoral and right ventricle heart blood) and tissue biopsy samples (lung, kidney, liver, spleen, thigh muscle and adipose tissue) were collected utilizing CT-guided biopsy. Approximately 24 h later (t2; mean 28 ± 15 h), during the autopsy, samples from the same body regions were collected manually and in addition brain tissue, gastric content, urine and left ventricle heart blood. Analysis was conducted with liquid chromatography tandem mass spectrometry. Significant time-dependent methadone concentration increases in femoral blood (pB) indicate the occurrence of PMR, however, ultimately not relevant for forensic interpretation. The main metabolite of methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), showed a less significant trend for PMR. Redistribution by passive diffusion along the muscle-to-pB concentration gradient seems likely for methadone, but not for EDDP. Results for fentanyl suggest extensive PMR. Other opioids such as tramadol, codeine, hydrocodone and oxycodone showed no consistent trend for significant PMR. Overall, CT-guided biopsy sampling proved to be a valuable tool for the investigation of PMR mechanisms.

Introduction

During the forensic postmortem investigation into the cause and manner of death, a forensic toxicologist aims to determine a legal or illegal drug intake or application prior to death and attempts to assess the contribution of a drug towards the cause and manner of death. The key concept in this context is whether or not the concentration of a drug in a postmortem sample accurately reflects the concentration at time of death (1). Besides antemortem- (reference values from living people) and perimortem factors (agonal phase), particularly postmortem factors may influence case interpretation (2, 3). The anatomical and physiological changes that can alter drug concentrations artificially after death are summarized in the term postmortem redistribution (PMR). Caused by diffusion processes, degradation or drug neo-formation driven by microorganisms, significant site- and time-dependent variations in drug concentrations may be observed compared to time of death (4, 5). Various studies report, that pH, volume of distribution (Vd), protein binding affinity, bacterial biotransformation action and lipophilicity may, upon others, influence the extent of PMR (4, 6, 7).

Opioids are widely used for their pain relieving effects but due to their highly addictive properties are prone to misuse. The high abusive potential of opioids makes them an important target in forensic toxicological analyses with a frequent contribution to cause of death.

According to the Centers for Disease Control and Prevention (CDC) on average more than 90 Americans died from an opioid overdose every day based on figures from 2015 (8, 9). Additionally, in October 2017 the ongoing opioid epidemic was declared a nationwide public-health emergency in the USA (10). Well known in this context is methadone that is used in methadone maintenance treatment. Further, particularly fentanyl was brought into the public attention in recent years as an extensively abused, extremely potent opioid (11, 12). More traditional substances or metabolites of these, acting on the opioid receptors are for example codeine, tramadol, hydrocodone and oxycodone. Only few studies focus on the tendency of opioids undergoing PMR, despite the fact that due to their physiochemical properties (e.g., large Vd) most opioids are thought to be prone to PMR. Generally, the predominant tool to predict the extent of PMR is the cardiac-to-femoral blood concentration ratio (C/P-ratio). This was also utilized by some research groups to assess PMR of methadone and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) (13–16). As an alternative approach the liver-to-femoral blood (L/P) ratio was previously used to estimate the extent of methadone PMR (4). This was based on the idea that the greater magnitude of the L/P-ratio compared to the C/P-ratio provides an advantage in interpreting a drug's potential for PMR (17). Particularly for fentanyl, the superiority of the L/P-ratio over the C/P-ratio has been recently addressed (12, 18). However, neither of the aforementioned ratios have a clear relationship to a drugs' physiochemical properties such as Vd, pKa and protein binding affinity that are thought to influence the PMR. Further, time-dependent concentration changes and the usefulness of alternative matrices are often neglected in current studies. Following this, the aim of this study was to clarify the potential of time-dependent PMR of opioids not only in blood but also in alternative matrices such as muscle, liver, kidney, lung, spleen and adipose tissue. In this context, the application of computed tomography (CT)-guided biopsy sampling hours before conventional autopsy should provide valuable information on drug concentration changes in alternative matrices (19, 20). Further, redistribution mechanisms should be investigated particularly for methadone and EDDP.

Materials and Methods

Chemicals and reagents

Methanolic solutions of methadone, EDDP, fentanyl, codeine, oxycodone, oxymorphone, dihydrocodeine, hydrocodone, tramadol and O-desmethyltramadol (ODMT) (1 mg/mL) and the deuterated internal standards (IS) methadone-d_9, fentanyl-d_5, codeine-d_3 (0.1 mg/mL) and tramadol-d_3 (1 mg/mL) were obtained from Cerilliant (delivered by Sigma-Aldrich, Buchs, Switzerland). Water was purified with a Purelab Ultra Millipore filtration unit (Labtech, Villmergen, Switzerland) and acetonitrile of high-performance liquid chromatography (HPLC) grade was obtained from Fluka (Buchs, Switzerland). All other chemicals used were from Merck (Zug, Switzerland) and of the highest grade available.

Postmortem sample collection

Samples from blood vessels and alternative matrices were collected at two time points (t1 and t2) after death according to Staeheli *et al.* (19) in the course of the routine toxicological investigation from 23 deceased. In short, after admission to the institute, immediately followed by postmortem CT-imaging (t1) on a 128-slice scanner (Somatom Definition Flash, Siemens Medical Solutions, Forchheim, Germany), introducer needles were placed into the right heart ventricle, the right lung, the right lobe of the liver, the right kidney, the spleen, subcutaneous adipose tissue of the waist, muscle tissue at the upper thigh and the right femoral vein using the virtobot system (19, 21). To verify the needle position, a second CT scan was performed. A 1 mL of blood was withdrawn from the right heart ventricle and the femoral vein. Triplicate biopsy samples were obtained from all alternative matrices detailed above (~20 mg/biopsy). These were immediately weighed into 2-mL Metal Bead Lysing Matrix tubes (MP Biomedicals, Illkirch, France). The body fluids were aliquoted into 2-mL Eppendorf Safe Lock Tubes (Schoenenbuch, Switzerland) at 20 µL each. Approximately 24 h after the virtobot sampling procedure (mean 28 ± 15 h), samples from the same body regions were collected during the autopsy (t2). Additionally, heart blood from the left ventricle, urine, gastric content, cerebrospinal fluid and cerebellum were collected and aliquoted into triplicates of 20 µL or 20 mg, respectively. All samples were stored at −20°C until analysis.

