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RESEARCH

Simultaneous determination of four ethanol biomarkers in blood: avalidated method for use in forensic toxicology in 257 postmortem samples

Kan örneklerinde dört farklı etanol biyobelirtecinin eş zamanlı tayini: 257 postmortem örnek ile adli toksikolojide kullanılmak üzere geliştirilen ve doğrulanan bir yöntem

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Abstract

Purpose: Ethyl alcohol is the most widely and legally available intoxicating substance. However, excessive consumption is associated with numerous negative social consequences, including the potential for significant health risks. Rapid and simple diagnosis of alcohol use is necessary to initiate appropriate and effective treatment and is critical in forensic toxicological analysis. Ethanol biomarkers have clinical utility in the detection, diagnosis, and treatment of alcohol use disorders.

Materials and Methods: An analytical method for the simultaneous determination of four different alcohol biomarkers ethyl glucuronide (EtG), ethyl sulphate (EtS), N-acetyltaurine (NAcT), and 16:0/18:1-phosphatidyl ethanol (PEth) in blood samples from forensic cases was developed, validated and verified for the accurate monitoring of alcohol abuse and dependence. Analyses were performed using a liquid chromatography-mass spectrometry system.

Results: 257 blood and vitreous samples were collected from 255 male and 2 female subjects aged 16-80 years (average 44±14.82) from the Forensic Medicine Council of Turkey and analyzed for ethanol biomarkers and calculated ethanol concentrations. A total of 257 blood samples were found to contain ethanol, with concentrations ranging from 12.0 to 444.0 mg/dL. Vitreous concentrations ranged from 23 mg/dL to 597 mg/dL. The limit of detection (LOD) for EtG, EtS, NAcT, and PEth were 3.1, 3.9, 7.3, and 5.7 ng/mL respectively in blood samples. The limit of quantification (LOQ) for EtG, EtS, NAcT, and PEth were 7.8, 8.4, 18.3, and 13.1 ng/mL, respectively in blood samples.

Conclusion: The method has potential in forensic toxicology as an invaluable tool for the accurate and

Öz

Amaç: Etil alkol en yaygın ve yasal olarak erişilebilen intoksikan maddedir. Ancak aşırı tüketimi, önemli sağlık riskleri de dahil olmak üzere çok sayıda olumsuz sosyal sonuçla ilişkilidir. Alkol kullanımının hızlı ve basit bir şekilde teşhis edilmesi, uygun ve etkili tedavinin başlatılması için gereklidir ve adli toksikolojik analizlerde kritik öneme sahiptir. Etanol biyobelirteçleri alkol kullanım bozukluklarının tespiti, teşhisi ve tedavisinde klinik faydaya sahiptir.

Gereç ve Yöntem: Adli vakalardan alınan kan örneklerinde dört farklı alkol biyobelirteci olan etil glukuronid (EtG), etil sülfat (EtS), N-asetiltaurin (NAcT) ve 16:0/18:1- fosfatidil etanolün (PEth) eşzamanlı tayini için bir analitik yöntem geliştirilmiş, alkol kötüye kullanımı ve bağımlılığının doğru bir şekilde izlenmesi için doğrulanmış ve onaylanmıştır. Analizler sıvı kromatografi-kütle spektrometresi sistemi kullanılarak gerçekleştirilmiştir.

Bulgular: Türkiye Adli Tıp Kurumu'ndan yaşları 16-80 arasında değişen (ortalama 44±14.82) 255 erkek ve 2 kadın olgudan 257 kan ve vitreus örneği toplanmış ve etanol biyobelirteçleri ve hesaplanan etanol konsantrasyonları için analiz edilmiştir. Toplam 257 kan örneğinin etanol içerdiği ve konsantrasyonlarının 12.0 ila 444.0 mg/dL arasında değiştiği tespit edilmiştir. Vitreus konsantrasyonları 23 mg/dL ile 597 mg/dL arasında değişmektedir. Kan örneklerinde EtG, EtS, NAcT ve PEth için tespit limiti (LOD) sırasıyla 3,1, 3,9, 7,3 ve 5,7 ng/mL idi. EtG, EtS, NAcT ve PEth için miktar belirleme limiti (LOQ) kan örneklerinde sırasıyla 7,8, 8,4, 18,3 ve 13,1 ng/mL'dir.

