

Analysis of Cholesterol Lowering Drugs (Statins) Using Dried Matrix Spots Technology and Poroshell 120 HPLC Columns

Ritu Arora, William Hudson, Ben Yong and Paul Boguszewski, Agilent Technologies, Lake Forest, California, USA.

Introduction

Dried blood spot (DBS) technology combined with the analytical capability of modern mass spectrometers (LC-MS-MS) has emerged as an important method for the quantitative bioanalysis of small molecules.

Four statins were analysed on novel non-cellulose based Dried Matrix Spotting (DMS) cards. These compounds, being acidic in nature, are challenging in terms of achieving lower detection limits, except for atorvastatin. Poroshell 120 is an excellent column choice for running the analysis, because it offers speed and high resolution advantages comparable to sub-2 μm columns, but is more forgiving for dirtier samples due to its standard 2 μm frits.

DMS procedure

Fresh human whole blood (990 μL) was spiked with 10 μL /each of four statins, at 100 \times concentrated working standard to create a calibration curve of 20, 50, 100, 200, 500 and 2000 ng/mL. After vortexing, 15 μL of each

concentration of spiked blood was spotted on Agilent Bond Elut DMS cards (Part #: A400150), which are non-cellulose in nature. For accuracy and precision, three replicates of blood concentrations at 20 ng/mL and 500 ng/mL were also prepared. Accuracy and precision studies were also extended to competitive cellulose-based cards. Cards, once spotted, were left overnight for drying. 3 mm disks were punched and placed in 2 mL vials. Each spot was dissolved in 300 μL desorption solvent (60% methanol with 1% ammonium hydroxide containing 0.5 ng/mL naproxen as an internal standard), and vortexed. Spots were left to soak in desorption solvent for \sim 2 hours, samples were then removed and put in conical vials, followed by evaporation to dryness. Samples were reconstituted in 100 μL of mobile phase (70% 5 mM Ammonium formate: 30% CH_3CN) and vortexed. Samples were subjected to LC-MS-MS analysis with appropriate CH_3OH blanks and mobile phase blanks run after calibration curve samples to prevent carry-over issues.

Figure 1: LC-MS-MS chromatogram of 50 ng/mL blood spiked with statins after DMS work-up.

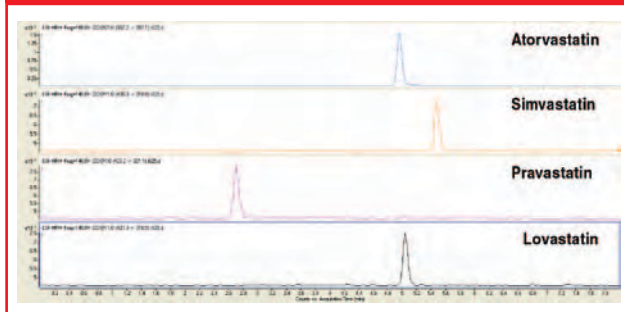


Figure 2: LC-MS-MS chromatogram of atorvastatin at 1 ng/mL spiked blood.

