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Article

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Methadone, Buprenorphine, Oxycodone, Fentanyl and Tramadol in Multiple Postmortem Matrices

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Abstract

Peripheral blood (PB) concentrations are generally preferred for postmortem toxicological interpretation, but some autopsy cases may lack blood for sampling due to decomposition or large traumas, etc. In such cases, other tissues or bodily fluids must be sampled; however, limited information exists on postmortem concentrations in matrices other than blood. Pericardial fluid (PF), muscle and vitreous humor (VH) have been suggested as alternatives to blood, but only a few studies have investigated the detection of opioids in these matrices. In this study, we aimed to investigate the detection of methadone, buprenorphine, oxycodone, fentanyl and tramadol in postmortem samples of PF, skeletal muscle and VH, in addition to PB and cardiac blood and if drug concentrations in these alternative matrices were comparable to those in PB and thereby useful for interpretation. In most of the 54 included cases, only one opioid was detected. Methadone, oxycodone, fentanyl and tramadol were detected in all of the alternative matrices in almost all cases, while buprenorphine was detected less often. For methadone, the concentrations in the alternative matrices, except in VH, were relatively similar to those in PB. Larger variations in concentrations were found for buprenorphine, oxycodone and tramadol. Quantitative analyses appeared useful for fentanyl, in all of the alternative matrices, but only four cases were included. Toxicological analyses of opioids in these alternative postmortem matrices can be useful for detection, but quantitative results must be interpreted with caution.

Introduction

Opioid use carries significant risk for addiction, morbidity and mortality, causing both an economic and a social burden on any population struggling with an opioid epidemic. In most European countries and in the USA, opioids appear to be the major culprit in drug-related deaths, and several countries have seen an increase in the number of opioid-related deaths over the past years (1–3).

Traditionally, heroin has been held responsible for most opioid-related deaths and still appears to be the main reported drug in such deaths in Europe (2). The long-acting opioids methadone and buprenorphine are used in substitution therapy for opioid addiction, most commonly heroin addiction, but these drugs also carry significant risks for abuse and overdose themselves. France reported more deaths involving methadone than heroin in 2017 (2, 4), and Finland experienced more abuse of buprenorphine than heroin as early as 2002 (5). Similarly, Norway has observed a decrease in deaths involving heroin over the past decade, while deaths related to several prescription opioids, such as oxycodone and methadone, appear to be rising (2). Tramadol, another prescription opioid, was recently shown to be present or implicated in at least 300 drug-induced deaths in Europe (2). Furthermore, both the USA and Australia have reported that deaths with opioids other than heroin are more common and that deaths involving synthetic opioids, like fentanyl (1, 6), are increasing. Monitoring deaths related to opioids is obviously an important part of tracking a country's potential for an opioid epidemic and relies heavily on the analyses and interpretation of postmortem blood samples.

Drug concentrations analyzed in postmortem blood samples are, however, not necessarily representative of those present in blood immediately prior to death. Following death, changes will occur in the body that may alter drug concentrations extensively (7, 8). Most importantly, drugs that are highly concentrated in visceral organs will redistribute to surrounding blood and tissues. This redistribution is most prominent in the thorax and abdomen, due to the locality of many visceral organs in these areas. Consequently, postmortem blood samples are preferentially collected from peripheral parts of the body, like the femoral vein. Many factors, like high temperatures, a long time span between death and autopsy, and trauma, including cardiopulmonary resuscitation, may exacerbate postmortem redistribution (9–13).

Drug concentrations in postmortem peripheral blood (PB) are commonly used to assess a drug's potential for toxicity and involvement in death. For many drugs, the amount of data on PB concentrations has become sufficient to allow for rather strong conclusions to be drawn. There are, however, forensic

autopsy cases where PB is lacking, and in these cases cardiac blood (CB) is often collected. Drug concentrations in CB are generally higher than in PB, due to postmortem redistribution, and CB is therefore considered less suited to estimate drug concentrations prior to death (14). In cases with no available blood, samples must be collected from other matrices. Many alternative tissues and bodily fluids have been suggested (14), but no obvious secondary choice for quantitative analysis has been identified as of yet.

Muscle is one of few matrices that are often available, even in severely decomposed bodies, making it an obvious choice for toxicological analyses in cases lacking blood. One challenge, however, is that muscle is a quite solid and inhomogeneous tissue, which in theory may contribute to larger discrepancies in the measurement of concentrations between muscle and PB than for more liquid matrices. Additionally, drug concentrations may vary between different muscle sampling sites, complicating their usefulness (15). Vitreous humor (VH) is located in a closed compartment and is thus less prone to postmortem redistribution (16). A few studies have compared concentrations in muscle or VH with blood for opioids other than morphine (17–30). Like VH, pericardial fluid (PF) is located in a more protected area but has a larger volume. Even though relatively good correlations have been observed between blood and PF concentrations, there appears to be little data on opioids in this matrix (31–34).

