Improved Sensitivity of Acidic Drugs in Dried Blood Spotting Through Optimized Desorption

Application Note

Pharmaceutical

Authors

Ritu Arora, William Hudson,
and Paul Boguszewski
Agilent Technologies, Inc.

Introduction

Dried matrix spotting (DMS) or dried blood spotting (DBS) is being adopted as a microsampling technique for pharmaceutical development and has been given a great deal of attention in recent years. 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 acidic analytes to reach optimal desorption conditions and the resulting impact on sensitivity.

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Experimental

Materials and methods

- Agilent Bond Elut Dried Matrix Spotting (DMS) cards, (p/n A400150)
- Agilent Poroshell 120 EC-C8, 150 × 2.1 mm, 2.7 µm (p/n 693775-906)
- Human whole blood (pooled, mixed gender) in Heparin was purchased from Biochemed Services.
- Chemicals: Atorvastatin, simvastatin, pravastatin, and lovastatin were purchased from Sigma Chemicals. Ammonium formate for preparation of buffer was purchased from Aldrich.
- Water and acetonitrile (LC-MS grade) were purchased from VWR.

Table 1 lists the acidic compounds used in this study.

Table 1. Acidic drugs (Statins) screened - general information.

Compound	Log P	pKa	Therapeutic use
Atorvastatin	6.36	4.46	Cholesterol reducer
Simvastatin	4.68	N/A	Cholesterol reducer
Pravastatin	2.18	4.70	Cholesterol reducer
Lovastatin	4.26	N/A	Cholesterol reducer

Desorption solvents

Two sets of experiments were conducted to determine the optimal desorption solvent needed for achieving best sensitivity for statins overall. Blood, pooled (in Heparin), was spiked with statins mix at 20 ng/mL, spotted on Agilent Bond Elut DMS cards, and dried overnight. Spots of 3 mm diameter were punched out. Each punch was soaked in 300 µL of desorption solvent and left to soak for approximately 2 hours. Samples were removed and put in conical vials, followed by evaporation to dryness, and reconstituted in 100 µL of mobile phase for LC/MS analysis.

Desorption solvents investigated included neat acetonitrile and methanol concentrations of 100% and 80%, with and without modifiers, such as ammonium formate and ammonium hydroxide. The modifiers were added such that the resulting solution promoted ionization of the acidic statin compounds. The following variations were explored:

- 100% CH_3OH
- 80% CH_3OH
- 100% CH_3OH with 10 mM ammonium formate
- 80% CH_3OH with 10 mM ammonium formate
- 100% CH_3OH with 1% NH_4OH
- 80% CH_3OH with 1% NH_4OH
- 100% CH_3CN
- 80% CH_3CN
- 80% CH_3CN with 10 mM ammonium formate (100% CH_3CN with 10 mM ammonium formate was insoluble)
- 100% CH_3CN with 1% NH_4OH
- 80% CH_3CN with 1% NH_4OH

After determining which of the above solvents resulted in best sensitivity, further experiments were carried out with 80% MeOH with 1% NH_4OH as the reference, and MeOH % varied from 80% to 40%. Thus, the following variations in desorption solvents were screened:

- 80% CH_3OH with 1% NH_4OH
- 60% CH_3OH with 1% NH_4OH
- 40% CH_3OH with 1% NH_4OH

This experiment was also conducted at 10 ng/mL.

Desorption methods

To assess centrifugation versus soak, 3 mm Agilent Bond Elut DMS blood spots were desorbed using 300 µL 60% MeOH with 1% NH_4OH. Samples were centrifuged at 15,000 rpm for 15 minutes, evaporated to dryness, and reconstituted in 100 µL of mobile phase. Another set of spots were desorbed in the same solvent and soaked for 1 hour before evaporation.

