Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Influence of postmortem phenomena on VAMS blood sampling in toxicology

Myriam Bertrand-Ndoye
m.bertrandndoye@chu-lille.fr
Centre Hospitalier Universitaire de Lille
Alexandr Gish
Centre Hospitalier Universitaire de Lille
Florian Hakim
Centre Hospitalier Universitaire de Lille
Caroline Ghoul
Centre Hospitalier Universitaire de Lille
Yann Delannoy
Centre Hospitalier Universitaire de Lille
Quentin Scanvion
Centre Hospitalier Universitaire de Lille
Valéry Hédouin
Unité de Taphonomie Médico-Légale
Hugo Girard
Centre Hospitalier Universitaire de Lille
Jean-Michel Gaulier
Centre Hospitalier Universitaire de Lille

Research Article

Keywords: VAMS, postmortem redistribution, forensic toxicology, dried blood sampling, postmortem interval

Posted Date: October 7th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11034034/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

Introduction. Postmortem redistribution and *in vitro* instability of xenobiotics limit interpretation of postmortem blood concentrations. The use of VAMS (Volumetric Absorptive MicroSampling) remains poorly studied in this field. This study evaluates concordance between an early sample taken at body recovery (T1) and a sample taken at autopsy (T2), according to the postmortem interval (PMI) and inter-sampling interval (Δt).

Materials and methods. 82 bodies were included (2021–2023). A dried blood microsample (VAMS Mitra® Neoteryx™, 10 µL, or DBS Hémaxis® for seven subjects) was collected from a peripheral vein at initial body recovery (T1) and again at autopsy (T2). EtG, PEth 16:0/18:1 and 16:0/20:4, cocaine, opiates, methadone/EDDP, tramadol, cannabinoids, benzodiazepines and their metabolites were quantified by LC-MS/MS and LC-HRMS. The C2/C1 ratio was studied against Δt and PMI using Spearman correlation (n ≥ 10), Passing-Bablok regression (n > 20) and Bland-Altman analysis (n ≥ 15).

Results. 34 subjects had at least one detected substance (mean PMI: 38.3 h; mean Δt: 44.5 h). Good inter-time stability was observed for PEth 16:0/18:1 (Spearman rho = 0.871; p < 0.001), methadone/EDDP and EME. Conversely, marked variability was noted for cocaine and benzoylecgonine beyond 50 h of Δt, a systematic increase in 6-MAM, and a biphasic kinetic for oxazepam and nordiazepam, suggestive of persistent postmortem biotransformation of diazepam. No significant correlation was found with total PMI.

Conclusion. VAMS provides exploitable information; no variation altered conclusions regarding cause of death, although inter-individual variability outweighs the effect of delay, supporting systematic early sampling.

Introduction

In the forensic context, when examining a deceased person, the toxicologist's objective is to estimate blood concentrations of toxic substances at the time of death in order to assess their possible role in that death. This role can range from a blood concentration that influences the circumstances of death to a clearly toxic, potentially fatal concentration that establishes the cause of death.

The forensic pathologist collects the samples needed for these analyses. These samples are therefore collected at a specific point during a period that begins with the cessation of cardio-respiratory activity and continues through the final *postmortem* processes. These processes alter the biological environments in which exogenous substances persist. Enzymatic activity continues, cell lysis is accompanied by the release of molecules or free radicals into the bloodstream, and changes in pH and bacterial activity—whether involving the production or consumption of substrate—are among the phenomena that interact with one another during this *postmortem* period. *Postmortem* blood is therefore more akin to a “serohematinous fluid mixture,” in which substances can form, degrade, or move from one organic compartment to another, thereby causing variations in their blood concentrations [1, 2]. Although analyzing a larger number of samples from different biological matrices may improve the estimation of a victim’s toxic exposure, *postmortem* blood is still considered the best reflection of *antemortem* blood for qualitative and quantitative interpretation [3, 4].

Theoretically, the *postmortem* interval (PMI) is the first variable determining the extent of *postmortem* redistribution (PMR). Putrefaction and xenobiotic redistribution phenomena begin within the very first hours after death and increase with a longer PMI. Redistribution from the lungs, with rising concentrations of exogenous substances, begins as early as the second hour after death in cardiac blood. Enzymatic activity ceases for some enzymes within 24 to 36 hours after death, but appears to persist longer for others [1, 5, 6]. In practice, given the time it takes to discover the body, transport it to a forensic medical institute, and schedule an autopsy, it may take several days before toxicological samples are collected. These samples are not systematically taken at the scene where the body was found, which means that only a fraction of the bodies sent to a forensic medical institute are subject to this procedure.

In addition to these phenomena occurring within the body after death (*in cadaver*), further changes occur within samples subjected to variable storage conditions. Sensitive analytical methods combined with innovative preparation techniques have improved sample analysis today. However, these analytical methods and processes do not fully address the *in vitro* instability of xenobiotics during the pre-analytical period of collected biological samples.

Thus, despite the progress made in the field of analysis, there is no guarantee that the qualitative and quantitative results obtained from *postmortem* blood tests reflect the actual conditions in the blood at the time of death.

In recent years, interest in microsampling using alternative sampling methods has grown, and its use in clinical and analytical toxicology is well documented. Volumetric Absorptive MicroSampling (VAMS) devices represent the latest generation of this approach

and are based on capillary absorption on an absorbent polymer. They show relative independence from sample hematocrit, thereby ensuring greater accuracy of the sampled volume and hence of the quantitative results obtained [7]. They are suited to broad screening of illicit substances in whole blood but can also be used for other biological matrices, such as urine, plasma, or saliva [8].

In clinical toxicology, methodological studies on the optimization and analytical validation of microsampling have been carried out. Several have examined the stability of multiple analytes under various temperature and storage conditions and reported good stability over periods exceeding one month. Some have also performed clinical validations, notably for drugs with a narrow therapeutic index. The results are promising in terms of concordance with conventional blood samples [9–11].

In analytical toxicology, VAMS use has also been evaluated, first through comparison with conventional Dried Blood Spot (DBS) cards. These comparisons demonstrated good concordance, or even an advantage, for VAMS [12, 13]. The influence of hematocrit, the variability between arterial, venous and capillary samples, and the comparison between blood and plasma samples have also been assessed [14, 15]. Moreover, VAMS use is documented for numerous classes of illicit substances, with a generally simple pre-analytical extraction step and satisfactory extraction yields [9, 10, 14–16].

Finally, in the *postmortem* field, application of blood microsampling remains limited, although a few studies exist. Nishio *et al.* demonstrated the feasibility of quantifying neuroleptic and antipsychotic drugs in *postmortem* cardiac blood samples collected on DBS at autopsy. Concentrations of all drugs stored at − 80°C on DBS remained nearly stable *in vitro* for at least 2 weeks [17].

Moretti *et al.* presented several studies aiming to identify cocaine and its metabolites, antidepressants and antipsychotics, and benzodiazepines on DBS. *In vitro* stability, assessed over a three-month period at room temperature, was very good, and this alternative blood sampling method was subsequently applied to real *postmortem* cases [18–20].

Dubois-Chabert *et al.* developed a toxicological screening method on DBS for various drugs and medications and their metabolites, then applied it to a hundred autopsy subjects. The study reported that DBS detected 20% more compounds than conventional blood sampling methods and concluded on a gain in sensitivity [21]. Ethanol consumption biomarkers have also been evaluated using VAMS in *postmortem* blood by Hakim *et al.* [22].

Finally, VAMS use for qualitative toxicological screening in a *postmortem* medico-legal context was applied to a real case in which VAMS samples were taken from several biological matrices. Targeted screening identified several psychoactive substances, notably methamphetamine, opioids, and a synthetic cathinone [23].

Although several studies have demonstrated the analytical feasibility of DBS and VAMS in the *postmortem* context, these studies mostly rely on samples collected at a single *postmortem* time point and have primarily focused on analyte storage stability. *Postmortem* changes occurring within the body (in cadaver) in blood concentrations, as assessed by microsampling, and their potential impact on toxicological interpretation, have not been addressed.

In this context, the present study aims to evaluate the concordance of VAMS concentrations between an early sample taken during initial body recovery and a subsequent sample taken at autopsy. The objective is to assess the value of toxicological information obtained through VAMS according to the PMI.

Materials and Methods

1. Recruitment

Inclusion criteria

Bodies taken into the custody of the medical examiner's office for an autopsy or external examination, including a toxicology sample, from a deceased adult whose presumed time of death (PMI) is within the last 96 hours.

Exclusion criteria

Putrefied bodies; those with an estimated PMI of more than 96 hours or an unknown time of death; autopsies or external examinations that did not result in a toxicological sample being collecte;, and cases where the expected interval between the T1 and T2 samples was less than 4 hours.

2. Equipment used for postmortem sampling

In 2021, microsamples were collected from capillary finger blood using a single-use sampling device: either a DBS Hémaxis® (n = 7).

From 2023 onward, a VAMS Mitra® Neoteryx™ device (10 µL) was used, with blood obtained either by skin puncture of a vein of the foot or ankle using a 20-gauge hypodermic needle, or, alternatively, by femoral puncture using a 14-gauge trocar needle mounted on a 20 mL syringe.

The VAMS device consists of a rigid plastic handle topped with a detachable absorbent tip made of a porous polymer whose exact composition, protected by patent, is not disclosed by the manufacturer. It is available in three calibrated blood sample volumes (10, 20 and 30 µL); only the 10 µL version was used in this study.

3. Sampling protocol

T1 samples

After opening the body bag and removing clothing covering the lower limbs, a visible superficial vein was located on the dorsum of the foot or in the peri-malleolar region. The skin was cleaned with a compress if soiled before puncture. A skin prick with a needle produced a drop of blood; if unsuccessful, another site on the foot or ankle was attempted.

As soon as a drop of blood appeared, two VAMS devices were applied to it until their absorbent tip was fully saturated. If this approach failed, femoral puncture with a needle-mounted syringe was performed instead. Each microsample was labelled “T1”, with the date and time of collection.

The two VAMS devices were then stored in their casing, itself placed in a bag left visible inside the body bag, before the bag was resealed and the body returned to refrigeration.

T2 samples

At the time of the external examination or autopsy, the body bag was reopened to retrieve the bag containing the devices. The two remaining, still unused VAMS devices were used following the same procedure as at T1, then labelled “T2” with the body's forensic institute reference number and the date and time of the second sample.

All microsamples were packaged together with the seals containing the routine *postmortem* toxicological samples (vitreous humor, bile, urine, liquid cardiac and femoral blood).

4. Data collection

The following parameters were systematically recorded: sex and age of the deceased; time of death (death certificate or, failing that, official report of body discovery); date and time of each of the two samples; anatomical site used (foot/ankle, femoral, or other); anamnestic context collected on admission (history of addiction, known pathologies, ongoing treatments, presence of alcohol or substances near the body, and any other circumstance deemed relevant); and autopsy conclusions regarding the cause of death, recorded on a standardized autopsy data collection form.

5. Toxicological analyses

After collection, samples were kept at 4°C at the forensic institute until their daily transfer to the toxicology laboratory, Monday to Friday. VAMS devices were then separated from conventional tubes and stored at room temperature, protected from light, with a desiccant. The mean pre-analytical delay observed was 6 days (median 3 days; standard deviation 10 days).