This study was part of a larger research project, from which concentrations of heroin metabolites in samples of PF, skeletal muscle and VH, in addition to PB and CB, collected from forensic autopsy cases, have already been reported (33). Our aim was to investigate the detection of methadone, buprenorphine, oxycodone, fentanyl and tramadol in postmortem samples of these same matrices. We also aimed to investigate whether the drug concentrations measured in these alternative matrices were comparable to those in PB and thereby useful for toxicological interpretation.

Materials and Methods

In this study, the detection of methadone, buprenorphine, oxycodone, fentanyl and tramadol, in multiple postmortem matrices was investigated in 54 autopsy cases. Cases were included based on the detection of these opioids in PB. Samples were also collected from CB, PF, psoas major muscle (PMM), vastus lateralis muscle (VLM) and VH.

Materials

Our study was part of a larger project, where matrices were sampled from 183 forensic autopsies, performed at Oslo University Hospital between June 2013 and November 2016. Upon detection of opioids in PB, analyses for the same opioid(s) were also performed in the other matrices. In this study, 54 cases were included where one or more of the following opioids were detected in PB: methadone ($n = 24$), buprenorphine ($n = 11$), oxycodone ($n = 8$), fentanyl ($n = 4$) and/or tramadol ($n = 11$). In addition, five separate autopsy cases, in which no drugs were detected by screening analysis in PB, were used for evaluation of matrix effects (ME) and extraction recoveries.

Cases were included in the project when the pathologist expected toxicological findings, based on the circumstances. Furthermore, cases were included when all the selected matrices were available. A few cases, however, did not have sufficient VH for the opioid analyses, having already been prioritized for other analyses. Limited case information from each autopsy was available to the study; the requisition form provided by the forensic pathologist generally included a preliminary cause of death and some specific case information (e.g., possible drug use).

Sampling and storage

Autopsies were performed between 0 and 8 days after death. Samples were collected from PB, CB, PF, PMM, VLM and VH. PB samples were collected from the femoral vein. The procedure for collection and preparation of the samples has previously been described in more detail by Øiestad et al. (35). In four of the methadone cases, two of the buprenorphine cases and one of the tramadol cases, there was insufficient VH for opioid analyses. One of the cases had enough VH for methadone analysis, but not for buprenorphine analysis.

Samples of PB, CB, PMM and VLM were collected in 25-mL Sterilin Tubes (Bibby Sterilin, Staffordshire, UK), containing 200 mg of potassium fluoride solution as a preservative. PF and VH (from one or both eyes) were sampled in 5-mL glass BD Vacutainer evacuated tubes (BD Diagnostics, Plymouth, UK), with 20 mg of sodium fluoride and 143 IU of heparin. After arriving at our laboratory, the samples were stored in a refrigerator at 4°C, and analyses were generally performed within 1–2 weeks.

Muscle tissue was homogenized before analysis. In short, approximately 3.5 g of each of the muscle samples were homogenized in approximately 14.0 mL of Type-1 water (18.2 MΩ·cm), obtained from an in-house Milli-Q Biocel from Millipore (Billerica, MA, USA), giving a muscle content of approximately 20% in the homogenate. The reported concentrations in muscle were corrected for the dilution of the samples as previously described by Øiestad et al. (35).

Analytical repertoire and methods

PB samples were screened for approximately 100 psychoactive pharmaceutical drugs and drugs of abuse, including alcohols, opioids, benzodiazepines, amphetamines, new psychoactive substances, anticonvulsants, antidepressants and antipsychotics.

Screening analyses for opioids in PB were performed using a previously published ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS-MS) method (36). Upon detection of methadone, buprenorphine, oxycodone, fentanyl or tramadol in PB, confirmatory analyses were performed in all matrices.

Confirmatory analyses for oxycodone in all matrices were performed by a previously published method (37) with modifications as described by Thaulow et al. for other opioids (33). Confirmatory analyses for fentanyl and buprenorphine were performed according to Berg et al. (38), except for the use of a reduced volume of organic solvent (1 mL) for the extraction. Methadone and tramadol were analyzed in the same manner as fentanyl and buprenorphine (method not published); however, a larger volume (200 µL) was used for solvation of the evaporate, the capillary voltage was set to

3 kV and the desolvation temperature was 650°C. The gradient was also slightly changed: gradient profile (percentage of B); 5% B in 0.0–0.15 min, 5–30% B in 0.15–0.30 min, 30–50% B in 0.30–2.70 min, 50–82% B in 2.70–3.58 min, 82% B in 3.58–4.40 min, 82–98% B in 4.40–4.50 min, 98% B in 4.50–6.30 min, 98–5% B in 6.30–6.40 min and 5% B in 6.40–7.0 min. Methadone-d_3 and ^{13}C-tramadol-d_3 were used as internal standards (ISs). For the first part of the study period (see Tables SI and SII), tramadol was analyzed as described by Amundsen et al. (39). The main active metabolite of tramadol, *O*-desmethyltramadol, was only analyzed by the latter method, and results for *O*-desmethyltramadol were therefore not included in this study. To minimize analytical variation, the samples from all matrices in each case were analyzed in the same analytical series. Analytical results below the lower limits of quantitation (LLOQs) are reported as zero.