Sonuç: Geliştirilen yöntem, farklı biyolojik örneklerde alkol kullanımı ve bağımlılığının biyobelirteçlerinin doğru

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simultaneous identification of biomarkers of alcohol use and dependence in different biological samples.
Keywords: Ethyl glucuronide (EtG), Ethyl sulfate (EtS), N-acetyltaurine (NAcT) 16:0/18:1-phosphatidylethanol (PEth), Alcohol biomarkers

ve eş zamanlı tanımlanması için adli toksikolojide değerli bir araç olarak potansiyel taşımaktadır.
Anahtar kelimeler: Etil glukuronid (EtG), Etil sülfat (EtS), N-asetiltaurin (NAcT) 16:0/18:1- fosfatidiletanol (PEth), Alkol biyobelirteçleri

INTRODUCTION

Compulsive drinking is a major global public health problem with a detrimental effect on the quality of life of individuals. Many health problems can result from alcohol dependence. Worldwide, 5.9% of deaths are directly related to binge drinking[1]. Therefore, it is important to monitor alcohol dependence and accurately diagnose alcohol abuse to provide functional treatment and rehabilitation perspectives[2].

The well-known alcohol markers PEth, EtS, and EtG in samples such as urine, hair and blood have been evaluated for the monitoring of alcohol abuse and the accurate and precise diagnosis of alcohol abuse[3]. However, the search for novel forensic markers continues due to the stability, selectivity, sensitivity, and limited detection window of accepted markers, which is key to both proving alcohol consumption in post-mortem individuals and developing productive treatment and rehabilitation approaches for dependent individuals [4,5]. This study aimed to develop and validate an analytical method for the simultaneous detection of alcohol consumption and alcohol dependence biomarkers in human blood. For a thorough assessment of alcohol consumption in medical and forensic applications, objective biomarkers are needed to assess either all drinking or the amount and time since last drinking[6].

Over the past 20 years, EtG has become the most commonly used direct alcohol biomarker to detect urinary and hairborne alcohol use[7]. In 2005, a promising alcohol biomarker was identified as ethyl sulphate, a minor metabolite of ethanol[8]. It has been found that detection windows of EtGs and EtSs in urines are extended at high alcohol levels and range between 40 and 130 h[9]. NAcT, another promising direct alcohol biomarker, has also been included in the method[2,4]. As there is limited research on this metabolite, its inclusion in the developed method provides an opportunity to compare NAcT with the other established alcohol biomarkers. NAcT is a by-product of alcohol metabolism and is a compound that increases with alcohol consumption[4]. PEth is a promising candidate as a phospholipid ethanol metabolite produced by chronic and excessive alcohol consumption and a long-term indicator of alcohol consumption, with a multi-day half-life in red blood cells and potential sensitivity to even low levels of alcohol consumption[10,11].

Most existing methods allow separate detection of markers for alcohol consumption and alcohol dependence. This study aims to develop a new analytical method for the simultaneous detection of alcohol biomarkers. The method used modern analytical techniques to confirm high sensitivity, validity, and reproducibility.

MATERIALS AND METHODS

Chemicals

EtG, EtS, NacT, PEth, pentadeuterated EtG, and EtS were purchased from TRC (Toronto, Canada). Ammonium acetate, methanol, ethanol, 1-propanol were obtained from Merck (Darmstadt, Germany). De-ionized water was produced in-house with a Millipore Direct Q8 UV system, (Darmstadt, Germany). All solvents were of LC-MS grade.

Authentic autopsy samples

Two hundred fifty-seven blood samples who has presence of alcohol consumption before death were collected during the autopsy performed in the Council of Forensic Medicine mainly from traffic accidents, suicide by hanging found death at home. Blood and intraocular fluid were analyzed for alcohol in each autopsy sample. Blood and intraocular fluid positive for ethanol were included in the study.

