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Frequency of postmortem ethanol formation in blood, urine and vitreous humor – Improving diagnostic accuracy with the use of ethylsulphate and putrefactive alcohols

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ABSTRACT

Purpose: This study aimed to compare the frequency of postmortem ethanol formation in blood, urine and vitreous humor according to negative ethylsulphate (EtS) in blood or positive putrefactive alcohols (PA's) in either medium. Furthermore, it aimed to evaluate the interpretational value of calculated ethanol ratios in relation to EtS and PA results.
Methods: Blood ethanol positive forensic cases were included; one dataset consisting of 2504 cases with EtS analysed in blood and another dataset with 8001 cases where PA's were analysed.
Results: PA's were found in 24.4% of cases. EtS was negative in 15.3%, 9.4% and 7.4% of cases that were positive for ethanol in blood, urine and vitreous humor, respectively. In EtS negative cases, the concentrations of ethanol in blood, urine and vitreous humor were lower than 0.20 g/kg in 51.3%, 67.4% and 77.8%, respectively. It was 1.0 g/kg or higher in blood in 4.2% of cases. More EtS negative and PA positive cases were seen in central compared to peripheral blood. Ethanol ratios between urine or vitreous humor and blood were significantly lower in both EtS negative and PA positive cases, but large variations were observed.
Conclusion: EtS and PA analysis improve the diagnostic accuracy of ethanol in postmortem cases. Postmortem ethanol formation in vitreous humor and urine were both more frequent than expected and we recommend the analysis of ethanol primarily in peripheral blood if available.

1. Introduction

Ethanol is amongst the most frequently occurring toxicological substances in postmortem cases [1]. Determining whether the measured ethanol concentration represents alcohol intake prior to death, postmortem formation, or a combination of the two, is important because it is possible ethanol may be either the causal or contributory factor of death. Verifying the intake of alcohol is important in medicolegal cases, such as those concerning sudden child deaths and accidents in aviation or traffic. Ratio calculations can be conducted by analysing ethanol in samples taken from multiple sites, preferably peripheral (or central) blood, urine and vitreous humor. These ratios are further compared with mean distribution ratios observed in the elimination phase of ethanol. Deviating ratios may indicate postmortem formation at one or more of the sample sites, however, this can also be caused by other factors. The interpretational value of using ethanol ratios alone is therefore limited [2–4].

Complementary analyses of putrefaction alcohols (PA's) in blood, such as 1-propanol and 1-butanol, strengthens the assumption that the ethanol concentration detected is partly or fully caused by postmortem formation [3]. In addition, the non-oxidative ethanol metabolites, ethylglucuronide (EtG) and ethylsulphate (EtS), have

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more recently become recognized as direct markers for alcohol intake. Their pharmacokinetics are well understood [5–10] and their appearance has been studied in postmortem blood, urine and vitreous humor [11–16], where negative results indicate that ethanol was not ingested before death.

The frequency of postmortem ethanol formation determined by negative EtS analyses was previously investigated in blood, but knowledge from urine and vitreous humor is very limited [17]. The primary aim of our study was to compare the frequency of postmortem ethanol formation in blood, urine and vitreous humor as determined by the absence of EtS in blood. The secondary aim was to compare frequencies of postmortem ethanol formation in blood sampled from various sample sites and frequencies within different ethanol concentration levels. This was determined through both the presence of PA's and the absence of EtS. Finally, the authors aimed to explore the interpretational value of ethanol ratios compared to that of PA's and EtS.

2. Materials and methods

2.1. Norwegian study population

Between the 6th of November 2011 and the 31st of December 2017, The Norwegian Institution received samples from 11010 forensic autopsy cases. Each case was analysed and interpreted with regards to available sample mediums and history of death. This study included all ethanol positive cases in autopsy blood that also had valid accompanying EtS and, in most cases, EtG analysis in blood (46 cases had missing or invalid EtG). Cases included were those labelled as "peripheral blood", "central blood" or "blood" by the pathologists. If available in each case, ethanol results for urine and vitreous humor were also included. In view of previous literature showing possible instability of EtG in blood [18–20] EtS is reported as the main result within this study.

2.1.1. Sampling

The blood and urine samples were collected in 25.0 mL Sterilin tubes (Bibby Sterilin, Staffordshire, UK) containing a preservative solution of 200 mg potassium fluoride. Vitreous humor was mainly collected in 5.0 mL glass BD Vacutainer evacuated tubes (BD Diagnostics, Plymoth, UK) containing a preservative of 20.0 mg of sodium fluoride and 143 IU of heparin. Depending on the amount sampled, vitreous humor was collected from one or both eyes. The samples were refrigerated at 4 °C upon arriving at the laboratory and until analysis.