LLOQs for the methods were 0.011 mg/L for methadone, 0.00094 mg/L for buprenorphine, 0.0095 mg/L for oxycodone, 0.00014 mg/L for fentanyl and 0.026 mg/L for tramadol. Due to the homogenization with dilution of the muscle samples, the measured concentrations in the homogenates were correspondingly lower than what would have been detected without the dilution. Only muscle homogenate concentrations measured above LLOQs were corrected for the dilution. The negative result in muscle in some of the cases might therefore be an outcome of the dilution, possibly causing a lower detection rate of opioids in the muscle samples.

Method validation

Extraction recoveries and ME in the different matrices were tested at two concentration levels using samples from five blank autopsy cases. ME were evaluated by the post-extraction addition approach (40), comparing peak heights of blank matrix samples spiked after extraction with peak heights of spiked neat standards. ME in percentage were calculated by dividing the mean peak height for the samples spiked after extraction (A) by the mean peak height found for the neat standards (B): $ME = (A/B) \times 100\%$. The neat standards were prepared in the solution used for reconstitution of the extract residue after evaporation. Extraction recovery was calculated by comparing peak heights obtained when compounds were added before and ISs were added after the extraction step, with those obtained when both compounds and ISs were added after extraction.

Statistical analysis

IBM SPSS Statistics 26, Sigma Plot 14.0 and Microsoft Excel were used to analyze the data. Histogram assessments and testing for normality with the Kolmogorov–Smirnov test showed that methadone, buprenorphine, oxycodone, fentanyl and tramadol concentrations were generally not normally distributed in the different matrices; therefore, median and range (minimum – maximum) values are reported, including cases with concentrations below LLOQ (reported as zero). Median concentration ratios for the opioids in the different matrices to PB were calculated, also including cases with concentrations below LLOQ (reported as zero). For matrices where the opioid was not detected in all cases, the median concentration ratios of the positive cases only were also calculated.

To investigate the relationships between the concentrations in PB and the alternative matrices, we utilized Spearman's rank correlation. P-values < 0.05 are reported as significant.

Table I. Methadone Concentrations in PB, CB, PF, PMM, VLM and VH from Autopsy Cases

Case number	PB (mg/L)	CB (mg/L)	PF (mg/L)	PMM (mg/kg)	VLM (mg/kg)	VH (mg/L)	Decomposition[a]
1	4.9	5.8	6.3	5.9	4.4	0.27	0
2	1.6	1.4	2.5	2.6	2.3	0.82	1
3	1.3	0.58	2.0	0.99	0.81	0.26	Missing
4	0.90	0.52	0.62	0.53	0.53	n.a.	1
5	0.67	0.83	0.63	0.56	0.49	0.14	0
6	0.67	0.37	0.98	0.71	0.71	0.093	1
7	0.64	0.58	0.95	0.59	0.57	0.95	0
8	0.60	1.27	1.43	0.97	0.88	0.28	0
9	0.59	0.28	1.12	0.59	0.46	0.18	0
10	0.45	0.54	0.70	0.54	0.42	0.15	0
11	0.44	0.50	0.92	0.42	0.47	0.082	1
12	0.35	0.44	0.53	0.53	0.57	0.083	0
13	0.26	0.15	0.33	0.25	0.17	0.049	0
14	0.24	0.12	0.46	0.20	0.18	0.068	0
15	0.24	0.43	0.98	0.52	0.39	n.a.	1
16	0.23	0.28	0.40	0.33	0.31	0.078	1
17	0.21	0.33	0.46	0.29	0.25	0.095	0
18	0.19	0.19	0.26	0.36	0.22	0.062	0
19	0.19	0.30	0.31	0.23	0.20	0.043	0
20	0.17	0.17	0.25	0.17	0.16	0.036	0
21	0.12	0.28	0.13	0.098	0.13	0.023	0
22	0.086	0.083	0.13	0.093	0.065	n.a.	0
23	0.049	0.057	0.10	0	0.064	n.a.	0
24	0.022	0.048	0.053	0	0	0.014	1

Extent of decomposition is also reported. Cases are sorted by descending concentrations in PB.
[a]0 = no decomposition, 1 = slight decomposition, 2 = moderate decomposition.
n.a. = (matrix) not available.

Table II. Buprenorphine Concentrations in PB, CB, PF, PMM, VLM and VH from Autopsy Cases

Case number	PB (mg/L)	CB (mg/L)	PF (mg/L)	PMM (mg/kg)	VLM (mg/kg)	VH (mg/L)	Decomposition[a]
25	0.020	0.024	0.030	0.12	0.13	0.0074	0
26	0.0088	0.0025	0.014	0.036	0.022	0.0018	1
27	0.0073	0.022	0.051	0	0	0	1
28	0.0034	0.022	0.0070	0.026	0.0091	0	0
29	0.0032	0	0	0	0	0	0
24	0.0022	0.0018	0.0022	0.027	0	n.a.	1
30	0.0012	0.0025	0.0021	0.0049	0.0046	0	Missing
31	0.0010	0.0011	0	0	0	0	0
32	0.0010	0	0	0	0	0	1
33	0.0009	0	0	0	0	n.a.	2
34	0.0009	0.0021	0.0026	0	0	0	2

Extent of decomposition is also reported. Cases are sorted by descending concentrations in PB. Cases with multiple opioids were only given one case number.
[a]0 = no decomposition, 1 = slight decomposition, 2 = moderate decomposition.
n.a. = (matrix) not available.