Sample preparation and quantitative liquid chromatography tandem mass spectrometry analysis

All samples belonging to one case were extracted and analyzed on the same day. Tissue and body fluid extraction as well as quantitative analysis was performed according to Staeheli *et al.* (22). In short, organ and tissue samples were first homogenized using a Fast Prep®-24 Instrument (MP Biomedicals, Illkirch, France). A two-step liquid–liquid extraction (LLE) was performed with butyl acetate/ethyl acetate (1:1, *v/v*), step 1 at pH 7.4 and step 2 at pH 13.5. After combination of the extracts, all samples were evaporated to dryness and reconstituted in 60 µL mobile phase. Quantitative analysis was carried out on a Thermo Fischer Ultimate 3000 UHPLC system (Thermo Fischer, San Jose, CA, USA) coupled to a Sciex 5500 QTrap linear ion trap quadrupole mass spectrometer (Sciex, Darmstadt, Germany). Instrument settings were adapted from Staeheli *et al.* (22), who previously validated a scheduled multiple reaction monitoring (MRM) method for 83 analytes in 11 postmortem matrices including femoral blood (pB), heart blood (HB), muscle, liver, kidney, spleen, lung, brain and adipose tissue. Validated lower limits of quantification (LLOQ) and linear calibration ranges for the studied analytes exemplified for blood were as follows: methadone 6 ng/mL (6–20,000 ng/mL), EDDP 2 ng/mL (2–16,000 ng/mL), fentanyl 0.8 ng/mL (0.8–2,000 ng/mL), tramadol 40 ng/mL (40–30,000 ng/mL), ODMT 12 ng/mL (12–16,000 ng/mL), codeine 2 ng/mL (2–1,600 ng/mL), oxycodone 2 ng/mL (2–2,000 ng/mL), hydrocodone 0.8 ng/mL (0.8–2,000 ng/mL), dihydrocodeine 12 ng/mL (12–10,000 ng/mL).

Data analysis

Methadone distribution within the body was assessed with the concentration ratio of each matrix to pB. Concentration differences between sampling points t1 and t2 for all analytes were calculated as percentage differences, defining the mean concentration at t1 as 100%. Statistical significance was assigned based on the student's *t*-test (two-tailed distribution, heteroscedastic, $P < 0.05$).

For evaluation of the redistribution mechanisms statistical data treatment was conducted. The percentage concentration change per hour relative to the concentration at t1 was calculated for pB, HB and spleen. Additionally, concentration ratios of adjacent matrices, potentially exhibiting a concentration gradient, were formed. These included ratios for muscle-to-pB, lung-to-HB, gastric content-to-spleen, lung-to-HB and HB (left ventricle)-to-HB (right ventricle). Further, the HB-to-pB ratio (representing the C/P-ratio) was formed. Nonparametric Spearman correlation coefficients (two-tailed, 95% confidence interval) were calculated using GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA, USA).

Results and Discussion

Assessment of time-dependent distribution and PMR of opioids was conducted within 12 cases of methadone intake (with detection of EDDP as metabolite), four cases of fentanyl use, four cases of tramadol ingestion (with detection of ODMT in two cases), and four, four, one and one cases in which codeine, oxycodone, hydrocodone and dihydrocodeine were quantified, respectively. Three cases within the methadone cohort and one case of codeine intake had to be excluded due to the inability to take blood samples at both time points in three cases and a systematic sampling error that occurred in one case. Raw concentrations for all analyzed samples can be found in Table S1 within the Supplementary material. The postmortem interval (PMI) in which all sampling procedures were carried out ranged from 4.5 to 93 h across all evaluated cases. Specific sampling time points and case circumstances as well as other detected drugs are summarized in Table I. The evaluation of the PMR behavior of non-opioid drugs was not part of the current study, so data is not shown. Additionally, alcohol concentrations were not measured in the sampled matrices so information was omitted for data evaluation.

Methadone and EDDP

Concentrations and redistribution of methadone

Methadone concentrations in pB at t1 ranged from 2.0 to 1,700 ng/mL at 4.5 to 59 h postmortem. Distribution across body regions was rather inhomogeneous, with highest concentrations detected in the liver, kidney, lung, gastric content and urine. This supports previous findings of Jantos and Skopp and summarized data by Baselt (14, 23). Muscle tissue concentrations were closest to pB concentrations (Figure 1) in all but one cases (muscle-to-pB-ratio: 18; classed as analytical outlier due to very low overall methadone concentrations) when comparing all sampled alternative matrices. This supports Holm and Linnet (13), who proposed muscle and brain tissue as potential alternative matrices in individual methadone cases where pB is not available.

Time-dependent concentration changes of methadone were evaluated by comparing concentrations at t1 and t2 (Figure 2). In most cases, either a significant increase ($P < 0.05$) or a minimal change in pB methadone concentrations was observed (range −9 to +71%; mean +20%; median +20%). This is suggestive of methadone being prone to PMR—despite observed inter-individual variability—and in line with current literature and its physiochemical properties (lipophilic, high protein binding, pKa 8.6, Vd 4–7 L/kg) (4, 13, 14, 24). When evaluating the concentration changes in the light of postmortem toxicological interpretation, however, no case interpretation had to be altered with respect to methadone concentrations at t1, so time-dependent concentration changes within the studied timeframe can be considered irrelevant.