Broad toxicological screening (library of approximately 1,700 compounds, including more than 700 novel psychoactive substances) was applied to all blood samples by LC-MS/MS and LC-HRMS, following a methodology previously published by our team [24, 25]. Additional targeted assays, covering various classes of medications, drugs of abuse and other toxicants, were also performed by LC-MS/MS.

The extraction protocol applied to VAMS devices consisted of placing the support containing 10 µL of dried blood into an Eppendorf tube, to which 100 µL of extraction solution was added (methanol/water 9:1, 0.1% formic acid, including internal standards). After vortexing (3 min) and sonication (10 min), the mixture was centrifuged for 14 minutes at 15,000 rpm. The resulting supernatant was diluted by half with a 5 mM ammonium formate buffer (pH 3), then 10 µL of this final extract were injected onto the LC-MS/MS (Xevo® TQ-S, Waters) and LC-HRMS (Xevo® G2-XS, Waters) systems.

The calibration range, together with the analytical trueness and precision parameters for microsamples and for conventional samples collected at autopsy, are provided in the Supplementary Data.

6. Statistical analysis

The number of molecules detected for each included case was determined. The mean, median and standard deviation were calculated for all *postmortem* intervals (PMI).

Descriptive statistics are expressed as median and interquartile range (IQR), with minimum and maximum values. For each analyte, paired T1 and T2 concentrations were summarized and the relative variation calculated using the formula (C2 − C1)/C1 × 100.

The seven subjects sampled on DBS Hémaxis® in 2021 were included in all concordance analyses, as both supports rely on the same principle of drying blood onto a solid matrix. For two of them, the PMI before the first sample could not be determined; these subjects were excluded solely from analyses involving this variable. For one subject from 2023, PMI and Δt data were missing; this subject was likewise excluded from analyses involving these variables.

Given the substantial disparity in sample sizes between analytes, the depth of statistical analysis was adapted to the number of available paired observations, according to three pre-defined thresholds.

Method comparison by Passing-Bablok regression was restricted to analytes with more than 20 paired observations. Only PEth 16:0/18:1 met this criterion. Constant and proportional bias between T1 and T2 measurements were assessed with 95% confidence intervals estimated by bootstrap resampling, and results were plotted graphically with the identity line as reference.

Bland-Altman analysis was performed for analytes with at least 15 paired observations.

Spearman correlations between the C2/C1 concentration ratio and the temporal variables were calculated for analytes with at least 10 valid ratio values.

Individual paired values are presented as connected-point plots. The C2/C1 ratio was calculated for each subject and plotted against the inter-sampling interval (Δt) and the PMI before the first sample (PMI). Total PMI, defined as the sum of the PMI before the first sample and the inter-sampling interval (PMI + Δt), was also calculated. The proportion of variance contributed by each of these two variables was estimated in the subgroup of subjects with both PMI and Δt available (n = 29). Spearman correlations between total PMI and each of its components, as well as between PMI and Δt themselves, were also calculated.

All analyses were performed using R software (version 4.6, R Foundation for Statistical Computing), using the mcr package for Passing-Bablok regression, BlandAltmanLeh for Bland-Altman analysis, and ggplot2 for graphical representations.

Results were also reviewed on a case-by-case basis, considering cases of multiple substance intoxication and the findings of the autopsy.

Results

A total of 82 bodies were included across the two inclusion periods: 22 in 2021 and 60 in 2023. Among these subjects, 35 underwent toxicological analysis as part of a medico-legal expert assessment. The remaining samples were either destroyed without analysis or retained pending a judicial request. Toxicological analysis identified at least one exogenous substance in 34 of the 35 subjects studied.

The mean PMI before the first sample was 38.3 hours (median: 35.5 hours; standard deviation: 21.3 hours). The mean interval between the T1 and T2 samples was 44.5 hours (median: 22.75 hours; standard deviation: 29.4 hours). The mean total PMI, i.e. the PMI at autopsy, was 81.1 hours (median: 76.5 hours; standard deviation: 27.8 hours).

Among the 29 subjects with both values available, Δt showed markedly greater dispersion than PMI (standard deviation 33.4 h versus 23.4 h), and contributed 67% of the variance of total PMI versus 33% for PMI. Total PMI was strongly correlated with Δt (rho = 0.74) and weakly with PMI (rho = 0.29). A negative correlation was also observed between PMI and Δt (rho = − 0.29), reflecting that bodies taken in charge later were autopsied within shorter intervals.

The substances mainly detected were as follows:

1. Alcohol consumption biomarkers: EtG, PEth 16:0/18:1 and PEth 16:0/20:4
2. Cocaine and its metabolites: ecgonine methyl ester (EME), benzoylecgonine (BZE) and cocaethylene;
3. Opiates: 6-monoacetylmorphine (6-MAM), morphine and codeine;
4. Methadone and its metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP);
5. Tramadol and its metabolites: O-desmethyltramadol and N-desmethyltramadol;
6. Δ9-tetrahydrocannabinol (THC) and its metabolites: 11-hydroxy-THC (THC-OH) and 11-nor-9-carboxy-THC (THC-COOH);
7. Benzodiazepines: alprazolam, bromazepam, clonazepam and its metabolite 7-aminoclonazepam, diazepam, lorazepam, nordiazepam, oxazepam and temazepam, as well as zopiclone.

Table 1 summarizes the median concentrations of exogenous substances detected among the 34 subjects according to sampling time. Median relative variations, minimum and maximum variations, and Spearman correlation coefficients are also indicated.

Table 1
Median concentrations (IQR) between T1 and T2, relative variations, and Spearman correlations according to Δt, PMI and total PMI

Group	Substance	n	C1 median µg/L (IQR)	C2 median µg/L (IQR)	Median rel. var. % (IQR)	Min-Max rel. var. %	Spearman Δt rho (p)	Spearman PMI rho (p)	Spearman total PMI rho (p)
Alcohol biomarkers	EtG	17	649 (1930)	611 (1932)	-14.8 (74.3)	-100-588.4	0.01 (p = 0.96, n = 15)	-0.05 (p = 0.858, n = 14)	-0.17 (p = 0.553, n = 14)
	PEth 16:0/18:1	27	114 (623.5)	73 (647.5)	-11.1 (66.5)	-100-166.7	-0.18 (p = 0.391, n = 24)	-0.28 (p = 0.188, n = 23)	-0.37 (p = 0.086, n = 23)
	PEth 16:0/20:4	12	58.5 (234.25)	60 (258.5)	-36 (130.8)	-100-2900	—	—	—
Opioids	Morphine	4	30.55 (31.17)	34.4 (45.27)	70.5 (136.4)	-75-258.3	—	—	—
	Codeine	4	4.25 (1.68)	5.85 (4.15)	35.3 (143.6)	-14.3-531.8	—	—	—
	6-MAM	3	3.8 (4.2)	13.4 (21.95)	252.6 (138.1)	93.8–370	—	—	—
	Tramadol	2	932 (878)	1310 (1100)	161 (127.9)	33.1-288.9	—	—	—
	N-desmethyltramadol	3	17 (400.75)	41 (564.75)	40.9 (70.6)	0-141.2	—	—	—
	O-desmethyltramadol	3	224 (271)	351 (161.5)	53.1 (45.9)	-35.2-56.7	—	—	—
	Methadone	8	82.2 (72.25)	79 (96.25)	5.8 (96.8)	-77.7-107.9	—	—	—
	EDDP	8	9 (27.35)	11.5 (41.23)	1.2 (60.3)	-65.1-35	—	—	—
Cocaine and metabolites	Cocaine	9	60 (71)	49 (367)	-8.5 (430)	-98.7-946.2	—	—	—
	EME	8	138.5 (370.75)	65 (228)	-2.1 (234)	-94-3900	—	—	—
	BZE	9	474 (823)	699 (507)	0 (119.9)	-38.9-380.8	—	—	—
	Cocaethylene	5	5.4 (6)	0.1 (19.3)	0 (84.7)	-100-640.7	—	—	—
Cannabinoids	THC	2	8.9 (8.1)	3.05 (2.05)	-22.5 (47.5)	-70-25	—	—	—
	THC-OH	2	1.6 (0.7)	1.3 (1.3)	-43.5 (56.5)	-100-13	—	—	—
	THC-COOH	3	6 (8.4)	7 (10)	16.7 (1.4)	15.4–18.2	—	—	—
Benzodiazepines and hypnotics	Alprazolam	3	48 (53)	35 (30.2)	-31.8 (30.2)	-35.4-25	—	—	—
	Bromazepam	2	983.5 (556.5)	736 (274)	-13.1 (21.3)	-34.4-8.2	—	—	—
	Clonazepam	1	29 (0)	7.3 (0)	-74.8 (0)	-74.8–74.8	—	—	—
	7-aminoclonazepam	1	9.5 (0)	32.4 (0)	241.1 (0)	241.1-241.1	—	—	—

Group	Substance	n	C1 median µg/L (IQR)	C2 median µg/L (IQR)	Median rel. var. % (IQR)	Min-Max rel. var. %	Spearman Δt rho (p)	Spearman PMI rho (p)	Spearman total PMI rho (p)
	Diazepam	7	58 (84.5)	44 (66.45)	0 (78.9)	-99.8-80.2	—	—	—
	Lorazepam	4	121 (63.47)	126.5 (89.75)	-6.6 (106.5)	-100-51.5	—	—	—
	Nordiazepam	9	155 (344.3)	115 (166.5)	13.6 (96)	-49.6-153.3	—	—	—
	Temazepam	2	23.2 (16.8)	24 (20)	-13.8 (23.8)	-37.5-10	—	—	—
	Oxazepam	11	50 (496.9)	45 (440.9)	-10 (31.4)	-100-66	0.52 (p = 0.126, n = 10)	—	—
	Zopiclone	4	11.5 (15.22)	5.55 (14.93)	-38 (105.3)	-100-92.9	—	—	—

IQR values are not interpretable for n < 3.

Alcohol biomarkers

EtG

The median relative variation of EtG also shows very good overall stability, with one larger, isolated variation of + 588.4%, corresponding to a change from 129 to 888 µg/L (Table 1).

The Spearman coefficient for Δt was non-significant (0.01; p = 0.96); however, visual inspection of the loess curve indicates a rise in the C2/C1 ratio up to 4 around 25 h of Δt, followed by a decrease and then a linear trend close to 1 after 50 h (Fig. 1**).**

The Spearman coefficient for PMI was non-significant (− 0.05; p = 0.85, n = 14), and visual inspection of the ratios does not reveal any identifiable kinetic pattern of its influence (**Online Resource 1**).

The Spearman coefficient for total PMI was also non-significant (-0.17; p = 0.55, n = 14). Analysis of the C2/C1 ratio as a function of total PMI reveals a kinetic pattern similar to that observed according to Δt, with an increase in the ratio up to 75-80h of total PMI, followed by a progressive decrease below 1 beyond 100h. (**Online Resource 2**).

Bland-Altman analysis revealed a mean bias of 1406.3 µg/L (T2 − T1), with limits of agreement ranging from − 4432.5 to + 7245.1 µg/L, reflecting substantial inter-individual variability between T1 and T2 concentrations, despite the absence of a major systematic bias (**Online Resource 3**).

