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 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
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, psos 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 VH were collected in 25-μL 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-μL 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, anti-
convulsants, antidepressants and antipsychotics.

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

Confirmatory analyses for oxycodone in all matrices were per-
formed 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-d3 and 13C-tramadol-d3
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-desmethy tramadol, was only analyzed by the latter method,
and results for O-desmethy tramadol 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 per-
centage 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) × 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 test-
ing for normality with the Kolmogorov–Smirnov test showed that
methadone, buprenorphine, oxycodone, fentanyl and tramadol con-
centrations were generally not normally distributed in the different
matrices; therefore, median and range (minimum – maximum) val-
ues 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 concen-
tration 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 correla-
tion. P-values < 0.05 are reported as significant.

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 Table VI. We detected methadone, oxycodone,
fentanyl and tramadol in all matrices in almost all cases. Buprenor-
phine 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, corre-
lations 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 ρ = 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 PB, which were more than six times higher than in PB. The four
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        | 0.14      | 0                |
| 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        | 0.59        | 0.57      | 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.      | 0                |
| 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           | 0.064     | n.a.             |
| 24          | 0.022     | 0.048     | 0.053     | 0           | 0           | 0         | 0.014            |

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                |
| 30          | 0.0022    | 0.0018    | 0.0022    | 0.027       | 0           | n.a.      | 0                |
| 31          | 0.0012    | 0.0025    | 0.0021    | 0.0049      | 0.0046      | 0         | Missing          |
| 32          | 0.0010    | 0.0011    | 0         | 0           | 0           | 0         | 0                |
| 33          | 0.0010    | 0         | 0         | 0           | 0           | 0         | 1                |
| 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.

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 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...
### 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* |
|-------------|-----------|-----------|-----------|-------------|-------------|-----------|---------------|
| 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        | 0.26        | 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        | 0.38        | 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.

*0 = no decomposition, 1 = slight decomposition, 2 = moderate decomposition.

### 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* |
|-------------|-----------|-----------|-----------|-------------|-------------|-----------|---------------|
| 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.

*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* |
|-------------|-----------|-----------|-----------|-------------|-------------|-----------|---------------|
| 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             |
| 48          | 2.8       | 3.7       | 4.6       | 3.8         | 3.7         | 3.2       | 0             |
| 49          | 1.8       | 2.8       | 4.1       | 2.6         | 1.4         | 1.6       | 1             |
| 50          | 1.5       | 1.4       | 1.8       | 1.2         | 1.4         | 1.2       | 0             |
| 51          | 0.67      | 0.45      | 0.63      | 0.46        | 0.44        | 0.48      | 0             |
| 52          | 0.48      | 1.8       | 3.2       | 0.66        | 0.55        | 0.51      | 1             |
| 53          | 0.17      | 1.7       | 4.3       | 0.53        | 0.14        | 0.071     | Missing       |
| 54          | 0.14      | 0.14      | 0.16      | 0.14        | 0.14        | 0.19      | 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.

*0 = no decomposition, 1 = slight decomposition, 2 = moderate decomposition.

n.a. = (matrix) not available.

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.
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<sup>a</sup> | 1.2 (0.46–2.4) | 1.6 (0.68–4.1) | 1.0 (0–2.1) | 1.1 (0.59–2.1) | 1.1 (0.59–1.6) |      |
| Median (range) concentration ratios<sup>b</sup> |      |      |      |      |      |      |
| **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) | 0.69 (0.019) | 0.73 (0.025) |
| Median (range) concentration ratios<sup>a</sup> | 1.1 (0–6.4) | 1.5 (0–7.0) | 0 (0–12) | 1.1 (0–3.2) | 1.1 (0–1.7) | 1.2 (0.19–7.5) |
| Median (range) concentration ratios<sup>b</sup> | 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<sup>a</sup> | 1.1 (0.14–5.1) | 1.2 (0.25–12) | 1.2 (0–3.2) | 1.1 (0–1.7) | 1.2 (0–1.7) |      |
| Median (range) concentration ratios<sup>b</sup> | 1.2 (0.17–3.2) | 1.2 (0.17–3.2) |      |      |      |      |
| **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) | 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<sup>a</sup> | 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<sup>b</sup> | 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.

<sup>a</sup>Concentration ratios including all values.

<sup>b</sup>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.
Methadone
For methadone, we observed median ratios close to unity and strong correlations for both CB and PF with PB, suggesting 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.

