Phosphatidylethanol (PEth) analysis for alcohol consumption monitoring: Integrated analytical solutions for sample collection and quality control

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APPLICATION HIGHLIGHTS

- HemaXis™ DB is a precise and accurate tool for PEth sampling
- PEth is stable for at least 6 months at 2-8°C in lyophilizate
- PEth is stable for at least 6 months at room temperature on HemaXis™ cards
- The DBS System/ACQ Science collaboration enabled the development of an integrated solution for PEth analysis

KEYWORDS

- Phosphatidylethanol (PEth)
- Alcohol consumption monitoring
- Dried Blood Spot (DBS) sampling
- HemaXis™ DB
- Quality controls

INTRODUCTION

Phosphatidylethanol (PEth) has recently gain great interest as a very sensitive and specific blood marker of alcohol consumption. This direct marker enables the discrimination of heavy or occasional drinkers and to monitor complete abstinence within a time window of 2 to 3 weeks. PEth analysis is consequently a very valuable asset in applications such toxicological investigations, addiction monitoring or transplantation candidate evaluation[1-3].

The major drawback of PEth is its lack of stability in blood. Studies showed its rapid enzymatic degradation over time when blood samples are not stored frozen[4,5]. Guaranteeing samples are frozen directly after patient blood draw and are kept frozen during shipment is a complex and expensive challenge. DBS sampling has been proposed by several groups as a practical and cheap alternative to solve the enzymatic degradation issue[5,6].

The increasing demand for blood PEth analysis created the need for standardized, straightforward yet reliable analytical solutions. The HemaXis™ DB collection device, developed by DBS System to standardize and simplify DBS sampling, is a very interesting tool to achieve this purpose. In order to propose an even more simplified and integrated commercial solution, DBS System and ACQ Science collaborated on the development of commercial PEth QC formulas under both lyophilizate and DBS forms.

In this application note, we describe the sampling performances of HemaXis™ DB devices for the collection of blood samples prior to PEth analysis, as well as the accuracy, precision and stability performances achieved with the QC formulas prepared by ACQ Science.

EXPERIMENTAL

QC preparation

Two levels of QC were prepared by ACQ Science from blank human whole blood by addition of 40 ng/mL and 300 ng/mL of PEth 16:0/18:1 from Enzo Life Sciences (ELS) AG, Switzerland (catalog number BML-ST400-0010).

Three QC formulas were then prepared by ACQ Science from both level spiked material:

- **Liquid QCs:** aliquots for both QC levels were placed at -20°C directly after preparation, and stored/shipped frozen (-20°C). Prior to analysis, both levels were let to thaw at room temperature. 10 µL spots were then prepared on HemaXis™ DB cards using a volumetric micropipette. Spots were let to dry for at least 60 minutes at room temperature before proceeding with the next analytical step.
- **Lyophilizate QCs:** 5 mL aliquots of both QC levels were lyophilizated following ACQ Science procedures. Lyophilizate QCs were then stored/shipped refrigerated (2-8°C). Prior to analysis, both levels were reconstituted with 5 mL of MilliQ™ water under gentle agitation for 20 minutes. 10 µL spots were then prepared on HemaXis™ DB cards using a volumetric micropipette. Spots were let to dry for at least 60 minutes at room temperature before proceeding with the next analytical step.
- **HemaXis™ QCs:** DBS samples were prepared using HemaXis™ DB. A drop of approximately 25 µL of spiked material was deposited onto paraffin film to mimic a drop obtained at the patient's fingertip after a finger prick. Sampling was then performed following HemaXis™ DB IFU. DBS QCs were then stored/shipped at room temperature.

LC-MS/MS analysis

Analysis was performed at the University Center of Legal Medicine in Geneva, on a Dionex RS/AS 3000 LC system coupled to a Sciex API 5000 mass spectrometer. Analytical parameters are detailed in table 1 and 2.

Table 1. Analytical parameters

Chromatographic column	Phenomenex Kinetex RP C18 50x2.1 mm, 2.6µm
Mobile phases	A: 2mM ammonium acetate/acetonitrile (30/70 v/v) B: isopropanol
Internal standard	PEth (16:0/18:1)-D5 (Chiron AS, Norway, catalog number C10943.39-100-CF)
SRM transitions	PEth: 701.4 → 255.1 (CE: -42V, DP: -100V) PEth-D5: 707.0 → 281.3 (CE: -46V, DP: -100V)
Calibration range	20 – 2000 ng/mL

Table 2. Elution gradient

Time (min)	%B	Flow (mL/min)
0	30	0.4
1.0	30	
4.0	80	
5.0	80	
5.1	30	
10.0	30	

Sample preparation

DBS samples were extracted using the "In-vial" extraction approach described by Déglon et al.[7],

Briefly:

1. The entire blood spot is punched out using a 7 mm punch
2. The disk is rolled into a cylinder and introduced into a glass injection vial with restrictor insert
3. 100 µL of methanol containing 40 ng/mL of internal standard is added
4. After capping and short mixing, the vial is placed into the LC system autosampler for injection