Table III. Oxycodone Concentrations in PB, CB, PF, PMM, VLM and VH from Autopsy Cases

Case number	PB (mg/L)	CB (mg/L)	PF (mg/L)	PMM (mg/kg)	VLM (mg/kg)	VH (mg/L)	Decomposition[a]
35	0.47	0.064	0.12	0.078	0.078	0.088	1
30	0.45	0.50	0.44	0.64	0.54	0.62	Missing
36	0.36	0.20	0.31	0.22	0.21	0.19	1
37	0.24	0.28	0.28	0.29	0.25	0.28	0
38	0.22	1.1	2.0	0.70	0.38	0.27	1
39	0.071	0.29	0.86	0.11	0.10	0.13	0
40	0.041	0.034	0.054	0.050	0.058	0.027	0
41	0.018	0.018	0.034	0	0	0.13	0

Extent of decomposition is also reported. Cases are sorted by descending concentrations in PB. Cases with multiple opioids were only given one case number.
[a]0 = no decomposition, 1 = slight decomposition, 2 = moderate decomposition.

Ethics

The study was approved by the Regional Committee for Medical Research Ethics (reference number 2012/2173) and by the Higher Prosecuting Authority (reference number 2012/02455). None of the cases in this study were listed in the National Registry of Withdrawal from Biological Research Consent, which allows people to reserve their right not to be included in biological research. From the middle of 2015, the next of kin was also given the opportunity to decline inclusion of the deceased's data into research projects.

Results

In 51 (94%) of the 54 cases, only one of the investigated opioids was detected in PB, while two opioids were detected in two cases and three opioids were detected in one case.

Individual opioid concentrations in PB, CB, PF, PMM, VLM and VH are presented in Tables I–V. We detected methadone, oxycodone, fentanyl and tramadol in all matrices in almost all cases. Buprenorphine was less often detected in the alternative matrices, with a particularly low detection rate in PMM (45%), VLM (36%) and VH (22%). The extent of decomposition is also shown in Tables I–V. Most cases ($n = 48$; 89%) had none or slight decomposition, and three cases (6%) had moderate decomposition. Decomposition was not graded by the forensic pathologist in three cases (Case numbers 3, 30 and 52).

Median opioid concentrations in the different matrices, correlations between concentrations in the alternative matrices and PB (excluding fentanyl due to the low number of cases), and median concentration ratios of the alternative matrices to PB are summarized in Table VI. Median concentration ratios for positive cases only, where relevant, are also presented in Table VI. Significant ($P < 0.001$ for all) and strong (Spearman's $\rho = 0.85$–0.94) correlations between methadone concentrations in PB and the alternative matrices were observed. Correlations for buprenorphine and tramadol were also significant for all matrices, but less strong for some. For oxycodone, no significant correlations were found between the concentrations in PB and any of the alternative matrices. Although most of the median concentration ratios for all of the opioids in the alternative matrices to PB were close to unity, the range in the ratios varied widely.

Individual case concentration ratios of the alternative matrices to PB for the different opioids are presented in Figure 1. The methadone concentration ratios presented within a relatively narrow range, while a larger variation in ratios was observed for buprenorphine, oxycodone and tramadol. For example, several of the buprenorphine cases presented with more than four times higher concentrations in the alternative matrices compared with PB, and three tramadol cases (including one outlier excluded from the figure) had concentrations in PF, which were more than six times higher than in PB. The four fentanyl cases showed quite low variation in the ratios, which can be observed in Figure 1d.

In the vast majority of cases, several additional psychoactive drugs were also detected (results not shown). In two cases, an opioid was the only drug detected. The most commonly detected additional drugs among the methadone

Table IV. Fentanyl Concentrations in PB, CB, PF, PMM, VLM and VH from Autopsy Cases

Case number	PB (mg/L)	CB (mg/L)	PF (mg/L)	PMM (mg/kg)	VLM (mg/kg)	VH (mg/L)	Decomposition[a]
42	0.018	0.018	0.023	0.014	0.018	0.0082	0
43	0.012	0.013	0.029	0.029	0.021	0.0095	1
44	0.011	0.0094	0.012	0.011	0.012	0.0094	0
45	0.0020	0.0032	0.0064	0.0043	0.0044	0.0030	1

Extent of decomposition is also reported. Cases are sorted by descending concentrations in PB. Cases with multiple opioids were only given one case number.
[a]0 = no decomposition, 1 = slight decomposition, 2 = moderate decomposition.

