Improving Sensitivity of Basic Drugs in Dried Blood Spotting through Optimized Desorption

Application Note

Pharmaceutical

Authors

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Introduction

Dried matrix spotting (DMS) or dried blood spotting (DBS) technology, combined with the analytical capability of modern mass spectrometers (LC/MS/MS), has recently emerged as an important method in the quantitative bioanalysis of small molecules. The great interest in DBS lies in the small volume of sample required, ease of collection, reduced sample shipping costs, and versatile storage conditions [1, 2, 3, 4].

As a relatively new technique in bioanalysis, investigating the impact of variables that may affect its overall efficiency is essential. Agilent Bond Elut Dried Matrix Spotting cards use a novel, noncellulose-based substrate for dried matrix and dried blood spotting. These were used to evaluate method development options available for basic analytes to reach optimal desorption conditions, and the resulting impact on mass spectrometric sensitivity.

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Experimental

Materials and Methods

- Agilent Bond Elut Dried Matrix Spotting (DMS) cards, (p/n A400150)
- Agilent Poroshell 120 EC-C18, 30 × 2.1 mm, 2.7 µm column (p/n 691775-902)
- Human whole blood (pooled, mixed gender) was purchased from Biochemed Services.
- Chemicals: Atenolol, pindolol, metoprolol, and propranolol were purchased from Sigma Chemicals.
- Water and methanol (LC/MS grade) were purchased from VWR.

Fresh human whole blood, pooled (in Heparin) was spiked with a mix of four basic pharmaceuticals, comprising β-blockers such as atenolol, pindolol, metoprolol, and propranolol, at a concentration of 20 ng/mL. The log P and pKa values of the four drugs screened are listed in Table 1. After vortexing, 15 µL of blood was aliquoted per spot on Agilent Bond Elut Dried Matrix Spotting cards followed by overnight drying. Circular punches of 3 mm diameter were taken from the DMS cards and used for the desorption studies.

Table 1. Basic drugs screened, general information.

Compound	Log P	pKa
Atenolol	0.5	9.6
Pindolol	1.9	8.8
Metoprolol	1.6	9.7
Propranolol	3.0	9.5

Desorption methods

Different techniques were tested to evaluate the best way to desorb the analytes from the membrane. Each test was compared to a standard and protein precipitated sample. All blood samples were evaporated and reconstituted in 100 µL mobile phase.

- **Standard:** By determining the ratio of the area of the punch to the area of the spot, the volume taken from a 3 mm punch can be determined. The actual blood volume sampled is 4 µL and is consistent regardless of the amount of volume spotted on the card [5]. A 4 µL amount of 20 ng/mL standard was desorbed with 300 µL of 0.1% formic acid 80:20 MeOH:H_2O, centrifuged at 15,000 rpm for 15 minutes, evaporated to dryness, and diluted to 100 µL with mobile phase.
- **Protein precipitation:** A 4 µL sample of blood was diluted to 100 µL with H_2O. Then, 300 µL of 0.1% formic acid in MeOH was used as a crash solvent (1:3 aqueous:organic crash) to precipitate the proteins. This was done to compare DMS extracts with the traditionally used protein crash technique.
- **Centrifugation (15 minutes):** A 3 mm punched DMS blood spot was desorbed using 300 µL of 0.1% formic acid 80:20 MeOH:H_2O. The sample was centrifuged at 15,000 rpm for 15 minutes.
- **Soak (1 hour):** A 3 mm DMS blood spot was desorbed using 300 µL of 0.1% formic acid 80:20 MeOH:H_2O. The sample was soaked for 1 hour before evaporation.

Centrifugation time

The centrifugation time was increased in 15 minute increments to test if recoveries/responses could be improved with higher centrifuge times. Spots of 3 mm diameter were taken from different 20 ng/mL spots and put into 2 mL centrifuge tubes. Then, 300 µL of 0.1% formic acid in 80:20 MeOH:H_2O was added to each spot and centrifuged for 15, 30, 45, and 60 minutes. Each sample was then evaporated and reconstituted in 100 µL of mobile phase.