Autopsies were performed according to Turkish Criminal Procedure 5271/87-89 in Ankara between 01.03.2023 and 01.11.2023. Ethical approval was obtained by the Ethics Committee of the Cukurova University (approval number: 129/06.01.2023). Forensic Medicine Council Consent Certificate (approval number: 21589509/2022/1068). Blank and postmortem blood samples were stored at -20^{o}C until the analysis. Analyses were performed at the council of Forensic Medicine, Ankara

HS-GC-FID sample preparation

The method was validated using blank human blood (free from ethanol, EtG, EtS and PEth) samples obtained from a regional blood donation center. Fresh working solutions were prepared daily. The internal standard (IS) solution of 1-propanol was prepared in water (0.01 M) and stored at +4°C. A postmortem blood or vitreus humor sample (200 µL) was added to 1000 µL of IS (1-propanol, 0.01 M) in a clean headspace vial, capped, and then transferred to the HS sampler.

Alcohol analysis of blood and vitreous humor with HS-GC-FID

Headspace gas chromatography/flame ionisation detection (HS-GC-FID) was used to analyse blood and vitreous samples. A Perkin Elmer Clarus 500 GC (Shelton, USA) and HS module were equipped with a flame ionisation detector. The columns were Elite BAC 1 Advantage (30 m×0.32 mm ID×1.8 µm) and B (Elite BAC 2 Advantage (30 m×0.32 mm ID×0.6 µm) were used. The oven, the transfer line, the needle, and the column temperature were set to 70 ˚C , 75 ˚C and 110 ˚C, and 220 ˚C, respectively. The injection time, the hold time and the cycle time and total GC run time were set 0.02, 0.2, 8.5, 10 minutes, respectively. The sampling rate was set 12.5 points per second. The gas flow for each detector was 450 mL/min of air and 45.0 mL/min of hydrogen. The LOD, LOQ were found 3.99 and 4.3 mg/dL in blood and 4.16 and 4.66 mg/dL in vitreous humor, respectively. The method was found linear in the range of 3.9 to 393.7 mg/dL for blood and vitreus humor (r2=0.9999).

Preparation of the sample for LC-MS/MS

To prepare the samples, 50 µL of IS mix (5 µg/mL) was added to 250 µL whole blood and then 750 µL of acetonitrile was added. Samples were vortexed and then centrifuged at 14,000 rpm for 10 minutes. Next, In a vacuum centrifuge, 700 µL of the supernatant was separated and evaporated under gentle nitrogen flow. These were reconstituted with 350 µL of mobile phase (A:B, 40:60) and injected. If necessary, samples were diluted 1:20 to ensure a concentration in the calibration range.

Method validation for LC-MS/MS

The analytical method wasvalidated according to Standard Practices for Method Validation in Forensic Toxicology by the Scientific Working Group for Forensic Toxicology (SWGTOX) [12]. The validation process included determination of selectivity, linearity, limits of detection and limits of quantification (LOD, LOQ), matrix effects, extraction efficiency, recovery, linearity and intra- and inter-day accuracy, precision and stability. Selectivity was assessed by analysing eight blank blood samples for endogenous and/or exogenous substances at the retention time of the analytes and internal standards. Calibration samples were spiked with working solutions containing EtS, EtG, NAcT, and PEth in methanol (1 and 10 µg/mL). A 6-point calibration was performed using the concentrations of EtG, EtS, NAcT, and PEth a range of 10 to 1000 ng/mL. The LOD and the LOQ were calculated with acceptable precision from the spiked blood samples (n=10). Precision and accuracy were determined by preparing whole blood samples at three concentrations: Low concentration (25 ng/mL), Medium concentration (250 ng/mL), and High concentration (750 ng/mL). Carryover was measured by injecting the blood calibrator sample at the highest concentration three times, followed by a blank blood sample containing IS to check for carryover. Matrix effects, recovery, and extraction efficiency were analyzed by preparing samples in whole blood at three concentrations (low: 25 ng/mL, medium: 250 ng/mL, high: 750 ng/mL)[13,14]. To measure autosampler stability of sprocessed samples were re-injected after 24 hours of storage in the sampler.