PEth 16:0/18:1

For PEth 16:0/18:1, variability between T1 and T2 is low, with a minority of moderate individual variations either upward or downward (median variation: −12 µg/L; min-max variation: [− 650; 1250] µg/L; the largest increase corresponding to a change from 750 to 2000 µg/L) (Table 1). There was no statistically significant influence of the T1-T2 interval or of PMI, with respective Spearman coefficients of − 0.18 (p = 0.39) and − 0.28 (p = 0.18, n = 23) (Table 1). The plot of the C2/C1 ratio against Δt shows a poorly homogeneous, uninformative dispersion (Fig. 2). The plot against PMI does not reveal any relationship between these two parameters, nor any combined effect of PMI and Δt (Fig. 3). Accounting for total PMI does not change this observation, with a non-significant coefficient of − 0.37 (p = 0.09, n = 23). The plot of the C2/C1 ratio against total PMI shows a progressive, regular decline of the loess curve, from a ratio close to 1.6 at the shortest total PMI values to values below 1 beyond 90 h, consistent with the negative Spearman coefficient observed (**Online Resource 4**).

Bland-Altman analysis revealed a mean bias of − 74.1 µg/L (T2 − T1), with limits of agreement ranging from − 827.0 to + 678.8 µg/L, reflecting inter-individual variability between T1 and T2 concentrations despite a moderate mean bias (Fig. 4).

Passing-Bablok regression showed no proportional bias (slope = 1.11; 95% CI 0.88–1.40), with a small constant bias (intercept = − 16.37 µg/L; 95% CI − 45.72 to − 2.71) of negligible magnitude relative to the concentrations measured (Fig. 5). A strong monotonic correlation was observed between T1 and T2 concentrations (Spearman rho = 0.871; p < 0.001), consistent with the absence of proportional bias shown by the Passing-Bablok regression.

PEth 16:0/20:4

For PEth 16:0/20:4, variability between T1 and T2 is low, with a minority of moderate individual variations either upward or downward. The largest decrease was − 100%, corresponding to a loss of 62 µg/L; the largest increase was + 2900%, corresponding to a change from 55 to 1650 µg/L. There is no visible influence of Δt, PMI or total PMI, with C2/C1 ratios close to 0 for all three temporal variables (Table 1, **Online Resource 5 and 6, Online Resource 7**).

Opioids

Morphine/codeine/6-MAM

For morphine and codeine, all four subjects showed variations either upward or downward, with a min-max variation of [− 75; 258.3]% for morphine and [− 14.3; 531.8]% for codeine, corresponding to absolute variations of [− 31.5; 72] µg/L and [− 0.7; 11.7] µg/L respectively.

For 6-MAM, all three concentrations were higher at T2 than at T1 (median variation: +252.6%, i.e. +13 µg/L). Given the small sample size, observations regarding the influence of Δt and PMI should be considered exploratory. The dispersion of the C2/C1 ratios for morphine, codeine and 6-MAM appears similar as a function of Δt. The dispersion of morphine and codeine ratios also appears similar as a function of PMI (only one subject with known PMI for 6-MAM).

Methadone and EDDP

For methadone and EDDP, concentrations showed very low median variability, + 5.8% and + 1.2% respectively (i.e. +2.5 µg/L and + 0.25 µg/L), with a larger, isolated decrease from 1000 to 396 µg/L (− 60.4%) for methadone and from 204 to 108 µg/L (− 47.1%) for EDDP, in the same subject. The C2/C1 ratio was very stable around 1 with no notable influence of Δt, particularly for methadone (**Online Resource 8**). No identifiable kinetic was observed regarding the influence of PMI (**Online Resource 9**). The C2/C1 ratio also remained stable around 1 as a function of total PMI for both molecules, with no identifiable kinetic (**Online Resource 10**).

Tramadol and metabolites

For tramadol, only two subjects were recorded, with concentrations changing from 1810 to 2410 µg/L and from 54 to 210 µg/L. Concentrations of N-desmethyltramadol and O-desmethyltramadol for these two subjects also showed slight increases between T1 and T2. A third subject was positive only for tramadol metabolites, with trace-level N-desmethyltramadol at both sampling times and relative stability between T1 and T2 for O-desmethyltramadol (574 vs 372 µg/L, i.e. −35.2%; Table 1).

Cocaine and metabolites

Cocaine concentrations showed substantial variability between T1 and T2 (median variation: −8.5%; min-max variation: [− 98.7; 946.2]%, i.e. −3.1 µg/L; absolute min-max variation: [− 987.4; 861] µg/L) (Table 1). The same pattern was observed for BZE. The C2/C1 ratio for both molecules was stable around 1 before 50 h of Δt; beyond this delay, a trend toward an increasing ratio was observed (Table 1, Fig. 6). The combined graph of the cocaine and BZE C2/C1 ratio as a function of PMI shows a profile comparable to that observed according to Δt: after higher values at the shortest PMIs (< 20h), the ratio stabilizes around 1 between 20 and 50h for both molecules, before a marked increase beyond this interval, more pronounced for cocaine than for BZE (**Online Resource 11**).The C2/C1 ratio plotted against total PMI showed a comparable pattern to that observed for Δt, with the ratio stable around 1 up to approximately 65 h of total PMI, followed by an increasing trend for both molecules (**Online Resource 12**)

For EME, two marked decreases were observed (1000 to 60 µg/L and 1000 to 401 µg/L) and one moderate increase (201 to 510 µg/L), against five subjects with very small variations. The C2/C1 ratio was very stable as a function of Δt and PMI. A major outlier was observed with a ratio of 40 at 20 h of PMI (**Online Resource 13**), corresponding to a change from a trace-level concentration to 10 µg/L, i.e. a variation of + 3900% (Table 1). As a function of total PMI, the C2/C1 ratio remained stable around 1, with the previously described outlier located at the shortest total PMI values (**Online Resource 14**).

THC and its metabolites

For THC, only two subjects were recorded, with concentrations changing from 0.8 to 1 µg/L and from 17 to 5.1 µg/L. Metabolite concentrations for these two subjects showed variations from 0.9 to 0 µg/L and from 2.3 to 2.6 µg/L for THC-OH, and from 6 to 7 µg/L and from 22 to 26 µg/L for THC-COOH. A third subject was positive only for THC-COOH, with a concentration of 5.2 µg/L at T1 and 6 µg/L at T2.

Benzodiazepines and hypnotics

Oxazepam

Individual variability of oxazepam concentrations between T1 and T2 was observed, with a median variation of − 10% and a min-max variation of [− 100; 66]% (i.e. −0.1 µg/L; absolute min-max variation: [− 551; 305] µg/L). A kinetic influenced by Δt was observed, with the loess curve turning negative between 0 and 50 h before turning positive, then flattening beyond 100 h (**Online Resource 15**). No identifiable kinetic was observed for the influence of PMI. As a function of total PMI, a biphasic kinetic was also observed, with the loess curve turning negative up to approximately 70 h before turning positive around 90–100 h, then returning toward 1 beyond 130 h (**Online Resource 16).**

Nordiazepam

For nordiazepam, variability between T1 and T2 was low, with a median variation of + 13.6% and a min-max variation of [− 49.6; 153.3]% (absolute variation: −16 µg/L; min-max: [− 37; 10] µg/L). The loess curve of the C2/C1 ratio as a function of Δt follows a kinetic similar to that of oxazepam (**Online Resource 15**). No identifiable kinetic was observed for the influence of PMI. The loess curve as a function of total PMI follows a kinetic similar to that of oxazepam, with a trough around 80 h and a positive peak around 100 h (**Online Resource 16**).

Diazepam

For diazepam, variability between T1 and T2 was low, with a median variation of 0% and a min-max variation of [− 99.8; 80.2]% (absolute min-max variation: [− 98; 211] µg/L). The loess curve as a function of Δt was little influenced by this delay, with dispersion around 1 and a slight downward trend between 20 and 60 h (**Online Resource 17**). No identifiable kinetic was observed for the influence of PMI. As a function of total PMI, the loess curve turns slightly negative around 70 h before progressively returning toward 1, with no marked trend (**Online Resource 18**).

Other benzodiazepines and related compounds

Alprazolam concentrations were very stable between T1 and T2, with a median variation of − 31.8% (absolute min-max variation: [− 42.6; 7] µg/L). The two bromazepam-positive subjects showed variations from 427 to 462 µg/L and from 1540 to 1010 µg/L (respective Δt: 16 h and 45 h). For clonazepam, the single subject showed a concentration of 29 µg/L at T1 versus 7.3 µg/L at T2, with no metabolite detected (Δt: 45.5 h). Another subject was positive for 7-aminoclonazepam without detectable clonazepam, with a concentration of 9.5 µg/L at T1 and 32.4 µg/L at T2 (Δt: 71 h). Median variability of lorazepam between T1 and T2 was + 10.45 µg/L, with an absolute min-max variation of [− 110; 68] µg/L. The two temazepam-positive subjects showed variations from 40 to 44 µg/L and from 6.4 to 4 µg/L (respective Δt: 20.5 h and 62 h).

Finally, for zopiclone, median variability was − 4.5 µg/L, with an absolute min-max variation of [− 35; 13] µg/L.

Overall, an undetectable T2 concentration was observed in several cases. For PEth 16:0/18:1, two subjects showed complete disappearance between T1 and T2, from T1 concentrations of 5 and 37 µg/L. For PEth 16:0/20:4, three subjects showed disappearance, from T1 concentrations of 2, 14 and 62 µg/L. For EtG, one subject showed disappearance from a T1 concentration of 23 µg/L. For cocaethylene, one subject showed disappearance from a T1 concentration of 6.1 µg/L. For THC-OH, one subject showed disappearance from a T1 concentration of 0.9 µg/L. For lorazepam, one subject showed disappearance from a T1 concentration of 22.1 µg/L. For oxazepam, one subject showed disappearance from a T1 concentration of 0.1 µg/L. For zopiclone, one subject showed disappearance from a T1 concentration of 9 µg/L.

Subject 2 showed disappearance for two different PEth homologues (PMI unknown; Δt = 18 h). Subject 17 showed disappearance for PEth 16:0/18:1 and oxazepam (PMI = 67 h; Δt = 21 h). Subject 22 showed disappearance for lorazepam and zopiclone (PMI = 26 h; Δt = 45.5 h). Subject 26 showed disappearance for PEth 16:0/20:4 (PMI unknown; Δt unknown). No correlation was found between these disappearances and Δt, PMI, or their sum.

No systematic relationship was observed between the T1 concentration level and the magnitude of the absolute variation between T1 and T2.

Discussion

1. Influence of postmortem phenomena

The influence of the *postmortem* interval on concentrations between two given sampling periods has been studied in previous work. In a liquid-blood protocol similar to ours, Han *et al.* (2012) compared samples taken at admission to the morgue and at autopsy in 149 cases. The mean interval between the two samples was 64 hours (0.5 to 164 hours). Overall, few drugs showed particularly large variations; among the 30 drugs studied, only 7 showed an increase greater than 20%. For a few molecules, variations reached up to 300%. Thus, for certain drugs, significant *postmortem* redistribution could occur even when peripheral samples are used, independently of PMI. No clear relationship was found between elapsed time and the extent of *postmortem* redistribution, and although predictive factors such as a high volume of distribution or strong lipophilicity exist, these alone do not predict the magnitude of concentration increases at autopsy [26].