Desorption solvents

Both MeOH and ACN were tested at various concentrations with 0.1% formic acid (FA), that is, 100%, 80%, 60%, and 40% organic. A concentration study of FA was also carried out with 80% MeOH and ACN: 0%, 0.1%, 0.5%, and 1% FA. Spots of 3 mm diameter were taken from different 20 ng/mL spots and put into 2 mL centrifuge tubes. They were then desorbed with 300 µL of each of the desorption solvents, centrifuged at 15,000 rpm for 15 minutes, evaporated, and reconstituted in 100 µL of mobile phase.

LC/MS conditions

Column:	Agilent Poroshell 120 EC-C18, 2.1 × 30, 2.7 µm (p/n 691775-902)	
Mobile phase:	A: 0.1% Formic acid in H_2O, B: MeOH	
Flow rate:	200 µL/min	
Gradient:	t_0	A: 80%, B: 20%
	$t_{1.0\text{-}2.0}$	A: 20%, B: 80%
	$t_{2.01\text{-}3.0}$	A: 80%, B: 20%
Run time:	3 min	
Gas temperature:	350 °C	
Gas flow:	10 L/min	
Nebulizer:	15 psi	
Sheath gas temperature:	250 °C	
Sheath gas flow:	7 L/min	
Polarity:	Positive	
Capillary:	4,000 V	
Instrument:	Agilent 1290 Infinity LC System, Agilent 6460 Series Triple Quadrupole LC/MS System	

LC/MS transitions

Compound	Parent ion	Product ion	CE (V)	Dwell time (ms)	Fragmentor (V)
Atenolol	267.2	145.1	22	200	140
Pindolol	249.2	116.1	14	200	100
Metoprolol	268.2	116.2	14	200	140
Propranolol	260.2	116.1	14	200	100

Results and Discussion

Figure 1 is an example of 20 ng/mL spiked blood after work-up with DMS cards. The column used for the analysis is an Agilent Poroshell 120 EC-C18 2.7 µm column based on a superficially porous microparticulate column packing. This particle technology is designed to give all the performance advantages of sub-2 µm particles with backpressures that are comparable to sub-3 µm particles. All four analytes separate with baseline resolution and good peak shapes on a short 30 mm column.

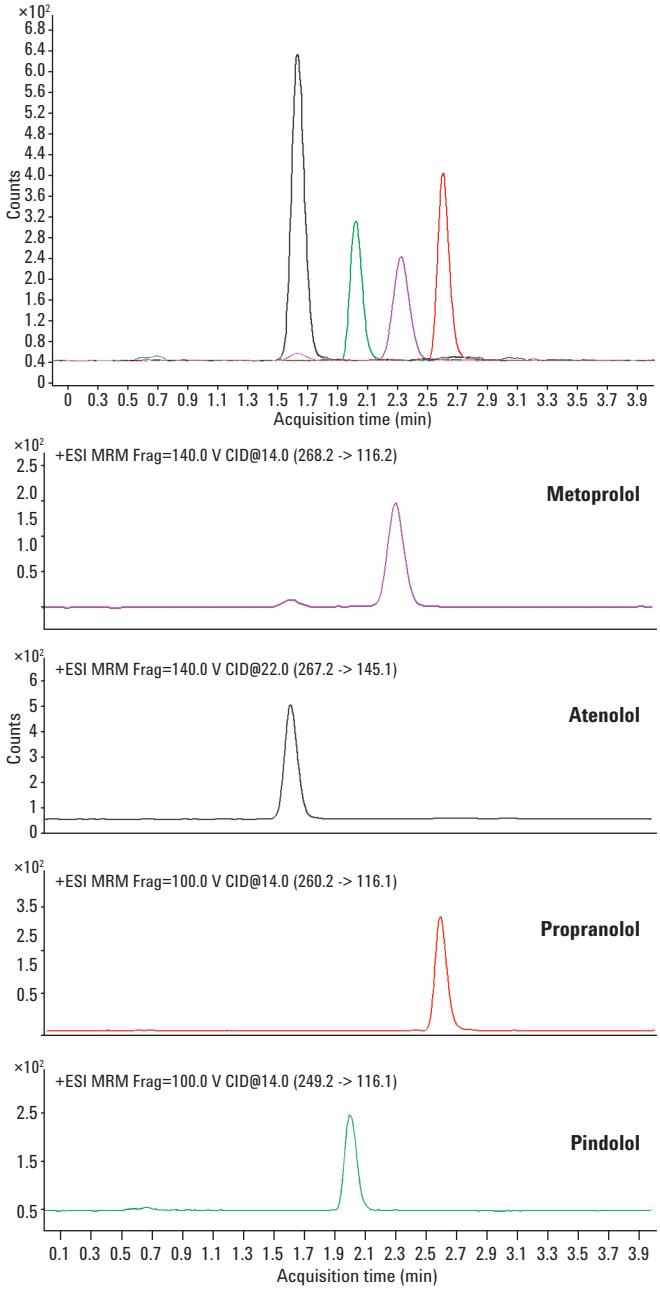

Figure 1. LC/MS/MS chromatogram of 20 ng/mL blood spiked with β-blockers after DMS work-up.

