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Can measurements of heroin metabolites in post-mortem matrices other than peripheral blood indicate if death was rapid or delayed?

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ABSTRACT

Background: In heroin-related deaths, it is often of interest to determine the approximate time span between intake of heroin and death, and to decide whether heroin or other opioids have been administered. In some autopsy cases, peripheral blood cannot be sampled due to decomposition, injuries or burns. The aim of the present study was to investigate whether measurements of heroin metabolites in matrices other than peripheral blood can be used to differentiate between rapid and delayed heroin deaths, and if morphine/codeine ratios measured in other matrices can separate heroin from codeine intakes.
Methods: In this study, we included 51 forensic autopsy cases where morphine was detected in peripheral blood. Samples were collected from peripheral and cardiac blood, pericardial fluid, psoas and lateral vastus muscles, vitreous humor and urine. The opioid analysis included 6-acetylmorphine (6-AM), morphine, morphine-3-glucuronide (M3G), morphine-6-glucuronide (M6G) and codeine. Urine was only used for qualitative detection of 6-AM. 45 heroin-intake cases were divided into rapid deaths (n = 24), based on the detection of 6-AM in blood, or delayed deaths (n = 21), where 6-AM was detected in at least one other matrix but not in blood. An additional 6 cases were classified as codeine-intake cases, based on a morphine/codeine ratio below unity (<1) in peripheral blood, without detecting 6-AM in any matrix.
Results: The median morphine concentrations were significantly higher in the rapid compared with the delayed heroin deaths in all matrices ($p = 0.004$ for vitreous humor and $p < 0.001$ for the other matrices). In the rapid heroin deaths, the M3G/morphine concentration ratios were significantly lower than in the delayed deaths both in peripheral and cardiac blood ($p < 0.001$), as well as in pericardial fluid ($p < 0.001$) and vitreous humor ($p = 0.006$), but not in muscle. The morphine/codeine ratios measured in cardiac blood, pericardial fluid and the two muscle samples resembled the ratios in peripheral blood, although codeine was less often detected in other matrices than peripheral blood.
Conclusions: Measurements of heroin-metabolites in cardiac blood, pericardial fluid and vitreous humor provide information comparable to that of peripheral blood regarding rapid and delayed heroin deaths, e.g. M3G/morphine ratios <2 indicate a rapid death while ratios >3 indicate a delayed death. However, considerable overlap in results from rapid and delayed deaths was observed, and measurements in muscle appeared less useful. Furthermore, matrices other than peripheral blood can be used to investigate morphine/codeine ratios, but vitreous humor seems less suited.

1. Introduction

Heroin or its metabolites are involved in the majority of fatal overdoses related to illicit drug use in Europe [1]. Interpretation of toxicological findings in these cases is often challenging. Heroin is, with a half-life of less than 5 min [2], rapidly metabolized to 6-acetylmorphine (6-AM) and then further to morphine, which is

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glucuronidated to morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) [3]. The half-life of 6-AM and morphine in blood is approximately 20–30 min [2] and 2–3 h [4], respectively. Codeine is often detected in low concentrations after heroin intake, because 6-acetylcodeine, which is a common byproduct of illegally produced heroin, is metabolized to codeine [5].

When interpreting toxicological findings in heroin-related deaths, it may be of great importance to assess the time span between administration of heroin and death [6]. Detection of 6-AM in blood has been suggested to indicate a rapid death [6–9], most likely within 1–2 h [9,10], or even as short as within 20–30 min [6], after intake of heroin. When 6-AM is detected in another matrix (e.g. urine or vitreous humor), but not in blood, a longer time span between heroin intake and death is likely [7,11–14]. However, the detection times in matrices other than blood and urine are sparsely investigated. Previous studies have found that deaths occurring rapidly after intake of heroin are characterized by higher morphine concentrations [6–8,15], lower morphine glucuronide concentrations [6] and lower morphine glucuronide/morphine concentration ratios in blood compared with that of more delayed deaths [6,16].

The morphine concentration ratios of vitreous humor to peripheral blood have also been reported to differ between rapid and delayed deaths [7]. It has previously been suggested that the same applies also to the drug concentration ratios of skeletal muscle to blood [17,18], or of pericardial fluid to blood [19], but this has not been thoroughly investigated.

In addition to estimating the approximate time span between intake of heroin and death, it may be essential to verify heroin use, and to differentiate intake of heroin from intake of other opioids, such as codeine, which is also metabolized to morphine. Detection of 6-AM shows unequivocally that heroin has been administered, but in cases where 6-AM cannot longer be detected in any matrix, the morphine/codeine ratio can be helpful. A morphine/codeine ratio above unity (>1) in blood has been shown to be a good marker of intake of heroin; whereas a ratio below unity (<1) indicates intake of codeine, both in living [10,20] and post-mortem cases [21–23]. Whether this also applies to other post-mortem matrices has been less studied, except from in vitreous humor [7,24,25] and urine [22].

Peripheral blood is not always available at forensic autopsies, for instance due to severe decomposition, injuries or burns. In such cases, the toxicological assessment relies on analyses performed in other matrices, like pericardial fluid, skeletal muscle or vitreous humor. Knowledge about concentrations of heroin metabolites in rapid compared with delayed deaths in different post-mortem matrices, and whether the findings in other matrices are comparable to the results from peripheral blood, is lacking.

The aim of this study was to investigate if concentrations of morphine and morphine glucuronides, as well as concentration ratios of M3G/morphine and M6G/morphine, in cardiac blood, pericardial fluid, psoas muscle, lateral vastus muscle and vitreous humor, can indicate whether death occurred rapidly or more delayed after intake of heroin. We also studied whether the morphine concentration ratios in the same matrices relative to peripheral blood differ between rapid and delayed deaths. Furthermore, we examined if morphine/codeine concentration ratios measured in cardiac blood, pericardial fluid, psoas muscle, lateral vastus muscle and vitreous humor resemble the ratios measured in peripheral blood.