2.1.2. Analytical methods

Ethanol in blood and vitreous humor was screened and quantified by two headspace gas chromatography flame ionization detector methods (HS-GC-FID) using two different capillary columns and two different internal standards [21]. Ethanol in urine was screened by one enzymatic method [22] at the start of the study period, followed by an enzymatic method using DRI® Ethyl Alcohol Assay according to the manufacturer specifications (Thermo Fisher Scientific, Waltham, MA, USA). Urine samples that screened positive (≥ 0.10 g/kg), were quantified by the two HS-GC-FID methods [21]. Positive analyses for ethanol in blood, urine and vitreous humor were defined as values equal to or higher than 0.10 g/kg. In cases with positive findings of ethanol in blood, EtG and EtS were quantified in blood by one ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method at the start of the study period [23], followed by a new UHPLC-MS/MS method [24]. Positive analyses for EtS and EtG were defined as values equal to or higher than their limits of quantification, 0.15 µM (0.019 mg/L) and 0.3 µM (0.067 mg/L), respectively.

2.2. Swedish study population

Between the 1st of January 2013 and the 31st of December 2017, the Swedish institution received samples from 24453 forensic autopsy cases. These were analysed for ethanol and other volatiles, including 1-propanol and 1-butanol. Cases were further classified as "putrefactive alcohol positive" (PA positive) if 1-propanol, 1-butanol or both were detected. This study included all cases positive for ethanol in blood and, if available in each case, ethanol results for urine and vitreous humor. Cases were stratified into three groups based on the blood sampling site (peripheral, central and unspecified).

2.2.1. Sampling

The blood and urine samples were mainly collected in 10 mL NUNC tubes (VWR International AB) containing a preservative solution of 80 mg potassium fluoride. Vitreous humor was mainly collected in 5 mL glass Venosafe tubes (Terumo Sweden AB) containing a preservative of 16 mg sodium fluoride and 60 USP of sodium heparin. Depending on the amount sampled, vitreous humor was collected from one or both eyes. The samples were refrigerated at 4 °C upon arriving the laboratory and until analyses.

2.2.2. Analytical method

Ethanol in blood, urine, and vitreous humor was quantified by HS-GC-FID using two different capillary columns and two different

Table 1
Number of ethylsulphate (EtS) negative cases within different ethanol levels in blood, urine and vitrous humor and number of putrefactive alcohol (PA) positive cases within different ethanol levels in blood.

Ethanol level in either medium (g/kg)	Blood		Blood		Urine		Vitreous humor	
	N	PA pos[a] (%)	N	EtS neg (%)	N	EtS neg (%)	N	EtS neg (%)
Total	**8001**	**1952 (24.4)**	**2504**	**382 (15.3)**	**1900**	**178 (9.4)**	**271**	**20 (7.4)**
2.00 ≤	1844	200 (10.8)	629	1 (0.2)	863	2 (0.2)	79	0 (0.0)
1.40 – 1.99	1189	152 (12.8)	441	4 (0.9)	308	1 (0.3)	51	0 (0.0)
1.20 – 1.39	436	94 (21.6)	146	5 (3.4)	82	1 (1.2)	10	0 (0.0)
1.00 – 1.19	402	86 (21.3)	147	6 (4.1)	72	2 (2.8)	11	0 (0.0)
0.90 – 0.99	249	68 (27.3)	80	5 (6.3)	47	2 (4.3)	4	0 (0.0)
0.80 – 0.89	288	90 (31.2)	66	4 (6.1)	49	0 (0.0)	17	1 (5.9)
0.70 – 0.79	296	112 (37.8)	92	10 (10.9)	50	3 (6.0)	11	1 (9.1)
0.60 – 0.69	319	122 (38.2)	103	19 (18.4)	44	3 (6.8)	14	0 (0.0)
0.50 – 0.59	390	145 (37.1)	110	24 (21.8)	62	15 (24.2)	13	0 (0.0)
0.40 – 0.49	441	185 (42.0)	133	50 (37.6)	69	13 (18.8)	9	0 (0.0.)
0.30 – 0.39	530	216 (40.8)	157	55 (35.0)	76	29 (38.2)	13	1 (7.7)
0.20 – 0.29	678	230 (33.9)	213	103 (48.4)	86	45 (52.3)	21	3 (14.3)
0.10 – 0.19	939	252 (26.8)	187	96 (51.3)	92	62 (67.4)	18	14 (77.8)