EtG, EtS, NAcT and PEth analysis with LC-MS/MS

A Shimadzu liquid chromatography module with a combined column oven (Shimadzu, Kyoto, Japan) maintained at 40°C was maintained with a (100×2.1 mm, 1.7 µm) Waters BEH C18 column. The mobile phase consisted of A (90% v/v methanol and 25 mm ammonium acetate in water) and B (methanol). The solvent was isocratic with 60% mobile phase B. The flow rate, injection volume, and autosampler temperature were set to 0.3 mL/min, 5 µL, and 8°C, respectively. Electrospray ionisation (ESI) negative and multiple reaction monitoring (MRM) modes were used for detection. The source voltage was set at 1.5 kV. The gas flows for nebulisation, heating, and drying was set to 3, 10, and 10 L/min respectively. Desolvation line, heat block, desolvation and interface temperatures were set to, 250, 400, 526 and 300°C respectively. The collision induced dissociation (CID) gas was set at 270 kPa. The pause

time and polarity change time were set to 1 ms and 5 ms respectively. The dwell time for the analytes was set between 5 and 13 ms. The MRM transitions and collision energies were optimised for each transition with infusion. The total run time was 6 min. The following precursor ion/product ion MS/MS transitions were used: EtG is quantified at m/z 221/75 and qualified at m/z 221/85. EtS is quantified with m/z 125/97 and qualified with m/z 125/80. NAcT is quantified at m/z 166/107 and qualified at m/z 166/80. PEth is quantified at m/z 701/281 and qualified at m/z 701/255. The deuterated internal standard are qualified at m/z 130/98 and m/z 130/80 for EtS d5 and 226/75 and 226/85 for EtG d5, respectively Figure 1 shows chromatograms (MRM) of all analytes with a concentration of 10 ng/mL in methanol.