Table V. Tramadol Concentrations in PB, CB, PF, PMM, VLM and VH from Autopsy Cases

Case number	PB (mg/L)	CB (mg/L)	PF (mg/L)	PMM (mg/kg)	VLM (mg/kg)	VH (mg/L)	Decomposition[a]
46	3.6	0.21	0.70	0.89	0.66	n.a.	2
42	3.3	4.5	5.8	3.9	3.2	2.9	0
47	2.8	3.7	4.6	3.8	3.7	3.2	0
48	1.8	2.8	4.1	2.6	1.4	1.6	1
49	1.5	1.4	1.8	1.2	1.4	1.2	0
50	0.67	0.45	0.63	0.46	0.44	0.48	0
51	0.48	1.8	3.2	0.66	0.55	0.51	1
52	0.17	1.7	4.3	0.53	0.14	0.071	Missing
53	0.14	0.14	0.16	0.14	0.14	0.19	0
24	0.090	0.19	0.24	0	0	0.18	1
54	0.040	0.087	0.42	0	0	0.071	0

Extent of decomposition is also reported. Cases are sorted by descending concentrations in PB. Cases with multiple opioids were only given one case number.
[a]0 = no decomposition, 1 = slight decomposition, 2 = moderate decomposition.
n.a. = (matrix) not available.

cases were clonazepam and/or its active metabolite 7-aminoclonazepam ($n = 20$; 83%), morphine ($n = 13$; 54%) and tetrahydrocannabinol ($n = 10$; 42%). Clonazepam and/or 7-aminoclonazepam ($n = 8$; 73%) was also the most common drug among the buprenorphine cases. The additional drugs found in the oxycodone, tramadol and fentanyl cases were quite variable.

Recovery and ME

Extraction recoveries and ME were investigated for all the matrices at two concentration levels in five different samples of each matrix. For the analytical method used to confirm tramadol at the beginning of the study period (old method), one level was investigated. The extraction recoveries for all compounds were 47–91% for blood and PF, 25–76% for muscle and 50–84% for VH, except for tramadol with the new analytical method for which the recoveries varied from 5 to 67%, as shown in Table SI along with the corresponding coefficients of variation (CVs) for each analyte. ME were tested in all six matrices using the post-extraction addition approach and found to be 84–115%. ME calculated both with and without corrections with an IS, as well as the corresponding CV for the corrected matrix effects are shown in Table SII. When calculated against an IS, the ME varied between 97 and 108%, except for tramadol in VH using the old analytical method where the matrix effect was 120%. This method, however, did not use isotope-labeled tramadol as IS. For the ME, the variation given as CV was low, whereas more variability was observed for the extraction recoveries, especially for buprenorphine and tramadol, and the use of stable isotope-labeled ISs is recommended. Based on the results, we believe it is feasible to compare concentrations for different matrices.

Discussion

This study showed that methadone, oxycodone, fentanyl and tramadol could be detected in all of the alternative matrices, in almost all of the included cases. Buprenorphine differed from the other opioids, by being detected less often. In most of the methadone cases, the measured concentrations in different matrices, except in VH, were relatively similar to those in PB. These findings indicate that quantitative analysis of methadone in CB, PF and muscle tissue can be useful. In the cases with buprenorphine, oxycodone and tramadol, we found larger variations in concentrations between the different matrices, indicating that quantitative analysis in matrices other than blood may be less useful for these opioids. For fentanyl, quantitative analyses in all of the investigated matrices appeared useful, but only four cases with fentanyl were included in our study.

Our findings are partly novel, as few studies have investigated the detection of these opioids in multiple matrices, including skeletal muscle and VH. Furthermore, the detection of methadone, buprenorphine and tramadol in PF has, to the best of our knowledge, not been previously investigated. Oxycodone and fentanyl have been investigated in PF in one study, but not previously detected in real-life samples (34).

Methadone

For methadone, we observed median ratios close to unity and strong correlations for both CB and PF with PB, suggesting

Table VI. Positive Cases (*n*) of the Included Cases (*N*) for Five Opioids in Multiple Matrices in 54 Autopsy Cases

