Homogeneity of Dried Matrix Spots

Application Note

Pharmaceuticals

Authors

William Hudson, Ben Yong,
Paul Boguszewski, Ritu Arora
Agilent Technologies, Inc.
25200 Commercentre Drive
Lake Forest, CA 92630
USA

Introduction

Dried blood spotting for bioanalytical analysis in clinical and preclinical pharmaceutical testing has gained in popularity in recent years. As drug studies require quick, high quality toxicokinetic and pharmacokinetic data, dried blood spotting offers a good alternative to traditional plasma extraction and analysis.

In this technique, blood spot quality is a critical issue. Since only a small portion of the spot is typically used, it is necessary to verify that the size of the blood spot is consistent as well as that the dispersion of the analyte throughout the spot is homogeneous. Whether the punch is taken from the center or toward the edge of the spot, the recoveries must be reproducible.

Experimental

Four analytes were chosen based on the availability of deuterated standards and their suitability as typical pharmaceutical small molecule drugs: clozapine, zolpidem, paroxetine, and nortriptyline.

1,000 µL of human blood was spiked to 20 ng/mL of the four analytes. A 20 µL drop of blood was spotted onto an Agilent Bond Elut DMS card. The blood spot was dried for two hours and five disks were punched out. A 1.5 mm disk was punched from the center, top, left, right and bottom left edge and each punch was placed into a single well of a 96-well 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 initial mobile phase.

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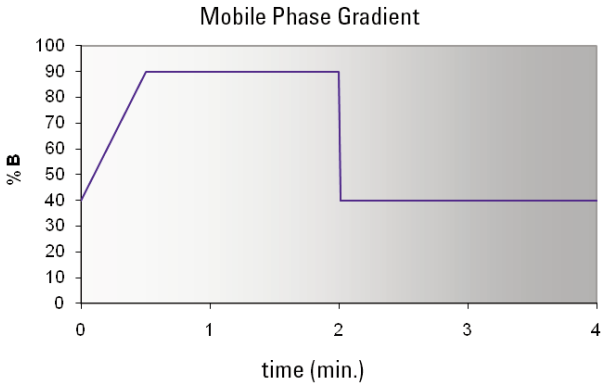
LC/MS conditions

Column	Pursuit XRS^{ultra} C18 50 mm x 2.0 mm	
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	

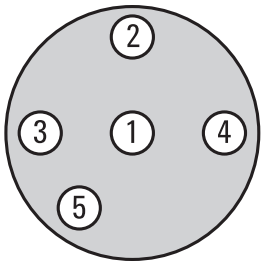


Compound	Q1 ion	Product ion	CE
Zolpidem	308.2	235.1	36 V
Zolpidem-D6	314.2	235.0	36 V
Clozapin	327.1	270.1	20 V
Clozapine-D4	331.2	272.1	20 V
Paroxetine	330.2	192.1	20 V
Paroxetine-D6	336.2	198.1	20 V
Nortriptyline	264.2	105.1	16 V
Nortriptyline-D3	267.2	233.0	8 V

Results and Discussion

From three different lots of material, the RSD values for the diameter of the spots were all 3% or lower.

Lot no.	1	2	3	4	Avg	RSD
80062	0.214″	0.218″	0.212″	0.211″	0.214″	1.4%
81121	0.224″	0.214″	0.219″	0.209″	0.217″	3.0%
80862	0.217″	0.205″	0.216″	0.210″	0.212″	2.6%



A 1.5 mm punch was taken out of a 20 µL blood spot in the marked locations. Concentration = 20 ng/mL in blood. The response of the analyte was consistent for each punch taken.

		1	2	3	4	5
Zolpidem	Avg response (n = 4)	11113	11641	11233	10807	10802
	CV (from center)		0.6%	1.1%	–2.7%	–2.8%
Clozapine	Avg response (n = 4)	6122	6462	6511	5972	5933
	CV (from center)		3.1%	3.5%	–1.3%	–1.7%
Paroxetine	Avg response (n = 4)	1480	1474	1462	1446	1532
	CV (from center)		–0.4%	–1.3%	–2.3%	3.5%
Nortriptyline	Avg response (n = 4)	5233	5100	5398	5431	5318
	CV (from center)		–2.5%	3.2%	3.8%	1.6%

Conclusions

Spot diameter was consistent across five spots and three different lots of material with RSD values of 3% or less.

Punches taken at the top, left, right and diagonally bottom were compared to the center punch and were consistent with center spot recovery.

Data demonstrates even distribution of the four different compounds within the blood spot.

Matrix spot homogeneity and consistent spot size ensure the analytical result will be consistent throughout any clinical study.

For More Information

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

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

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