[a] 1-propanol and/or 1-butanol

internal standards as described by Thelander et al. [25]. In the same method, the two PA's 1-propanol and 1-butanol were qualitatively identified based on retention time and, if one or both of the compounds were identified in one or both mediums, it was assigned as PA-positive cases. Positive results were defined as values equal to or higher than the lower limit of quantification for ethanol (0.10 g/kg) and the limit of detection for both of the two PA's (0.02 g/kg).

2.3. Ethics and statistics

The Norwegian data set was handled according to the current data processing agreement between the Department of Forensic Sciences, Oslo University Hospital and the Norwegian Higher Prosecuting Authority. The latter is the owner of forensic data in Norway. The authors received access to anonymous data exclusively, hence, case histories and/or circumstances of death were not provided. The Swedish data set was handled according to ethics approval 2016/489–31 from the Regional Ethics Committee in Linkoping.

SPSS® Software version 25.0 (IBM Corporation) was used for statistical analyses and Student's *t*-test was used to compare differences between groups. Peripheral, central, and unspecified blood were merged into "blood" for most of the statistical analyses.

3. Results

The total number of cases included in the Norwegian data set was 2504. The mean age was 51.6 years (the age was unknown in 67 cases). 75.3% (n = 1886) were males and 24.6% (n = 615) were females (the gender was unknown in 3 cases). The total number of cases included in the Swedish data set was 8001. The mean age was 56.5 years (the age was unknown in 11 cases). 75.3% (n = 6027) were males and 24.6% (n = 1908) were females (the gender was unknown in 66 cases).

From the Norwegian data set, 15.3% of all ethanol positive blood samples, 9.4% of all ethanol positive urine samples and 7.4% of all ethanol positive vitreous humor samples were EtS negative.

Table 1 presents different ethanol concentration levels in these blood, urine and vitreous humor samples. It is worth noting that frequencies for blood, urine and vitreous humor within each concentration level presented in Table 1, do not necessarily connect to the same case. For all three mediums, the frequency of EtS negative cases decreased with increasing ethanol concentrations. Most of the vitreous humor ethanol concentrations (77.8%) were lower than 0.20 g/kg, compared to urine (67.4%) and blood (51.3%).

Table 1 also shows that 24.4% of the blood ethanol positive cases found within the Swedish data set were PA positive. Although to a lesser extent, the frequency of cases that were positive for PA's, decreased with increasing ethanol concentration, as with EtS negative cases. Less cases were PA positive at the lowest ethanol levels, compared to EtS negative.

Table 2 shows EtS and PA results at the three different blood sampling sites. EtS was negative in 55.2% of ethanol positive central blood samples, 13.5% of peripheral blood samples and 8.9% of unspecified blood samples. The frequencies of PA detections were 59.6% in central blood and 21.4% in peripheral blood.

Table 2
Distribution of ethylsulphate (EtS) negative and putrefactive alcohol (PA) positive cases between different blood sampling sites.

Postmortem formation marker result	Peripheral blood	Central blood	Unspecified blood
EtS positive (%)	1892 (86.5)	56 (44.8)	174 (91.1)
EtS negative (%)	296 (13.5)	69 (55.2)	17 (8.9)
PA negative (%)	5793 (78.6)	184 (40.4)	72 (40.9)
PA positive (%)	1576 (21.4)	272 (59.6)	104 (59.1)

Table 3A
Ethanol ratios in paired samples of urine and blood, and vitreous humor and blood according to ethylsulphate (EtS) results.

	Urine/blood ethanol ratio (n = 1918)		Vitreous humor/blood ethanol ratio (n = 309)	
	EtS positive (n = 1724)	EtS negative (n = 194)	EtS positive (n = 263)	EtS negative (n = 46)
Mean (SD)	1.48 (0.94)	1.12 (1.54)[a]	1.15 (0.43)	0.29 (0.54)[b]
10 perc	0.99	0.20	0.70	0.00
25 perc	1.17	0.50	1.04	0.00
50 perc (Median)	1.33	0.91	1.17	0.00
75 perc	1.50	1.18	1.30	0.36
95 perc	2.57	3.00	1.73	1.47
Lowest	0.00	0.00	0.00	0.00
Highest	21.00	18.00	3.50	2.96