	PB	CB	PF	PMM	VLM	VH
Methadone						
n/*N*	24/24	24/24	24/24	22/24	23/24	20/20 (4 missing)
Median (range) concentrations	0.31 (0.022–4.9)	0.35 (0.048–5.8)	0.57 (0.053–6.3)	0.47 (0–5.9)	0.41 (0–4.4)	0.088 (0.014–0.95)
Spearman's ρ (*P*-value)		0.85 (<0.001)	0.90 (<0.001)	0.94 (<0.001)	0.92 (<0.001)	0.88 (<0.001)
Median (range) concentration ratios[a]		1.2 (0.46–2.4)	1.6 (0.68–4.1)	1.0 (0–2.1)	1.0 (0–1.6)	0.26 (0.050–1.5)
Median (range) concentration ratios[b]				1.1 (0.59–2.1)	1.1 (0.59–1.6)	
Buprenorphine						
n/*N*	11/11	8/11	7/11	5/11	4/11	2/9 (2 missing)
Median (range) concentrations	0.0022 (0.0009–0.020)	0.0021 (0–0.024)	0.0022 (0–0.051)	0 (0–0.12)	0 (0–0.13)	0 (0–0.0074)
Spearman's ρ (*P*-value)		0.70 (0.017)	0.72 (0.012)	0.69 (0.019)	0.69 (0.019)	0.73 (0.025)
Median (range) concentration ratios[a]		1.1 (0–6.4)	1.5 (0–7.0)	0 (0–12)	0 (0–6.6)	0 (0–0.37)
Median (range) concentration ratios[b]		1.6 (0.28–6.4)	1.8 (1.0–7.0)	6.0 (4.1–12)	3.3 (2.5–6.6)	0.29 (0.20–0.37)
Oxycodone						
n/*N*	8/8	8/8	8/8	7/8	7/8	8/8
Median (range) concentrations	0.23 (0.018–0.47)	0.24 (0.018–1.1)	0.29 (0.034–2.0)	0.17 (0–0.70)	0.15 (0–0.54)	0.16 (0.027–0.62)
Spearman's ρ (*P*-value)		0.36 (0.39)	0.29 (0.49)	0.48 (0.23)	0.55 (0.16)	0.38 (0.35)
Median (range) concentration ratios[a]		1.1 (0.14–5.1)	1.2 (0.25–12)	1.2 (0–3.2)	1.1 (0–1.7)	1.2 (0.19–7.5)
Median (range) concentration ratios[b]				1.2 (0.17–3.2)	1.2 (0.17–1.7)	
Fentanyl						
n/*N*	4/4	4/4	4/4	4/4	4/4	4/4
Median (range) concentrations	0.012 (0.0020–0.018)	0.011 (0.0032–0.018)	0.018 (0.0064–0.029)	0.012 (0.0043–0.029)	0.015 (0.0044–0.021)	0.0088 (0.0030–0.0095)
Median (range) concentration ratios		1.0 (0.84–1.6)	1.8 (1.1–3.2)	1.6 (0.75–2.3)	1.4 (1.0–2.2)	0.80 (0.45–1.5)
Tramadol						
n/*N*	11/11	11/11	11/11	9/11	9/11	10/10 (1 missing)
Median (range) concentrations	0.67 (0.040–3.6)	1.4 (0.087–4.5)	1.8 (0.16–5.8)	0.66 (0–3.9)	0.55 (0–3.7)	0.50 (0.071–3.2)
Spearman's ρ (*P*-value)		0.66 (0.026)	0.64 (0.035)	0.88 (<0.001)	0.88 (<0.001)	0.92 (<0.001)
Median (range) concentration ratios[a]		1.4 (0.06–10)	1.8 (0.19–26)	1.1 (0–3.2)	0.86 (0–1.3)	0.99 (0.43–2.0)
Median (range) concentration ratios[b]				1.2 (0.25–3.2)	0.89 (0.18–1.3)	

Median and range (minimum – maximum) concentrations and concentration ratios (matrix/PB) are reported. Concentration ratios for positive cases only are also reported, where applicable. Correlations (Spearman's ρ and *P*-value) between concentrations in alternative matrices and PB are reported for all drugs, except for fentanyl. Concentrations are reported in mg/L for PB, CB, PF and VH and in mg/kg for PMM and VLM.

[a]Concentration ratios including all values.

[b]Concentration ratios including positive cases only.