LC/MS conditions

Column:	Agilent Poroshell 120 EC-C8, 2.1 × 150 mm, 2.7 µm (p/n 693775-906)
Mobile phase:	A: 5 mM ammonium formate B: CH_3CN
Flow rate:	200 µL/min
Gradient:	t_0 A: 70%, B: 30% $t_{5.0}$ A: 25%, B: 75% $t_{5.5}$ A: 25%, B: 75% $t_{5.6}$ A: 70%, B: 30% $t_{8.0}$ A: 70%, B: 30%
Colum temperature:	30 °C
Run time:	8 min
Gas temperature:	275 °C
Gas flow:	10 L/min
Nebulizer:	10 psi
Sheath gas temperature:	250 °C
Sheath gas flow:	7 L/min
Polarity:	Negative
Capillary:	3500 V
Instrument:	Agilent 1290 Infinity LC System/Agilent 6460 Triple Quadrupole LC/MS Sytem

LC/MS transitions

Compound	Parent ion	Product ion	CE (V)	Dwell time (ms)	Fragmentor (V)
Atorvastatin	557.2	397.1	27	100	180
Simvastatin	435.3	318.9	11	100	140
Pravastatin	423.2	321.1	7	100	140
Lovastatin	421.3	318.9	11	100	140

Results and Discussion

Desorption solvents

Figure 1 is an example of 50 ng/mL spiked blood after work-up with DMS cards. The column used for the analysis is an Agilent Poroshell 120 EC-C8 2.7 µm column based on a superficially porous microparticulate column packing. This new particle technology is designed to generate high efficiency separations at lower backpressures. Backpressures of 394 bar for this separation on a 150 mm × 2.1 mm column format on an ultra high pressure system, such as an Agilent 1290 Infinity LC System, are impressive.

Poroshell 120 EC-C8 is an endcapped bonded phase which helps in providing excellent peak shapes of all analytes and being a C8, is less retentive for non-polar analytes (all in the current mix except pravastatin). Among all the compounds examined, atorvastatin was the most sensitive. It could be detected easily at 1 ng/mL [5].

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 which 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 [5, 6].

The cards display excellent spot homogeneity with reproducible extractions, at higher recoveries, across a range of hematocrit levels [7, 6, 8, 9]. 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 suppresion, and analyte recovery [10]. Bond Elut DMS is amenable to a broad range of biological matrices including plasma [11].

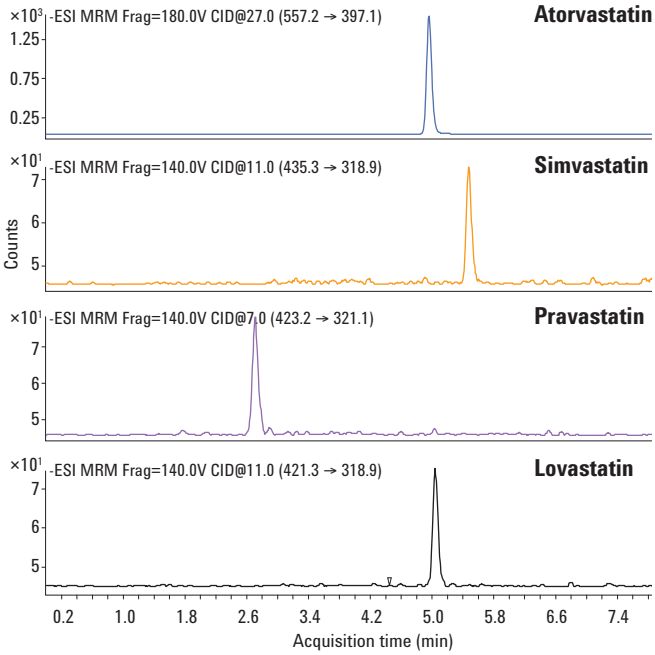

Figure 1. An example of 50 ng/mL spiked blood after work-up with DMS cards.