However, one case showed a 45% decrease of methadone pB concentration over time (case 6; 230–130 ng/mL within 18 h). Compared to the other methadone cases, the latter had a significantly later first sampling point (PMI of 59 h in comparison to 4.5–30 h; Figure 2). It is well recognized that decomposition of a body is a dynamic process that commences almost immediately after death (5). At first, within the fresh stage, the disintegration of cellular membranes and the release of cellular fluids in the body (autolysis) is the main driving force. As early as

Table I. Case circumstances including the postmortem interval (PMI) between death and t1, time between sampling points (dt), cause of death, detected opioid and other drugs detected; cases 7, 8 and 12 were excluded for evaluation

Case	PMI t1 (h)	dt (h)	Cause of death	Opioid	Other detected drugs
1	20	41	Internal bleeding	Methadone	Diazepam
2	30	23	Opioid intoxication	Methadone	Pipamperone, quetiapine, fluoxetine, diazepam
3	16	18	Acute heart failure	Methadone, morphine	Cocaine
4	6	18	Combined opioid and cocaine intoxication	Methadone	Cocaine, zopiclone
5	27	29	Opioid intoxication	Methadone, morphine, 6-MAM	Cocaine, alprazolam, flunitrazepam
6	59	18	Combined drug intoxication	Methadone	Methylphenidate, alprazolam, citalopram, quetiapine, olanzapine, pipamperone
7	11	18	Central respiratory paralysis after combined drug intoxication	Methadone, morphine	Midazolam, diphenhydramine, 2-MAPB, MDAI
8	13	20	Hypoxic brain damage	Methadone, morphine, codeine	Midazolam, cocaine
9	5	23	Combined drug intoxication	Methadone, hydrocodone	Oxazepam, metamizol, fluoxetine
10	4.5	19	Combined drug intoxication	Methadone, morphine, 6-MAM, codeine	Cocaine, diazepam, methylphenidate
11	28	46	Combined drug intoxication	Methadone, morphine, 6-MAM	Citalopram, methylphenidate, trazodone
12	30	16	Combined drug intoxication	Methadone	Diazepam, temazepam
13	63	25	Opioid intoxication	Fentanyl, morphine	Midazolam, citalopram, lorazepam, metamizol
14	42	51	Acute heart failure	Fentanyl, morphine, oxycodone	Zolpidem
15	33	24	Combined drug intoxication	Fentanyl	Cocaine, bupropion, flunitrazepam, levomepromazine, midazolam
16	5	22	Acute heart failure	Fentanyl	
17	13	65	Opioid intoxication	Tramadol	Oxazepam, lorazepam, zopiclone
18	20	20	Acute heart failure	Tramadol	
19	7	67	Central regulatory failure after headshot	Tramadol, oxycodone	Bromazepam, diazepam
20	5	19	Hypoxic brain damage	Tramadol	Lorazepam
21	15	21	Acute heart failure	Codeine, oxycodone	
22	32	23	Combined drug intoxication	Codeine, morphine, 6-MAM	Quetiapine, trazodone
23	28	26	Combined drug intoxication	Oxycodone, morphine	Midazolam, quetiapine

6-MAM, 6-monoacetylmorphine; 2-MAPB, 1-(Benzofuran-2-yl-)-*N*-methylpropan-2amin; MDAI, 5,6-Methylendioxy-2-aminoindan.

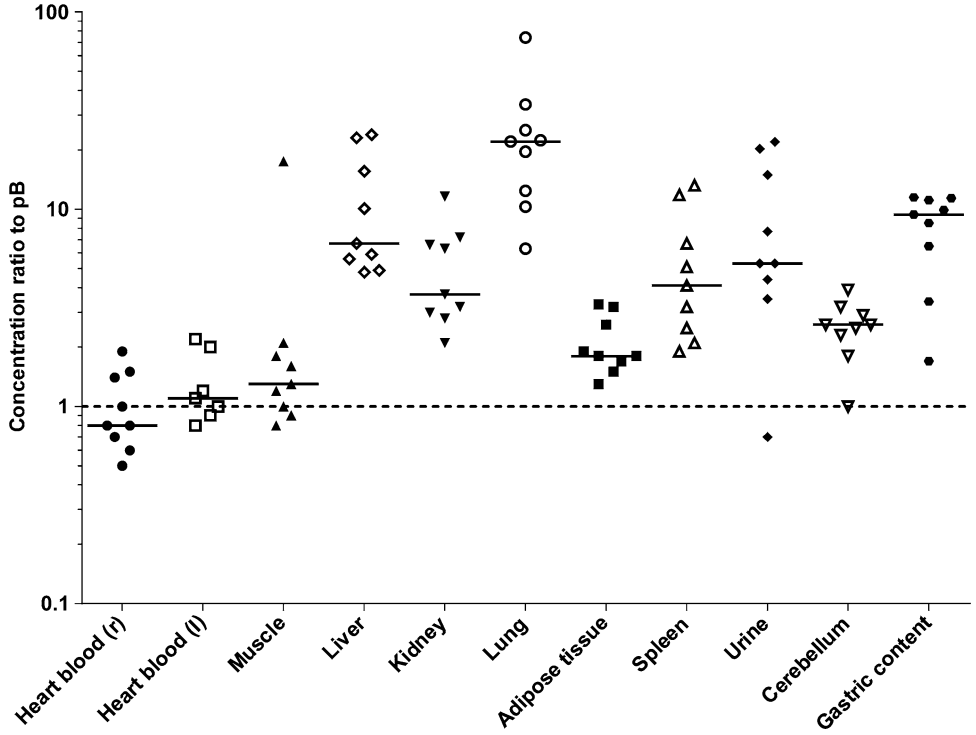

Figure 1. Distribution of methadone across sampled tissues; displayed as concentration ratio to femoral blood (pB); each dot represents one case; the line represents the median ratio; in cases where a concentration ratio at t1 was not available, concentration ratio at t2 was displayed (HB (l), urine, cerebellum and gastric content); the dotted line indicates equal concentration to pB; cases 7, 8 and 12 were excluded for evaluation.