2. Materials and methods

2.1. Materials

This study is part of a larger project that collected samples from several post-mortem matrices at 173 forensic autopsies performed between June 2013 and June 2016. The forensic pathologists mainly included cases where toxicological findings were likely based on the circumstances; such as information about an overdose or the presence of drug paraphernalia on the scene.

The present study investigated 66 cases where morphine was detected above the limit of quantification (LOQ) in peripheral blood, of which a total of 51 cases were included in the present material, as presented in Fig. 1. The remaining 15 morphine-positive cases were excluded, as intake of heroin or codeine could not be verified. Based on the detection of 6-AM, 45 cases were categorized as heroin-intake cases, and the concentrations of heroin metabolites in the different matrices in these cases have previously been published [26]. In the present study, these 45 cases were further classified into rapid heroin deaths (<1–2 h after intake of heroin), based on the detection of 6-AM in peripheral or cardiac blood, or delayed heroin deaths (>1–2 h after intake of heroin), if 6-AM was detected in any other matrix but not in blood, as shown in Fig. 1. An additional 6 cases were classified as codeine-intake cases, based on a morphine/codeine ratio <1 in peripheral blood without detecting 6-AM in any matrix.

2.2. Sampling and analytical methods

The autopsies of the 51 included cases were performed 0–6 (median 2) days after the assumed day of death. The degree of decomposition was graded by the forensic pathologist who performed the autopsy. No cases with severe decomposition were included, as all the selected matrices were not available in these cases.

Samples were collected during autopsies from peripheral blood, cardiac blood, pericardial fluid, psoas muscle, lateral vastus muscle, vitreous humor and urine. The sampling is described in more detail in Oiestad et al. [27]. In 2 of the 66 assessed cases vitreous humor was not available, and in 7 other cases urine was not available. The standard requisition form submitted with the samples contained a limited amount of circumstantial information.

The analytical methods are described in detail in Thaulow et al. [26]. Briefly, peripheral blood samples were screened for approximately 100 different psychoactive substances. Only cases with morphine detected above LOQ in peripheral blood were included in this study. The confirmation analyses of 6-AM, morphine, codeine, M3G and M6G in all matrices were performed using modified versions [26] of a previously published UHPLC-MS/MS method [28]. Protein precipitation was followed by filtration on Captiva ND lipids filter plates (Agilent Technologies, Santa Clara, California, USA) for all matrices, except urine which was only filtrated. The LOQ was 0.0033 mg/L and 0.0086 mg/L for 6-AM and morphine, respectively, 0.0090 mg/L for codeine, and 0.014 mg/L for M3G and M6G in blood, pericardial fluid and vitreous humor. In muscle, the LOQ was 0.0033 mg/kg and 0.0086 mg/kg for 6-AM and morphine, respectively, 0.0090 mg/kg for codeine, and 0.014 mg/kg for M3G and M6G. Only qualitative detection of 6-AM in urine was assessed, because quantitative results were considered less relevant.

2.3. Statistical analyses

The statistical analyses were performed using IBM SPSS Statistics 23. Generally, the concentrations of 6-AM, morphine, codeine, M3G and M6G had skewed distributions, assessed by the histograms, boxplots and Q-Q plots. Therefore, non-parametric tests were used, and the median and range values are reported. The concentrations of morphine, M3G and M6G, as well as the concentration ratios of M3G/morphine and M6G/morphine in the different matrices were compared between rapid and delayed heroin deaths using the Mann-Whitney test. The ratios of the morphine concentrations in the different matrices relative to

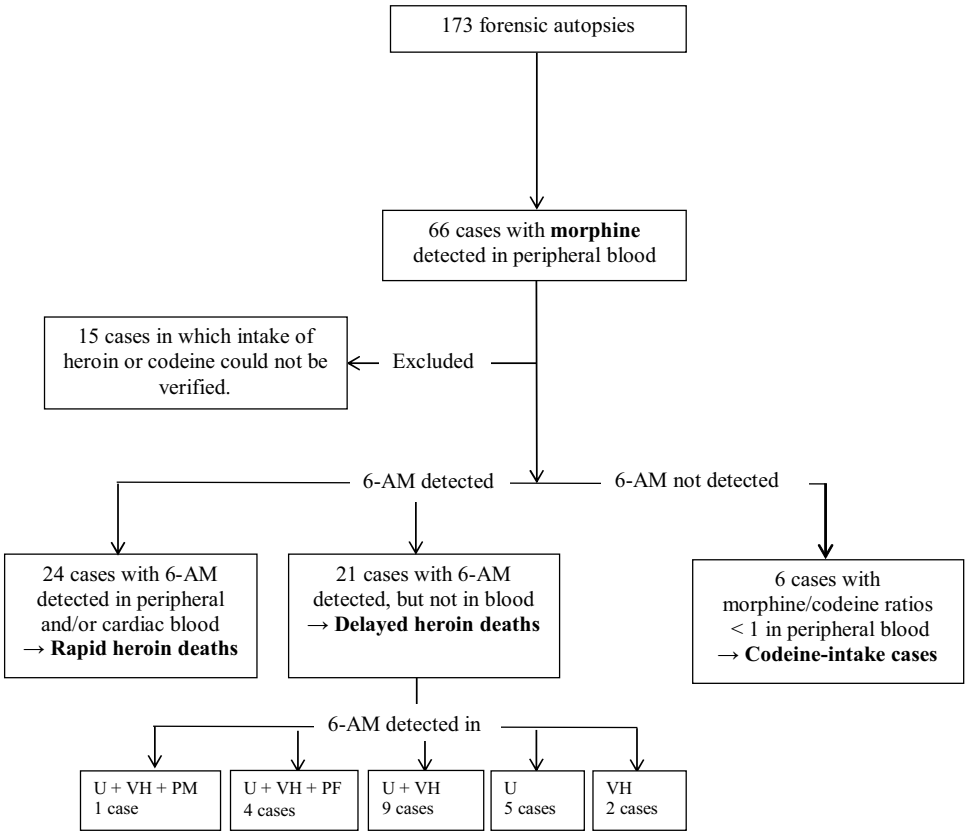

Fig. 1. A flowchart of the investigated cases. U = urine, VH = vitreous humor, PM = psoas muscle, PF = pericardial fluid.

those of peripheral blood, the number of days between death and autopsy, and the concentrations of the most common additional drugs in peripheral blood, were also compared between rapid and delayed heroin deaths using the Mann-Whitney test.