[a] $p < 0.001$ compared to EtS positive cases,
[b] $p < 0.001$ compared to EtS positive cases

In the Norwegian data set, ethanol analysis was performed in 1918 urine samples and 309 vitreous humor samples, supplementary to blood. Ethanol concentration ratios between urine or vitreous humor and blood were calculated. Table 3A presents these ratios in paired samples in relation to their blood EtS results. Among the EtS positive cases, the mean ethanol ratios with blood were significantly higher both for urine (1.48) and vitreous humor (1.15) (p-value < 0.001 for both) compared to EtS negative cases (mean ratios of 1.12 and 0.29, respectively). They were however within wider ranges, especially the urine to blood ratios. It is important to note that blood ethanol was positive in all cases (according to inclusion criteria), while urine and vitreous humor ethanol could be negative, explaining why the median ratio for vitreous humor was 0.00.

In the Swedish data set, ethanol analysis was performed in 5881 urine samples and 931 vitreous humor samples supplementary to blood. Table 3B presents the urine or vitreous humour to blood ethanol ratios divided into PA negative and positive cases. The mean ratios were significantly higher in PA negative cases (p-value < 0.001 for both urine and vitreous humor).

All EtS negative cases with urine ethanol concentrations equal to or higher than 0.60 g/kg and vitreous humor ethanol concentrations equal to or higher than 0.10 g/kg are presented in Table 4. Amongst these cases, the highest urine ethanol concentration was 2.90 g/kg with a urine to blood ratio of 5.80. In vitreous humor, the highest value was 0.80 g/kg with a ratio of 2.96.

Table 3B
Ethanol ratios in paired samples of urine and blood, and vitreous humor and blood according to putrefactive alcohol (PA) results.

	Urine/blood ethanol ratio (n = 5881)		Vitreous humor/blood ethanol ratio (n = 931)	
	PA negative (n = 4738)	PA positive (n = 1143)	PA negative (n = 783)	PA positive (n = 148)
Mean (SD)	1.39 (0.78)	1.20 (0.70)[a]	1.06 (0.48)	0.60 (0.84)[b]
10 perc	0.000	0.000	0.000	0.000
25 perc	0.839	0.838	0.839	0.846
50 perc (Median)	1.214	1.213	1.214	1.215
75 perc	1.408	1.408	1.408	1.409
95 perc	2.162	2.161	2.162	2.159
Lowest	0.00	0.00	0.00	0.00
Highest	13	9.07	4.67	8.24

[a] $p < 0.001$ compared to PA negative cases,
[b] $p < 0.001$ compared to PA negative cases

Table 4
Individual results of paired samples in cases that were ethylsulphate (EtS) negative in blood (which all were also ethylglucuronide (EtG) negative). For vitreous humor, all EtS negative cases are shown, while for urine, only those EtS negative cases with ethanol concentrations equal to or higher than 0.60 g/kg, are shown.

Case number	Ethanol concentration (g/kg)				Ethanol ratio	
	Urine (n = 14)	Vitreous humor (n = 20)	Blood		Urine/ blood	Vitreous humor/ Blood
1	2.90	n.a.	0.50	Pb	5.80	–
2	2.50	n.a.	1.60	Cb	1.56	–
3	1.80	n.a.	0.10	Cb	18.00	–
4	1.20	n.a.	1.20	Pb	1.00	–
5	1.10	n.a.	1.00	Cb	1.10	–
6	1.10	n.a.	0.40	Pb	2.75	–
7	0.90	n.a.	0.70	Pb	1.29	–
8	0.90	n.a.	0.40	Pb	2.25	–
9	0.70	n.a.	0.66	Pb	1.06	–
10	0.70	n.a.	0.19	Pb	3.68	–
11	0.70	n.a.	0.40	Pb	1.75	–
12	0.60	n.a.	1.80	Cb	0.33	–
13	0.60	n.a.	0.60	Pb	1.00	–
14	0.60	n.a.	0.60	Pb	1.00	–
15	n.a.	0.80	0.27	Pb	–	2.96
16	n.a.	0.70	0.50	Pb	–	1.40
17	n.a.	0.30	0.20	Pb	–	1.50
18	n.a.	0.20	0.30	Pb	–	0.67
19	n.a.	0.20	0.38	Pb	–	0.53
20	n.a.	0.20	0.47	Pb	–	0.43
21	n.a.	0.10	0.10	Pb	–	1.00
22	n.a.	0.10	0.13	Pb	–	0.77
23	n.a.	0.10	0.15	Pb	–	0.67
24	n.a.	0.10	0.20	Pb	–	0.50
25	n.a.	0.10	0.23	Pb	–	0.43
26	n.a.	0.10	0.30	Pb	–	0.33
27	n.a.	0.10	0.30	Pb	–	0.33
28	n.a.	0.10	0.30	Pb	–	0.33
29	n.a.	0.10	0.36	Cb	–	0.28
30	n.a.	0.10	0.39	Pb	–	0.26
31	n.a.	0.10	0.40	Ub	–	0.25
32	n.a.	0.10	0.40	Pb	–	0.25
33	n.a.	0.10	0.50	Pb	–	0.20
34	n.a.	0.10	0.51	Pb	–	0.20

