Analysis of Amitriptyline, Fluoxetine, Quetiapine, and Sertraline Using Dried Blood Spots

Application Note

Pharmaceutical

Authors

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Introduction

Dried blood spotting (DBS) has gained in popularity with pharmaceutical laboratories for pre-clinical and clinical trials for new chemical entities in the pharmaceutical industry. Fifteen microliters of blood is spotted onto a blood spotting card and allowed to dry for at least 2 hours. A 2–4 mm core is then punched from the spot. Analytes are desorbed from the punched spot using solvent, then analyzed by LC-MS/MS. Cellulose materials have typically been used for these applications, but a new non-cellulose material is now available. This Dried Matrix Spotting (DMS) media can also be used for other biological fluids such as plasma, urine, and spinal fluid.

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Experimental

Four analytes were chosen: quetiapine, amitriptyline, sertraline, and fluoxetine. Fluoxetine-D6 was readily available as an internal standard, so it was used for all compounds. Precision and accuracy recoveries were calculated based on the linear regression of the calibration standards. The accuracy was calculated at a low level (0.5 ng/mL), a mid-level (5.0 ng/mL), and high level (500 ng/mL).

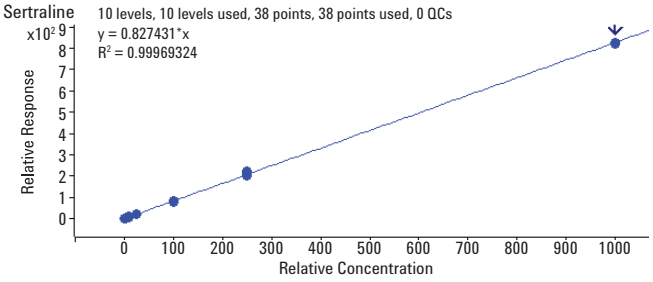
LC/MS conditions

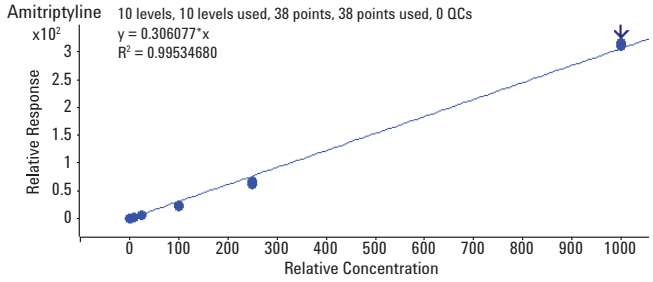
Column	Agilent Poroshell 120 EC-C18 3.0 mm x 50 mm 2.7µm	
Mobile phase	A: 0.1% Aqueous formic acid	
	B: ACN	
Pump program	Flow rate 300 µL/ min	
	t_0	A: 50%, B: 50%
	$t_{1.5-2.0}$	A: 10%, B: 90%
	$t_{2.01-300}$	A: 50%, B: 50%
Run time	3:00 minutes	
Gas temp	350 °C	
Gas flow	10 L/min	
Nebulizing	20 psi	
Pol	Pos	

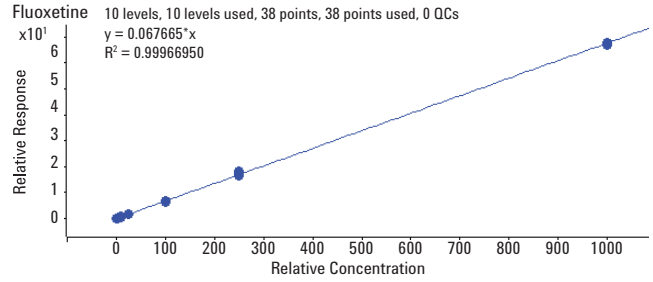
Compound	Q1 ion	Product ion	CE
Quetiapine	384.0	253.1	18 V
Amitriptyline	278.2	105.1	22 V
Sertraline	306.1	159.01	26 V
Fluoxetine	310.1	163.0	26 V
Fluoxetine-D6	316.1	154.1	2 V