References
1. Centers for Disease Control and Prevention. (2020) Opioid Overdose. https://www.cdc.gov/drugoverdose/epidemic/index.html (accessed Dec 20, 2020).
2. European Monitoring Centre for Drugs and Drug Addiction. (2019) Drug-Related Deaths and Mortality in Europe. https://www.emcdda.europa.eu/publications/srapid-communications/dru RELATED-DEATHS-IN-EUROPE-2018 (accessed Dec 20, 2020).
3. European Monitoring Centre for Drugs and Drug Addiction. (2020) European Drug Report 2020: Trends and Developments. https://www.emcdda.europa.eu/publications/cdr/trends-developments/2020_en (accessed Dec 20, 2020).
4. European Monitoring Centre for Drugs and Drug Addiction. (2017) European Drug Report 2017: Trends and Developments. http://www.emcdda.europa.eu/cdr/2017_en (accessed Dec 20, 2020).
5. Partanen, A., Mäki, J. (2004) Buprenorphine more common as a problem drug in Finland. Nordic Studies on Alcohol and Drugs, 21, 156–161.
6. Australian Institute of Health and Welfare. (2020) Alcohol, Tobacco & Other Drugs in Australia. https://www.aihw.gov.au/reports/alcohol/alcohol-tobacco-other-drugs-australia/contents/impacts/health-impacts (accessed Dec 20, 2020).
7. Pounder, D.J., Jones, G.R. (1990) Post-mortem drug redistribution—a toxicological nightmare. Forensic Science International, 45, 253–263.
8. Pelissier-Alicot, A.L., Gaulier, J.M., Champsaur, P., Marquet, P. (2003) Mechanisms underlying postmortem redistribution of drugs: a review. Journal of Analytical Toxicology, 27, 533–544.
9. Prouty, R.W., Anderson, W.H. (1990) The forensic science implications of site and temporal influences on postmortem blood-drug concentrations. Journal of Forensic Sciences, 35, 243–270.
10. Hilberg, T., Rogde, S., Morland, J. (1999) Postmortem drug redistribution—human cases related to results in experimental animals. Journal of Forensic Sciences, 44, 3–9.
11. Skopp, G. (2004) Preanalytic aspects in postmortem toxicology. Forensic Science International, 142, 75–100.
12. Kennedy, M.C. (2010) Post-mortem drug concentrations. Internal Medicine Journal, 40, 183–187.
13. Gerostamoulos, D., Beyer, J., Staikos, V., Taylor, P., Woodford, N., Drummer, O.H. (2012) The effect of the postmortem interval on the redistribution of drugs: a comparison of mortuary admission and autopsy blood specimens. Forensic Science, Medicine, and Pathology, 8, 373–379.
14. Drummer, O.H., Gerostamoulos, J. (2002) Postmortem drug analysis: analytical and toxicological aspects. Therapeutic Drug Monitoring, 24, 199–209.
15. Langford, A.M., Taylor, K.K., Pounder, D.J. (1998) Drug concentration in selected skeletal muscles. Journal of Forensic Sciences, 43, 22–27.
16. Bevalot, F., Cartiser, N., Bottinelli, C., Fanton, L., Guittton, J. (2016) Vitreous humor analysis for the detection of xenobiotics in forensic toxicology: a review. Forensic Toxicology, 34, 12–40.
17. Ziminski, K.R., Wemyss, C.T., Bidanseat, J.H., Manning, T.J., Lukash, L. (1984) Comparative study of postmortem barbiturates, methadone, and morphine in vitreous humor, blood, and tissue. Journal of Forensic Sciences, 29, 903–909.
18. Drummer, O.H., Syrris, M.L., Phelan, M., Cordner, S.M. (1994) A study of deaths involving oxycodone. *Journal of Forensic Sciences*, 39, 1069–1075.

19. Anderson, D.T., Muto, J.J. (2000) Duragesic transdermal patch: post-mortem tissue distribution of fentanyl in 25 cases. *Journal of Analytical Toxicology*, 24, 627–634.

20. Anderson, D.T., Fritz, K.L., Muto, J.J. (2002) Oxycontin: the concept of a “ghost pill” and the postmortem tissue distribution of oxycodone in 36 cases. *Journal of Analytical Toxicology*, 26, 448–459.

21. Couper, F.J., Chopra, K., Pierre-Louis, M.L. (2005) Fatal methadone intoxication in an infant. *Forensic Science International*, 153, 71–73.

22. Bynum, N.D., Poklis, J.A., Gaffney-Mraft, M., Garsiade, D., Roper-Miller, J.D. (2005) Postmortem distribution of tramadol, amitryptiline, and their metabolites in a suicidal overdose. *Journal of Analytical Toxicology*, 29, 401–406.