In our study, observations regarding the exploitability of VAMS according to PMI were likewise nuanced depending on the substance. All kinetics should be interpreted cautiously given the width of the confidence intervals of the loess curves.

Some variations may be attributed to *postmortem* phenomena, as for cocaine and BZE. Analysis of their C2/C1 ratio according to Δt suggests stability before 50 h, followed by an increase in concentrations for both molecules. This kinetic for cocaine could be explained by late passive redistribution phenomena from cocaine-rich organs (liver, lung, spleen) toward peripheral blood, amplified by tissue decomposition [27]. Our result is consistent with the study by Zilg et al., demonstrating no significant difference between central and peripheral concentrations for a short PMI (0–49 h, mean ratio = 0.8), against larger differences between central and peripheral concentrations, and between arterial and venous concentrations, for a long PMI (100–216 h, mean ratio = 2.0) [28].

Regarding the similarity of the loess curves of the C2/C1 ratios according to Δt between cocaine and BZE, it should be recalled that BZE is produced through enzymatic and non-enzymatic hydrolysis of cocaine. Degradation of cocaine into BZE may therefore continue after death. Spontaneous hydrolysis producing BZE occurs at a rate of approximately 4.8% of present cocaine per hour at pH 7.4 and 37°C [29]. While refrigerated storage of bodies ensures a stable temperature, metabolic changes cannot be excluded. In addition, PMI showed no statistically significant relationship with the cardiac/peripheral blood (C/P) ratios of cocaine, BZE, or EME, suggesting that the relationship between cardiac and peripheral blood does not depend solely on *postmortem* redistribution and confirming that it may also be influenced by cocaine degradation phenomena [27]. One may therefore hypothesize that the increase in the BZE C2/C1 ratio after 50 h reflects continued BZE production through *postmortem* hydrolysis of residual tissue cocaine, which would explain why BZE increases even as cocaine degrades. Although BZE, being less lipophilic, would be expected to be less subject to PMR, its own PMR and its production through hydrolysis are likely to coexist and mutually reinforce one another.

Other degradation kinetics complicate the interpretation of concentration variations under *postmortem* conditions. In our study, 9 cases showed the concomitant presence of a parent benzodiazepine with one or more of its active metabolites, and 5 cases showed an isolated benzodiazepine. The two loess curves of the C2/C1 ratio according to Δt were similar for oxazepam and nordiazepam, with a negative peak around 40 hours and a positive peak around 80 hours. This latter peak finding diverges from previous literature, which describes general trends of significant, time-dependent decrease for these two molecules. This phenomenon could, however, be explained by the *postmortem* metabolic conversion of diazepam into nordiazepam and then into oxazepam. The presence of only two lorazepam-oxazepam associations in our study may have made their effect on oxazepam kinetics negligible [30].

For other molecules such as cocaethylene, degradation in liquid blood is well known. Degradation, or even complete disappearance for a low T1 concentration, was also found in our study.

6-MAM shows even greater instability, requiring the development of detection protocols on vitreous humor, cerebrospinal fluid, or even gastric mucosa [26, 31, 32]. Our parallel study comparing VAMS with standard blood tubes demonstrated stabilization of 6-MAM on the microsample: three cases were positive for 6-MAM in the microsamples, whereas only one was positive in standard tube samples. In this latter case, the concentration differed by − 93.61% between the standard tube and the microsample. One case in this parallel study had a storage period of two years [33]. However, this finding does not fully explain the slight increase observed between T1 and T2 in the present study. Attributing the systematic increase seen in the 3 cases of 6-MAM in our study to postmortem redistribution

phenomena cannot be confirmed given the small sample size of our study. An increasing trend was also observed for tramadol and its metabolites. Although peripheral blood sampling, as opposed to central blood, reduces postmortem redistribution, it does not eliminate it entirely, as potential substance sources such as the bladder, skeletal muscle, and adipose tissue may still influence it [26].

Furthermore, some results from our study are not precisely explained by current knowledge: although the median percentage variation of EtG was negative between C1 and C2, the Bland-Altman analysis showed a positive mean bias with a high upper limit. In detail, several marked increases were observed around 25 h of Δt, alongside a majority of stable, or even slightly decreasing, concentrations before and after this delay. These findings suggest that EtG concentrations tend, at least at times, to increase overall between T1 and T2. This is biologically plausible under the hypothesis of *postmortem* neoformation. *In cadaver*, EtG has been shown to be broken down by bacteria, although significant degradation is unlikely over short intervals without decomposition or elevated temperature [34, 35]. *In cadaver*, neoformation may explain an increase through continued ethanol metabolism [36, 37]. *In vitro*, general stability is the norm; H. Schloegl *et al.* reported relatively constant concentrations at 4°C in sealed tubes, and at room temperature over 5 weeks. Variations ranged from a 30% decrease to an 80% increase, averaging + 37.5%, which, despite differing study conditions, remains consistent with our results [38]. As ethanol is volatile on VAMS, EtG stability is expected on VAMS.

For most molecules in our study, no identifiable trend was observed as a function of Δt. Major inter-individual variability is known for morphine. When examining the mean of a cohort, individual differences are smoothed out, yielding an overall profile of minimal *postmortem* changes [30]. Here again, no correlation was found between the extent of changes and the pre-autopsy interval. Several factors could inform reflection on this phenomenon, such as oral administration or the presence of resuscitation, both of which would increase the C/P ratio [39]. In our study, the four morphine-positive cases are the same as the codeine-positive cases. Among these four cases, three were also positive for 6-MAM. This presence of morphine is therefore interpreted here as a product of heroin and codeine degradation pathways. Regarding codeine, it is plausible that it was absorbed through heroin powder use rather than independent intake. Codeine/morphine/6-MAM interactions in cadaver have been studied in the context of heroin intoxication [32]. The amount of 6-MAM converted into morphine is thought to be too small to produce a measurable increase in morphine concentration between the two samples [26]. However, morphine-codeine interactions *in cadaver* are complex. *In vivo*, the blood codeine/morphine ratio is subject to inter-individual metabolism (CYP2D6 genetics), and in cadaver and in vitro, an increase in morphine may be explained by hydrolysis of glucuronide-conjugated morphine forms, even in cases where only codeine intake was suspected [40, 41]. The similarity between the loess curves for morphine and codeine is therefore not interpretable here, given the complexity of the interactions between these two molecules and the small size of our study.

Finally, results demonstrate good stability for PEth, methadone, EDDP and EME. Regarding PEth, its stability on VAMS has been demonstrated in recent years [42]. In vitro PEth formation, linked to the presence of alcohol in blood as shown in previous studies and liable to produce artificially elevated results, can therefore be avoided [43].

Methadone and its metabolite were also stable, although with a major, isolated decrease common to both molecules in the same subject. Neither the data collected on this subject nor the literature explains this degradation (subject no. 22: PMI = 26 h, Δt = 45.5 h, BMI = 21.7 kg/m², no trauma) [44]. The same applies to EME, with two marked decreases from 1000 to 60 (subject no. 5: PMI = 46.5 h, Δt = 14.5 h, BMI = 24.3 kg/m², no trauma) and from 1000 to 401 µg/L (subject no. 22) [27].

Overall, *postmortem* redistribution phenomena were observed in our study, and, for some molecules, an identifiable kinetic of the C2/C1 ratio was found as a function of the interval elapsed between the two samples. However, no correlation was found between the magnitude of *postmortem* redistribution and total PMI. Kinetics observed according to this variable broadly reproduced those described according to Δt. This convergence may be explained by the construction of total PMI, of which Δt accounts for two-thirds of the variance in our series. This finding would reinforce the value of early sampling.

Not all overall variation trends are explained, and even for stable molecules, strong inter-individual variations exist. Although there is general consensus that certain physicochemical properties of drugs contribute to their propensity for PMR, drug properties represent only part of the toxicological picture. PMR and postmortem changes in drug concentrations result from a complex interaction between the drug, the deceased, and case circumstances such as body mass index, the degree of decomposition at autopsy, the position of the body at the time of discovery, and the presence of any trauma or resuscitation attempt [44].

2. Impact on toxicological interpretation

Alcohol consumption markers make up the largest subset in our study. They do not estimate *perimortem* consumption, but allow an estimate of consumption over the days and weeks preceding sampling. The variations observed therefore have no impact on cause of

death; the results of this study provide more insight into the practical applicability of VAMS than into their forensic interpretation.

The strong monotonic correlation observed between T1 and T2 concentrations of PEth 16:0/18:1 (rho = 0.871; $p < 0.001$), together with the absence of proportional bias on Passing-Bablok regression, suggests good stability of this biomarker in cadaver over the interval studied, despite the increase in PMI between the two sampling times. This finding is notable given that the literature reports analytical instability of PEth in stored liquid blood, with concentration increases of up to 20–70% depending on storage duration [43]. By stabilizing the sample from the moment of collection through drying onto a solid support, VAMS use overcomes this analytical confounder and allows more specific assessment of in cadaver biological changes in PEth. The good concordance observed in our study may thus reflect genuine *in vivo/in cadaver* stability of PEth 16:0/18:1, independent of the tube degradation artifacts previously described in liquid blood.

This good group-level concordance should not, however, obscure the inter-individual variability shown by the Bland-Altman analysis, whose wide limits of agreement [− 827.0; +678.8 µg/L] reflect heterogeneity in individual behaviour, independent of any mean bias. PEth 16:0/18:1 is a cumulative biomarker allowing quantitative estimation of alcohol consumption over the month preceding sampling. In the absence of international consensus, several studies pooled in a review agree on two discriminating threshold values for consumption type, set at 20 and 200 µg/L (< 20 µg/L: low consumption or abstinence; 20–200 µg/L: moderate consumption, i.e. 2–4 standard drinks/day several days a week; > 200 µg/L: excessive consumption, *i.e.* more than 4 standard drinks/day several days a week). Despite the statistical inter-individual variability, in our study 4 of 27 subjects moved from “moderate” to “low or abstinent” consumption, and 1 subject moved from “low or abstinent” to “moderate”.

The second homologue, PEth 16:0/20:4, despite limited literature, appears to have a half-life allowing a detection window of approximately one week. Inter-individual variability similar to that of PEth 16:0/18:1 was observed; however, no interpretive threshold value has been reported for this homologue, precluding clinical interpretation. The same applies to EtG, for which threshold values have been established for urinary EtG over the preceding 24 to 48 hours [45, 46].

Our results suggest that interpretation remains reliable for benzodiazepines at high concentrations, as none of the observed variations altered medico-legal interpretation. VAMS appears globally reliable at the population level, but case-by-case interpretation should remain cautious, as it does not fully overcome inter-individual variability, which outweighs the effect of delay, both for molecules with overall stability, as with methadone, where subject no. 22 shows differing exposure levels between T1 and T2 [47–49], and for molecules with substantial inter-individual variability, as with morphine in subject no. 6 (C1 = 68 to C2 = 140 µg/L) [47]. This same subject showed an increase in tramadol concentration between T1 and T2 (1810 to 2410 µg/L).

Interpretation should remain cautious for molecules undergoing *postmortem* redistribution, such as cocaine after 50 h: three subjects showed decreases (no. 5: 1000 to 12.6 µg/L) or increases (no. 20: 91 to 952 µg/L and no. 12: 77 to 400 µg/L) that could alter the interpretation of exposure levels.