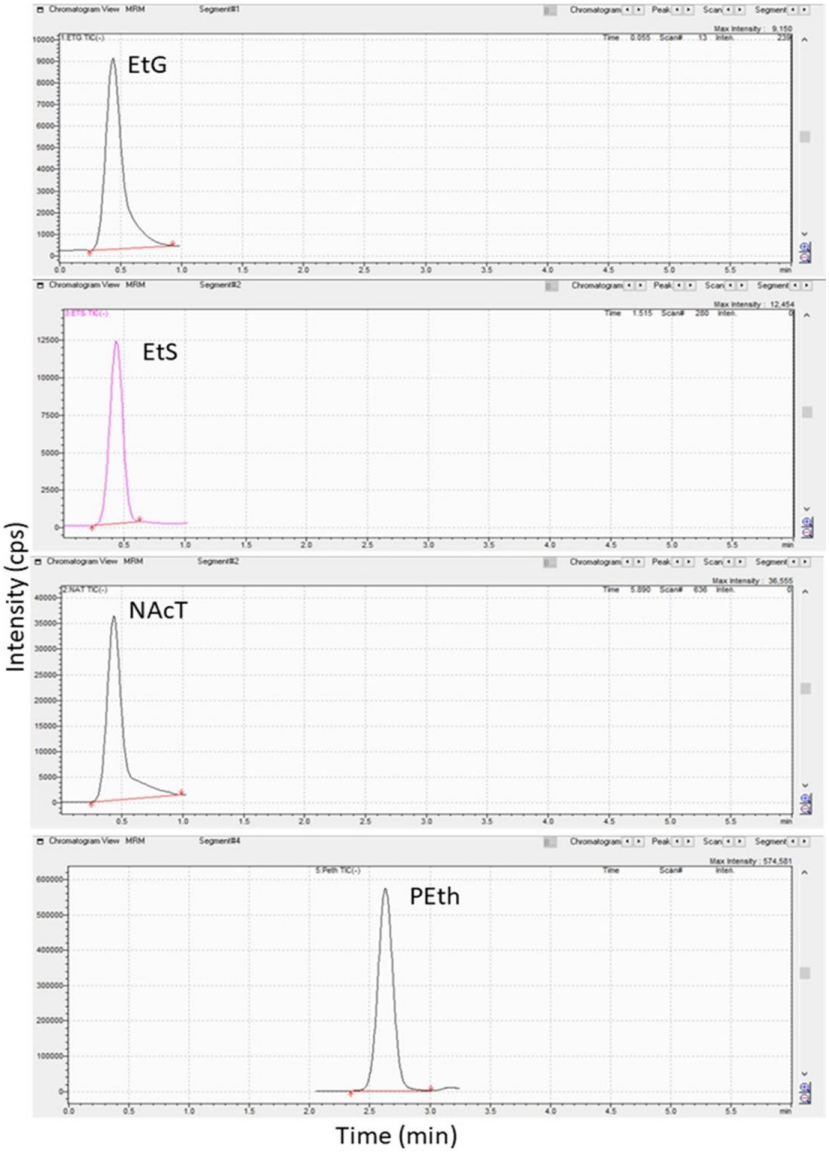

Figure 1. MRM chromatograms of EtG, EtS NAcT and PEth. Electro sprey negative ionisation and multiple reaction monitoring mode (MRM) total ion chromatograms for analytes.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software, version 17.0, from SPSS Inc. (Chicago, IL). One-way Kruskal-Wallis analysis of variance was used to assess differences between groups. This non-parametric test was used to determine whether there were statistically significant differences in the distribution of at least one biomarker among the samples. It is a suitable choice because the normality of data distribution was not assumed in our study on alcohol biomarkers.

The Mann-Whitney U test was used for post hoc analysis. Results are presented as mean ± standard deviation (SD). P values were considered statistically significant if they were less than 0.05. This non-parametric post-hoc test was used to identify pairwise comparisons between groups that showed significant differences in the Kruskal-Wallis test. It helps pinpoint which specific groups differed from each other in terms of biomarker levels.

RESULTS

Validation results

Table I presents the results of the method validation. Interfering peaks were evaluated on six blank blood samples at the retention times of the analytes and no interfering compounds were detected. NAcT was detected in every sample in this experimental setting due to its endogenous formation. To assess the linearity of the developed method, we used a linear calibration model with a 1/x regression model (x, concentration) and spiked concentrations within the calibration range. The correlation coefficient (R2) was greater than 0.995. Precision and accuracy were within acceptable limits, with all spiked samples at low, medium and high concentrations having measured concentrations within ±15% of the target, in accordance with guidelines. The method demonstrated intra- and inter-assay precisions of 3.3-9.1% and 3.8-9.7%, respectively. Accuracy values ranged from 98.8-105.6%. No relevant peaks were observed in the blanks during the carryover study. The limit of detection (LOD) for EtG, EtS, NAcT and PEth were 3.1, 3.9, 7.3, and 5.7 ng/mL, respectively. The limit of quantification (LOQ) for EtG, EtS, NAcT and PEth were 7.8, 8.4, 18.3, and 13.1 ng/mL, respectively. Table 1 shows the recovery (91.7-103.8%), and matrix effect (89.2 and 96.1%). The autosampler was used to monitor low, medium, and high spiked blood samples and stock solutions at 8°C. The results were within the acceptance criteria (±10%) by comparing the peak areas in the samples after 0, 2, 4, 8, 12, and 24 hours to the peak areas in the first process examples. The analytes were stable under the test conditions and the results were within the acceptance criteria.

Table 1. Validation results

Validation parameters/substances	EtG	EtS	NAcT	PEth
Linearity range (ng/mL)	10-1000 ng/mL			
Intraday precision low (%)	9.1	7.6	4.9	7.5
Intraday precision medium (%)	5.3	4.9	5.3	6.8
Intraday precision high (%)	4.3	3.3	4.6	5.7
Interday precision low (%)	8.3	9.7	3.8	8.3
Interday precision medium (%)	6.4	7.6	4.3	7.6
Interday precision high (%)	6.9	6.3	4.8	7.3
Limit of detection(LOD) (ng/mL)	3.1	3.9	7.3	5.7
Limit of quantification (LOQ) (ng/mL)	7.8	8.4	18.3	13.1
Accuracy Low (%)	103.2	104.1	101.2	105.6
Accuracy Medium (%)	102.8	101.3	103.1	99.6
Accuracy High (%)	101.8	98.8	102.8	102.8
Recovery low (%)	98.7	101.3	99.6	103.8
Recovery medium (%)	96.7	98.5	95.6	94.2
Recovery high (%)	93.1	96.8	91.7	95.2
Matrix Effect Low (%)	94.3	96.1	91.3	93.1
Matrix Effect Medium (%)	93.2	94.6	90.6	94.3
Matrix Effect High (%)	90.8	93.9	89.2	95.7