23. Coompan, V., Cordonnier, J., Pien, K., Van Varenbergh, D. (2007) LC-MS/MS analysis of fentanyl and norfentanyl in a fatality due to application of multiple duraogesic transdermal therapeutic systems. *Forensic Science International*, 169, 223–227.

24. Knitel, J.L., Clay, D.J., Bailey, K.M., Gebhardt, M.A., Kramer, J.C. (2009) Comparison of oxycodone in vitreous humor and blood using EMIT screening and gas chromatographic-mass spectrometric quantitation. *Journal of Analytical Toxicology*, 33, 433–438.

25. Olson, K.N., Luckenbill, K., Thompson, J., Middleton, O., Geiselhart, R., Mills, K.M., et al. (2010) Postmortem redistribution of fentanyl in blood. *American Journal of Clinical Pathology*, 133, 447–453.

26. Jantos, R., Skopp, G. (2013) Postmortem blood and tissue concentrations of R- and S-enantiomers of methadone and its metabolite EDDP. *Forensic Science International*, 226, 254–260.

27. Poklis, J., Poklis, A., Wolf, C., Mainland, M., Haiz, L., Devers, K., et al. (2015) Postmortem tissue distribution of acetyl fentanyl, fentanyl and their respective nor-metabolites analyzed by ultrahigh performance liquid chromatography with tandem mass spectrometry. *Journal of Analytical Toxicology*, 25, 435–441.

28. Holm, K.M., Linnet, K. (2015) Distribution of enantiomers of methadone and its main metabolite EDDP in human tissues and blood of postmortem cases. *Journal of Forensic Sciences*, 60, 95–101.

29. Brockbals, L., Stæheli, S.N., Gascho, D., Ebert, L.C., Kraemer, T., Steuer, A.E. (2018) Time-dependent postmortem redistribution of opioids in blood and alternative matrices. *Journal of Analytical Toxicology*, 42, 365–374.

30. Isikierka, M., Zawadzki, M., Sipot, P., Jurek, T. (2021) Detection of drugs in postmortem specimens of blood, vitreous humor and bone marrow aspirate. *Journal of Analytical Toxicology*, 45, 348–355.

31. Monya, F., Hashimoto, Y. (1999) Pericardial fluid as an alternative specimen to blood for postmortem toxicological analyses. *Legal Medicine*, 1, 86–94.

32. Tominaga, M., Mochue, T., Ishikawa, T., Kawamoto, O., Oritani, S., Ikeda, K., et al. (2013) Postmortem analyses of drugs in pericardial fluid and bone marrow aspirate. *Journal of Analytical Toxicology*, 37, 423–429.

33. Thaulow, C.H., Øststad, Å.M.L., Rogde, S., Karinen, R., Brochmann, G.W., Andersen, J.M., et al. (2018) Metabolites of heroin in several different post-mortem matrices. *Journal of Analytical Toxicology*, 42, 311–320.

34. Ferreira, E., Corté Real, E., Pinho, E.M.T., Margallo, C. (2020) A novel bioanalytical method for the determination of opioids in blood and pericardial fluid. *Journal of Analytical Toxicology*, 44, 754–768.

35. Øststad, Å.M.L., Karinen, R., Rogde, S., Nilsen, S., Boye Eldor, K.B., Brochmann, G.W., et al. (2018) Comparative study of postmortem concentrations of antidepressants in several different matrices. *Journal of Analytical Toxicology*, 42, 446–458.

36. Øststad, E.L., Johansen, U., Øststad, Å.M., Christophersen, A.S. (2011) Drug screening of whole blood by ultra-performance liquid chromatography-tandem mass spectrometry. *Journal of Analytical Toxicology*, 35, 280–293.

37. Berg, T., Lundanes, E., Christophersen, A.S., Strand, D.H. (2009) Determination of opiates and cocaine in urine by high pH mobile phase reversed phase UPLC-MS/MS. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 877, 421–432.

38. Berg, T., Jørgenrud, B., Strand, D.H. (2013) Determination of buprenorphine, fentanyl and LSD in whole blood by UPLC-MS/MS. *Journal of Analytical Toxicology*, 37, 159–165.

39. Amundsen, I., Øiestad, Å.M., Ekeberg, D., Kristoffersen, L. (2013) Quantitative determination of fifteen basic pharmaceuticals in ante- and post-mortem whole blood by high pH mobile phase reversed phase ultra high performance liquid chromatography-tandem mass spectrometry. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 927, 112–123.