It should be noted that subjects no. 22 and 6 illustrate inter-individual variability across several molecules, suggesting that this phenomenon is not restricted to a single pharmacological class in a given individual.

For 6-MAM, its presence confirms heroin use, but its concentrations do not reflect exposure levels, and thus do not change the interpretation of cause of death. Although the three subjects were detected at both T1 and T2 in our study, its instability in cadaver should be recalled, and undetectable concentrations were observed at T2 for other molecules, although this occurred only from low C1 concentrations.

Although some cases showed substantially different exposure levels between T1 and T2 for a given molecule, these variations did not alter the overall medico-legal interpretation of the case in our series, cause of death most often relying on a context of poly-intoxication.

3. Limitations

3.1 Sample size

Our inclusion yield, between the bodies initially enrolled and the toxicological assessments actually performed, was approximately 50%. This sample is too small to yield statistically significant results for most substances.

3.2 Reproducibility and missing data

Although all VAMS samples were collected from peripheral blood (femoral or ankle), microsampling was not systematically performed at the same site for every body. Sample storage time, although documented (median 3 days), was not standardized and was not included as a covariate in the analyses. Capping PMI at 96 hours restricts its dispersion, whereas Δt was subject to no upper bound, unbalancing the respective weight of the two variables within total PMI. Three subjects were excluded from analyses of the temporal variables due to missing data.

3.3 Equipment

A switch from DBS to VAMS use occurred in 2023; samples collected on both supports were included together in the concordance analyses. The difference in matrix between the porous VAMS polymer and DBS card cellulose should be borne in mind [50], this heterogeneity of support constituting a limitation to the interpretation of the observed variations. Despite standardization, DBS volumes remain imprecise, with blood spreading unevenly and forming serum rings, thereby increasing hematocrit-related bias. DBS were originally designed for whole blood, which posed a challenge for postmortem samples. As with most DBS studies, our results are therefore primarily qualitative, or at best semi-quantitative [52]. Microsampling methods such as DBS and VAMS offer the advantage of a small blood volume but require highly sensitive analytical techniques such as LC-MS/MS. The pre-analytical phase is generally more complex and time-consuming than conventional sampling [52].

Analytical variability and the extraction process may have influenced the concentration difference between VAMS and whole blood, particularly for hydrophobic substances such as methadone, with an relative standard deviation (RSD) greater than 15%.

4. Perspectives

Given that current data linking PMR to specific variables are often insufficient, it remains difficult to formulate practical recommendations enabling toxicologists to interpret postmortem data based on current study findings [44]. Our results underscore the value of early sampling. Recent literature identifies several important methodological needs in PMR research, notably multi-timepoint, multi-matrix sampling. Our study directly addresses this first need by proposing repeated sampling in the same subject at two distinct time points of the postmortem period (early T1, autopsy T2), allowing intra-individual concentration kinetics to be captured rather than being limited to single-timepoint group comparisons. Using VAMS as an additional sample could provide a better understanding of the relationships between decedent-related variables, still insufficiently studied, and *postmortem* concentration changes, by stabilizing molecules *in vitro*.

Conclusion

Our study provides exploratory observations on the usability of VAMS within the studied time window. It provides data on the concordance of blood concentrations obtained by VAMS between an early sample (T1) and a sample taken at autopsy (T2), in a *postmortem* forensic context. By proposing repeated sampling in the same subject at two distinct time points, it allows intra-individual concentration kinetics to be captured, thereby complementing the literature, which is mostly based on single-timepoint group comparisons.

Results show good inter-time stability for several biomarkers and substances, notably PEth 16:0/18:1, for which the strong correlation (rho = 0.871; $p < 0.001$) and the absence of proportional bias on Passing-Bablok regression suggest robust stability in cadaver, independent of the tube degradation artifacts described in liquid blood. Results are also encouraging for methadone, EDDP, EME, and benzodiazepines at high concentrations. Conversely, variability was observed for cocaine and BZE beyond 50 hours of inter-sampling interval, and a biphasic kinetic was observed for the oxazepam/nordiazepam pair. No significant correlation was found between total PMI and the extent of the variations observed, consistent with the literature emphasizing the multifactorial nature of postmortem redistribution.

Regarding forensic interpretation, these variations did not alter the conclusions on cause of death for the subjects studied, although 6 of them showed substantially different exposure levels between T1 and T2 for certain molecules; this finding underscores the caution required in case-by-case interpretation, as inter-individual variability often outweighs the effect of delay, including for molecules that are stable overall at the group level.

This study confirms the feasibility and value of VAMS as a tool for micro-sampling in postmortem forensic investigations, while reaffirming the consensus regarding the need to collect samples as soon as possible after the body is discovered. However, the small

sample sizes—which stem from the rarity of certain toxicological associations—limit the statistical significance of our results for many substances and call for caution when generalizing them..

Systematic VAMS sampling from the time of body recovery, combined with multicentre studies involving larger sample sizes and incorporating multiple sampling time points and matrices, would help consolidate these exploratory observations and refine practical recommendations for forensic toxicologists. It could also help further the study of the relationships between decedent-related variables, still insufficiently studied, and postmortem concentration changes.

Declarations

Competing interests.

The authors declare no competing interests.

Ethics approval.

This study was conducted on biological samples collected within the framework of judicial requisitions (réquisitions judiciaires) ordered by the local Public Prosecutor (Procureur de la République), covering both conventional postmortem toxicological sampling and the VAMS micro-sampling protocol. No additional ethics committee approval was required, in accordance with French legal provisions governing medico-legal autopsies performed under judicial authority.

Consent to participate.

As samples were collected within the framework of judicial requisitions (réquisitions judiciaires) ordered by the Public Prosecutor for medico-legal death investigation purposes, individual or family consent was not required under French law. No identifying personal data are reported in this manuscript.

Funding.

The authors did not receive support from any organization for the submitted work.

Author Contribution

Author contributions. Conceptualization: JM.G; Methodology: M.BN ; Investigation: A.G, F.H (toxicological analyses), C.G, Y.D (sample collection, pilot phase); Formal analysis: M. BN; Resources: V. H; Supervision: H.G (PhD supervision), Q.S(initial phase), JM.G; Writing – original draft: M. BN; Writing – review & editing: JM.G.All authors read and approved the final manuscript.

Data Availability

The datasets generated and analyzed during the current study are not publicly available due to the confidential and judicial nature of forensic autopsy data, obtained under judicial requisition. Data may be made available by the corresponding author upon reasonable request and subject to authorization from the relevant judicial authorit

References

1. Pélissier-Alicot AL, Gaulier JM, Champsaur P, Marquet P (2003) Mechanisms Underlying Postmortem Redistribution of Drugs: A Review. J Anal Toxicol 1 nov 27(8):533–544. 10.1093/jat/27.8.533
2. Pélissier-Alicot AL (2016) La redistribution post mortem: état des lieux en 2016. Toxicol Anal et Clinique juin 28(2):103–114. 10.1016/j.toxac.2015.12.004
3. Truver MT, Chronister CW, Davis GG, Gray TR, Hartman RL, Kahl JH et al (2025) Application of professional best practices in postmortem forensic toxicology. J Anal Toxicol 1 oct 49(8):529–541. 10.1093/jat/bkaf061

4. Flanagan RJ, Connally G, Evans JM (2005) Analytical Toxicology. Toxicol Rev 1 mars 24(1):63–71. 10.2165/00139709-200524010-00005
5. Butzbach DM (2010) The influence of putrefaction and sample storage on post-mortem toxicology results. Forensic Sci Med Pathol 1 mars 6(1):35–45. 10.1007/s12024-009-9130-8
6. Yarema MC, Becker CE (2005) Key Concepts in Postmortem Drug Redistribution. Clin Toxicol janv 43(4):235–241. 10.1081/CLT-58950
7. Xie I, Xu Y, Anderson M, Wang M, Xue L, Breidinger S et al (2018) Extractability-mediated stability bias and hematocrit impact: High extraction recovery is critical to feasibility of volumetric adsorptive microsampling (VAMS) in regulated bioanalysis. J Pharm Biomedical Anal 15 juill 156:58–66. 10.1016/j.jpba.2018.04.001
8. Denniff P, Spooner N (2014) Volumetric Absorptive Microsampling: A Dried Sample Collection Technique for Quantitative Bioanalysis. Anal Chem 19 août 86(16):8489–8495. 10.1021/ac5022562
9. Mercolini L, Protti M, Catapano MC, Rudge J, Sberna AE (2016) LC–MS/MS and volumetric absorptive microsampling for quantitative bioanalysis of cathinone analogues in dried urine, plasma and oral fluid samples. J Pharm Biomedical Anal 10 mai 123:186–194. 10.1016/j.jpba.2016.02.015
10. Pires B, Catarro G, Soares S, Gonçalves J, Rosado T, Barroso M et al (2025) Volumetric Absorptive Microsampling in Toxicology. Toxics janv 13(1):25. 10.3390/toxics13010025
11. Tagwerker C, Baig I, Brunson EJ, Dutra-Smith D, Carias MJ, de Zoysa RS et al (2021) Multiplex Analysis of 230 Medications and 30 Illicit Compounds in Dried Blood Spots and Urine. J Anal Toxicol 1 juill 45(6):581–592. 10.1093/jat/bkaa125
12. Nugraha RV, Yunivita V, Santoso P, Hasanah AN, Aarnoutse RE, Ruslami R (2023) Analytical and Clinical Validation of Assays for Volumetric Absorptive Microsampling (VAMS) of Drugs in Different Blood Matrices: A Literature Review. Molecules 14 août 28(16):6046. 10.3390/molecules28166046
13. Chang WCW, Cowan DA, Walker CJ, Wojek N, Brailsford AD (2020) Determination of anabolic steroids in dried blood using microsampling and gas chromatography-tandem mass spectrometry: Application to a testosterone gel administration study. J Chromatogr 27 sept 1628:461445. 10.1016/j.chroma.2020.461445
14. Protti M, Rudge J, Sberna AE, Gerra G, Mercolini L (2017) Dried haematic microsamples and LC–MS/MS for the analysis of natural and synthetic cannabinoids. J Chromatogr B 15 févr 1044–1045:77–86. 10.1016/j.jchromb.2016.12.038
15. Al-Qurain AA, Williams DB, Mackenzie L, Roberts MS, Wiese MD (2021) Simultaneous LC-MS/MS quantification of oxycodone, tramadol and fentanyl and their metabolites (noroxycodone, oxymorphone, O- desmethyltramadol, N- desmethyltramadol, and norfentanyl) in human plasma and whole blood collected via venepuncture and volumetric absorptive micro sampling. J Pharm Biomedical Anal 5 sept 203:114171. 10.1016/j.jpba.2021.114171
16. Mandrioli R, Mercolini L, Protti M (2020) Blood and Plasma Volumetric Absorptive Microsampling (VAMS) Coupled to LC-MS/MS for the Forensic Assessment of Cocaine Consumption. Molecules 26 févr 25(5):1046. 10.3390/molecules25051046
17. Nishio T, Toukairin Y, Hoshi T, Arai T, Nogami M (2025) Determination of multiple psychostimulants and antipsychotics in postmortem dried blood spot samples by liquid chromatography–tandem mass spectrometry. Legal Med 1 juill 76:102650. 10.1016/j.legalmed.2025.102650
18. Moretti M, Visonà SD, Freni F, Tomaciello I, Vignali C, Groppi A et al (2018) A liquid chromatography–tandem mass spectrometry method for the determination of cocaine and metabolites in blood and in dried blood spots collected from postmortem samples and evaluation of the stability over a 3-month period. Drug Test Anal 10(9):1430–1437. 10.1002/dta.2399
19. Moretti M, Freni F, Valentini B, Vignali C, Groppi A, Visonà SD et al (2019) Determination of Antidepressants and Antipsychotics in Dried Blood Spots (DBSs) Collected from Post-Mortem Samples and Evaluation of the Stability over a Three-Month Period. Molecules janv 24(20):3636. 10.3390/molecules24203636
20. Moretti M, Freni F, Tomaciello I, Vignali C, Groppi A, Visonà SD et al (2019) Determination of benzodiazepines in blood and in dried blood spots collected from post-mortem samples and evaluation of the stability over a three-month period. Drug Test Anal 11(9):1403–1411. 10.1002/dta.2653
21. Dubois-Chabert D, Flament E, Hoizey G, Chatenay C, Fanton L, Bottinelli C (2025) Development of a dried blood spot screening test using gas chromatography coupled to high-resolution mass spectrometry: application in authentic postmortem samples. J Anal Toxicol 15 févr bkaf011. 10.1093/jat/bkaf011 PubMed PMID: 39953782
22. Hakim F, Ghoul C, Wiart JF, Richeval C, Delannoy Y, Hedouin V et al Added value evidences of a simultaneous determination of two phosphatidylethanol isoforms and ethylglucuronide in dried blood spots in forensic contexts. Toxicologie Analytique et Clinique. 1