EtG: Ethyl glucuronide, EtS: Ethyl sulfate, NAcT: N-acetyltaurine, PEth: 16:0/18:1-phosphatidylethanol

Blood samples from forensic cases

257 blood and vitreous samples were collected from 255 male and 2 female subjects aged 16-80 years range (mean 44± 9.44) and analysed for ethanol. Of these, 257 blood samples tested positive for ethanol with concentrations ranging from 12.0 to 444.0 mg/dL. Vitreous concentrations ranged from 23 mg/dL to 597 mg/dL. Blood samples were also tested for EtG, EtS, NAcT and PEth concentrations. EtG concentrations ranged from 390 ng/mL to 18007 ng/mL with a mean of 3131.0± 624.6 ng/mL. EtS concentrations ranged from 191 ng/mL to 16811 ng/mL with a mean of 2614.6 ±563.8 ng/mL. The mean measured concentration of NAcT in blood was 198.8± 58.3ng/mL (range: 37.0-1514.3 ng/mL). Total PEth concentrations ranged from 1.0 to 314.0 ng/mL with a mean of 105.6 ±11.1 ng/mL. Figure 2 shows extracted ion chromatograms of EtG, EtS, NAcT, and PEth in postmortem blood sample.

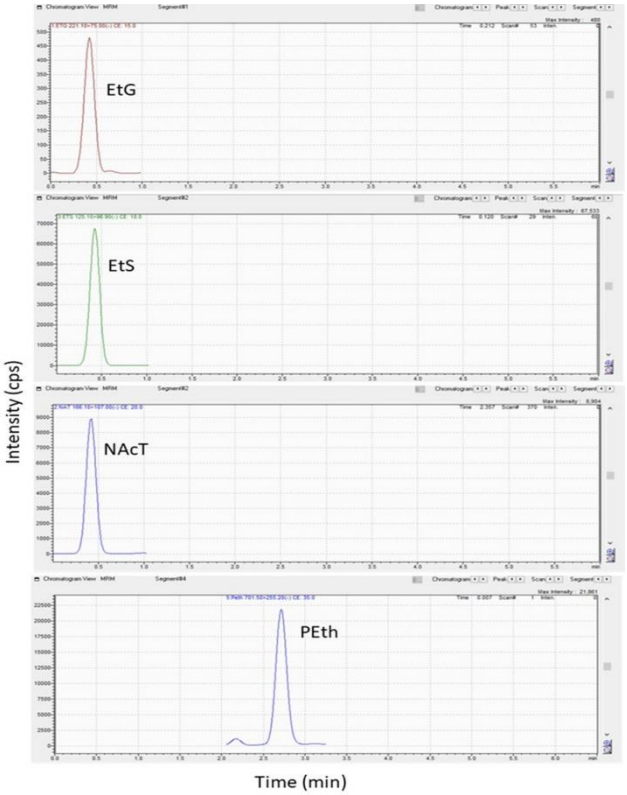

Figure 2. Extracted ion chromatograms of EtG, EtS, NAcT, and PEth in postmortem blood sample.

DISCUSSION

The analytical method developed for the simultaneous detection of NacT, ETG, Peth, and ETS in human blood and intraocular fluid has demonstrated high accuracy, sensitivity and reproducibility. The method was validated according to international guidelines and showed excellent linearity, selectivity, limits of quantification and detection, matrix effects, extraction efficiency, sensitivity and recovery. A good correlation was observed between postmortem blood and vitreous humor (Spearman rank correlation test: correlation blood/vitreous humor rs = 0.986) for ethanol concentration. The quantification results for vitreous humor were found to be slightly higher than for blood.

Hofmann et al. reported a LOD and LOQ of 2.6 and 7.7 ng/mL, respectively, for EtG in postmortem blood[5]. Hoiseth et al. also reported a LOD and LOQ of 20 and 60 ng/mL, respectively, for EtG in postmortem blood [15]. For EtS, a LOD of 4.7 and 13 ng/mL and a LOQ of 8.3 and 28 ng/mL were

reported[15,16]. Hofmann et al. reported a LOD and LOQ of 12.5 and 28.9 ng/mL for NAcT in postmortem blood[5]. Schröck et al. and Hofmann et al. reported LOD and LOD of 6.9 and 10.0 and LOQ of 11.3 and 12.0 ng/mL, respectively[15,17]. In the present study, LOD and LOQ were found to be 3.1, 3.9, 7.3, and 5.7 ng/mL and 7.8, 8.4, 18.3 and 13.1 ng/mL, respectively. The LODs of the developed method were found to be sufficiently low for EtG, EtS, NAcT, and PEth.