The decomposition was categorized as either "no decomposition" or "slight or moderate decomposition", and the Fisher's exact test was used for comparison between the rapid and delayed death groups. The number of cases with detection of the most common additional drugs was compared between rapid and delayed deaths using the Chi-square test.

P-values <0.05 were considered significant. When multiple tests were performed, correction according to the Bonferroni method [29] was used for each group of tests. Only p-values that reached significance after correction for multiple testing are reported as significant.

2.4. Ethics

The Regional Committee for Medical Research Ethics (reference number 2012/2173) and the Higher Prosecuting Authority (reference number 2012/02455) have approved this study.

3. Results

A flowchart of the investigated cases is shown in Fig. 1. Twenty-four cases were classified as rapid heroin deaths, and 21 cases as delayed heroin deaths. An additional 6 cases were categorized as codeine-intake cases.

3.1. Rapid versus delayed heroin deaths

The median number of days between assumed death and autopsy was not significantly different between the rapid (median 2, range 0–5 days) and the delayed (median 3, range 1–6 days) death groups ($p = 0.34$). The number of decomposed cases was also not significantly different between the two groups ($p = 0.71$). In the rapid deaths there was no visible decomposition in 20 of the cases, while 2 cases had slight and 2 other cases had moderate decomposition. In the delayed deaths, 15 and 5 of the cases had none and slight decomposition, respectively. In 1 case in the delayed death group, the decomposition was not graded by the forensic pathologist. The extent of case information provided on the requisition form filled in by the forensic pathologists varied, and did not include details regarding the time span between intake of heroin and death. However, information on the form indicated that the deceased was found dead in 29 (64%) of the cases. The presence of a syringe at the scene was described in 16 of the cases (9 of 24 rapid deaths, and 7 of 21 delayed deaths).

The median morphine concentrations were significantly higher in the rapid compared with the delayed heroin deaths in all matrices, as shown in Table 1a. The median concentrations of M3G and M6G were not significantly different (after Bonferroni correction) between the rapid and the delayed heroin deaths in any of the matrices, except for a higher concentration of M6G in cardiac blood in the rapid deaths, as shown in Tables 1b and 1c. The morphine glucuronides, particularly M6G, were detected in muscle in only a few cases (4 and 2 M6G-positive cases in psoas and lateral vastus muscle, respectively, in the rapid and delayed deaths combined).

The M3G/morphine and M6G/morphine concentration ratios in the rapid and delayed heroin deaths are presented in Fig. 2a and b. The median M3G/morphine ratio was significantly lower in the rapid compared with the delayed death group in peripheral and cardiac blood ($p < 0.001$), pericardial fluid ($p < 0.001$) and vitreous humor ($p = 0.006$), but not in muscle ($p = 0.03$ and $p = 0.14$ for psoas and vastus muscle, respectively) after correcting for multiple

Table 1a
Median concentrations (conc.) and ranges of morphine in the different matrices in rapid (6-AM detected in blood) versus delayed deaths (6-AM detected, but not in blood). Number of cases analyzed (n) = 24 for rapid deaths in all matrices, except for vitreous humor that had n = 22. N = 21 for all matrices in the delayed death group. The rapid and delayed groups were compared using the Mann–Whitney test.

Morphine			
	Rapid deaths	Delayed deaths	P-value
Peripheral blood conc. (mg/L)	0.26 (0.031–1.1)	0.088 (0.0096–0.48)	<0.001*
Cardiac blood conc. (mg/L)	0.45 (0.042–1.5)	0.067 (0–0.73)	<0.001*
Pericardial fluid conc. (mg/L)	0.44 (0.035–1.6)	0.11 (0.012–0.67)	<0.001*
Psoas muscle conc. (mg/kg)	0.34 (0.047–1.1)	0.11 (0–0.62)	<0.001*
Lateral vastus muscle conc. (mg/kg)	0.26 (0–1.4)	0.058 (0–0.80)	<0.001*
Vitreous humor conc. (mg/L)	0.075 (0.035–0.46)	0.044 (0.01–0.15)	0.004*

* Significant after Bonferroni correction (corrected significance level <0.0083).

Table 1b
Median concentrations (conc.) and ranges of morphine-3-glucuronide (M3G) in the different matrices in rapid (6-AM detected in blood) versus delayed deaths (6-AM detected, but not in blood). Number of cases analyzed (n) = 24 for rapid deaths in all matrices, except for vitreous humor that had n = 22. N = 21 for all matrices in the delayed death group. The rapid and delayed groups were compared using the Mann–Whitney test.

Morphine-3-glucuronide			
	Rapid deaths	Delayed deaths	P-value[a]
Peripheral blood conc. (mg/L)	0.30 (0.037–2.4)	0.22 (0.036–0.63)	0.44
Cardiac blood conc. (mg/L)	0.56 (0.079–1.9)	0.27 (0.020–0.68)	0.014
Pericardial fluid conc. (mg/L)	0.60 (0.090–5.1)	0.60 (0.17–1.4)	0.75
Psoas muscle conc. (mg/kg)	0.078 (0–0.52)	0.11 (0–0.37)	0.74
Lateral vastus muscle conc. (mg/kg)	0 (0–1.2)	0.065 (0–0.26)	0.64
Vitreous humor conc. (mg/L)	0.094 (0–2.0)	0.11 (0–0.44)	0.32

[a] No significant differences (corrected significance level <0.0083).