Previous studies with this group of statins and Agilent Bond Elut DMS cards indicated that atorvastatin was the most sensitive among all screened [5]. Hence, average response on this analyte was given more focus as opposed to the other statins. Figure 2 illustrates the effect of different desorption solvents on the average response of atorvastatin at 20 ng/mL. Methanol worked significantly better when compared to acetonitrile for most of the 100% experiments, probably due to a solubility issue with pure acetonitrile. Methanol 80% neat, and with modifiers, yielded improved responses compared to their acetonitrile counterparts. Ammonium hydroxide worked better than ammonium formate buffer with both 80% and 100% organic solvents, most likely due to better ionization offered by a higher overall solvent pH for these acidic analytes.

No clear trend was observed with either methanol or acetonitrile-based solvent systems in the case of simvastatin, although 80% methanol with 10 mM ammonium formate yielded the best response (Figure 3). Spots desorbed with 100% acetonitrile containing 10 mM ammonium formate were insoluble and, hence, not analyzed. Methanol clearly worked better than acetonitrile for the more hydrophilic pravastatin (Figure 4). Ammonium hydroxide worked better than ammonium formate buffer with 80% methanol.

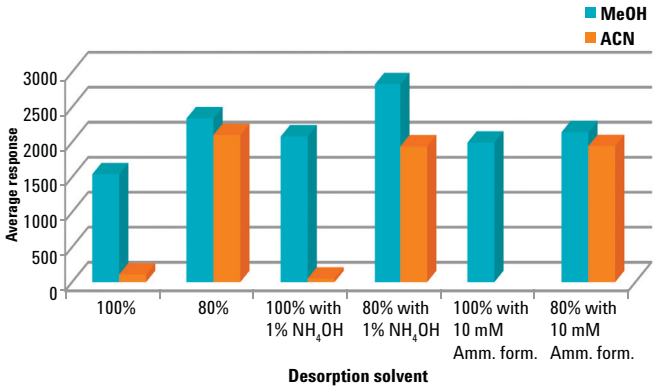

Figure 2. Effect of methanol and acetonitrile-based desorption solvents on sensitivity of atorvastatin.

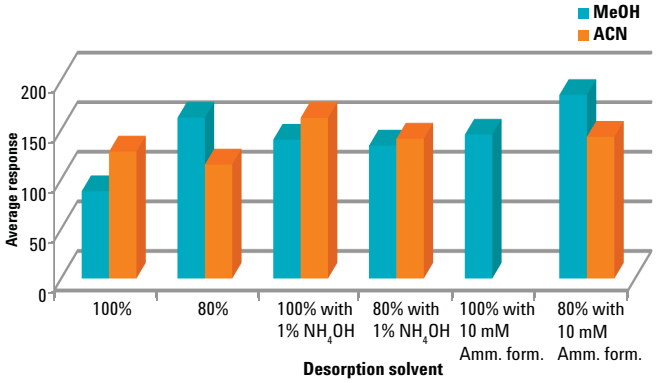

Figure 3. Effect of methanol and acetonitrile-based desorption solvents on sensitivity of simvastatin.

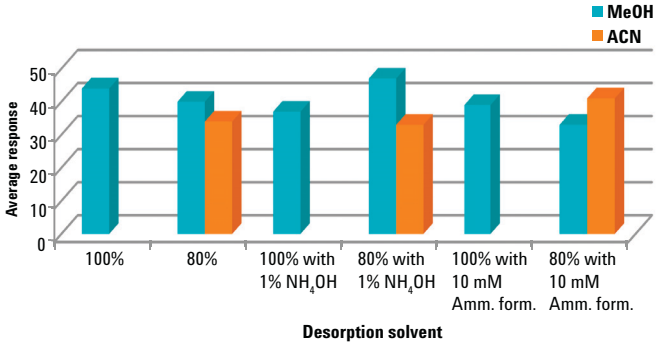

Figure 4. Effect of methanol and acetonitrile-based desorption solvents on sensitivity of pravastatin.