36–72 h after death, the additional destruction of soft tissues by microorganisms, such as bacteria and fungi, becomes the dominant decomposing factor within the bloated stage (25). These different stages of decomposition within the specified timeframe can be brought into play when evaluating the difference between case 6 and the remaining sample cohort. It indicates that, depending on the progress of decomposition, varying concentration change phenomena might be observed. This stresses the importance of having reliable data on time of death and sampling time available to prevent misinterpretation.

In adipose tissue samples, all but one case (case 3; +32%) showed a methadone concentration decrease between t1 and t2 (range: −40 to −13%; mean: −23%; median: −19%). Within the following matrices, methadone concentration increases as well as decreases were observed over time with no clear trend (Figure 2): HB (range: −16 to +78%; mean +23%; median +19%), muscle (range: −85 to +33%; mean: −8%; median: −4%), liver (range: −21 to +29%; mean: +3%; median: +2%), lung (range: −68 to +62%; mean: −4%; median: −11%) and spleen (range: −46 to +28%; mean: +6%; median: +6%). Kidney methadone concentration changes between t1 and t2 also differed within the case-cohort (range: −68 to +29%; mean: −21%; median: −17%), but showed a stronger trend for concentration decreases over time compared to the aforementioned matrices. However, as proposed by Staeheli *et al.* (20), general drug concentration difference between kidney medulla and cortex could be observed . Due to the applied sampling workflow in this study, distinction between cortex and medulla during biopsy sampling was not possible, which might influence the extent of observed time-dependent concentrations changes, leading to potential variations.

Concentrations and redistribution of EDDP

In all but cases 11 of the methadone sample-cohort, the pharmacologically inactive metabolite EDDP has been quantified as well. EDDP pB concentrations at t1 ranged from 1.0 to 195 ng/mL. Similar to methadone, EDDP peak concentrations across the studied matrices were found in kidney, liver and urine in line with Jantos and Skopp (14). Contrary to methadone, less significant ($P < 0.05$) time-dependent concentration changes were observed for EDDP (Figure 3). Despite a visible trend of drug concentration increases over time (range −3 to +206%; mean +51%; median +32%), the current data cannot support a strong tendency for PMR. The most extensive concentration increase between t1 and t2 was observed for case 5 (+206%). However, the overall EDDP concentration in this case was very low (mean over triplicates = 1 ng/mL), so time-dependent concentration changes cannot entirely be attributed to potential PMR, but analytical fluctuation close to the LLOQ has to be considered as a potential reason for variability. Additionally, five other cases showed non-significant concentration changes, as either variability within triplicates were too big or EDDP concentration increases were only minimal. Following this, EDDP time-dependent concentration changes can also be classified as non-relevant for toxicological case interpretation.

Analogous to methadone, the following matrices showed EDDP concentration increases as well as decreases over time with no clear trend: muscle (range: −70 to +82%; mean: +19%; median: +32%), liver (range: −26 to +524%; mean: +85%; median: +17%), kidney (range: −70 to +46%; mean: −17%; median: −19%), lung (range: −53 to +103%; mean −5%; median: −24%), adipose tissue (range: −44 to +183%; mean: +43%; median: +25%), spleen (range: −36 to +87%; mean: +25%; median: +7%). The majority of HB concentrations increased between t1 and t2 (range: +16 to +104%; mean: +34%; median: +20%), with only case 2 decreasing in the studied time period (−24%).

Redistribution mechanisms

Based on the observed significant time-dependent concentration changes of methadone within the sample cohort, potential redistribution mechanisms