Agilent Bond Elut DMS cards use an innovative, noncellulose technology that delivers significantly improved analytical sensitivity, reproducibility, and ease-of-use. The improved MS signal results from a cellulose-free format that has reduced nonspecific binding with no impregnated chemical reagents. This feature makes the noncellulose product exhibit better quality data for desorption compared to traditional cellulose cards [6, 7].

The cards display excellent spot homogeneity with reproducible extractions, at higher recoveries, across a range of hematocrit levels [8, 7, 9, 10]. The effect of hematocrit on assay bias was examined across a wide range of hematocrit levels. The cards generated a narrow assay bias in all the three critical parameters affecting overall performance, namely, spot area, ion suppression, and analyte recovery [11]. Bond Elut DMS is amenable to a broad range of biological matrices including plasma [12].

Analyte desorption was measured in terms of MS response for each analyte. The effect of different desorption methods, centrifugation times, and desorption solvents on analyte sensitivity is presented to reflect the overall efficiency of the technique.

Figure 2 illustrates that centrifuging the sample for 15 minutes gave the best overall results, especially for the more hydrophobic, higher Log P analyte, propranolol. Atenolol, which is the most polar, had the poorest recovery. In the protein precipitated sample, propranolol yields recoveries higher than the standards, most likely due to co-extraction of hydrophobic endogenous interferences, because the technique does not provide any sample clean-up. Centrifugation for 15 minutes yields higher responses than soak for 60 minutes for three out of the four drugs screened. This implies significant reduction in sample processing time once the spots are punched, leading to increased throughput.

Figure 3 compares response in 15 minute increments when the blood spots were desorbed and centrifuged at 15,000 rpm for 15-60 minutes. Centrifugation for 15 minutes appeared to be sufficient. Propranolol response decreased as centrifugation time increased, probably due to more interferences being desorbed with time.

Desorption methods

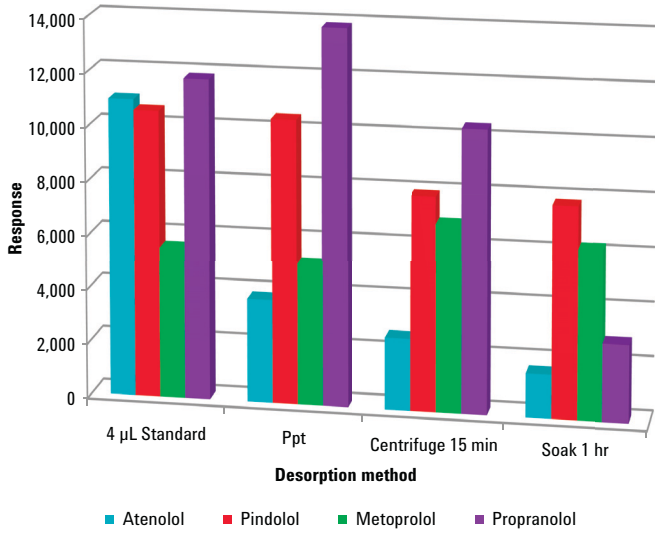

Figure 2. Effect of desorption methods on the response of β-blockers (n = 4).

Centrifugation time

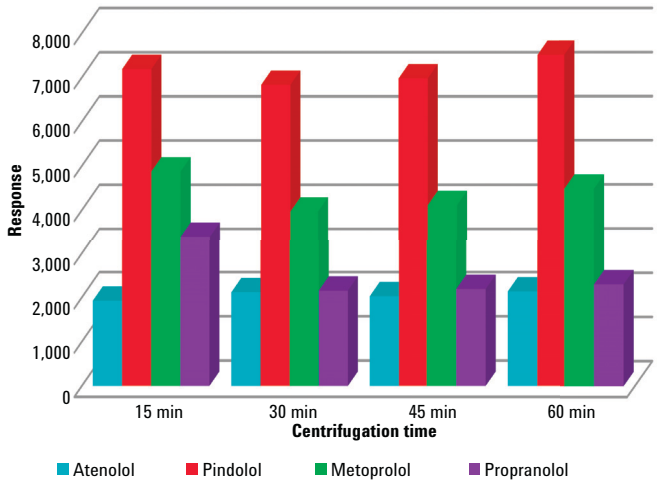

Figure 3. Effect of centrifugation times on the response of β-blockers (n = 4).