Table 1c
Median concentrations (conc.) and ranges of morphine-6-glucuronide (M6G) in the different matrices in rapid (6-AM detected in blood) versus delayed deaths (6-AM detected, but not in blood). Number of cases analyzed (n) = 24 for rapid deaths in all matrices, except for vitreous humor that had n = 22. N = 21 for all matrices in the delayed death group. The rapid and delayed groups were compared using the Mann–Whitney test.

Morphine-6-glucuronide			
	Rapid deaths	Delayed deaths	P-value
Peripheral blood conc. (mg/L)	0.035 (0–0.58)	0.036 (0–0.13)	0.94
Cardiac blood conc. (mg/L)	0.12 (0.021–0.63)	0.051 (0–0.10)	0.006*
Pericardial fluid conc. (mg/L)	0.12 (0.020–1.2)	0.12 (0.030–0.32)	0.86
Psoas muscle conc. (mg/kg)	0 (0–0.12)	0 (0–0.081)	0.93
Lateral vastus muscle conc. (mg/kg)	0 (0–0.22)	0 (0–0)[a]	0.18
Vitreous humor conc. (mg/L)	0 (0–0.31)	0.014 (0–0.10)	0.23

* Significant after Bonferroni correction (corrected significance level <0.0083).
[a] No cases positive for M6G.

testing. The median M6G/morphine ratio was significantly lower in the rapid death group compared with the delayed death group in peripheral blood ($p = 0.002$) and pericardial fluid ($p < 0.001$), but not in cardiac blood ($p = 0.012$), muscle ($p = 0.95$ and $p = 0.66$ for psoas and lateral vastus muscle, respectively) or vitreous humor ($p = 0.097$) after correcting for multiple testing.

The morphine concentration ratios of the different matrices to peripheral blood are shown in Table 2. After correcting for multiple testing, only the median morphine ratio of vitreous humor/peripheral blood was significantly ($p = 0.003$) lower in the rapid heroin deaths (0.29, range 0.15–1.2) compared with the delayed deaths (0.64, range 0.21–3.2).

3.2. Morphine/codeine ratios

The morphine/codeine ratios in the different matrices in the verified heroin cases (6-AM detected in any matrix, n = 45) and in the codeine-intake cases (morphine/codeine <1 in peripheral blood, n = 6) are presented in Table 3. In the heroin-intake cases, the morphine/codeine ratios were all >1 in peripheral blood, cardiac blood, pericardial fluid, psoas muscle and lateral vastus muscle, although codeine was less often detected in the other matrices than in peripheral blood, particularly in muscle, and the ratios could therefore not be calculated in all cases. Two of the heroin-intake cases had morphine/codeine ratios <1 in vitreous humor, while the ratios in peripheral blood were both >1. All six codeine-intake cases had ratios <1 in all matrices, except for one case in which codeine was not detected in either of the muscle samples.

3.3. Additional drugs

Poly drug use was found in the vast majority of the heroin-intake cases (n = 43, 96%) and in all the codeine-intake cases (n = 6, 100%). The number of cases detecting the most common additional drugs (clonazepam and/or its metabolite 7-aminoclonazepam, amphetamine and/or methamphetamine and tetrahydrocannabinol) was not significantly different between the rapid and the delayed heroin deaths, and the concentrations of these drugs were also not significantly different between the two groups (data not shown). In the codeine-intake cases (n = 6), the most commonly detected additional psychoactive drugs were oxazepam (n = 4) and zopiclone (n = 3).