NAcT can be detected between 500-2,300 ng/ml in the blood after exposure to ethanol [4]. Blood levels of NAcT ranged from 37 to 1514.3 ng/ml, indicating both acute and chronic alcohol consumption. Hoffman et al. reported a blood NAcT concentration of 12.5 ng/ml[5]. Blood NAcT concentrations of 13-31 ng/mL after 2 weeks of abstinence were reported by Luginbühl et al[4,13]. A moderate increase in NAcT between 27-57 ng/ml was observed due to drinking activity. In a recent study, blood NacT concentrations ranged from 37.0 to 1514.3 ng/mL (mean: 198.8 ng/mL).

Drinking habits were categorized by PEth concentration according to the Basel 2022 Consensus. Low ethanol consumption was assumed if the PEth concentration was less than 20 ng/mL, moderate in the range 20 ng/mL to 200 ng/mL, and high 200 ng/mL and above[5]. Consistent with this categorisation, PEth concentrations of less than 20 ng/mL were found in BACs of less than 100 mg/dL, with the exception of four cases associated with chronic alcohol use. PEth concentrations below 200 ng/ml were found with BACs between 100 and 444.0 mg/dL. Only two cases were found above 200 ng/ml, one methanol intoxication and one carbon monoxide intoxication.

A limitation of this study is that we were only able to access ethanol levels at the time of death. Due to the retrospective nature of the study, it was not possible to obtain detailed information regarding individual drinking patterns, frequency of alcohol consumption, and the alcohol consumption and consumption amount in each case.

The concentration of EtG is mainly dependent on the dose of ethanol consumed, the peak BAC reached and the time after drinking that urine is excreted[18]. In addition, the EtG curve is shifted in time by several hours and can be detected for much longer than ethanol itself [15]. EtG and Ets can be detected in blood soon after alcohol consumption (<45 minutes). EtG can be detected in serum for up to 8 hours longer than ethanol[19]. EtS can be detected in serum for approximately twice as long as ethanol[20].

The concentrations of case EtG, EtS, NAcT, and PEth in blood samples were found to be related to alcohol consumption habits through the analysis of forensic cases. Higher concentrations of case EtG, EtS, NAcT, and PEth were associated with chronic alcohol consumption, while lower concentrations were associated with a short interval between alcohol consumption and death. The current findings in this study are consistent with previous research on EtG, EtS, NAcT, and Peth levels in cases related to drinking habits.

For those engaged in the fields of health and forensic science, this method offers considerable potential. The analysis of NAcT and PEth in blood samples from forensic cases yielded valuable insights into patterns of alcohol consumption and dependence in the studied population. The concentrations of both markers exhibited considerable variability, reflecting the differences in alcohol consumption and dependence between individuals. The presence of EtG and EtS in high concentrations is indicative of recent alcohol consumption, whereas the presence of PEth is indicative of chronic and excessive alcohol consumption. The NAcT levels provide a comprehensive overview of alcohol use in the studied population, thereby supporting the assessment of alcohol consumption.

The method developed has the potential to be applied in forensic toxicology, providing a valuable tool for the precise and simultaneous identification of biomarkers of alcohol consumption and dependence in different biological samples. Nevertheless, further research and validation in larger populations are required to establish the effectiveness and dependability of the method for routine use in forensic investigations and clinical practice. Moreover, the integration of novel biomarkers and sophisticated analytical techniques may facilitate the identification and monitoring of alcohol use disorders, ultimately leading to enhanced public health outcomes and individual well-being.