sept 2022;30th meeting of SFTA- 59th meeting of TIAFT - September 202234(3, Supplement):S36. 10.1016/j.toxac.2022.06.033

23. Magny R, Adell J, Labat L, Ludes B, Dufayet L, Houzé P (2026) Volumetric absorptive microsampling device for forensic Toxicologic screening: A case report as proof-of-concept. Forensic Sci Int 1 oct 387:113028. 10.1016/j.forsciint.2026.113028
24. Gish A, Richeval C, Wiart JF, Hennart B, Hakim F, Demarly C et al (2023) Interpretation of hair and nails findings in an infant death case related to maternal addiction to tramadol. Drug Test Anal 15(9):1022–1026. 10.1002/dta.3485
25. Gicquel T, Richeval C, Mesli V, Gish A, Hakim F, Pelletier R et al (2021) Fatal intoxication related to two new arylcyclohexylamine derivatives (2F-DCK and 3-MeO-PCE). Forensic Sci Int juill 324:110852. 10.1016/j.forsciint.2021.110852
26. Han E, Kim E, Hong H, Jeong S, Kim J, In S et al (2012) Evaluation of postmortem redistribution phenomena for commonly encountered drugs. Forensic Sci Int 10 juin 219(1):265–271. 10.1016/j.forsciint.2012.01.016
27. Emaus RA, Borra LCP, Van Der Hulst R, Kloos DP, Rijken DJ, Elsinga PH et al (2023) Postmortem redistribution of cocaine and its metabolites, benzoylecgonine and ecgonine methyl ester in humans: Important variables that might be influencing the central blood / peripheral blood ratio. Forensic Sci Int juill 348:111707. 10.1016/j.forsciint.2023.111707
28. Zilg B, Thelander G, Giebe B, Druid H (2017) Postmortem blood sampling—Comparison of drug concentrations at different sample sites. Forensic Sci Int sept 278:296–303. 10.1016/j.forsciint.2017.07.006
29. Moriya F, Hashimoto Y (1996) Postmortem Stability of Cocaine and Cocaethylene in Blood and Tissues of Humans and Rabbits. J Forensic Sci 1 juill 41(4):612–616. 10.1520/JFS13963J
30. Brockbals L, Wartmann Y, Mantinieks D, Glowacki LL, Gerostamoulos D, Kraemer T et al (2021) Postmortem Metabolomics: Strategies to Assess Time-Dependent Postmortem Changes of Diazepam, Nordiazepam, Morphine, Codeine, Mirtazapine and Citalopram. Metabolites sept 11(9):643. 10.3390/metabo11090643
31. Al-Asmari A, Alharbi H, Zughaibi T (2022) Post-Mortem Analysis of Heroin Biomarkers, Morphine and Codeine in Stomach Wall Tissue in Heroin-Related Deaths. Toxics 14 août 10(8):473. 10.3390/toxics10080473
32. Wyman J, Bultman S (2004) Postmortem Distribution of Heroin Metabolites in Femoral Blood, Liver, Cerebrospinal Fluid, and Vitreous Humor*. J Anal Toxicol 1 mai 28(4):260–263. 10.1093/jat/28.4.260
33. Bertrand- Ndoye M, Girard H, Hakim F, Gish A, Ghoul C, Delannoy Y et al (2026) Use of blood micro-samples in forensic thanatology. Forensic Sci Int 1 févr 380:112798. 10.1016/j.forsciint.2025.112798
34. Baranowski S, Serr A, Thierauf A, Weinmann W, Groβe Perdekamp M, Wurst FM et al (2008) In vitro study of bacterial degradation of ethyl glucuronide and ethyl sulphate. Int J Legal Med 1 sept 122(5):389–393. 10.1007/s00414-008-0229-3
35. Høiseth G, Karinen R, Johnsen L, Normann PT, Christophersen AS, Mørland J (2008) Disappearance of ethyl glucuronide during heavy putrefaction. Forensic Sci Int 7 avr 176(2):147–151. 10.1016/j.forsciint.2007.08.002
36. Vezzoli S, Bernini M, De Ferrari F (2015) Ethyl glucuronide in vitreous humor and blood postmortem specimens: analysis by liquid chromatography-electrospray tandem mass spectrometry and interpreting results of neo-formation of ethanol. Ann Ist Super Sanita 51(1):19–27 doi:10.4415/ANN_15_01_05 PubMed PMID: 25857380
37. Palmer RB A review of the use of ethyl glucuronide as a marker for ethanol consumption in forensic and clinical medicine. Seminars in Diagnostic Pathology. 1 févr 2009;Toxicologic/Environmental Pathology26(1):18–27. 10.1053/j.semdp.2008.12.005
38. Schloegl H, Dresen S, Spaczynski K, Stoertzel M, Wurst FM, Weinmann W (2006) Stability of ethyl glucuronide in urine, post-mortem tissue and blood samples. Int J Legal Med mars 120(2):83–88. 10.1007/s00414-005-0012-7
39. Kamphuis AEM, Borra LCP, Van Der Hulst R, Kloos DP, Rijken DJ, Bosman IJ et al (2021) Postmortem redistribution of morphine in humans: Important variables that might be influencing the central blood/peripheral blood ratio. Forensic Sci Int déc 329:111094. 10.1016/j.forsciint.2021.111094
40. Romberg RW, Lee L (1995) Comparison of the Hydrolysis Rates of Morphine-3-Glucuronide and Morphine-6-Glucuronide with Acid and β-Glucuronidase. J Anal Toxicol 1 mai 19(3):157–162. 10.1093/jat/19.3.157
41. Berg-Pedersen RM, Ripel Å, Karinen R, Vevelstad M, Bachs L, Vindenes V (2014) Codeine to Morphine Concentration Ratios in Samples from Living Subjects and Autopsy Cases after Incubation. J Anal Toxicol 1 mars 38(2):99–105. 10.1093/jat/bkt099
42. Van Uytfanghe K, Ramirez Fernandez M, del Vos M, Wille A, Stove SMR (2021) Quantitation of phosphatidylethanol in dried blood after volumetric absorptive microsampling. Talanta 1 févr 223:121694. 10.1016/j.talanta.2020.121694
43. Hose J, Andresen-Streichert H, Thevis M, Rothschild MA, Juebner M (2025) Phosphatidylethanol in post-mortem blood: A comparative study of blood matrices and its stability at routine storage conditions. J Forensic Sci 70(5):1963–1972. 10.1111/1556-4029.70106

44. Borra LCP, van der Hulst R, Elsinga PH, Boersma HH, Bosman IJ, Touw DJ (2026) 50 Years of postmortem redistribution research: What have we learned? Forensic Sci Int 1 févr 378:112699. 10.1016/j.forsciint.2025.112699
45. Hakim F (2022) Développement, validation, mise en œuvre et applications de méthodes de dosage de biomarqueurs de la consommation d'éthanol : phosphatidyléthanol sanguin [Doctorat ès Pharmacologie/Toxicologie, PhD] [Internet]. Université de Lille (2022-..); [cité 1 sept 2026]. Disponible sur: https://theses.fr/2022ULILS037 10.70675/c14c15ccz90efz4347za5d7z198cb3dd3c96
46. Gaulier JM, Gish A, Ghoul C, Bertrand-Ndoye M, Girard H, Scanvion Q et al (2023) Étude pilote du projet IN CADAVER: preuve de concept d'une voie future de la toxicologie post-mortem (et nouveau paradigme ?). Toxicol Anal et Clinique mai 35(2):S25. 10.1016/j.toxac.2023.03.031
47. Schulz M, Iwersen-Bergmann S, Andresen H, Schmoldt A (2012) Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics. Crit Care 26 juill 16(4):R136. 10.1186/cc11441
48. Uges DRA et al (2011) TIAFT reference blood level list of therapeutic and toxic substances. TIAFT, 22 juillet., ;
49. Eysseric H (2019) Toxicologie médico-légale et agonistes des récepteurs GABA. Revue Francophone des Laboratoires déc 2019(517):57–65. 10.1016/S1773-035X(19)30527-1
50. Mestad IO, Gjelstad A, Pedersen-Bjergaard S, Øiestad EL (2021) Green and sustainable drug analysis -- Combining microsampling and microextraction of drugs of abuse. Sustainable Chem Pharm 1 déc 24:100517. 10.1016/j.scp.2021.100517
51. Ben Yahyaten F, Labat L, Houzé P (2021) Nouvelles applications des systèmes alternatifs de micro-prélèvements. Revue Francophone des Laboratoires oct 2021(535):54–64. 10.1016/S1773-035X(21)00253-7

