# Direct Quantitation of Phosphatidylethanol (PEth) in Volume-Controlled Dried Blood Spots using the Fully Automated Transcend DSX-1 System

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## ABSTRACT

**Goal:** Demonstrate a complete and fully automated workflow for dried blood spot analysis of the alcohol-specific biomarker phosphatidylethanol (PEth) 16:0/18:1.

**Methods:** The analytical method was developed on the Thermo Scientific™ Transcend™ DSX-1 system consisted of a dried matrix spot module coupled with Thermo Scientific™ TurboFlow™ technology and a triple quadrupole mass spectrometer.

**Results:** High-throughput 8-min quantification of PEth 16:0/18:1 in dried blood spot were achieved with linearity ($R^2$>0.99) across 20 ng/mL to 2000 ng/mL, % RSD <10% to satisfy different cut-off needs in clinical settings.

## INTRODUCTION

Phosphatidylethanols (PEth) are phospholipids that only form in the presence of ethanol. Due to the relatively longer half-life, PEth is used as mid-term biomarker for alcohol consumption.[1] The most abundant homologue of PEth, PEth 16:0/18:1 (Figure 1), is usually quantified via liquid chromatography tandem mass spectrometry (LC-MS/MS) in the whole blood to reflect repeated alcohol usage. PEth can rapidly degrade in blood after sample collection if not stored frozen.[2] In contrast, PEth is stable once prepared as dried blood spots (DBS) as the drying process stops any enzymatic degradation. DBS also is minimally invasive and only requires 10 to 20 µL sample volume. Thus, quantifying PEth in DBS cards has gained popularity in recent years.

Here, we describe a complete and fully automated workflow to rapidly extract and quantify PEth 16:0/18:1 in DBS via Transcend DSX-1 system, which combines a dried matrix spot module with innovative flow-through desorption (FTD™) technology and TurboFlow LC-MS/MS. Ten-microliter whole blood was precisely spotted on the DBS cards using the volume-controlled HemaXis™ DB10 sample collection device (DBS System SA, Switzerland) (Figure 2).

## MATERIALS AND METHODS

### Sample Preparation

All samples were prepared by DBS System SA. PEth-free whole blood was collected from volunteers, checked for PEth, and used for calibration samples. Lyophilized QC samples were obtained from ACQ Science (Germany) and reconstituted according to the instruction. DBS samples were prepared using HemaXis DB10 device. The DBS cards were dried at room temperature and placed directly onto the cardholder in the dried matrix spot module.

**Figure 1. The structures of PEth 16:0/18:1 and its internal standard (IS) PEth 16:0/18:1-$d_5$, with the positions for deuterium is indicated in red circles.**

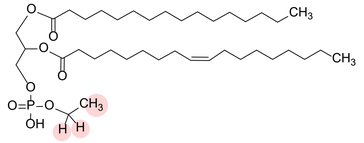
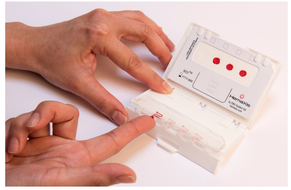


**Figure 2. HemaXis DB10 blood collection device (courtesy of DBS System SA).**



## MATERIALS AND METHODS (cont')

### Fully automated sample extraction.

PEth 16:0/18:1 was extracted from DBS cards with a 6 mm clamp using mobile phase A (Table 2) with HotCap™ enabled at 100 °C. IS was introduced using the built-in IS pump in the DMS module that overfilled an IS loop to ensure robust and reproducible IS addition (automated IS addition, AISA™. Every sample spot was photographed with the Intelligent Vision Camera (IVC™) prior to and after each run for spot recognition and sample traceability.

### Online SPE-Liquid chromatography.

Automated online SPE cleanup and chromatographic separation was performed on a Thermo Scientific™ Vanquish™ UHPLC system. The TurboFlow system was configured with 'Focus mode', and the analysis process and flow path are shown in Figure 4. After loading the extracted samples onto the TurboFlow column (Figure. 4A), the analytes were eluted using the high organic eluant stored in the "transfer loop" and refocused on the analytical column (Figure. 4B). The analyte separation was performed on the analytical column while TurboFlow column washed (Figure. 4C). To prepared for the subsequent analysis, the transfer loop was filled with eluant while analytical column was washed and equilibrated (Figure. 4D). The gradient, mobile phases, clamp washes, and columns used are described in Table 2.

### Mass spectrometry.

PEth quantification was performed using a Thermo Scientific™ TSQ Altis™ Plus mass spectrometer with a heated electrospray ionization probe in the negative mode. The capillary voltage was -3500 V and MS parameters such as selected reaction monitoring (SRM) are shown in Table 1.

