

## 1 Introduction

We demonstrate quantitation of a therapeutic drug in blood from dried blood spots (DBS) with direct flow-through LC-MS, including an examination of extraction methods, varied hematocrit levels, matrix effects and interferences.

DBS are a versatile means of sample collection to support pharmacokinetic studies. Typical analysis requires off-line punching and extraction of DBS. In contrast, direct flow-through elution from DBS cards coupled to online LC-MS can greatly streamline sample workflows and reduce potential handling errors.

We used accurate mass time-of-flight MS for the multiplexing advantage of both allowing quantitation of the drug and detection of a wide range of components eluting from DBS.

## 2 Methods

- A range of hematocrit levels were prepared by mixing appropriate volumes of plasma and erythrocyte fractions obtained from healthy donor blood.

- A prototype DBS clamping device (2 mm diameter clamping region), ACE™ automatic cartridge exchanger, and HPD™ high pressure dispenser (Spark Holland) were used to extract DBS. Isotopically-labeled internal standard was added by loop injection during DBS desorption.

- Exact mass electrospray time-of-flight (TOF) LC-MS analysis was performed with a PerkinElmer AxION 2 TOF mass spectrometer and Flexar UHPLC system.

- Datasets were analyzed with AviatorBatch software developed internally for rapid, automated data processing.

- Extracted ion chromatograms were processed and peak areas were reported using a narrow mass tolerance ( $\pm 0.01$  Da) to ensure specificity.

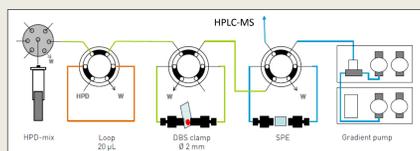


Figure 1. Schematic of the flow-through device and the PerkinElmer AxION 2 time-of-flight mass spectrometer.



## 3 Results

### Method Development

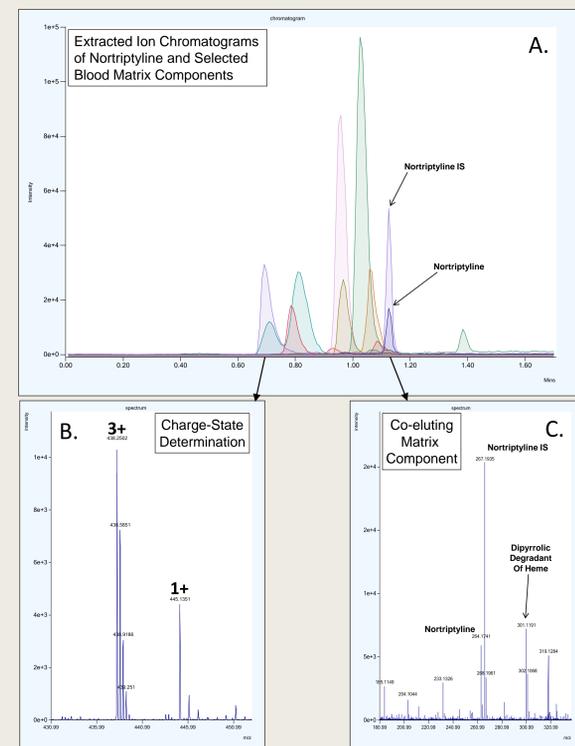


Figure 2. Selected extracted ion chromatograms (A) and mass spectra (B, C) of nortriptyline and matrix components eluting during LC-MS of a desorbed dried blood spot.

- Rapid separation of analytes from the complex matrix of the DBS eluate is facilitated with high resolution and high mass accuracy TOF analysis at fast data acquisition rates.

- Multiple charge states are readily determined with high resolution TOF data (Fig. 2B) aiding in identification of matrix components such as peptides.

- High mass accuracy detection (Fig. 2C) of nortriptyline, nortriptyline IS, as well as a co-eluting heme degradation product.<sup>‡</sup>

### Carryover

- Assay carryover was studied by examining the presence of nortriptyline from flow-through analysis of blank DBS cards acquired following analysis of highly concentrated samples (1000 ng/mL of nortriptyline).

- No significant carryover (0.02%) was observed in blank samples (triplicate) which suggests that the online flow-through device and the method developed here minimizes carryover from the clamping device.

### Linearity

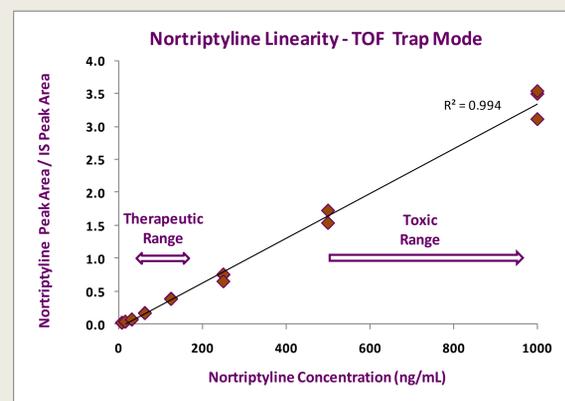


Figure 3. Nortriptyline linearity evaluated using the Spark Holland flow through device coupled online with the PerkinElmer AxION 2 LC-TOF MS

- The online sample extraction LC-TOF assay provides wide linear dynamic range from 7.81 to 1000 ng/mL (Fig. 3) which encompasses the clinical range for therapeutic drug monitoring.

