Decreasing DBS assay bias with Pipette tip cleanup of Dried Blood Spots

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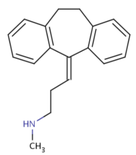
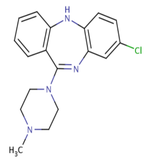
Introduction

Dried Blood spotting for bioanalytical analysis is gaining much popularity in recent years. Small sample volumes, inexpensive shipping and other areas of cost savings in preclinical and clinical trials have many companies exploring dried blood spotting. Most protocols desorb the analyte directly from the dried blood and inject directly into the LC/MSMS system. While this is a very simple protocol there is no sample clean up or matrix removal which leaves the analyst vulnerable to assay bias, inaccurate and unrepeatable results. These disadvantages can greatly offset the cost benefits. Pipettes are commonly used in laboratory analysis to transfer samples from one container to another. By inserting SPE material into a pipette tip, a cleanup of the sample can simultaneously be performed during this transfer. A reversed phase pipette tip was used to extract the analytes and remove matrix interferences prior to injection.

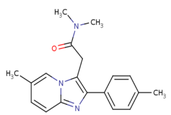
Analytes



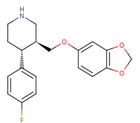
Nortriptyline
LogP: 4.7 pKa: 9.7



Clozapine
LogP: 2.7 pKa: 7.5



Zolpidem
LogP: 1.2 pKa: 6.2



Paroxetine
LogP: 3.1 pKa: 10.3

Standards were ordered at a concentration of 1.0 mg/mL in methanol from Cerilliant and diluted to the appropriate concentration for sample spiking

Experimental

Human blood was spiked to an appropriate concentrations of Nortriptyline, Clozapine, Zolpidem and Paroxetine. 15 µL spots were dried on a DMS (Dried Media Spotting) card. Each spot was allowed to dry overnight. A three millimeter punch was taken out of each spot. The spots were reconstituted in 100 µL of 80:20 MeOH:H_2O with 0.1% formic acid. A pipette tip with conditioned sorbent is used to transfer the eluted analyte. The desorbed blood spot is transferred into a centrifuge tube using a pipette tip filled with reversed phase sorbent. The spots were analyzed by LC/QQQ and the responses of the analytes were compared to non-treated blood spots

LC-MS Conditions

Agilent 1260 LC / 6460 QQQ

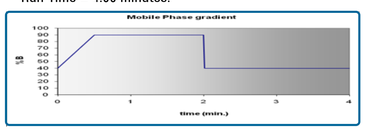
Column – Poroshell 120 SB-C18
2.1 x 50mm 2.7 µm
Mobile Phase –
A: 0.1% Aqueous Formic Acid B: MeOH
Pump Program
Flow rate 200 µL/ min.

t_0 A: 60%, B: 40%

$t_{0.5-2.0}$ A: 10%, B: 90%

$t_{2.01-4.00}$ A: 60%, B: 40%

Run Time = 4:00 minutes.



Gas Temp: 350 °C
Gas Flow: 10 l/min
Nebulizing: 20 psi
Pol: Pos

Compound	Q1 ion	Product ion	CE
Zolpidem	308.2	235.1	36 V
Zolpidem-D6	314.2	235.0	36 V
Clozapine	327.1	270.1	20 V
Clozapine-D4	331.2	272.1	20 V
Paroxetine	330.2	192.1	20 V
Paroxetine-D6	336.2	198.1	20 V
Nortriptyline	264.2	105.1	16 V
Nortriptyline-D3	267.2	233.0	8 V

Results and Discussion

OMIX Tip Clean up

- 100 µL C18 OMIX tip were used to cleanup the reconstituted dry blood spots
- 15 µl of human blood was spotted onto the DMS card and allowed top dry overnight
- A 3 mm punch was taken out of the center of the dried spot and transferred to a 2 mL centrifuge tube.
- 100 µL of 0.1% formic acid 80:20 Methanol: Water was added to the tube and centrifuged for 15 minutes at 15,000 rpm
- A 100µL C18 OMIX tip was conditioned by aspirating and discarding 100 µL of methanol.
- The sample was then aspirated out of the centrifuge tube and dispensed into a conical vial.
- 100 µL of 0.1% aqueous formic acid was added to the vial 20 µL was injected into the LC/QQQ