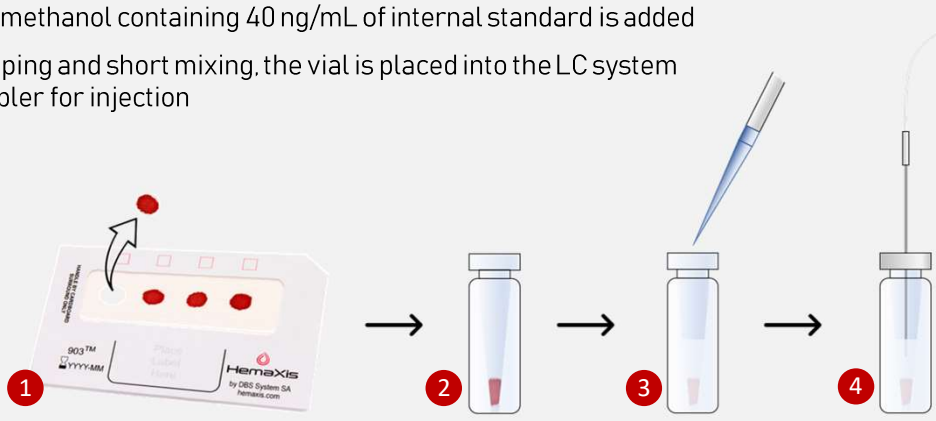

Figure 1. Sample preparation steps

EXPERIMENTAL (continued)

Accuracy and precision assessment

The impact of the QC preparation procedure on the recovered PEth values and on precision was assessed by running replicates of both levels of the three QC formulas within the same analytical run. For Liquid and Lyophilizate QCs, five replicates were generated using the same thawed/reconstituted bottle. For the HemaXis™ QCs, 10 spots from 10 different HemaXis™ DB cards were used.

Stability assessment

The stability of PEth in the three QC formulas was assessed by measuring five replicates of both levels 14, 47, 88 and 196 days after preparation by ACQ Science. Liquid QCs were stored at -20°C and Lyophilizate QCs at 2-8°C. A new bottle was thawed/reconstituted for each time point for the Liquid and Lyophilizate QCs, respectively. HemaXis™ QCs were stored at room temperature. Because the QC material was not prepared directly in the laboratory, the baseline concentrations could not be measured the day of the QC preparation.

RESULTS & DISCUSSION

Accuracy and precision assessment

The measured concentrations for each QC formulas and the observed CVs are presented in table 3 and 4, respectively.

The mean concentrations measured for Liquid and HemaXis™ QCs were statistically identical, and the observed CVs were comparable. This shows that the use of HemaXis™ DB to collect patient samples is equivalent in terms of accuracy and precision to the use of a conventional micropipette.

Table 3. Measured PEth concentrations

	Liquid QC	Lyophilizate QC	HemaXis™ QC
Level 1 40 ng/mL	37.82	35.38	38.55
Level 2 300 ng/mL	272.00	237.60	271.70

Table 4. Observed CV

	Liquid QC (n=5)	Lyophilizate QC (n=5)	HemaXis™ QC (n=10)
Level 1 40 ng/mL	1.31%	2.17%	2.51%
Level 2 300 ng/mL	2.79%	2.83%	3.43%

The concentrations measured for the Lyophilizate QCs were slightly lower than the Liquid QCs (-7.5% for level 1 and -12.5% for level 2). This can be explained by the fact that PEth is relatively hydrophobic, which can result in an incomplete solubilization of the analyte of interest when the lyophilizate material is reconstituted with pure water. However, the precisions observed for those two formulas were very comparable. This indicates the Lyophilizate QC is a very good surrogate to frozen liquid material, providing the target concentration values are assessed.

Stability assessment

The mean concentrations (n=5) measured 14, 47, 88 and 196 days after QC preparation are presented in figure 2.

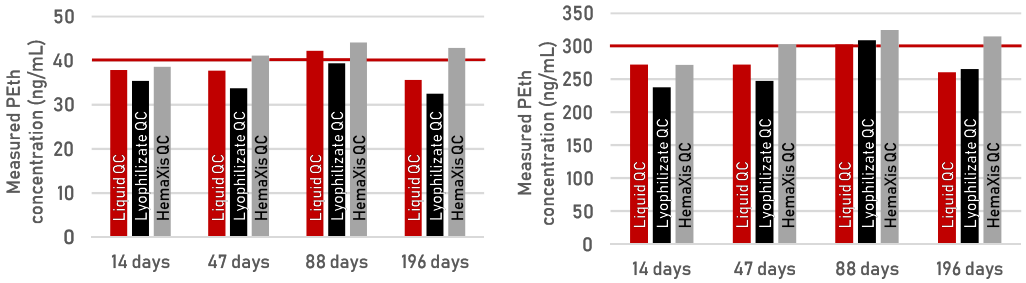

Figure 2. Mean concentrations (n=5) measured after various storage period. Target values are indicated by a red line

The absolute bias between the mean concentration of day 14 and day 196 for each formula was below 20%. The highest bias was observed for HemaXis™ QC Level 2 at day 88: +15.9%. All three formulas were then found to be stable for at least 6 months in their respective storage conditions. The HemaXis™ QCs closer to the target gravimetric values after 196 days, with a maximum bias of +5.6% (Level 1), against -13.2% for the Liquid QC (Level 2) and -18.8% for the Lyophilizate QC (Level 1).

CONCLUSION

The evaluation described in this application note showed that HemaXis™ DB is as precise and accurate than a conventional micropipette. Additionally, thanks to the collaboration between DBS System and ACQ Science, reliable and stable HemaXis™ QCs can now be commercially proposed as an interesting alternative to in-house QCs for laboratories analyzing PEth in DBS using HemaXis™ DB.

Our results also showed that Lyophilizate QCs are stable for at least 6 months at 2-8°C. This material is a very interesting alternative to in-house QCs for laboratories analyzing PEth in whole blood.

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ACKNOWLEDMENTS

DBS System thanks Dr Marc Augsburger and Estelle Lauer from the Unit of Forensic Toxicology and Chemistry of the University Center of Legal Medicine in Geneva for providing advices on the preparation of the QC formulas and for running all the analyses presented in this application note.

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