DBS Cards: PerkinElmer 226 Bioanalysis cards, 20 µL blood per spot.  
Linearity DBS: Whole blood spiked with nortriptyline in a 2-fold dilution series from 1000 to 3.91 ng/mL.  
Hematocrit DBS: Blood adjusted to hematocrits 25 to 65%, all spiked with nortriptyline to 80 ng/mL.  
Desorption: 5% methanol in water + 0.1% formic acid.  
SPE: 2 x 10 mm 7 µm HySphere C18 HD.  
Column: 2 x 30 mm 2.7 µm Brownlee SPP C18.  
Gradient: 10-100% MeOH + 0.1% FA over 1.5 min, 0.5 mL/min.

### Hematocrit Variability

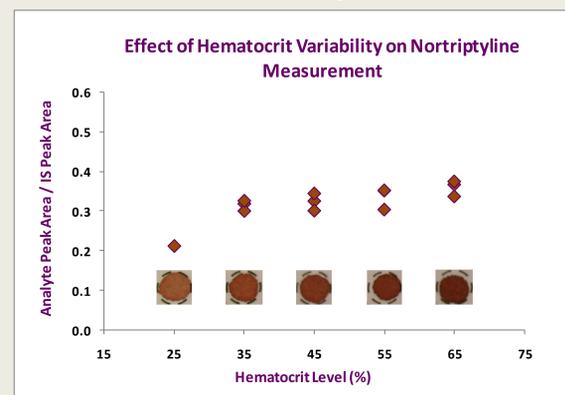


Figure 4. Effect of variation in % hematocrit on nortriptyline measurement

- Hematocrit levels can vary from 35 – 60% with gender, age, and disease state. Blood viscosity varies with hematocrit levels which may affect spreading of blood when applied to DBS cards and subsequent measured analyte concentration in the desorbed region.

- As seen in Figure 4, nortriptyline measurement was relatively unaffected in the 35 – 65% hematocrit range.

### Reproducibility Optimization

- DBS were desorbed with either high or low methanol concentrations.

- Flow from desorbed DBS was diluted online with 0.1% formic acid in water to allow binding to SPE cartridges.

- SPE cartridges were then switched in-line with the HPLC column for gradient separation.

DBS Cards: Whatman 903 paper, 25 µL blood per spot.  
DBS: Whole Blood Spiked with nortriptyline to 80 ng/mL.  
Desorption: 95% or 20% methanol with 0.1% formic acid.  
SPE: 2 x 10 mm 7 µm HySphere C18 HD.  
Column: 2 x 20 mm packed with 5 µm Restek Ultra C18.  
Gradient: 20-100% MeOH + 0.1% FA over 1 min, 0.7 mL/min.

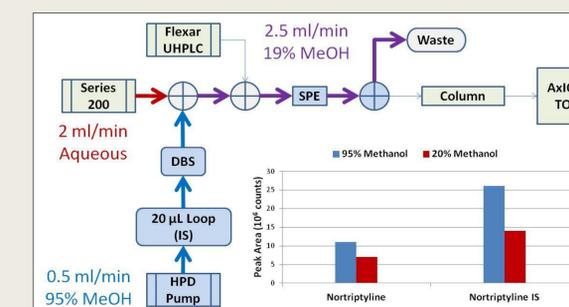


Figure 5. System schematic and, analyte and IS peak area with varied desorption solvent

Solvent n	Nortriptyline		Nortriptyline IS		Analyte/IS Ratio	
	95% MeOH	20% MeOH	95% MeOH	20% MeOH	95% MeOH	20% MeOH
SCV	8.5	16.7	4.8	7.4	5.5	13.2

Table 1. Analyte and IS reproducibility with varied desorption solvent

- Nortriptyline recovery (Fig. 5) and reproducibility (Table 1) were improved with high methanol concentration in the desorption solvent.

## 4 Summary

- Direct online flow-through SPE-LC-TOF-MS analysis provides a robust automated method for dried blood spot analysis.

- High resolution and high mass accuracy ESI-TOF-MS analysis allows rapid LC separations while maintaining specificity.

- The nortriptyline method described here shows good analytical performance in the clinical range and gives consistent results over a wide range of hematocrit values.

- Method development is in progress to determine optimal desorption conditions for improved reproducibility.

- The authors thank Eric Jappe for coding the custom data processing application.

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<sup>‡</sup>Francesco De Matteis et al., Rapid Communications in Mass Spectrometry, 2006; 20: 1209-1217.