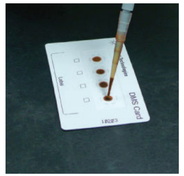
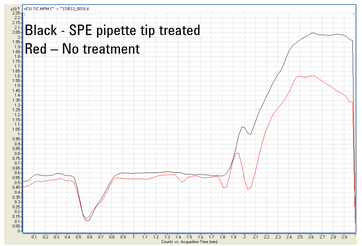
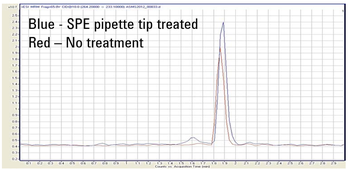


Figure I – Ion Suppression /Post column infusion



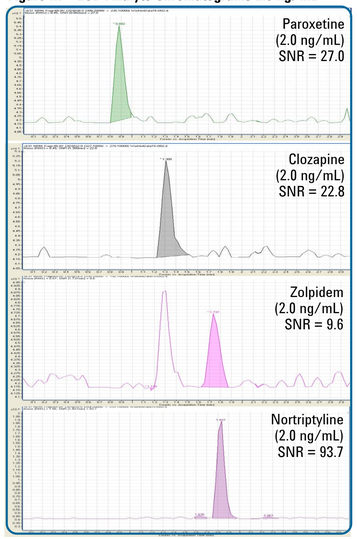
Less ion suppression is observed in the sample cleaned up by transferring with the OMIX tip

Figure II – Nortriptyline response comparison



Nortriptyline shows improved signal with the OMIX cleanup

Figure III – LOD Analyte Chromatograms 2.0 ng/mL



SNR calculated using peak height with RMS times 5.
Good analyte response was achieved at the 2.0ng/mL LOQ with signal to noise rations better than twenty to one.

Results and Discussion

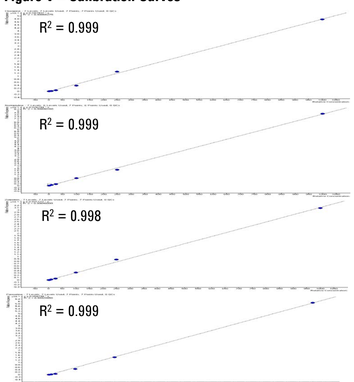
Figure IV – Analyte Recovery

Analyte Recovery		
5.0 ng/mL	DBS	DBS w/OMIX
Zolpidem	82%	95%
Clozapine	87%	93%
Paroxetine	82%	94%
Nortriptyline	31%	31%

A blood spot standard was prepared by spiking an aqueous standard to 5.0 ng/mL. 4 µL (the calculated volume based on the area of a 15 µL spot compared to the area of the 3mm punch) was diluted to 200 µL (The extract final volume) and injected into the LC/QQQ.

$$\% \text{ recovery} = \frac{\text{Extracted Analyte Response}}{\text{Standard Analyte Response}}$$

Figure V – Calibration Curves



In order to verify a good analytical method was being used, a calibration curve was analyzed from 2.0 ng/mL to 200 ng/mL. A first order regression was applied and the correlation coefficient was better than 0.998 for all four analytes.

Figure VI – Accuracy and Precision

Accuracy and Precision (n = 12)			
500 ng/mL	Avg amt	Accuracy	RSD
Zolpidem	458	92%	8%
Clozapine	441	88%	4%
Paroxetine	461	92%	6%
Nortriptyline	475	95%	7%

Accuracy and Precision studies were analyzed to verify curve fit and reproducibility. Six replicates at 500 ng/mL were spotted, extracted and analyzed. The recoveries were calculated based on linear fit of analyte the relative response

Conclusions

•An accurate and reproducible method was developed for the desorption, simple cleanup and analysis of 4 compounds in a dried blood spot. (Figure I&II)

•Post column infusion data demonstrated matrix interferences were removed by passing the blood through a reversed phase pipette tip. (Figure I)

•Nortriptyline showed significant improvement in signal response as demonstrated by the chromatogram and recovery table. (Figure II & IV)

•Good signal to noise at 2.0 ng/mL (> 8) was achieved for all compounds (Figure III)

•Good chromatography (Figure V) was achieved and linear calibration curves each having a correlation coefficient better than 0.995 (linear fit)for all four compounds (Figure IV)

•Analysis was accurate and reproducible (Figure VI). All recoveries were within 12% of the true value the spiked standard. Six replicates were analyzed at each level with RSDs below 8% for all compounds.

•Removing sample matrix inferences that can cause assay bias is critical to insure quality data. Dried blood spotting has advantages for sample handling and storage but present challenge for data quality. Using a simple pipette tip cleanup matrix interferences were removed and signal response was improved