Figures

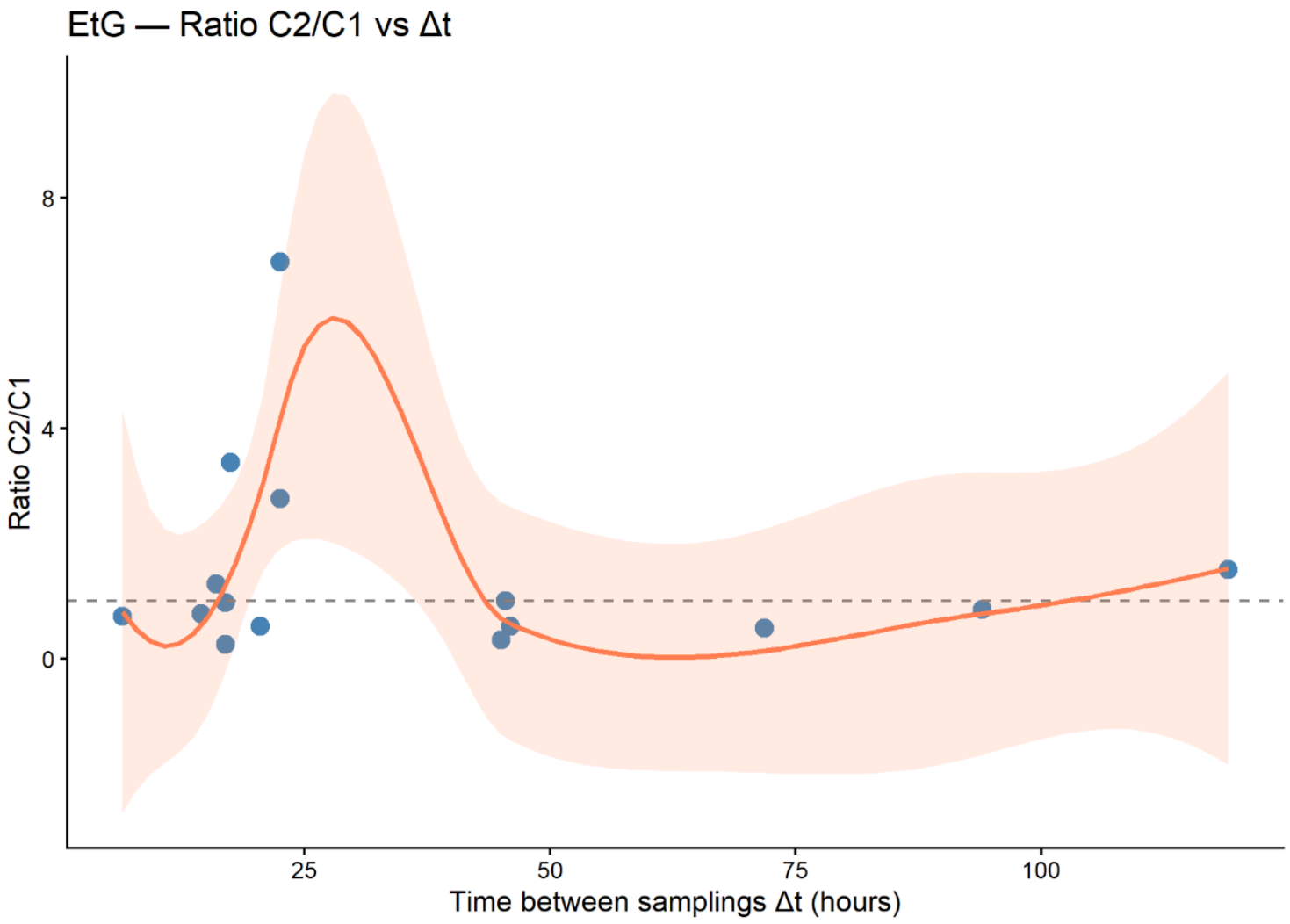


Figure 1

Evolution of the EtG C2/C1 concentration ratio according to the inter-sampling interval (Δt) — loess fit curve

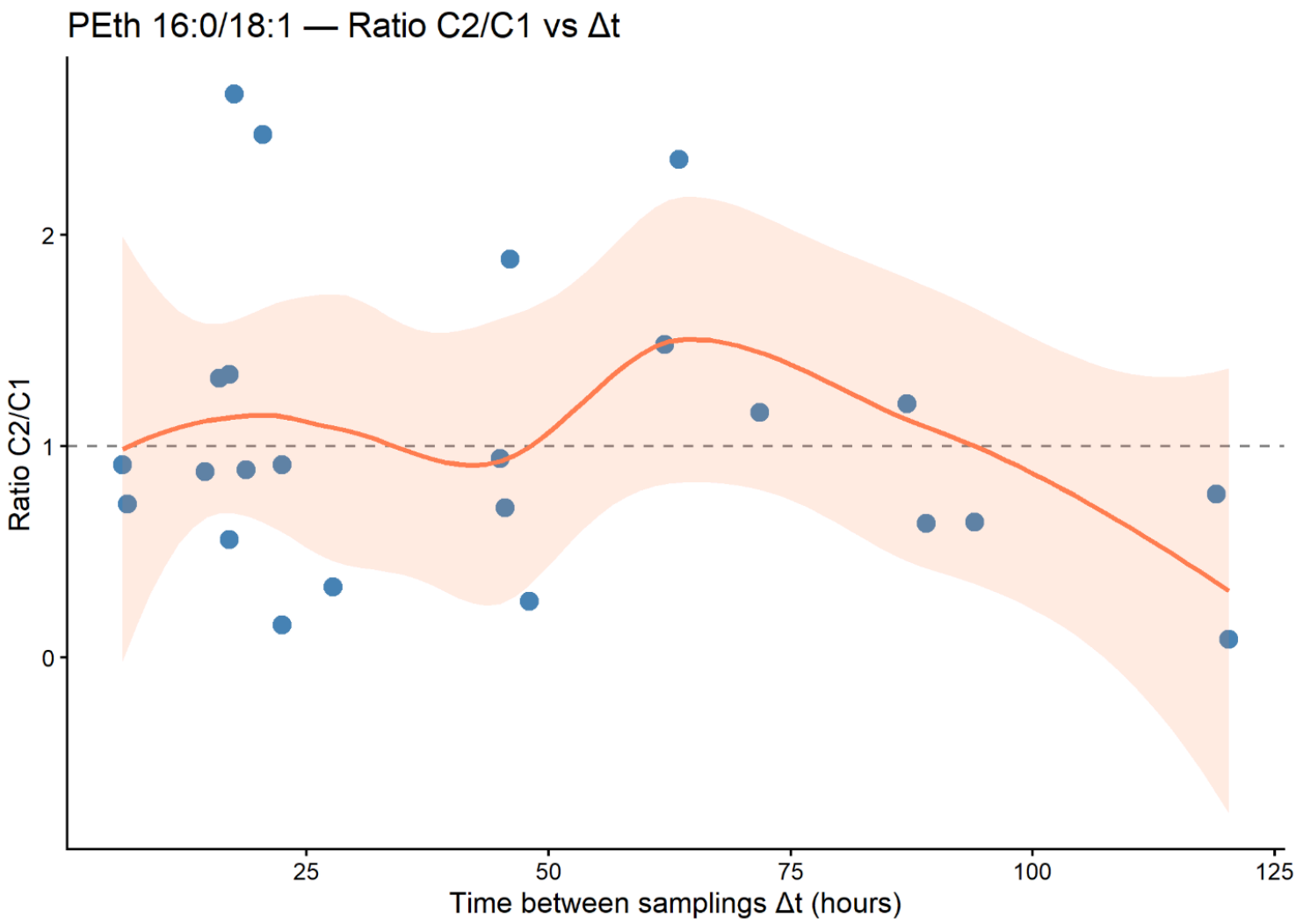


Figure 2

Evolution of the PEth 16:0/18:1 C2/C1 concentration ratio according to the inter-sampling interval (Δt)