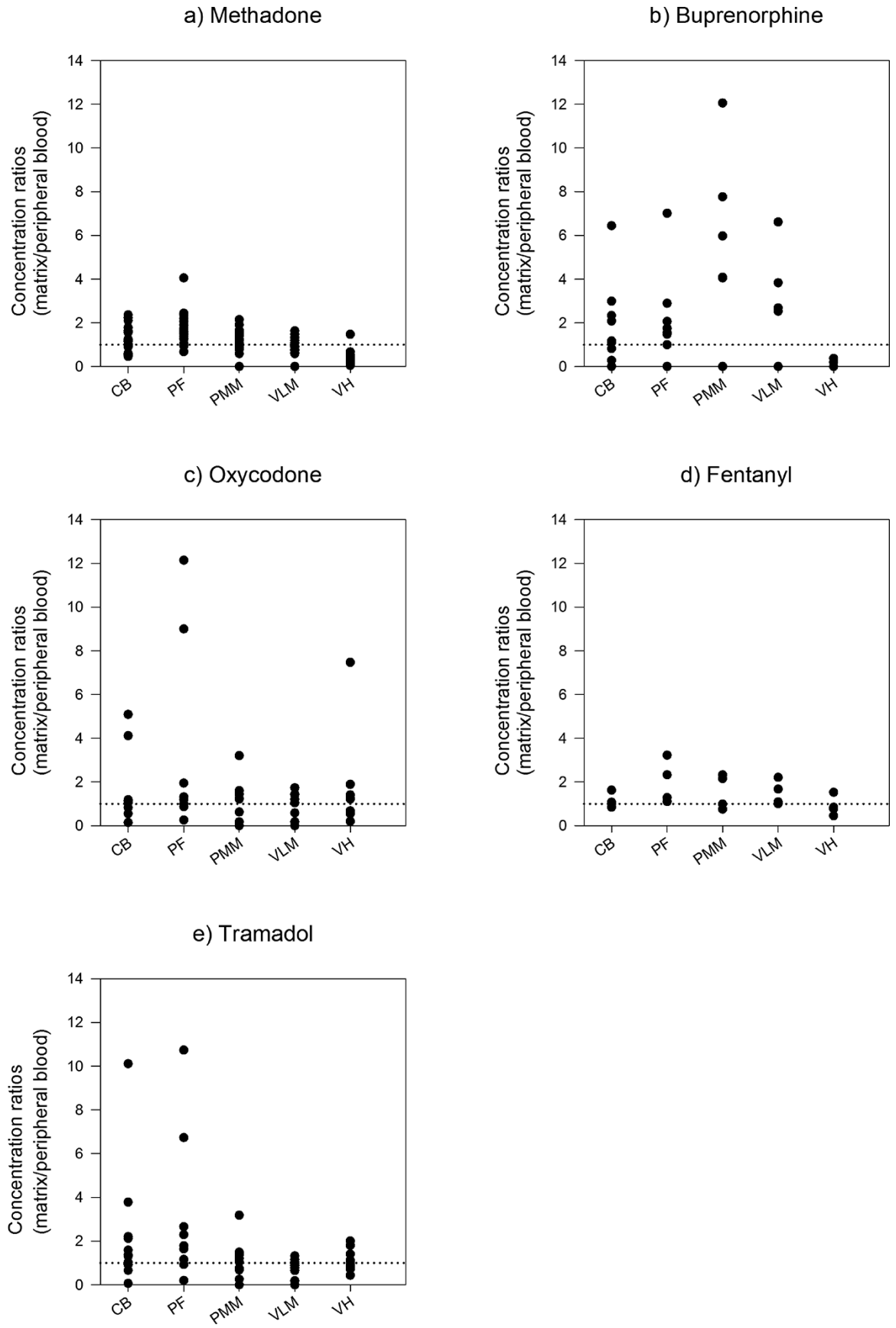

Figure 1. Concentration ratios (matrix/PB) for (a) methadone, (b) buprenorphine, (c) oxycodone, (d) fentanyl and (e) tramadol in 54 autopsy cases. The dots represent individual concentration ratios for CB, PF, PMM, VLM and VH, in each case. The dotted line represents a concentration ratio of one. One outlier in Figure 1e, with a PF/PB concentration ratio of 26, was excluded for better visualization.

these matrices as adequate alternatives to PB for the quantification of methadone in autopsy cases. A number of studies have also investigated methadone concentrations in CB (9, 26, 28, 29, 41–43). Like us, these studies found CB concentrations that were both higher and lower than those observed in PB, and, in general, an average CB to PB ratio above one. Jantos and Skopp (26) have suggested postmortem release of methadone from lung tissue as a probable explanation for this observation.

In muscle, the median methadone concentration ratios were close to unity with strong correlations for both muscle samples with PB, which has also been observed in previous studies including muscle samples from the same locations as our study (28, 29). Another study, however, found a lower median concentration in muscle than in PB, but did not disclose which muscles were sampled (26). It should be noted that methadone was not detected in one or both muscle samples in two of the methadone cases included in our study. These two cases had the lowest methadone concentrations in PB, and it is likely that the dilution of the muscle samples, in the homogenization process before analysis, contributed to the lack of detection of methadone in muscle.

Methadone concentrations were lower in VH than in PB in all cases but one, similar to findings in three previous studies (17, 21, 30), although these studies only included a total of seven cases with VH. Methadone is highly protein bound in blood, at 85–90% (44), which could explain the lower concentrations observed in VH. Only the free fraction of a drug can distribute from blood to VH. Drug concentrations measured in VH are therefore more likely to correlate with the free fraction of the drug in blood (16, 45). In addition, a lag in the transport across the blood–vitreous barrier and an accumulation of the drug in VH with regular use have been suggested for other drugs, like morphine (46), which may also be the case for methadone. Our findings indicate that VH can be suited for qualitative detection of methadone but that measured concentrations do not necessarily reflect concentrations in PB.

Buprenorphine

The reason for the low detection rate of buprenorphine in the alternative matrices is not obvious. One explanation could be that buprenorphine is a potent opioid and therefore normally present in low concentrations in blood. In our study, the concentrations of buprenorphine in PB were only slightly above the LLOQ in 5 of the 11 cases. Our finding of a lower number of cases with buprenorphine than with methadone is somewhat surprising, since the use of buprenorphine is more common than methadone in opioid maintenance treatment in Norway (47). There are also more people using buprenorphine than methadone for pain management in Norway (48), but the blood concentrations observed with this indication are usually very low and often below the LLOQ used in our study (49, 50).

Buprenorphine is highly protein bound in blood, at 96% (44), which likely contributed to the low detection in VH. When buprenorphine was detected in the alternative matrices the concentration ratios were also quite variable, suggesting that these alternative matrices may be less useful, both for detection and quantification of buprenorphine. Very few studies have addressed postmortem concentrations of buprenorphine in multiple matrices, and, to the best of our knowledge, none have studied the alternative matrices included in our study.