Desorption solvents

Figure 4 reflects that atenolol yields the best response at 60% ACN:0.1% FA or 40% MeOH:0.1% FA. Previous published work with basic analytes and dried blood spot technology cites the use of 80% MeOH with 0.1% FA as a generic desorption solvent resulting in high and reproducible recoveries [5, 7-12]. Thus, even though 80% organic did not generate the highest response when the organic composition was varied, it was still chosen as the composition in which FA concentrations were varied and the corresponding responses examined. In the FA concentration study experiments, the best response was obtained with 0.5% FA in 80% ACN, followed closely by 0% FA in 80% MeOH.

For pindolol, 60% ACN:0.1% FA generated the best response. No FA in 80% ACN or MeOH worked best in the FA concentration studies (Figure 5).

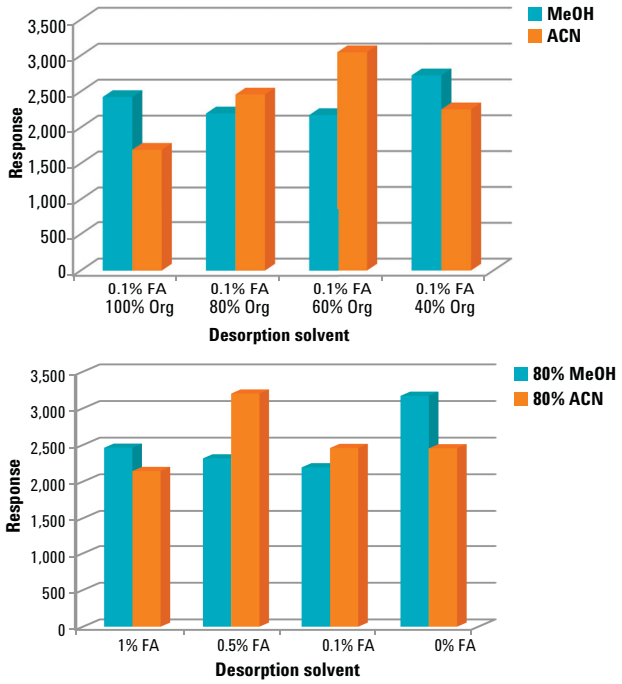

Figure 4. Effect of methanol and acetonitrile-based desorption solvents on the sensitivity of atenolol (n = 4).

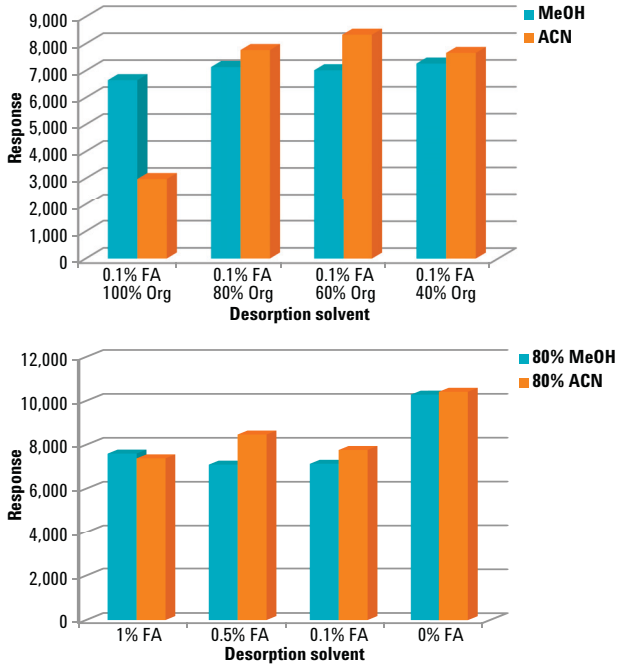

Figure 5. Effect of methanol and acetonitrile-based desorption solvents on the sensitivity of pindolol (n = 4).