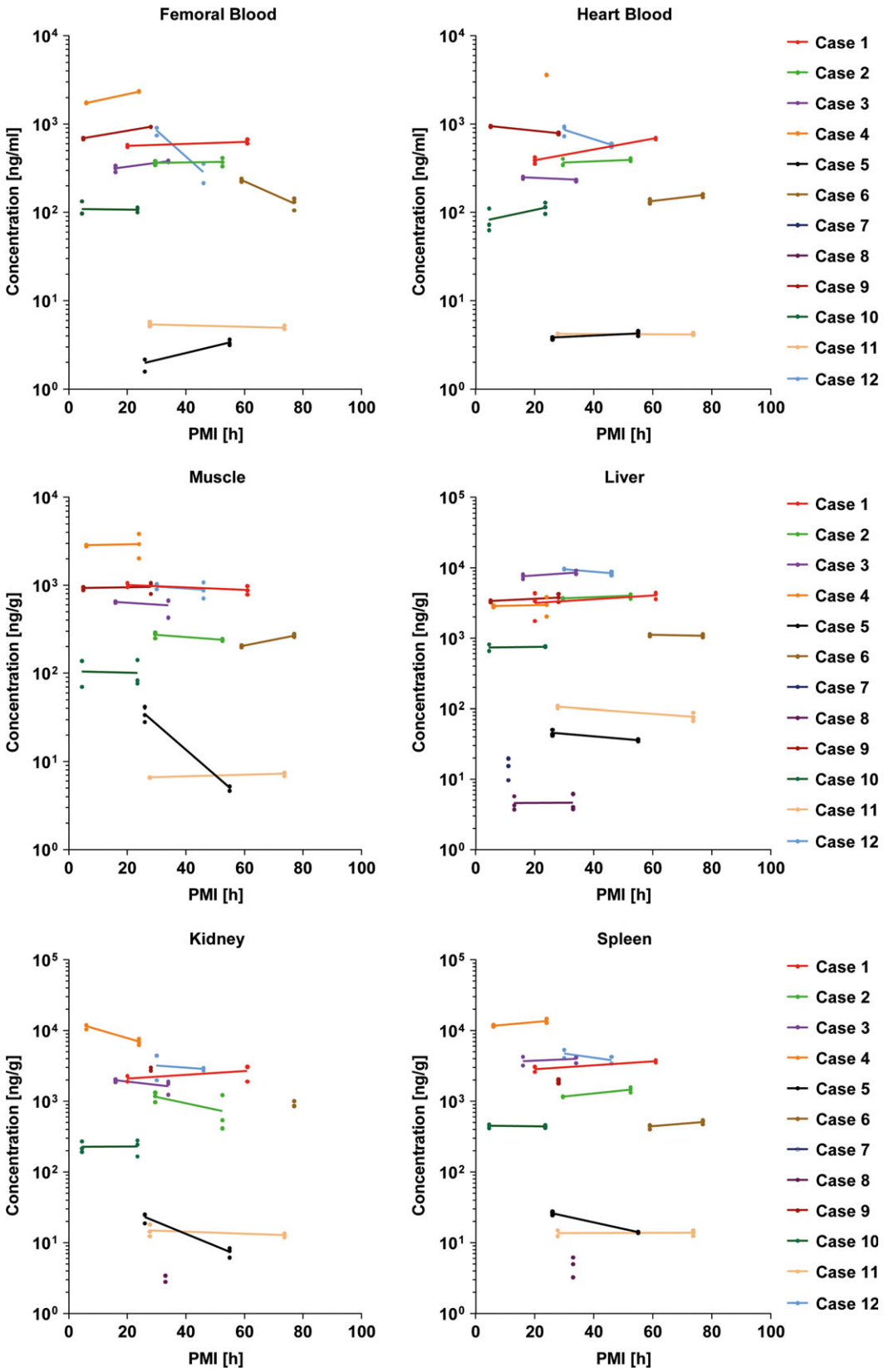

Figure 2. PMR of methadone in femoral blood, heart blood (right ventricle), muscle, liver, kidney, spleen, lung and adipose tissue displayed as concentration vs. postmortem interval (PMI). Each dot represents one sample of the triplicate measurements. The mean concentrations at each sampling time point were connected with a line in each case.