4. Discussion

Defined by negative blood EtS results, this study examined the frequency of postmortem formation of ethanol in urine and vitreous humor in addition to blood. The frequency proved to be relatively high in both urine and vitreous humor, especially at lower ethanol levels. The frequency of EtS negative cases increased with decreasing ethanol concentration levels. The findings conform with current accepted consensus that ethanol concentrations of 1.00 g/kg and greater are more likely to represent alcohol ingestion prior to death [2].

Ethanol is formed post-mortem by microbial activity [26]. The frequency of postmortem ethanol formation was found to be highest in blood, followed by urine and then vitreous humor. A possible explanation is the protected environment of the vitreous humor and, to a certain degree, also urine, which makes postmortem ethanol formation unlikely in urine and even less likely in vitreous humor [4,27]. Ethanol concentrations originating from postmortem formation were generally lower in urine than blood and lowest in vitreous humor. Various factors such as trauma to the eye or pelvis and its surrounding structures, urinary tract infections and glycosuria may facilitate postmortem formation [3,27–30]. Nevertheless, the frequencies observed within this study raise the question on whether postmortem formation in vitreous humor and, to some extent also in urine, is perhaps more common than previously assumed.

The difference in the pharmacokinetics of ethanol and its metabolites must be considered when ethanol is positive in blood, urine or vitreous humor and EtS is negative in blood. However, the lack of alcohol intake prior to death is the most probable explanation. Blood ethanol concentrations reach a maximum about 20–80 min after intake of a single dose [31], although detectable levels are reached more rapidly. After alcohol intake, EtS (and EtG) has a delay of approximately 15–30 min before they are detectable in blood. This difference in detection time may be a source of error if death occurred very shortly after the start of intake, allowing time for ethanol to be absorbed before EtS is formed. However, such a rapid death, particularly with high ethanol concentrations, would seem less likely. Instability of EtS, EtG or metabolic disorders could also theoretically cause false results, but discrepancies between the results for EtG and EtS were found in only very small numbers of cases within this study (detailed data not shown). Instability of EtG, but not EtS, in postmortem blood samples, has previously been verified by several studies [18,19]. Although EtG was mainly not reported in this study, its formation might theoretically be impaired in case of glucuronidation failure (Gilberts syndrome), although this is not documented [32].

Ethanol formed in blood and surrounding tissues could result in internal contamination of urine or vitreous humor [3]. Hence, ethanol detected in urine or vitreous humor in EtS negative cases could theoretically have diffused from surrounding tissues to the site where the sample was collected through leaking or damaged membranes. The present study design does not provide information that would allow such distinction of the ethanol detected in each sample.

This study showed that postmortem ethanol formation was more frequent in central than peripheral blood, both according to EtS results and PA results. Although this is not comprehensively documented, previous studies have reported a higher frequency of postmortem ethanol formation in central blood based on more rapid microbiological contamination and the theory that glycogen rich tissues, such as the heart, liver and lungs, provide a substrate for postmortem ethanol formation [2].