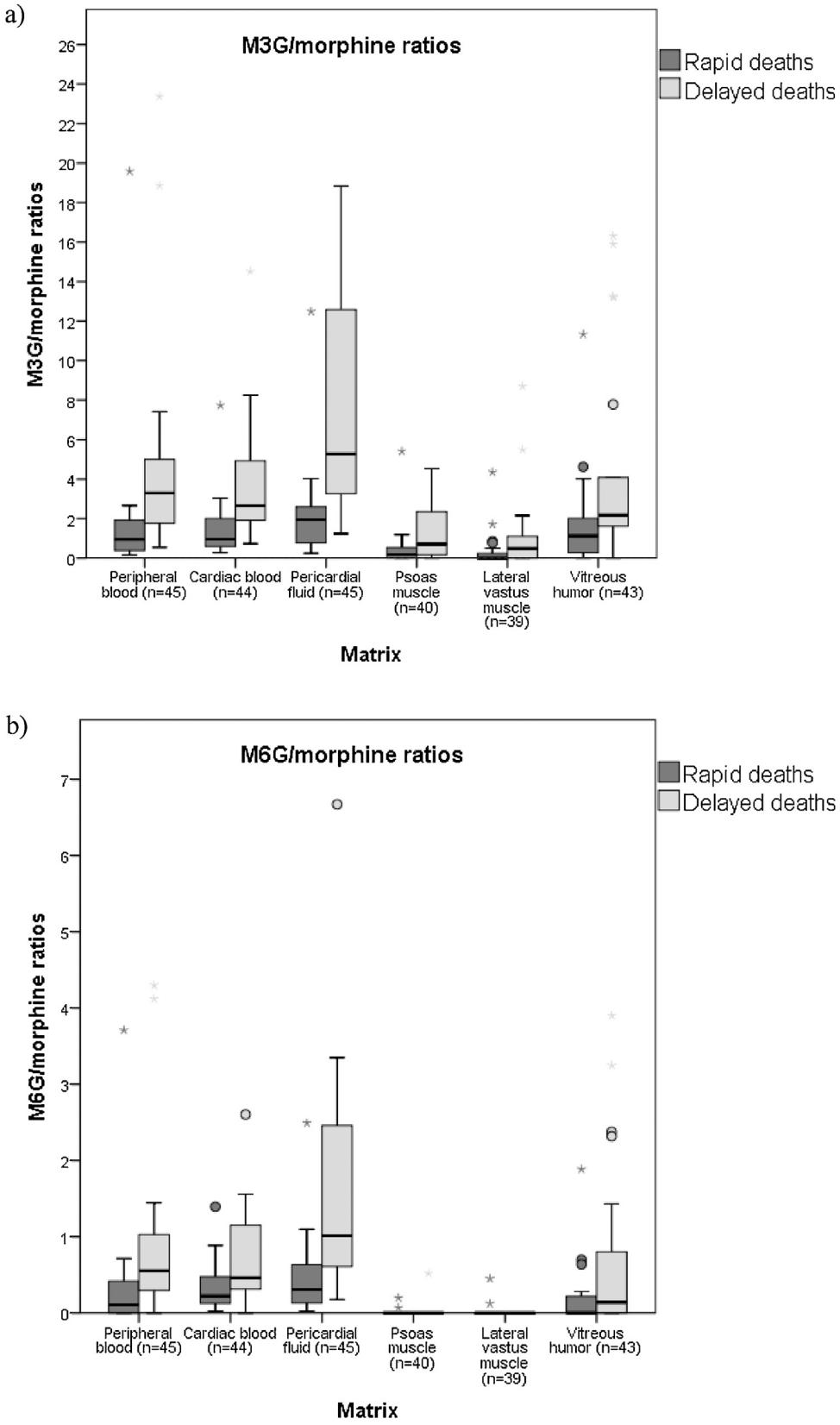

Fig. 2. Concentration ratios of (a) morphine-3-glucuronide (M3G)/morphine and (b) morphine-6-glucuronide (M6G)/morphine in rapid (6-AM detected in blood) versus delayed (6-AM detected, but not in blood) deaths in peripheral blood, cardiac blood, pericardial fluid, psoas muscle, lateral vastus muscle and vitreous humor. The boxes represent the interquartile range (25th-75th percentile), whereas the whiskers represent the smallest and largest values within 1.5 times the interquartile range. The horizontal line in each box is the median value. Circles (outliers) and asterisks (extreme points) represent values exceeding 1.5 and 3 times the interquartile range, respectively. Two cases with M3G/morphine ratios >25 in pericardial fluid were excluded from (a) and one case with a M6G/morphine ratio >7 in pericardial fluid was excluded from (b) for better visualization.

Table 2
Median ratios and ranges of morphine in cardiac blood, pericardial fluid, psoas muscle, lateral vastus muscle and vitreous humor relative to peripheral (periph.) blood in rapid (6-AM detected in blood) versus delayed deaths (6-AM detected, but not in blood). Number of cases analyzed (n) = 24 for rapid deaths in all matrices, except for vitreous humor that had n = 22. N = 21 for all matrices in the delayed death group. The rapid and delayed groups were compared using the Mann–Whitney test.

Ratios of morphine in matrix/peripheral blood			
	Rapid deaths	Delayed deaths	P-value
Cardiac blood/periph. blood			
Median ratio	1.4 (0.09–3.6)	1.1 (0–2.8)	0.031
Pericardial fluid/periph. blood			
Median ratio	1.6 (0.07–5.3)	1.4 (0.64–3.6)	0.24
Psoas muscle/periph. blood			
Median ratio	1.1 (0.14–1.9)	1.3 (0–19.2)	0.95
Lateral vastus muscle/periph. blood			
Median ratio	1.1 (0–1.5)	0.99 (0–1.7)	0.46
Vitreous humor/periph. blood			
Median ratio	0.29 (0.15–1.2)	0.64 (0.21–3.2)	0.003*

* Significant after Bonferroni correction (corrected significance level <0.01).

Table 3
The median morphine/codeine concentration ratios and ranges, as well as the number of codeine positive cases (N) are shown for all the matrices among the heroin-intake cases (6-AM detected in any matrix) versus the codeine-intake cases (morphine/codeine ratio <1 in peripheral blood).

Morphine/codeine ratios		
	Heroin cases (n = 45)	Codeine cases (n = 6)
Peripheral blood		
Median ratio	11 (1.2–17)	0.017 (0.01–0.8)
N	35	6
Cardiac blood		
Median ratio	11 (1.7–18)	0.016 (0–0.09)
N	32	6
Pericardial fluid		
Median ratio	9.2 (1.6–14)	0.015 (0–0.08)
N	34	6
Psoas muscle		
Median ratio	10 (1.9–13)	0.011 (0.004–0.10)
N	10	5
Lateral vastus muscle		
Median ratio	11 (2.4–13)	0.019 (0–0.10)
N	9	5
Vitreous humor		
Median ratio	3.9 (0.3[a]–6.6)	0.008 (0–0.03)
N	30	6

[a] Two heroin-intake cases had morphine/codeine ratios <1 in vitreous humor.

4. Discussion

In this study, we divided morphine-positive cases into rapid heroin deaths, based on the detection of 6-AM in blood, and delayed heroin deaths, if 6-AM was detected in matrices other than blood. Higher median morphine concentrations were found in all matrices in the rapid compared with the delayed heroin deaths, as seen in Table 1a. These results are in concordance with previous studies in blood [6–8,15]. We also found significantly lower M3G/morphine ratios in the rapid compared with the delayed deaths in peripheral blood, cardiac blood, pericardial fluid and vitreous humor, but not in muscle. 6-AM is reported to be unstable in blood [30,31]. There were, however, no significant differences in corpse decomposition, or in the number of days between death and autopsy, when comparing the rapid and delayed death groups; indicating that differences in post-mortem degradation of 6-AM does not explain our results. Still, we cannot exclude that some rapid death cases were misclassified as delayed deaths, due to post-mortem degradation of 6-AM in blood. The circumstantial information provided to us was not useful in discerning between rapid and delayed heroin deaths. Although not systematically reported, we have information that in 64% of the cases the person was found dead at the scene, implying that details regarding the time span between intake of heroin and death is most likely lacking. Furthermore, syringes were often observed at the scene, but did not seem to be associated with rapid deaths only.