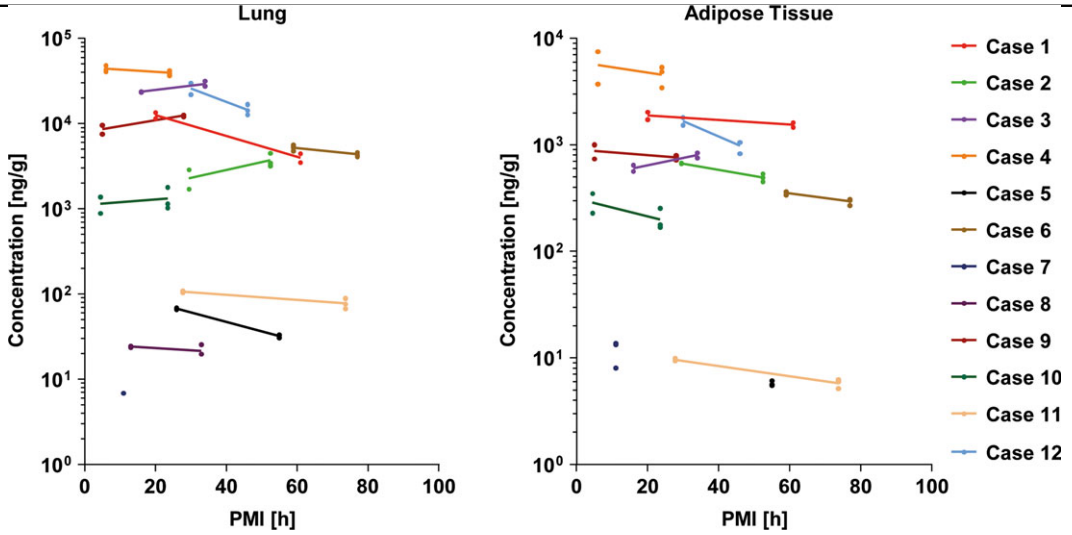

Figure 2. *Continued*

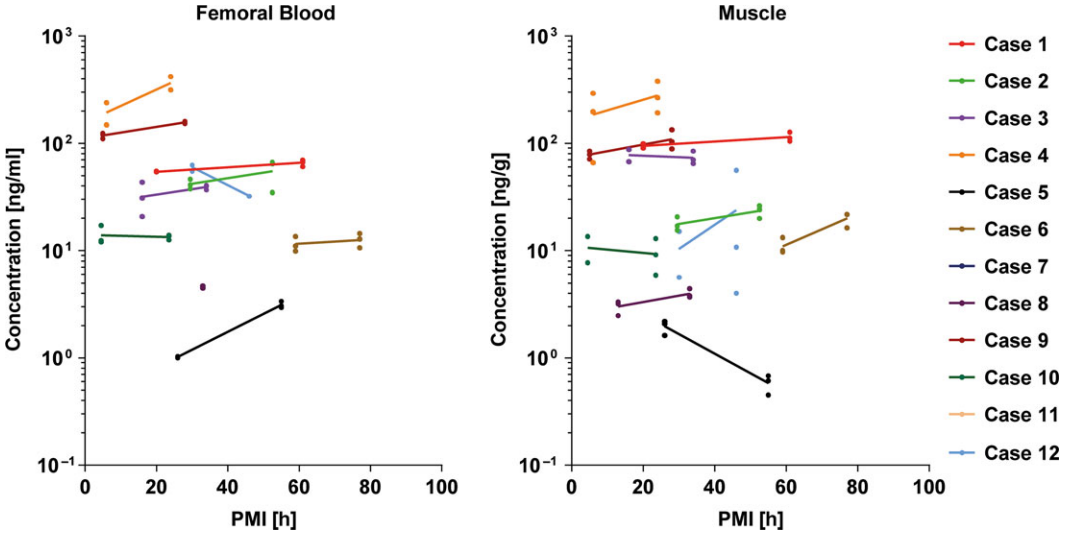

Figure 3. PMR of EDDP in femoral blood and muscle displayed as concentration vs. postmortem interval (PMI). Each dot represents one sample of the triplicate measurements. The mean concentrations at each sampling time point were connected with a line in each case.

were evaluated. A frequently used marker for the potential occurrence of PMR is a C/P-ratio >1 (6). Correlating the C/P-ratio of methadone to the concentration change in pB within the current sample cohort, a strong positive correlation was found (Figure 4a). However, only two cases at t1 showed a C/P-ratio >1 (case 5: 1.95; case 9: 1.36), indicating a weak overall potential of methadone to undergo PMR. This suggests that the C/P- ratio alone might not be a valuable indicator for postmortem concentration changes in case of methadone, as it omits time-dependency.

To evaluate the findings of recent studies, particularly the possibility of methadone being redistributed by passive diffusion should be assessed with the current dataset (14, 16). Concentration ratios of adjacent tissues were correlated to methadone concentration changes (Figure 4). Adapted from Staeheli *et al.* (20), inter-individual PMI differences were compensated by dividing concentration changes through the corresponding sampling interval, despite the fact that linearity of concentration changes across varying PMIs could not be assumed. As a distinct concentration gradient between muscle samples from the upper thigh and pB was found in the majority of cases (Figure 1), observed pB concentration increases over time might have been caused by methadone diffusion from adjacent muscle tissue (1). Spearman correlation of the muscle-to-pB-ratio at t1 against concentration changes in pB over time, supports this hypothesis, as a strong positive correlation was observed (Figure 4b). Even excluding cases with very low overall methadone concentrations (cases 5 and 11) as potential analytical outliers, a strong positive correlation between the two variables was still found. However, diffusion along the blood vessels cannot be excluded as concentrations distal or proximal to the sampling point were not investigated (20). Further sources of PMR that are discussed in literature are concentration gradients leading to potential passive diffusion between lung and cardiac vessels, and from gastrointestinal tract into surrounding organs (e.g., spleen) (1, 16). Spearman correlations were carried out