40. Matuszewski, B.K., Constantz, M.L., Chavez-Eng, C.M. (2003) Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Analytical Chemistry*, 75, 3019–3030.

41. Levine, B., Wu, S.C., Dixon, A., Smialek, J.E. (1995) Site dependence of postmortem blood methadone concentrations. *The American Journal of Forensic Medicine and Pathology*, 16, 97–100.

42. Lemaire, E., Schmidt, C., Denooz, R., Charlier, C., Boxho, P. (2016) Popliteal vein blood sampling and the postmortem redistribution of diazepam, methadone, and morphine. *Journal of Forensic Sciences*, 61, 1017–1028.

43. Lemaire, E., Schmidt, C., Dubois, N., Denooz, R., Charlier, C., Boxho, P. (2017) Site-, technique-, and time-related aspects of the postmortem redistribution of diazepam, methadone, morphine, and their metabolites: interest of popliteal vein blood sampling. *Journal of Forensic Sciences*, 62, 1559–1574.

44. IBM Watson Health. (2020) Micromedex® (Electronic Version). https://www.micromedexsolutions.com/ (accessed Nov 17, 2020).

45. Holmgren, P., Druid, H., Holmgren, A., Ahlner, J. (2004) Stability of drugs in stored postmortem femoral blood and vitreous humor. *Journal of Forensic Sciences*, 49, 820–825.

46. Rees, K.A., Pounder, D.J., Osselton, M.D. (2013) Distribution of opiates in femoral blood and vitreous humour in heroin/morphine-related deaths. *Forensic Science International*, 226, 152–159.

47. SERAF (2013) LAR som det vil bli fremover? https://www.med.uio.no/klinmed/forskning/centre/seraf/publikasjoner/rapporter/2013/medlastinger/se-raf-rapport-nr-1-2013-statusrapport-2012.pdf (accessed Nov 17, 2020).

48. Norwegian Institute of Public Health. (2020) Norwegian Prescription Database. www.reseptregisteret.no (accessed Dec 20, 2020).

49. Andresen, T., Upton, R.N., Foster, D.J., Christrup, L.L., Arendt-Nielsen, L., Drewes, A.M. (2011) Pharmacokinetic/pharmacodynamic analyses of transdermal buprenorphine and fentanyl in experimental human pain models. *Basic and Clinical Pharmacology and Toxicology*, 108, 274–284.

50. Olesen, A.E., Olofson, E., Andresen, T., Graversen, C., Drewes, A.M., Dahan, A. (2015) Stochastic pharmacokinetic-pharmacodynamic analysis of the effect of transdermal buprenorphine on electroencephalogram and analgesia. *Anesthesia and Analgesia*, 121, 1165–1175.

51. European Monitoring Centre for Drugs and Drug Addiction. (2020) *Fentanyl Drug Profile*. https://www.emcdda.europa.eu/publications/drug-profiles/fentanyl_en (accessed Dec 20, 2020).

52. Concheiro, M., Chesser, R., Pardi, J., Cooper, G. (2018) Postmortem toxicology of new synthetic opioids. *Frontiers in Pharmacology*, 9, 1210.

53. Moore, K.A., Cina, S.J., Jones, R., Selby, D.M., Levine, B., Smith, M.L. (1999) Tissue distribution of tramadol and metabolites in an overdose fatality. *The American Journal of Forensic Medicine and Pathology*, 20, 98–100.

54. Musshoff, F., Madea, B. (2001) Fatality due to ingestion of tramadol alone. *Forensic Science International*, 116, 197–199.

55. Costa, I., Oliveira, A., Guedes De Pinho, F., Teixeira, H.M., Moreira, R., Carvalho, E., et al. (2013) Postmortem redistribution of...
56. Levine, B., Ramcharitar, V., Smialek, J.E. (1997) Tramadol distribution in four postmortem cases. *Forensic Science International*, 86, 43–48.

57. Simonsen, K.W., Edvardsen, H.M., Thelander, G., Ojanpera, I., Thordardottir, S., Andersen, L.V., et al. (2015) Fatal poisoning in drug addicts in the Nordic countries in 2012. *Forensic Science International*, 248, 172–180.

58. Edvardsen, H.E., Tverborgvik, T., Frost, J., Rogde, S., Morild, I., Waal, H., et al. (2017) Differences in combinations and concentrations of drugs of abuse in fatal intoxication and driving under the influence cases. *Forensic Science International*, 281, 127–133.