We did not find significant differences in the absolute concentrations of morphine glucuronides in most of the matrices between rapid and delayed heroin deaths, as opposed to what has been demonstrated in a previous study in blood [6]. The concentrations of morphine glucuronides and the morphine glucuronides/morphine concentration ratios are expected to be higher in delayed compared with rapid deaths, because the glucuronidation has proceeded for a longer time period in the delayed death cases [6,32,33]. Several studies have also found higher free morphine/total morphine ratios in blood in rapid deaths compared with the more delayed deaths [7,8,34], which also indicates less glucuronidation in the rapid death cases. In the present study, we found significantly lower M3G/morphine ratios in the rapid compared with the delayed death group in all matrices, except for in muscle, which is in concordance with previous studies in blood [6,16]. Thus, our results indicate that the M3G/morphine ratios in cardiac blood, pericardial fluid and vitreous humor generally show the same differences between rapid and delayed deaths as those of peripheral blood, and could be useful when assessing whether death occurred rapidly or more delayed after intake of heroin. When observing the matrices other than muscle in Fig. 2a, M3G/morphine ratios approximately <2 suggest a rapid death after intake of heroin, which has also been proposed in previous studies regarding morphine glucuronide/morphine ratios in blood [32,33]. Furthermore, M3G/morphine ratios >3 suggest a more delayed death, although considerable overlap in the ratios was observed, as seen in Fig. 2a. Regarding the M6G/morphine ratios (Fig. 2b), our findings were less clear than the M3G/morphine ratios, but this could perhaps be caused by the generally lower concentrations of M6G compared with M3G in all matrices. We have no explanation for the single finding of higher M6G concentrations in cardiac blood in the rapid death group. The strength of using ratios between metabolite and parent drug, lies in that the ratios to a lesser extent, than that of the absolute concentrations, depend on the administered dose of heroin [32]. However, it must be noted that morphine glucuronides can accumulate in blood with repeated use of heroin or morphine [7,34], particularly in those with renal failure [35,36], and the concentrations can also change after death [37,38]. We have assumed that morphine glucuronide accumulation was roughly the same between rapid and delayed deaths, but accumulation could perhaps explain the high morphine glucuronide/morphine ratios found in some of the rapid death cases.

The median morphine concentration ratio of vitreous humor/peripheral blood was significantly lower in rapid compared with delayed deaths. This is in agreement with a previous study which suggested that in rapid deaths there may not be sufficient time for morphine to completely distribute from blood into vitreous humor [7]. A delayed transport of morphine into vitreous humor after administration of heroin has also been demonstrated in living pigs [12]. Interpretation of concentrations in vitreous humor is further complicated by the possibility of accumulation of morphine in vitreous humor with chronic use of heroin or morphine [7]. The morphine concentration ratios of pericardial fluid, psoas muscle and lateral vastus muscle relative to peripheral blood did not differ significantly between the rapid and delayed deaths, suggesting that the transport of morphine from blood into these matrices is faster than the transport into vitreous humor. Our results from muscle differ from those of Williams et al. [18] and Garriott et al.

[17] who suggested that the muscle/blood ratio depend on the time span between drug administration and death. However, the study by Williams et al. did not include any cases with morphine, and the study by Garriott et al. used central (aortic) blood instead of peripheral blood and these ratios could thus be more affected by post-mortem redistribution than our results.

Cases where 6-AM was not detected in any matrix, combined with a morphine/codeine ratio below unity in peripheral blood, were classified as codeine-intake cases. None of the heroin-intake cases (6-AM detected), had a morphine/codeine ratio below unity in peripheral blood, supporting the theory that the morphine/codeine ratio in peripheral blood is a good indication of whether codeine or heroin has been used [21–23]. Our results show that the morphine/codeine ratios in cardiac blood, pericardial fluid and both muscles correspond to the ratios in peripheral blood. However, codeine was less often detected in other matrices than blood, particularly in muscle, and it was therefore not possible to calculate the ratio for all the matrices in every case. To the best of our knowledge, morphine/codeine ratios in skeletal muscle or pericardial fluid have not previously been investigated in heroin-related deaths. Frost et al. examined morphine/codeine ratios in muscle in codeine-related fatalities, and found that the ratios in muscle appeared slightly higher than in blood, but with a large spread in data [39]. When using only vitreous humor for calculating the morphine/codeine ratios, we found that two of the cases would have been classified as codeine-intake cases, had it not been for the detection of 6-AM in any matrix. This indicates that morphine/codeine ratios in vitreous humor may not be comparable to the other matrices. Our finding differs from previous studies, which have found morphine/codeine ratios in vitreous humor that resembled the ratios found in blood in all heroin-intake cases [7,24,25]. It is, however, important to keep in mind that combined use of e.g. prescribed morphine and codeine, can give rise to ratios that resemble those of heroin use. Therefore, interpretation of morphine/codeine ratios in any matrix must be done with caution.

Our study did not aim to investigate the cause of death, but rather whether concentrations and ratios were comparable between the different matrices. Therefore, we chose to categorize all the cases where 6-AM was detected as “heroin deaths”, regardless of the assessment given by the forensic pathologist. This simplification implies that in some cases, heroin could have been less important in causing death, compared with the other drugs detected. However, because additional drugs are detected in the majority of heroin-related deaths [9,40,41] our results are expected to be relevant for heroin-intake cases in general. It is also worth mentioning that some of the 15 excluded morphine-positive cases could be a result of a “very delayed” heroin death, even though 6-AM was not detected, since intake of heroin is more common than intake of morphine among illicit drug users in Europe [1].

The strength of the present study lies in that several post-mortem matrices were collected in each case. However, changes in concentrations between the different matrices due to e.g. severe decomposition or great blood loss have not been investigated in our study. Since we now know that concentrations in other matrices can provide valuable information regarding heroin use, more complicated cases need to be investigated in future studies. The number of suspected codeine-intake cases in our study was low, and the use of morphine/codeine ratios measured in other matrices than blood should be further investigated in larger studies.