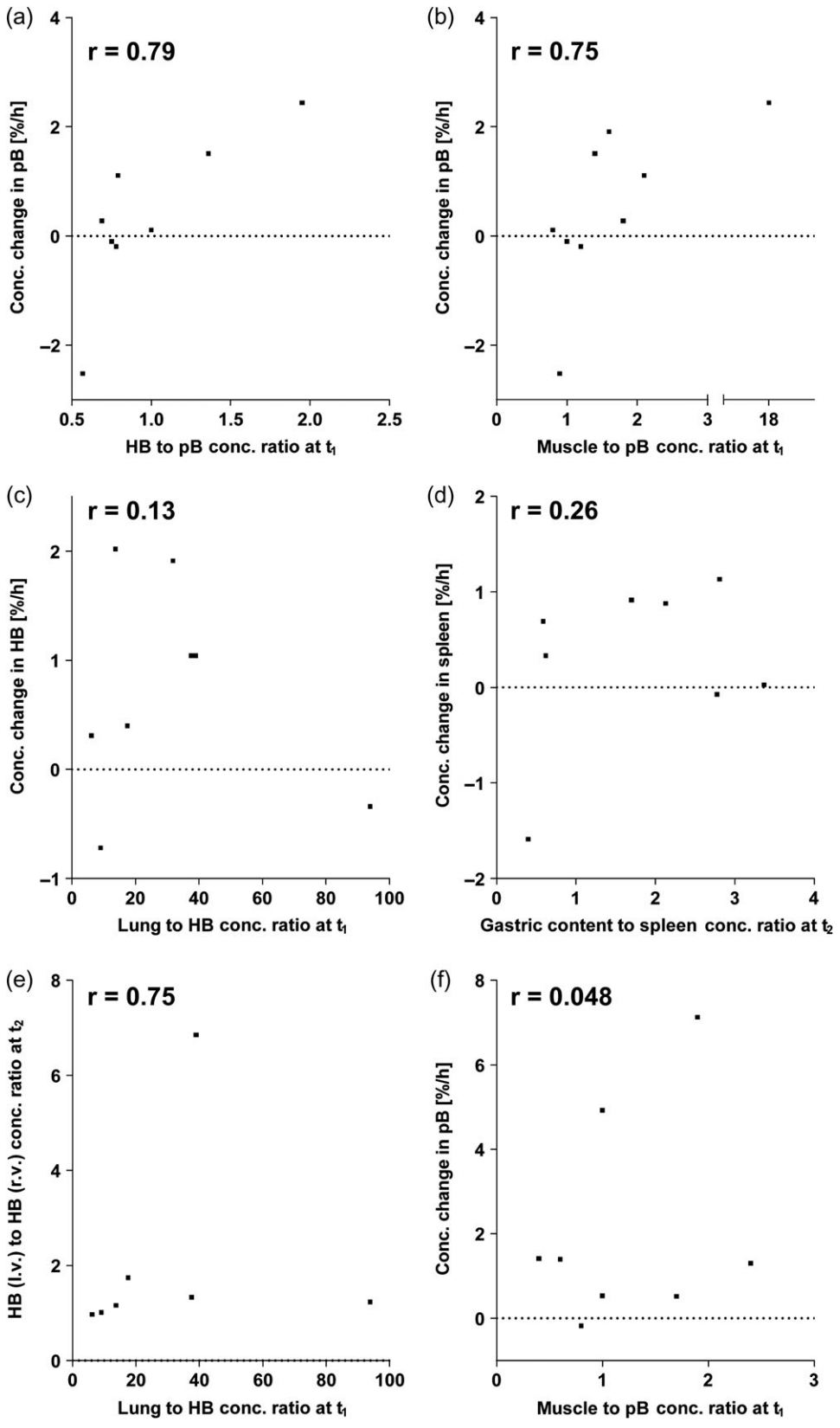

Figure 4. Evaluation of redistribution mechanisms of methadone in femoral blood (pB), displayed as the concentration change in pB [in percent per hour (%/h)] against the C/P-ratio (a) and the muscle-to-pB concentration ratio (b). Evaluation of redistribution mechanisms of methadone in heart blood (HB), displayed as the concentration change in HB against the lung-to-HB concentration ratio (c) and the HB concentration ratio between left and right ventricle against lung-to-HB concentration ratio (e). Further, the methadone concentration change in spleen was displayed against the gastric content-to-spleen ratio (d). Additionally, evaluation of the redistribution mechanism of EDDP in pB is shown as the concentration change in pB against the mucle-to-pB concentration ratio (f).

and are displayed in Figure 4. A very weak positive correlation was found for the concentration ratio between lung and HB against time-dependent concentration changes in HB from the right ventricle (Figure 4c). This suggests redistribution from lung tissue to the right heart ventricle is possible but might not be occurring extensively. In contrast to this, correlation of the lung-to-HB-ratio with the concentration ratio between HB from the left and the right ventricle showed a strong positive correlation (Figure 4e). This supports the theory of redistribution from the lung to the left heart via the pulmonary veins as proposed by Jantos and Skopp (14). Diffusion from gastric content to spleen as a closely situated organ to the stomach also seems possible based on a weak positive correlation found by spearman correlation (Figure 4d), although this is likely to not be relevant in most case interpretations.

In contrast to methadone, spearman correlation showed only a very weak/no potential for EDDP to be redistributed after death from thigh muscle tissue to pB ($r = 0.048$; Figure 4e). Further investigation of distribution mechanisms utilizing spearman correlation showed non-significant correlation between the tested variables (data not shown). This supports the overall finding detailed above, that a strong tendency of EDDP to undergo PMR cannot be supported. Although Jantos and Skopp concluded from their study, that EDDP does undergo PMR similar to methadone, they also proposed a weakness of EDDP to pass cell barriers (14). Based on the blood brain barrier this hypothesis can be supported by the current study, as the EDDP concentrations found in the brain were very low in comparison to the methadone concentration found in the same brain regions (taking into account the overall methadone/EDDP ratio within the body). Nevertheless, besides diffusion processes, putrefaction bacteria can also significantly affect drug concentration (26). The potential effect of these on EDDP time-dependent concentration changes were not studied in the course of this project.

Fentanyl

Within the four evaluated cases of fentanyl use, the concentration range in pB at t1 was between 0.4 and 11.1 ng/mL across a PMI between death and t1 of 5–63 h with 22–51 h between sampling points. The mode of fentanyl application for cases 14 and 15 was a continuous patch, whereas in cases 13 and 16 fentanyl was given during intensive care medical treatment several hours before death. Exact timings are not known, but complete distribution of fentanyl within the body was assumed at time of death. In accordance with the findings of Olson *et al.* (11), fentanyl peak tissue concentrations were found in the liver and kidney. Based on the physiochemical properties of fentanyl (Vd of 3–8 L/kg, pKa of 8.4, 80–86% protein bound, highly lipophilic) (18, 27), an extensive redistribution of the drug after death is expected, which is supported by our findings. As displayed in Figure 5, fentanyl showed significant time-dependent concentration increases in the peripheral blood in three out of four cases (range: +24 to +117%; mean: +62%; median: +74%). This confirms a high tendency for PMR even in peripheral sites as also reported in previous studies (11, 12, 18, 27, 28). Several publications discuss the unsuitability of the C/P-ratio in the context of fentanyl and propose the use of a liver-to-peripheral blood ratio (L/P-ratio) as a better marker to assess PMR (12, 18). This can only be supported in parts with the current study, as a high C/P-ratio of 2.5 at t1 and of 2.0 at t2 was already suggestive of an extensive PMR. However, it has to be stated that the L/P-ratio was considerably higher with 10.1 at t1 and 8.0 at t2, which does not contradict the aforementioned publications.