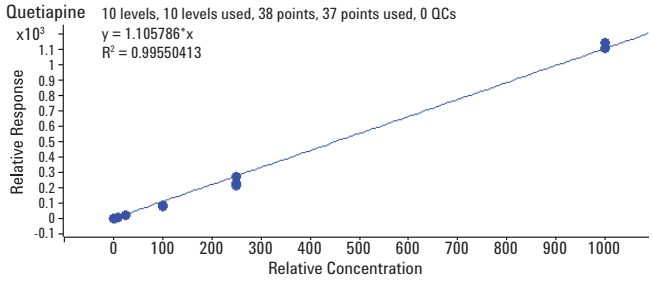
Results and Discussion

1 mL of human blood was spiked with 10 µL of each working standard to create a calibration curve of 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 20, 50, 200, 500 and 2,000 ng/mL. Fifteen µL of each of these standards was spotted onto an Agilent DMS card.





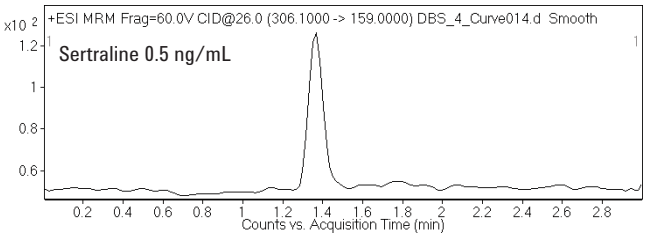


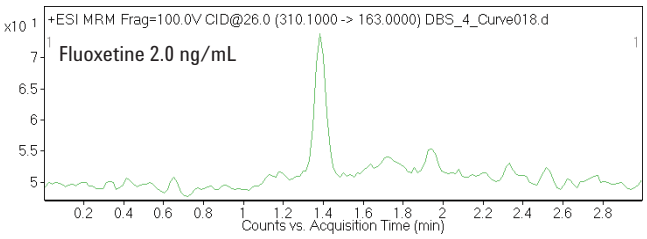


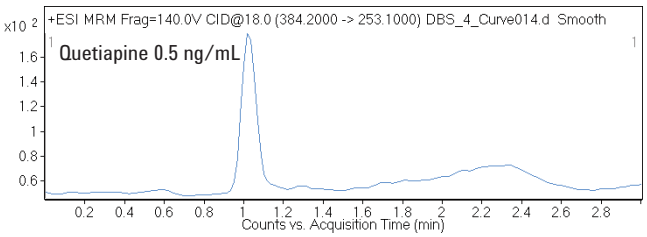
A 3 mm disk was punched from each dried spot and placed into a 96-well collection plate.

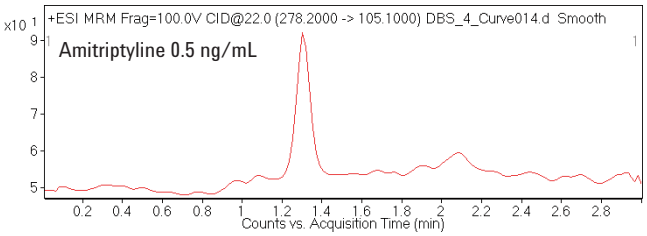
300 µL of 0.1% formic acid in 80% methanol (with 0.066 ng/mL of deuterated internal standard mix) was added to each well and vortexed.

The samples were evaporated to dryness and reconstituted in 100 µL of mobile phase.









Analyte Recoveries (n=6)

	0.5 ng/mL		5.0 ng/mL		500 ng/mL	
	% Rec	RSD	% Rec	RSD	% Rec	RSD
Quetiapine	91%	12%	87%	7%	99%	8%
Amitriptyline	90%	7%	95%	13%	92%	2%
Fluoxetine	105%	10%	104%	16%	101%	2%
Sertraline	90%	11%	100%	5%	97%	2%

Conclusions

Four compounds in blood were successfully desorbed and analyzed by LC-MS/MS. Good detection levels were achieved using an Agilent 1290 LC system and an Agilent 6460 mass spectrometer. Linearity was demonstrated using a 1st order regression and correlation coefficients were better than 0.995. Relative recoveries were within 10% of the true value with RSD values all less than 10%. DMS proved to be an effective means of storing plasma samples and protein removal for a simple approach to sample preparation prior to LC-MS/MS.

For More Information

Bond Elut DMS cards are intended for use in DMPK/ADME research applications only. They should not be used in diagnostic procedures.

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