### Data analysis.

Post-acquisition data analysis was carried out using Thermo Scientific™ TraceFinder™ software.

**Table 1. MS parameters**

| | Precursor m/z | Product m/z | CE (V) |
|---|---|---|---|
| PEth 16:0/18:1 | 701.5 | 255.3[2] | 33.5 |
| | | 281.3[1] | 31.2 |
| PEth 16:0/18:1-$d_5$ | 706.5 | 255.3[2] | 33.5 |
| | | 281.3[1] | 31.2 |

1. Quantifier
2. Qualifier

| | | | |
|---|---|---|---|
| Polarity | (-) | Cycle Time (seconds) | 0.4 |
| Sheath Gas (Arb) | 50 | Q1 Resolution (FWHM) | 0.7 |
| Aux Gas (Arb) | 10 | Q3 Resolution (FWHM) | 1.2 |
| Sweep Gas (Arb) | 0 | Source Fragmentation | 0 |
| Ion Transfer Tube Temp. (°C) | 325 | Chromatographic Peak Width (seconds) | 6 |
| Vaporizer Temp. (°C) | 350 | CID Gas (mTorr) | 1.5 |

**Figure 5. Representative chromatograms of PEth 16:0/18:1 and its IS.**

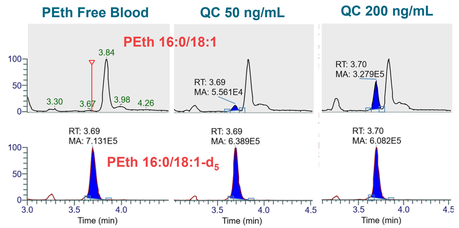



**Figure 3. Fully Automated Transcend DSX-1 System**


## Innovative technologies

- **Volume-Controlled Spots** • Precise Sampling
- **HotCap** • Heated Extraction
- **FTD (Flow-Through Desorption)** • Direct Analyte Desorption and Extraction
- **IVC (Intelligent Vision Camera)** • Spot Recognition, Sample Traceability, Chain of Custody
- **AIS (Automated IS Addition)** • Precise IS Addition
- **96 DBS Cards Capacity**
- **TurboFlow** • Online Sample Cleanup
- **Aria MX Software** • Integrated Software Control

## RESULTS

The most abundant PEth homologue, PEth 16:0/18:1, was quantified using an automated IS delivery module in the dried matrix spot module. PEths have very high hydrophobicity and similar structures, which make them challenging to separate chromatographically while maintaining a minimum carryover. The analyte separation was achieved on a Thermo Scientific™ Hypersil GOLD™ C8 column, which was selected over a C18 column to mitigate carryover. In addition, rapid aqueous/organic switching steps were also used to wash the clamp, TurboFlow column, and analytical column to minimize analyte carryover further.

Calibration and QC samples were spotted in triplicates via HemaXis DB10 device, where a precise 10 µL was loaded on the DBS cards. Accuracy and precision data of QC samples at two levels were compiled from two days, with % accuracy within 100 ± 10% and % RSD below 10% (Table 3). The results are comparable with those from the manual disc-punch method of the same QC samples performed by our collaborators in Switzerland (Table 3). Representative chromatograms of PEth quantification in PEth-free and QC samples are shown in Figure 5.

Calibration curves were built using a weighting factor of 1/x from a lower limit of quantification of 20 ng/mL to an upper limit of quantification of 2000 ng/mL with $R^2$ values greater than 0.99, % RSD and % Diff < 15% (Figure 6).