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REFERENCES

1. WHO. Alcohol: Global Status Report On Alcohol and Health. Geneva, World Health Organizationi, 2014.
2. Luginbühl M, Rutjens S, König S, Furrer J, Weinmann W. N-Acetyltaurine as a novel urinary ethanol marker in a drinking study. Anal Bioanal Chem. 2016;408:7529-36.
3. Schröck A, Thierauf A, Wurst FM, Thon N, Weinmann W. Progress in monitoring alcohol consumption and alcohol abuse by phosphatidylethanol. Bioanalysis. 2014;6:2285-94.
4. Luginbühl M, König S, Schürch S, Weinmann W. Evaluation of N-acetyltaurine as an ethanol marker in human blood. Alcohol. 2017;65:11-8.
5. Hofmann V, Sundermann TR, Schmitt G, Bartel M. Development and validation of an analytical method for the simultaneous determination of the alcohol biomarkers ethyl glucuronide, ethyl sulfate, N-acetyltaurine, and 16:0/18:1-phosphatidylethanol in human blood. Drug Test Anal. 2022;14:92-100.
6. Javors MA, Hill-Kapturczak N, Roache JD, Karns-Wright TE, Dougherty DM. Characterization of the pharmacokinetics of phosphatidylethanol 16:0/18:1 and 16:0/18:2 in human whole blood after alcohol consumption in a clinical laboratory study. Alcohol Clin Exp Res. 2016;40:1228-34.
7. Trius-Soler M, Praticò G, Gürdeniz G, Garcia-Aloy M, Canali R, Fausta N et al. Biomarkers of moderate alcohol intake and alcoholic beverages: a systematic literature review. Genes Nutr. 2023;18:7.
8. Walsham NE, Sherwood RA. Ethyl glucuronide. Ann Clin Biochem. 2012;49:110-7.
9. Andresen-Streichert H, Müller A, Glahn A, Skopp G, Sterneck M. Alcohol biomarkers in clinical and forensic contexts. Dtsch Arztebl Int. 2018;115:309-15.
10. Fleming MF, Smith MJ, Oslakovic E, Lucey MR, Vue JX et al. Phosphatidylethanol detects moderate-to-heavy alcohol use in liver transplant recipients. Alcohol Clin Exp Res. 2017;41:857-62.
11. Perilli M, Toselli F, Franceschetto L, Cinquetti A, Ceretta A, Cecchetto G et al. Phosphatidylethanol (PEth) in blood as a marker of unhealthy alcohol use: a systematic review with novel molecular insights. Int J Mol Sci. 2023;29:12175.
12. Scientific Working Group for Forensic Toxicology. Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method validation in forensic toxicology. J Anal Toxicol. 2013;37:452-74.
13. Peters FT, Drummer OH, Musshoff F. Validation of new methods. Forensic Sci Int. 2007;17:216-24.
14. Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem. 2003;75:3019-30.
15. Høiseth G, Karinen R, Christophersen AS, Olsen L, Normann PT, Mørland J. A study of ethyl glucuronide in post-mortem blood as a marker of ante-mortem ingestion of alcohol. Forensic Sci Int. 2007;165:41-5.
16. Schröck A, Thierauf-Emberger A, Schürch S, Weinmann W. Phosphatidylethanol (PEth) detected in blood for 3 to 12 days after single consumption of alcohol-a drinking study with 16 volunteers. Int J Legal Med. 2017;131:153-60.
17. Luginbühl M, Wurst FM, Stöth F, Weinmann W, Stove CP, Van Uytfanghe K. Consensus for the use of the alcohol biomarker phosphatidylethanol (PEth) for the assessment of abstinence and alcohol consumption in clinical and forensic practice (2022 Consensus of Basel). Drug Test Anal. 2022;10:1800-2.
18. Fosen JT, Mørland J, Høiseth G. The relationship between ingested dose of ethanol and amount of ethyl glucuronide formed in blood. J Anal Toxicol. 2020;44:861-3.
19. Schmitt G, Droenner P, Skopp G, Aderjan R. Ethyl glucuronide concentration in serum of human volunteers, teetotalers, and suspected drinking drivers. J Forensic Sci. 1997;42:1099-102.
20. Halter CC, Dresen S, Auwaerter V, Wurst FM, Weinmann W. Kinetics in serum and urinary excretion of ethyl sulfate and ethyl glucuronide after medium dose ethanol intake. Int J Legal Med. 2008;122:123-8.