Figure 5 is a study of varying methanol composition in desorption solvents containing 1% ammonium hydroxide in 20 ng/mL spiked blood DMS extracts. Methanol 60% with 1% ammonium hydroxide appeared to be the optimal desorption solvent for all 4 acidic statins. Simvastatin and lovastatin yielded the best response with this solvent. Atorvastatin was extremely ionizable and sensitive and generated a much higher response than the other analytes, even though this was not the best solvent for this compound. Pravastatin gave a measurable response at this concentration of methanol. Pravastatin, being the most hydrophilic of all, yielded the best results with the highest aqueous solvent, 40% methanol. Nevertheless, it was not chosen as higher aqueous desorption solvents would result in dirtier extracts.

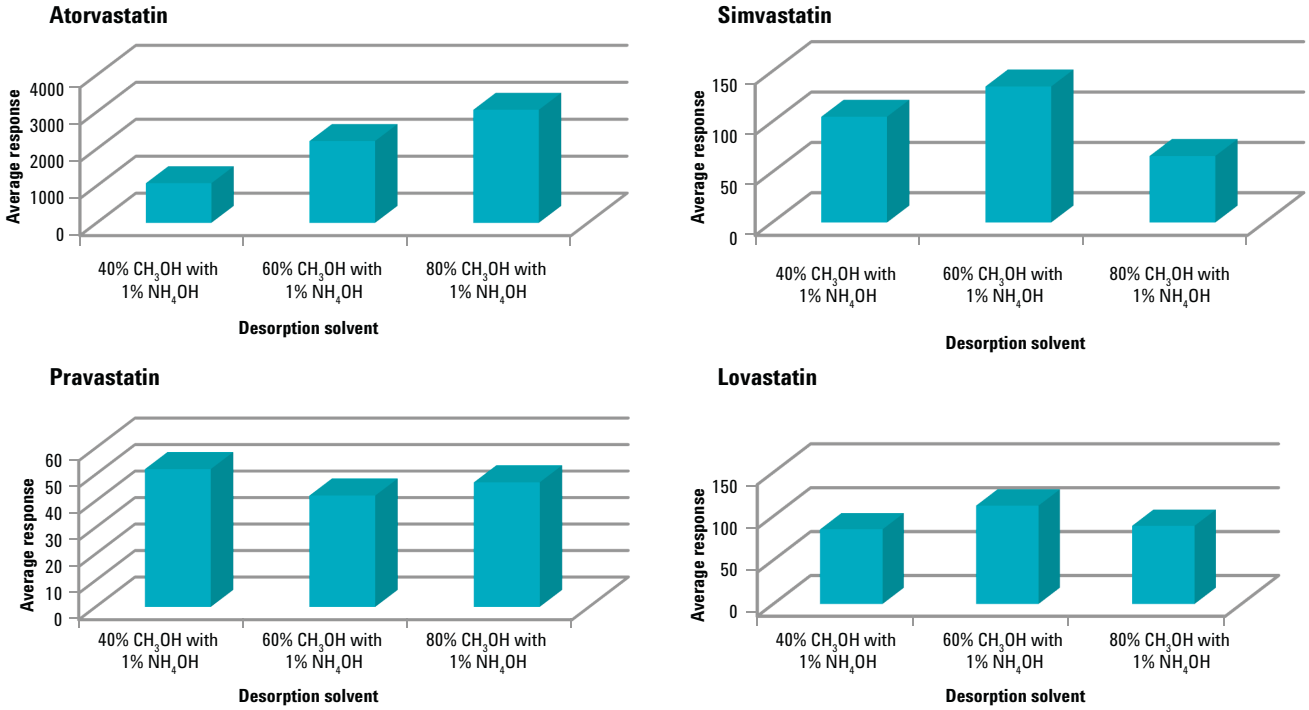

Figure 5. Effect of methanol composition on sensitivity of atorvastatin, simvastatin, pravastatin, and lovastatin using 20 ng/mL spiked blood DMS extracts.

Figure 6 shows comparisons of data generated with different methanol compositions at 10 ng/mL and 20 ng/mL. It is clear that the sensitivity of atorvastatin is much higher compared to the other 3 statins and can be detected at much lower limits of quantification (LOQs) than the rest [5].