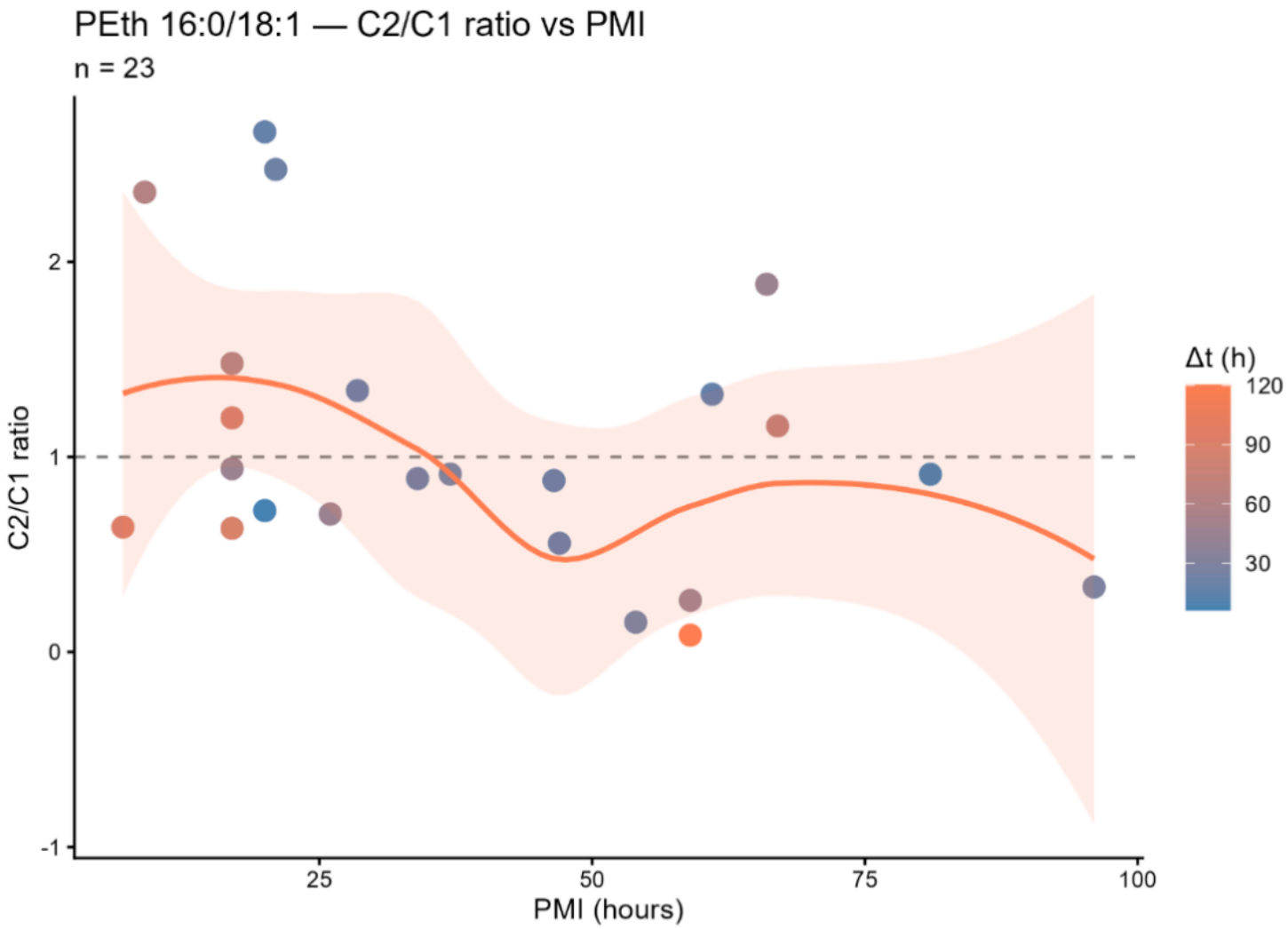


Figure 3

Evolution of the PEth 16:0/18:1 C2/C1 concentration ratio according to PMI

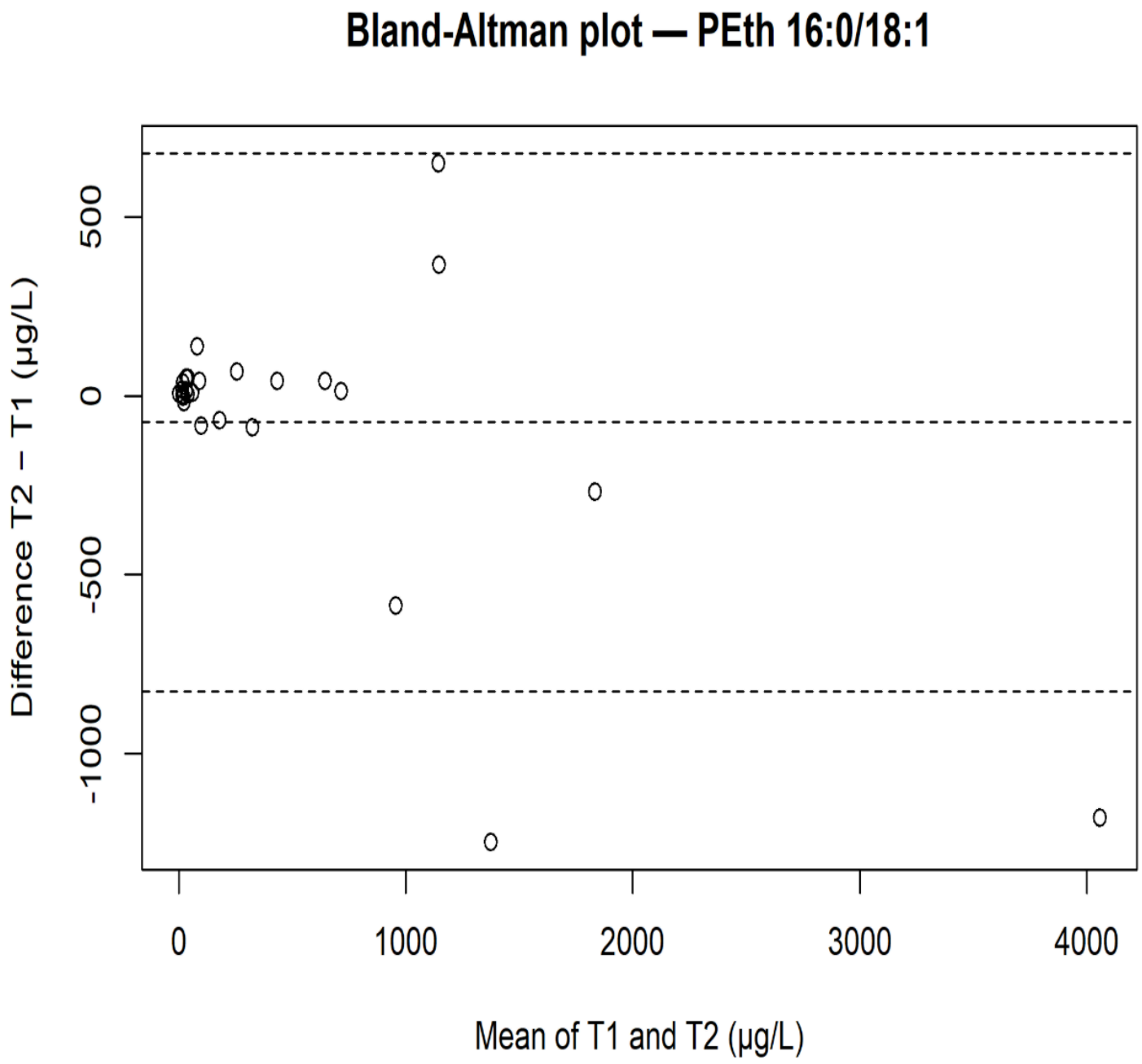


Figure 4

Bland-Altman analysis of PEth 16:0/18:1 concentrations between T1 and T2

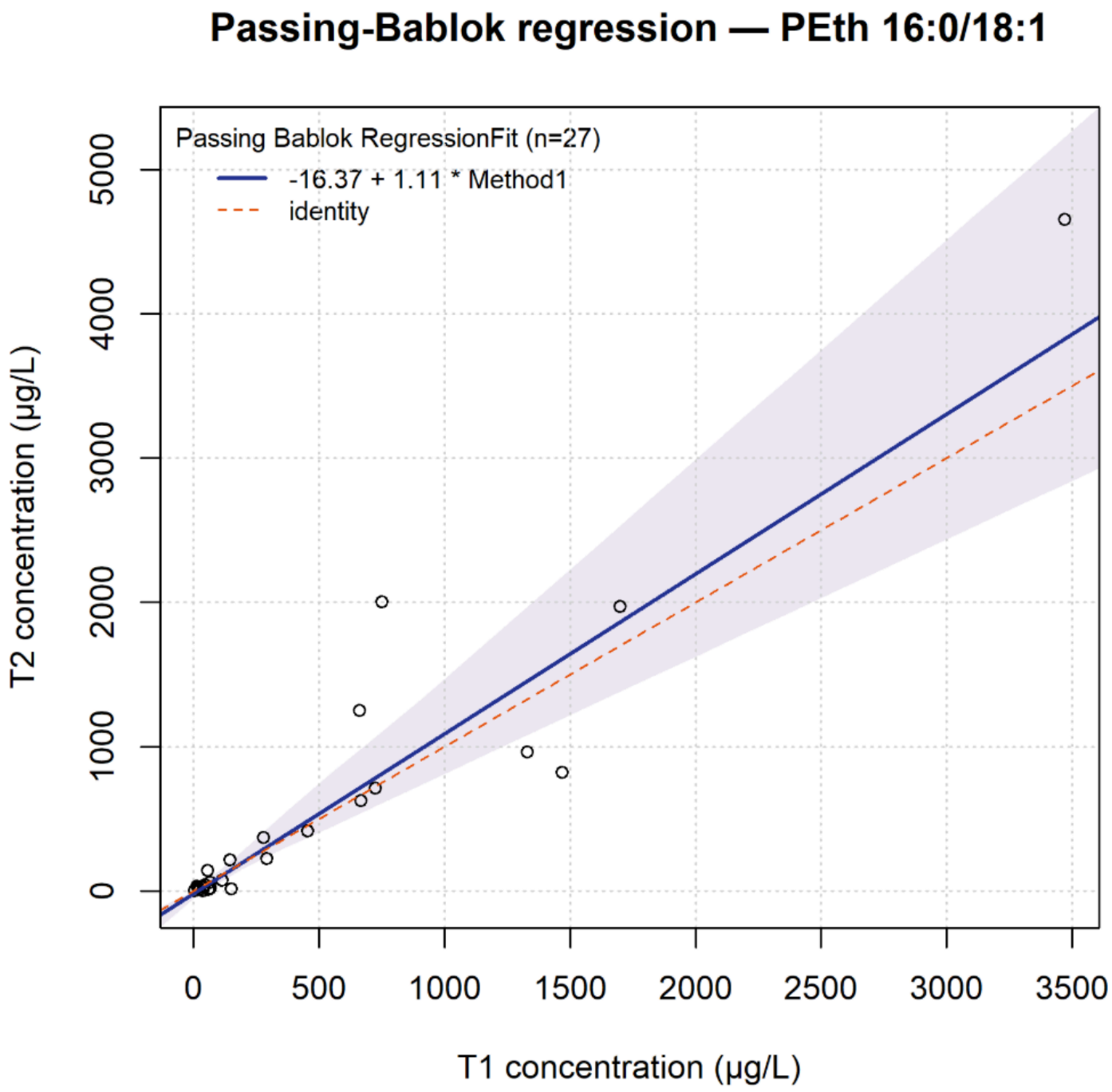


Figure 5

Passing-Bablok regression of PEth 16:0/18:1 concentrations between T1 and T2

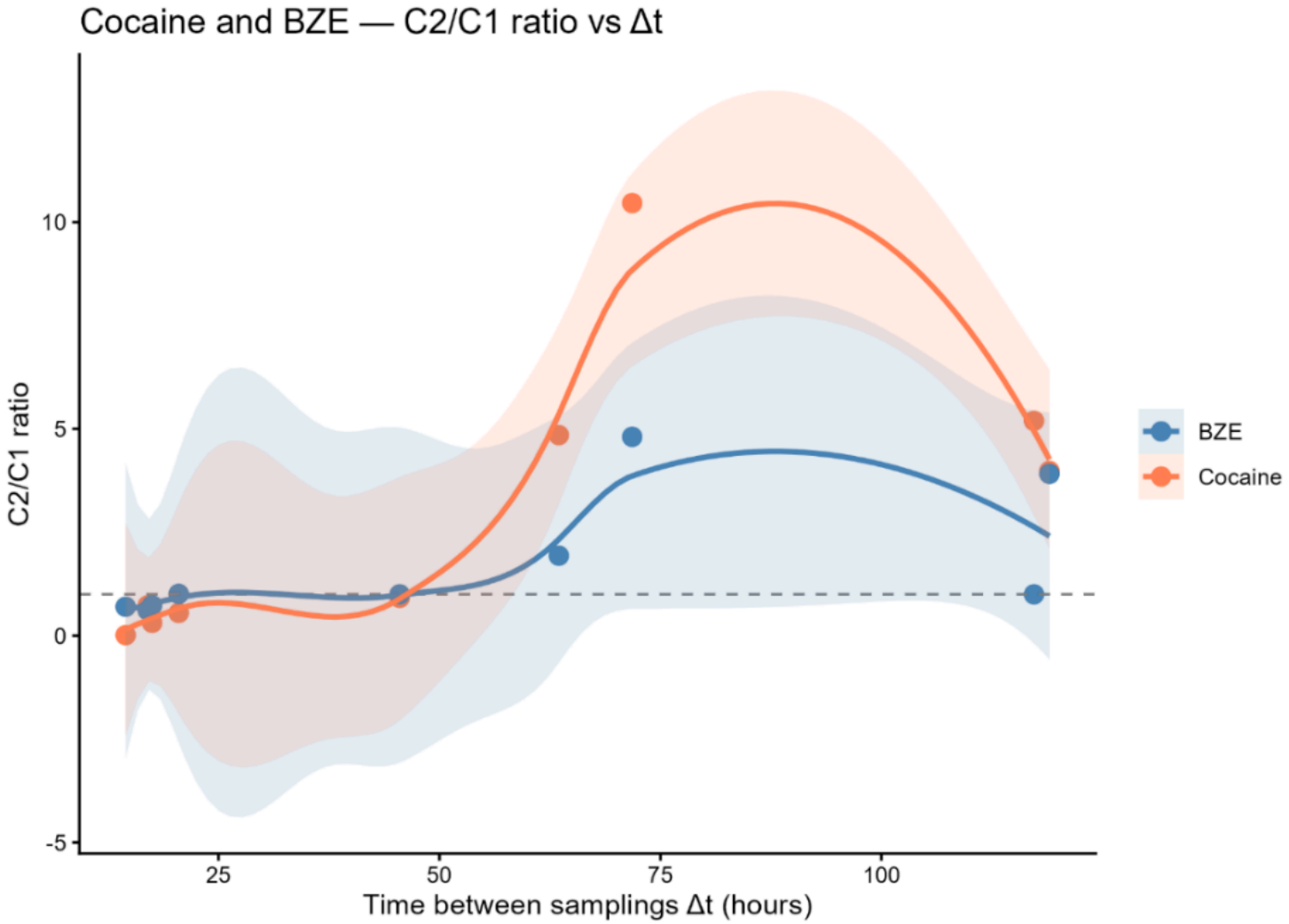


Figure 6

Evolution of the cocaine and benzoylecgonine (BZE) C2/C1 concentration ratio according to the inter-sampling interval (Δt)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ESMeng.docx