In the case of metoprolol, 100% ACN:0.1% FA yielded the best response. In general, ACN worked better than MeOH for every organic % tried. In the FA concentration study, the best response resulted from using 0.5% FA in 80% ACN (Figure 6).

For the more hydrophobic propranolol, MeOH worked better than ACN in general, with 80% MeOH:0.1% FA yielding the highest response. In the FA concentration study, 0.1% FA in 80% MeOH was far above the other concentrations investigated (Figure 7).

When selecting desorption solvents for basic compounds, such as β-blockers, there was no generic method for all compounds. Polar compounds, such as atenolol, pindolol, and metoprolol, worked better with acetonitrile, while nonpolar analytes like propranolol yielded better responses with methanol. Pindolol worked best with no FA, but propranolol response was very poor.

Conclusions

Analyte desorption and its resulting impact on sensitivity was improved when centrifugation was used, in comparison to soak for 1 hour, for basic analytes such as β-blockers. A 15 minute centrifugation time was adequate, and longer centrifugation times did not offer increased sensitivity. By replacing a soak step of 60 minutes with a short 15 minute centrifugation, there was a significant reduction in sample processing time, leading to overall increased throughput. Among the variety of solvents tried, the best overall desorption solvent that resulted in improving sensitivity used 80% MeOH with 0.1% FA. This was the best solvent for propranolol, and it provided sufficient response for the other compounds.

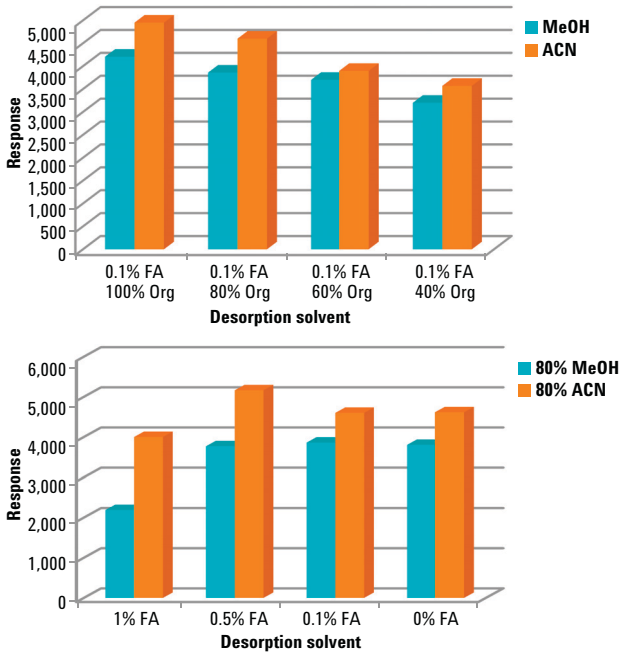

Figure 6. Effect of methanol and acetonitrile-based desorption solvents on the sensitivity of metoprolol (n = 4).

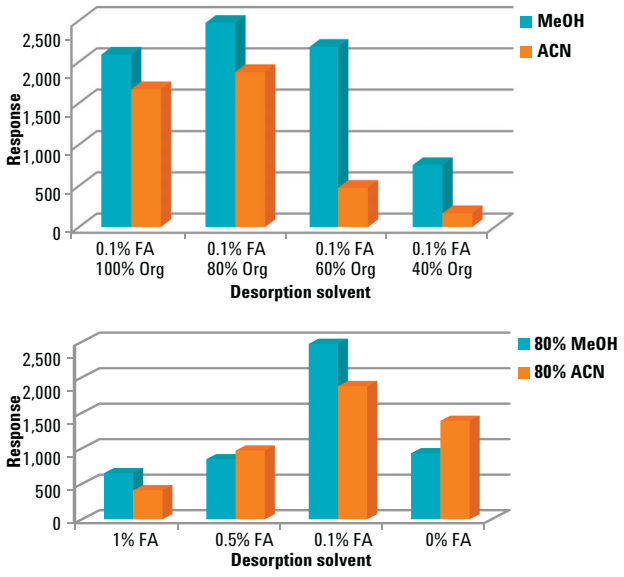

Figure 7. Effect of methanol and acetonitrile-based desorption solvents on the sensitivity of propranolol (n = 4).

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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. 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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