The frequency of EtS negative cases in the Norwegian study population was compared to detections of PA's in the Swedish study population. The two approaches to detect postmortem ethanol formation showed similar results at low concentration levels. At upper concentration levels, the frequency of postmortem formation was higher when identified in the presence of PA's. Although EtS negative cases and PA positive cases both indicate that postmortem ethanol formation has occurred, it is important to highlight that their interpretation differs. Negative EtS indicates that there was no alcohol intake prior to death; hence the total detected ethanol must have formed postmortem. Positive EtS indicates that ethanol was ingested, but does not exclude that postmortem formation has also occurred. On the contrary, positive PA's indicate that postmortem ethanol formation has occurred, but does not exclude that ethanol ingested prior to death may be present in addition. If the sensitivities of EtS and PA's were equal, one would expect a higher percentage of PA positive cases than EtS negative cases. Previous results have indicated that the sensitivity of PA's is high [33]. Based on results of this study and if PA's do not overestimate the number of cases where ethanol is formed postmortem, one could estimate the amount of cases representing a combination of alcohol intake and postmortem formation constituting up to 9% of all ethanol positive cases. The ethanol concentrations that result from postmortem formation are however expected to be low.

Most laboratories routinely analyse ethanol in more than one medium, typically urine and vitreous humor in addition to blood, and calculate ethanol concentration ratios. When death occurs sometime after alcohol intake, expected ratios are about 1.3 and 1.2 between urine or vitreous humor and blood, respectively [2,3,34,35]. Although the use of ratios allows some careful observational based assumptions, findings of the present study also point to certain

limitations with regards to their interpretation value. The ethanol ratios with blood were generally lower within EtS negative cases compared to the EtS positive cases, as well as within PA positive cases compared to PA negative cases. On a case level however, the ratios varied considerably, as seen in Table 4.

The main weakness of the present study was that EtS and PA´s was not analysed in the same cases and that comprehensive information about circumstances of death, post mortem interval and other details was not available. Also, PA´s might theoretically be present as congeners in alcoholic beverages, but then in lower concentrations than the present cut-off limits. Also, although presence of EtG and EtS indicates that ethanol was present in the total body before death, not analysing EtS and EtG in urine and vitreous humor in addition to blood could be a weakness of the present study. Analysing EtS (and/or EtG) in urine could however not be used to conclude whether the deceased was under the influence at the time of death due to the much too long detection time of both metabolites in urine compared to ethanol. It should be noted that EtS and EtG were analysed in urine additional to blood in a few cases (n = 15) in the present study. The results were similar to those in blood (data not presented). The main strength of the present study is the large material and the possibility to compare different matrices and the two methods for interpreting post mortem formation of ethanol.

5. Conclusions

Based on these two independent data sets, we conclude that postmortem formation of ethanol is common. Identified in the presence of PA's, 24.4% of all cases with positive blood ethanol are likely to have formed ethanol post-mortem. Based on negative EtS results, ethanol was formed entirely post-mortem in 15.3% of all cases with positive blood ethanol. The blood ethanol concentrations were lower or equal to 0.2 g/kg in the majority of cases where ethanol was formed entirely post-mortem, but concentrations of 1.0 g/kg or higher were seen in 4.2% of cases. We consider this significant blood ethanol concentrations.

Although less EtS negative cases were seen when ethanol was detected in urine and vitreous humor (9.4% and 7.4%, respectively), all three mediums were susceptible to postmortem formation of ethanol, hence we recommend using caution when interpreting low ethanol concentrations not only in blood, but also in urine and vitreous humor.

Calculated ethanol ratios differed significantly in EtS positive compared to EtS negative cases and in PA positive compared to PA negative cases, but ratios that are compliant with alcohol intake cannot rule out postmortem formation. The interpretation of ethanol detections in postmortem samples will most likely be more accurate by additionally analysing EtS and PA´s.

We also conclude that peripheral blood is less susceptive to postmortem ethanol formation than central blood, therefore we recommend that ethanol is analysed primarily in peripheral blood when available.

Conflicts of interest/Competing interests

The authors declare no conflict of interest.

Declarations

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CRediT authorship contribution statement

KO participated in planning the study, performed the statistical analyses, drafted the tables and manuscript and coordinated feedback from all co-authors. RK and FK joined planning the study, analysed parts of the data and participated in preparing the manuscript. LK and JM joined planning the study and participated in preparing the manuscript. GH joined planning the study, supervised KO in preforming statistical analyses, drafting the manuscript, coordinating feedback from all co-authors and with the correspondence of the manuscript. All authors approved the last version of the manuscript.

Ethics approval

The Norwegian study material was handled according to the current data processing agreement between the Department of Forensic Sciences, Oslo University Hospital and the Norwegian Higher Prosecuting Authority. The latter is the owner of forensic materials in Norway. The authors got access to anonymous data exclusively. The Swedish study material was handled accordingly to ethical approval 2016/489–31 from the Reginal Ethics Committee in Linköping, Sweden.

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