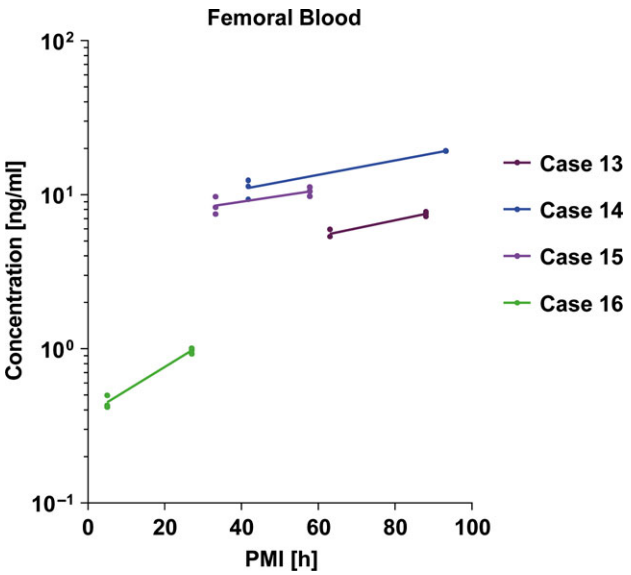

Figure 5. PMR of fentanyl in femoral blood displayed as concentration vs. postmortem interval (PMI). Each dot represents one sample of the triplicate measurements. The mean concentrations at each sampling time point were connected with a line in each case.

Other opioids

Other opioids such as tramadol, codeine, hydrocodone and oxycodone showed no clear trend in terms of PMR within the current evaluated dataset. Significant time-dependent tramadol concentration increases as well as decreases in pB were observed (+32%, +24%, +19%, −51%). In line with Han *et al.* (6), this suggests non-consistent PMR changes. Generally, the literature proposes a low-to-moderate tendency for tramadol to undergo PMR (6, 29, 30), which is in line with the extent of the measured concentration changes. Additionally as proposed by Costa *et al.* (29), a low tendency of ODMT (the main active metabolite of tramadol) for PMR can be concluded for the studied PMI timeframe; ODMT concentration changes of +20, +7 and −2% were detected in pB over time.

Similarly, the four cases positive for codeine in the current sample cohort, showed a concentration increase (+17%) and concentration decreases (−18%, −29%, −46%) in pB between sampling points. In three out of four cases the metabolite morphine was also detected. The PMR of morphine was already evaluated by Staeheli *et al.* and will not be extensively discussed here. Interestingly, morphine was found to undergo significant postmortem changes, which was not observed for codeine, despite structural similarities between the two analytes. Overall only a weak tendency of codeine to undergo time-dependent PMR in three out of four cases was found, although the case number is currently too small to draw definite conclusions. Contrary, the mean C/P-ratio was 2.4, suggesting that codeine might exhibit PMR. A comparable C/P-ratio (2.6) of codeine was previously found by Tolliver *et al.* (31), who concluded that five of their seven studied codeine cases were affected by PMR. The discrepancy between time-dependent concentration changes and measured C/P-ratio again raises the question of the suitability of the C/P-ratio when describing a time-dependent phenomenon like PMR. This is already proposed by Han *et al.* (6), who found a high C/P-ratio for codeine, but pointed out that a high C/P-ratio does not always reflect extensive PMR.

Saitman *et al.* (32) followed the same opinion when stating that there is little agreement as to what the C/P-ratio defines in terms of

PMR. Therefore, they focused on the L/P-ratio, when evaluating the potential for redistribution of hydrocodone. Based on this measure they found a low potential of hydrocodone to exhibit PMR (32). The one hydrocodone case in the current sample cohort supports these findings, as no concentration change in pB over time was observed (c(t1) = 1.9 ng/mL, c(t2) = 1.9 ng/mL) with a C/P-ratio of 1.2 and a L/P-ratio of 1.5 at t1. Dihydrocodeine, a commonly encountered metabolite of hydrocodone, was also detected in the aforementioned case. Here, a non-significant concentration increase between sampling point 1 and 2 of +10% was observed (c(t1) = 28 ng/mL, c(t2) = 31 ng/mL), with a C/P-ratio of 1.6 and a L/P-ratio of 2.2. Based on Saitman *et al.*, this again indicates a low tendency of dihydrocodeine to undergo PMR (32).

To the best of our knowledge, there is currently no publication available that focuses on the likelihood of oxycodone to exhibit PMR. Han *et al.* (6) report one case of oxycodone detection with a C/P-ratio of 1.04. The dataset of the current study includes four oxycodone cases, which showed no consistency in terms of PMR. pB concentrations showed increases as well as decreases over time (+93%, +14%, +10% (below LLOQ), −5%) but no definite conclusion could be drawn due to the small case number.

Limitations of the study

Overall, the current study is not without limitations, which include a small case number per analyte and slightly varying sampling time points across cases. By evaluating the data in a time-dependent manner, the latter was attempted to be corrected for. Further it has to be noted that due to organizational reasons the very early postmortem phase could not be studied (i.e., the timeframe between death and admission to the institute) and storage conditions between t1 and t2 might not accurately represent authentic environmental conditions of a deceased as body's were kept at 7°C between sampling points. Nevertheless, the presented results provide valuable information that aid in the understanding of PMR of opioids and are a unique possibility to study time-dependency of redistribution mechanisms within the human body.

Conclusion

Significant time-dependent methadone concentration increases in pB indicate the occurrence of PMR, however, concentration changes were regarded as not relevant with respect to forensic case interpretation. This also holds true for EDDP, which showed a less significant trend for PMR. Investigating potential redistribution mechanisms, passive diffusion along the muscle-to-pB concentration gradient seems likely for methadone, but not for EDDP. Results for fentanyl support the current literature and suggest that extensive PMR of the drug is expected. Other opioids such as tramadol, codeine, hydrocodone and oxycodone show no consistent trend for significant PMR. Overall, CT-guided biopsy sampling proved to be a valuable tool for the investigation of PMR mechanisms.

Supplementary data

Supplementary material is available at *Journal of Analytical Toxicology* online.

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Compliance with ethical standards

Declaration of no objection for ethical approval was obtained by the cantonal ethics committee of Zurich, Switzerland (KEK Waiver no. 42.2005).

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