5. Conclusions

This study shows that measurements of heroin metabolites in cardiac blood, pericardial fluid and vitreous humor, but less in muscle, provide information comparable to that of peripheral blood regarding rapid versus delayed heroin deaths, e.g. M3G/morphine ratios <2 indicate a rapid death, while ratios > 3 indicate a delayed death. These results could be useful when assessing whether death occurred rapidly or was more delayed after intake of heroin, also in cases where blood is not available. As always with post-mortem toxicological assessments, the concentrations and their ratios should be interpreted with great caution. A morphine/codeine ratio above unity, as measured in peripheral blood, cardiac blood, pericardial fluid or skeletal muscle, could possibly be regarded as an indication of heroin intake, while a morphine/codeine ratio measured in vitreous humor seems less useful.

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Declaration of interest

None.

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References

[1] European Monitoring Centre for Drugs and Drug Addiction (2017), European Drug Report 2017: Trends and Developments, Publications Office of the European Union, Luxembourg, https://doi.org/10.2810/144609.
[2] E.J. Rook, J.M. van Ree, W. van den Brink, M.J. Hillebrand, A.D. Huitema, V.M. Hendriks, J.H. Beijnen, Pharmacokinetics and pharmacodynamics of high doses of pharmaceutically prepared heroin, by intravenous or by inhalation route in opioid-dependent patients, Basic Clin. Pharmacol. Toxicol. 98 (1) (2006) 86–96.
[3] E.J. Rook, A.D. Huitema, W. van den Brink, J.M. van Ree, J.H. Beijnen, Pharmacokinetics and pharmacokinetic variability of heroin and its metabolites: review of the literature, Curr. Clin. Pharmacol. 1 (1) (2006) 109–118.
[4] R.C. Basel, Disposition of toxic drugs and chemicals in man, tenth ed., Biomedical Publications, Seal Beach, CA, 2014, pp. 992–995.
[5] A. Maas, B. Madea, C. Hess, Confirmation of recent heroin abuse: accepting the challenge, Drug. Test. Anal. 10 (1) (2018) 54–71.
[6] S. Darke, J. Duflou, The toxicology of heroin-related death: estimating survival times, Addiction 111 (9) (2016) 1607–1613.
[7] K.A. Rees, D.J. Pounder, M.D. Osselton, Distribution of opiates in femoral blood and vitreous humour in heroin/morphine-related deaths, Forensic Sci. Int. 226 (1–3) (2013) 152–159.
[8] B.A. Goldberger, E.J. Cone, T.M. Grant, Y.H. Caplan, B.S. Levine, J.E. Smialek, Disposition of heroin and its metabolites in heroin-related deaths, J. Anal. Toxicol. 18 (1) (1994) 22–28.
[9] A.W. Jones, A. Holmgren, J. Ahlner, Heroin poisoning deaths with 6-monoacetylmorphine in blood: Demographics of the victims, previous drug-related offences, polydrug use, and free morphine concentrations in femoral blood, Forensic Toxicol. 30 (1) (2012) 19–24.
[10] G. Ceder, A.W. Jones, Concentration ratios of morphine to codeine in blood of impaired drivers as evidence of heroin use and not medication with codeine, Clin. Chem. 47 (11) (2001) 1980–1984.
[11] M.L. Smith, E.T. Shimomura, J. Summers, B.D. Paul, D. Nichols, R. Shippee, A.J. Jenkins, W.D. Darwin, E.J. Cone, Detection times and analytical performance of commercial urine opiate immunoassays following heroin administration, J. Anal. Toxicol. 24 (7) (2000) 522–529.
[12] A. Gottas, M. Arnestad, P.S. Halvorsen, L.C. Bachs, G. Hoiseth, Pharmacokinetics of heroin and its metabolites in vitreous humor and blood in a living pig model, Forensic Toxicol. 34 (2) (2016) 277–285.
[13] E.J. Cone, P. Welch, J.M. Mitchell, B.D. Paul, Forensic drug testing for opiates: I. Detection of 6-acetylmorphine in urine as an indicator of recent heroin exposure; drug and assay considerations and detection times, J. Anal. Toxicol. 15 (1) (1991) 1–7.
[14] F. Pragst, K. Spiegel, U. Leuschner, A. Hager, Detection of 6-acetylmorphine in vitreous humor and cerebrospinal fluid–comparison with urinary analysis for proving heroin administration in opiate fatalities, J. Anal. Toxicol. 23 (3) (1999) 168–172.
[15] J.C. Garriott, W.Q. Sturner, Morphine concentrations and survival periods in acute heroin fatalities, N. Engl. J. Med. 289 (24) (1973) 1276–1278.
[16] M.J. Bogusz, R.D. Maier, S. Driessen, Morphine, morphine-3-glucuronide, morphine-6-glucuronide, and 6-monoacetylmorphine determined by means