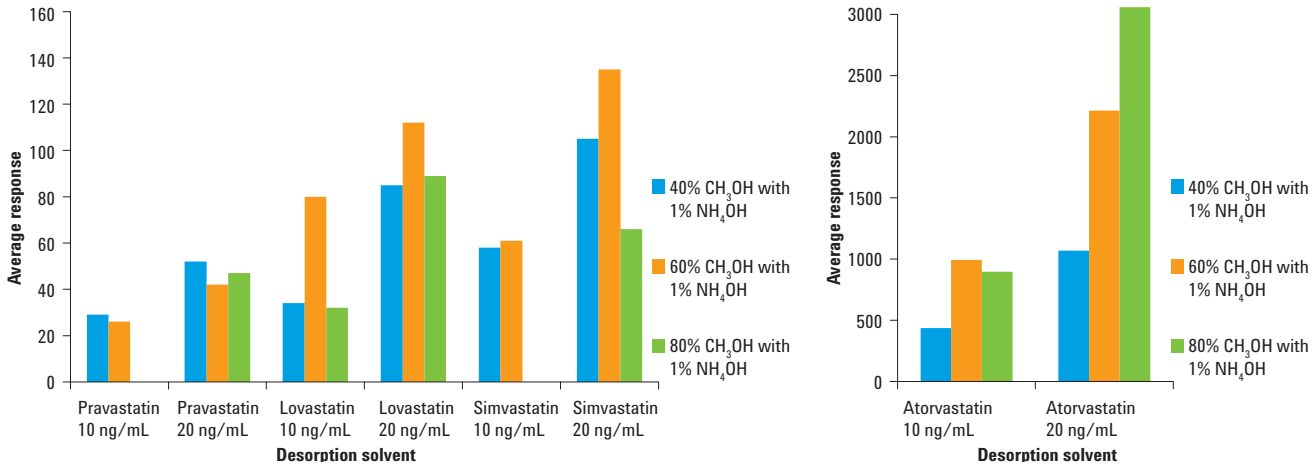

Figure 6. Effect of methanol composition on sensitivity of statins using 10 ng/mL and 20 ng/mL spiked blood DMS extracts.

Desorption methods

When blood spiked with statins mix at 20 ng/mL was spotted on DMS cards, the punched spots were desorbed using 60% MeOH containing 1% NH_4OH, the optimal desorption solvent resulting from the preceding desorption solvent studies. When experiments were conducted comparing centrifugation versus soak, centrifugation for 15 minutes yielded a better response compared to soak for 60 minutes for atorvastatin (Figure 7). This would translate to significant reduction in sample processing time, once the spots are punched, leading to increased throughput.

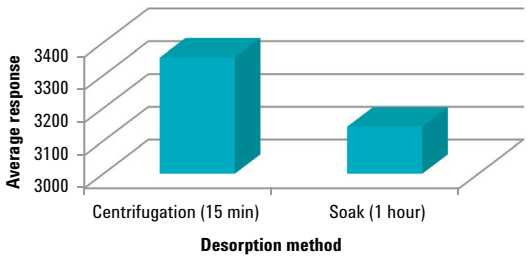

Figure 7. Effect of desorption method (centrifugation versus soak) on sensitivity of atorvastatin.

Conclusions

Analyte desorption and its resulting impact on sensitivity for acidic analytes, such as statins, was investigated with respect to desorption solvents and methods used for desorption. Various methanol and acetonitrile-based solvents tried with and without different modifiers revealed that the optimal desorption solvent that yielded the best sensitivity overall with Agilent Bond Elut DMS cards was 60% MeOH with 1% NH_4OH. This promoted the best ionization of these compounds, which, in turn, helped in improving sensitivity. Desorption through centrifugation for 15 minutes generated improved response when compared to soaking the samples for 60 minutes. By replacing a soak step of 60 minutes with a short 15 minute centrifugation, there is significant time-savings and overall reduction in processing time of punched extracts, leading to increased throughput and productivity. Atorvastatin had, undoubtedly, better sensitivity compared to the rest of the statins examined, making it the preferred analyte in this compound set for LOQ determinations.

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