Oxycodone

Although oxycodone was detected in all of the alternative matrices in most of the cases, we found a large range in the concentration ratios and no significant correlations between the concentrations in the different matrices and PB. Interestingly, one case (number 35) presented with a much higher concentration in PB than in any of the alternative matrices, but the reason for this pattern remains unclear. Still, for many of the other cases, the concentrations in the various matrices were close enough to those in PB to provide similar toxicological interpretation. For example, in five of the eight cases the muscle sample concentrations gave relatively good indications of those observed in PB. One study on postmortem oxycodone also found quite similar results for PB, CB and muscle, but reported concentrations from these matrices in only two cases (29). Oxycodone is 45% protein bound in blood (44), which is less than most of the other opioids, and could have contributed to the higher median concentration ratio observed for VH, compared with several of the other opioids. One previous study, which included 36 cases with CB and 7 cases with VH (20), and another study, which included 30 cases with VH (24), found variable results. The number of oxycodone cases in our study was only eight, and the number of previous studies on oxycodone is low, warranting further studies in alternative matrices for this drug.

Fentanyl

Fentanyl was detected in all of the alternative matrices in the four cases included in our study. Fentanyl is reported to be at least 80 times more potent than morphine (51), and it is normally detected in very low concentrations in blood. To overcome the challenge of low concentrations, the LLOQ (0.00014 mg/L) for fentanyl in our method was particularly low.

Fentanyl is highly lipophilic, meaning that the drug distributes easily across membranes (52), which could explain the relatively similar concentrations in blood, muscle and PF in all of our cases. On the other hand, fentanyl is highly protein bound, at 80–86% (44), which could be the reason for the slightly lower concentrations observed in VH in three of the four cases; similar to what has previously been observed in one study with three cases (30). Fentanyl may exhibit substantial postmortem redistribution into blood, including PB (29). Our study, however, was not designed to evaluate which matrix may best represent antemortem concentrations.

Tramadol

Tramadol was detected in all of the alternative matrices in all of the tramadol cases, except for in muscle in the two cases with the lowest tramadol concentrations in PB. The lower detection in muscle could, as previously discussed for methadone, be explained by the homogenization process, which results in a dilution of the samples.

The correlations between the concentrations measured in the alternative matrices and PB were significant and relatively strong for tramadol. Although the median concentration ratios for all of the matrices were close to unity, there

were also a few cases with large discrepancies. Similar to what has been previously reported (53–55), we found a median CB to PB concentration ratio of 1.4. One study, however, found a somewhat lower median CB to PB ratio (56), while another study reported a single tramadol case with a much higher central blood to PB ratio of 4.9 (22). In our study, we also had one case (number 52) with a very high CB to PB ratio of 10, demonstrating that large variations in ratios can occur and must be kept in mind during interpretation.

The tramadol concentrations in VH generally corresponded well with those in PB, as previously observed in a total of five cases in two studies (22, 30). These results could perhaps be explained by tramadol being only 20% protein bound in blood (44). Unfortunately, the case with the highest tramadol concentration in PB (case number 46), which had very large discrepancies in concentrations between PB and the alternative matrices, did not have enough VH for analysis. This signifies why the small volume is an important limitation of VH as an alternative matrix. To the best of our knowledge, only one case study has reported a tramadol concentration in muscle (22) and none have studied tramadol in PF.

Limitations

In this study, we only included cases where all of the alternative matrices were available for sampling, thereby excluding cases lacking blood (e.g., severely decomposed cases). It is uncertain to what degree our results are applicable to these latter types of cases.

The opioid concentrations were investigated independently of cause of death and circumstantial information. It should, however, be noted that the forensic pathologists selected the cases for this study upon suspicion of drug detection, which may have resulted in a higher share of cases with polydrug use being included than otherwise would have been observed in a more general population. Still, our finding of polydrug use in the vast majority of cases has also been observed in other studies (57, 58). The presence of additional drugs is not considered to affect the distribution of opioids between the matrices and is therefore not expected to influence our results.

For all of the opioids but methadone, there were a limited number of cases, especially for fentanyl, which was only detected in four cases. Similar results for opioids with few cases are not necessarily representative of all types of cases and must be applied with caution. Further studies are needed to provide firmer conclusions to assist with interpretation of these opioids in alternative postmortem matrices.

Conclusions

Our study shows that CB, PF, PMM, VLM and VH can be useful for qualitative detection of methadone, oxycodone, fentanyl and tramadol. For buprenorphine, there seems to be a considerable risk of not detecting the drug in these alternative matrices even when the drug is present in PB.

For methadone and fentanyl, quantitative results in these alternative matrices, except for methadone in VH, may also be useful, but larger studies are required to elucidate these suggestions further. For the other investigated opioids, the measured concentrations were generally more variable. Nevertheless, quantitative results from the alternative matrices could provide for similar interpretation to those of PB in many of the cases.

Toxicological analyses of opioids in alternative postmortem matrices can be useful for detection, but any interpretation of a quantitative result must be performed with considerable caution.

Supplementary data

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

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Data Availability Statement

The data that support the findings of this study are presented in the manuscript.

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