**Figure 4. TurboFlow Technology for Sample Cleanup (Focus Mode)**

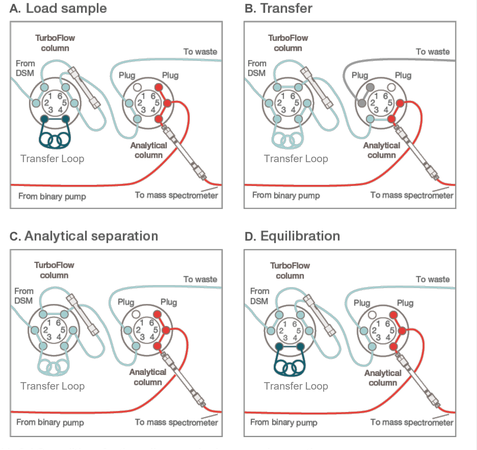



**Table 2. LC conditions for the online sample cleanup and separation**

| | TurboFlow Column | | | | | | | Analytical Column | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time (min) | Flow Rate (mL/min) | % A | % B | %C | %D | Tee | Loop | Flow Rate (mL/min) | %A | %B |
| 0 | 0.2 | 100 | - | - | - | ==== | out | 0.5 | 100 | - |
| 0.2 | 0.0 | 100 | - | - | - | ==== | out | 0.5 | 100 | - |
| 0.3 | 0.2 | 100 | - | - | - | ==== | out | 0.5 | 100 | - |
| 0.5 | 0.6 | 100 | - | - | - | ==== | out | 0.5 | 100 | - |
| 1.1 | 0.1 | 100 | - | - | - | T | in | 0.4 | 100 | - |
| 2.1 | 4 | 100 | - | - | - | ==== | in | 0.5 | 36 | 64 |
| 2.2 | 4 | - | 100 | - | - | ==== | in | 0.5 | 24 | 76 |
| 2.95 | 3 | - | - | 100 | - | ==== | in | 0.5 | 12 | 88 |
| 3.7 | 1 | - | - | - | 100 | ==== | in | 0.5 | - | 100 |
| 4.45 | 2.5 | 100 | - | - | - | ==== | in | 0.5 | - | 100 |
| 4.7 | 4 | - | 100 | - | - | ==== | in | 1.0 | 100 | - |
| 6.7 | 2 | 100 | - | - | - | ==== | out | 0.5 | 100 | - |
| Clamp Washes | Wash 1: 0.1% formic acid in water<br>Wash 2: acetonitrile/Isopropanol/Acetone, 3/3/4 (v/v/v)<br>Wash 3: Isopropanol | | | | | | | | | |
| Mobile Phases | A: 10 mM ammonium formate, 0.05% formic acid in water/acetonitrile, 3/7 (v/v)<br>B: 10 mM ammonium formate, 0.05% formic acid in methanol<br>C: acetonitrile/Isopropanol/Acetone, 2/2/1 (v/v/v)<br>D: Isopropanol | | | | | | | A: 10 mM ammonium formate, 0.05% formic acid in water/acetonitrile, 3/7 (v/v)<br>B: acetonitrile/isopropanol, 1/1 (v/v) | | |
| Columns | C8-XL Turbo column, 50 x 0.5 mm at room temperature | | | | | | | Hypersil Gold C8, 50 x 2.1 mm, 5 µm, 23 ° C | | |

**Figure 6. Calibration curves of PEth 16:0/18:1 and the RSD of IS from day-1**

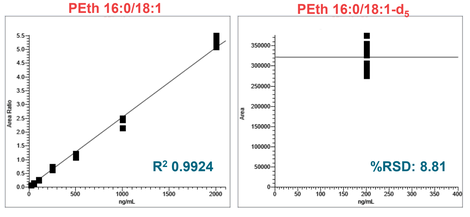



**Table 3. Precision and accuracy results in QC samples**

| Target (ng/mL) | Transcend DSX-1 | | | | | | | Disc-Punch and Manual Extraction |
|---|---|---|---|---|---|---|---|---|
| | Rep. (N=3) | Within-Day | | | Between-Day | | | |
| | | Mean | %Acc. | %RSD | Mean | %Acc. | %RSD | |
| 50 | Day-1 | **48.0** | 96.0 | 1.2 | | | | **47** (%RSD 5.7, N=19) |
| | Day-2 | **48.9** | 97.8 | 3.5 | **48.4** | 96.8 | 1.3 | |
| 200 | Day-1 | **208.0** | 104.0 | 7.4 | | | | **210** (%RSD 4.2, N=20) |
| | Day-2 | **214.0** | 107.0 | 0.5 | **211.0** | 105.5 | 2.0 | |

## CONCLUSIONS

- Transcend DSX-1 combines a dried matrix spot module and the TurboFlow LC-MS/MS, and provides a complete workflow for fast and robust quantification of small molecules in dried matrix spots.
- The association of volume-controlled HemaXis blood collection device together with the current DSX-1 method provides accurate and efficient quantification of alcohol-specific biomarker PEth in blood.

## REFERENCES

1. Javors, M. A., et. al. Alcohol Clin. Exp. Res. 2016 Jun, 40(6), 1228-1234.
2. Viel, G., et. al. Int. J. Mol. Sci. 2012 Nov, 13(11), 14788–14812.

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## TRADEMARKS/LICENSING

Disclaimer: For Research Use Only – Not for use in diagnostic procedures.

Specifications subject to change. Availability of product in each country depends on local regulatory marketing authorization status.



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