of atmospheric pressure chemical ionization-mass spectrometry-liquid chromatography in body fluids of heroin victims, J. Anal. Toxicol. 21 (5) (1997) 346–355.
[17] J.C. Garriott, Skeletal muscle as an alternative specimen for alcohol and drug analysis, J. Forensic Sci. 36 (1) (1991) 60–69.
[18] K.R. Williams, D.J. Pounder, Site-to-site variability of drug concentrations in skeletal muscle, Am. J. Forensic Med. Pathol. 18 (3) (1997) 246–250.
[19] M. Tominaga, T. Michiue, T. Ishikawa, O. Kawamoto, S. Oritani, K. Ikeda, M. Ogawa, H. Maeda, Postmortem analyses of drugs in pericardial fluid and bone marrow aspirate, J. Anal. Toxicol. 37 (7) (2013) 423–429.
[20] A.W. Jones, A. Holmgren, F.C. Kugelberg, Driving under the influence of opiates: concentration relationships between morphine, codeine, 6-acetyl morphine, and ethyl morphine in blood, J. Anal. Toxicol. 32 (4) (2008) 265–272.
[21] A.W. Jones, A. Holmgren, Concentration ratios of free-morphine to free-codeine in femoral blood in heroin-related poisoning deaths, Legal Med. 13 (4) (2011) 171–173.
[22] S.V. Konstantinova, P.T. Normann, M. Arnestad, R. Karinen, A.S. Christophersen, J. Morland, Morphine to codeine concentration ratio in blood and urine as a marker of illicit heroin use in forensic autopsy samples, Forensic Sci. Int. 217 (1-3) (2012) 216–221.
[23] A.D. Ellis, G. McGwin, G.G. Davis, D.W. Dye, Identifying cases of heroin toxicity where 6-acetylmorphine (6-AM) is not detected by toxicological analyses, Forensic Sci. Med. Pathol. 12 (3) (2016) 243–247.
[24] D.L. Lin, C.Y. Chen, K.P. Shaw, R. Havier, R.L. Lin, Distribution of codeine, morphine, and 6-acetylmorphine in vitreous humor, J. Anal. Toxicol. 21 (4) (1997) 258–261.
[25] J. Wyman, S. Bultman, Postmortem distribution of heroin metabolites in femoral blood, liver, cerebrospinal fluid, and vitreous humor, J. Anal. Toxicol. 28 (4) (2004) 260–263.
[26] C.H. Thaulow, A.M.L. Oiestad, S. Rogde, R. Karinen, G.W. Brochmann, J.M. Andersen, G. Hoiseth, M. Handal, J. Morland, M. Arnestad, E.L. Oiestad, D.H. Strand, V. Vindenes, Metabolites of Heroin in Several Different Post-mortem Matrices, J. Anal. Toxicol. 42 (5) (2018) 311–320.
[27] A.M.L. Oiestad, S. Rogde, S. Nilsen, K.B.B. Eldor, G.W. Brochmann, M. Arnestad, E.L. Oiestad, M.D. Peres, L. Kristoffersen, V. Vindenes, Comparative study of post-mortem concentrations of antidepressants in several different matrices, J. Anal. Toxicol. (2018), doi:http://dx.doi.org/10.1093/jat/bky030 in press.
[28] T. Berg, E. Lundanes, A.S. Christophersen, D.H. Strand, Determination of opiates and cocaine in urine by high pH mobile phase reversed phase UPLC-MS/MS, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 877 (4) (2009) 421–432.
[29] D.L. Streiner, G.R. Norman, Correction for multiple testing: is there a resolution? Chest 140 (1) (2011) 16–18.
[30] G. Hoiseth, B. Fjeld, M.L. Burns, D.H. Strand, V. Vindenes, Long-term stability of morphine, codeine, and 6-acetylmorphine in real-life whole blood samples, stored at −20 degrees C, Forensic Sci. Int. 239 (2014) 6–10.
[31] I. Papoutsis, P. Nikolaou, C. Pistos, A. Dona, M. Stefanidou, C. Spiliopoulou, S. Athanaselis, Stability of morphine, codeine, and 6-acetylmorphine in blood at different sampling and storage conditions, J. Forensic Sci. 59 (2) (2014) 550–554.
[32] R. Aderjan, S. Hofmann, G. Schmitt, G. Skopp, Morphine and morphine glucuronides in serum of heroin consumers and in heroin-related deaths determined by HPLC with native fluorescence detection, J. Anal. Toxicol. 19 (3) (1995) 163–168.
[33] R.E. Aderjan, G. Skopp, Formation and clearance of active and inactive metabolites of opiates in humans, Ther. Drug Monit. 20 (5) (1998) 561–569.
[34] V. Spiehler, R. Brown, Unconjugated morphine in blood by radioimmunoassay and gas chromatography/mass spectrometry, J. Forensic Sci. 32 (4) (1987) 906–916.
[35] R. Osborne, S. Joel, K. Grebenik, D. Trew, M. Slevin, The pharmacokinetics of morphine and morphine glucuronides in kidney failure, Clin. Pharmacol. Ther. 54 (2) (1993) 158–167.
[36] E. Bodd, D. Jacobsen, E. Lund, A. Ripel, J. Morland, E. Wiik-Larsen, Morphine-6-glucuronide might mediate the prolonged opioid effect of morphine in acute renal failure, Hum. Exp. Toxicol. 9 (5) (1990) 317–321.
[37] G. Skopp, L. Potsch, A. Klingmann, R. Mattern, Stability of morphine, morphine-3-glucuronide, and morphine-6-glucuronide in fresh blood and plasma and postmortem blood samples, J. Anal. Toxicol. 25 (1) (2001) 2–7.
[38] G. Skopp, R. Lutz, B. Ganssmann, R. Mattern, R. Aderjan, Postmortem distribution pattern of morphine and morphine glucuronides in heroin overdose, Int. J. Legal Med. 109 (3) (1996) 118–124.
[39] J. Frost, T.N. Lokken, A. Helland, I.S. Nordrum, L. Slordal, Post-mortem levels and tissue distribution of codeine codeine-6-glucuronide, norcodeine, morphine and morphine glucuronides in a series of codeine-related deaths, Forensic Sci. Int. 262 (2016) 128–137.
[40] A. Fugelstad, J. Ahlner, L. Brandt, G. Ceder, S. Eksborg, J. Rajs, O. Beck, Use of morphine and 6-monoacetylmorphine in blood for the evaluation of possible risk factors for sudden death in 192 heroin users, Addiction 98 (4) (2003) 463–470.
[41] H.E. Edvardsen, T. Tverborgvik, J. Frost, S. Rogde, I. Morild, H. Waal, T. Clausen, L. Slordal, V. Vindenes, Differences in combinations and concentrations of drugs of abuse in fatal intoxication and driving under the influence cases, Forensic Sci. Int. 281 (2017) 127–133.