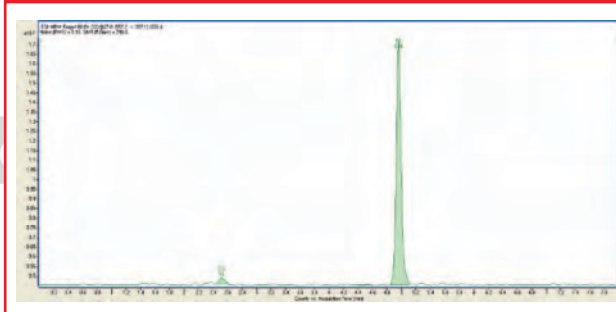
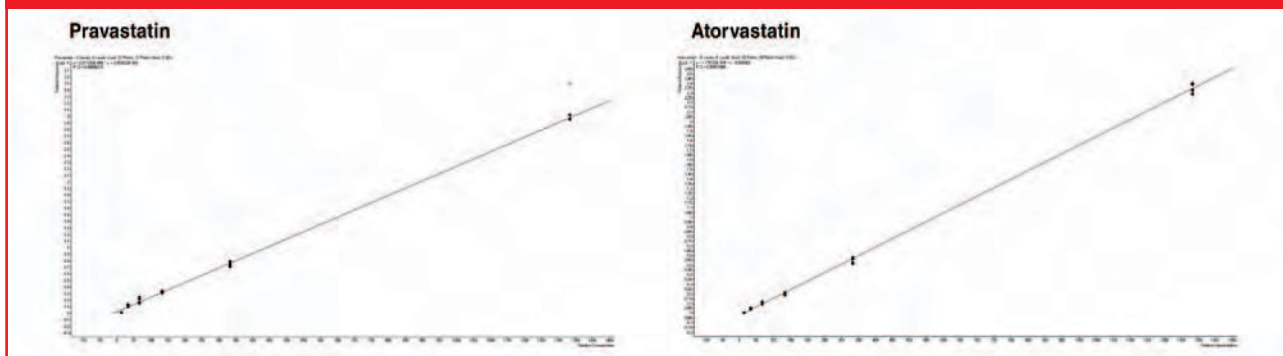


Figure 3: Representative calibration curves of statins in spiked blood.



Results and Discussion

Figure 1 is an example of 50 ng/mL spiked blood after work-up with DMS cards. The superficially porous particle technology of the Poroshell 120 column is designed to generate high efficiency separations at lower back-pressures. Back-pressures of 394 bar for this separation on a 150 × 2.1 mm column format on an ultra high pressure system such as an Agilent 1290 Infinity are impressive. Poroshell 120 EC-C8, an endcapped bonded phase, delivers excellent peak shapes for all analytes and is less retentive for non-polar analytes (all in the current mix except pravastatin). Amongst all the compounds examined, atorvastatin was the most sensitive. It could be detected easily at 1 ng/mL with a SNR of 797 (Figure 2), while others could barely be seen at 20 ng/mL.

LC-MS conditions

Column: Poroshell 120 EC-C8, 2.7 μ m, 150 × 2.1 mm (Part #: 693775-906)
 Mobile phase: A: 5 mM Ammonium formate, B: CH₃CN
 Flow-rate: 200 μ L/min
 Gradient: t₀ 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%

Table 1: MS-MS transition parameters of statins screened.

Compound	Parent ion	Daughter ion	Collision energy (V)
Atorvastatin	557.2	397.1	27
Simvastatin	435.3	318.9	11
Pravastatin	423.2	321.1	7
Lovastatin	421.3	318.9	11
Naproxen (IS)	229.1	169	27

The data presented is generated by the Agilent Masshunter software.

Column temp: 30 °C
 Back pressure: 394 bar
 Run time: 8 min
 Instrument: Agilent 1290 LC / 6460 QQQ
 Gas temp: 275 °C
 Gas flow: 10 L/min
 Nebulizer: 10 psi
 Sheath gas temp: 250 °C
 Sheath gas flow: 7 L/min
 Polarity: Negative

Linearity was observed in the calibration curves for six levels for pravastatin and atorvastatin using linear regression with correlation coefficients better than 0.998 (Figure 3). Lovastatin and simvastatin curves were non-linear at the highest concentration and yielded quadratic regression with correlation coefficients better than 0.999.

Table 3 lists relative recoveries of statins from blood after DMS desorption from Bond Elut DMS cards and competitive cards. The results indicate that there is ion-enhancement occurring with blood constituents when the cellulose product is used, resulting in artificially high recoveries. This data supports that the non-cellulose product exhibits better quality data for desorption compared to traditional cellulose cards.

Conclusions

A simple and rapid method has been developed for the analysis of an acidic group of compounds in human DMS samples by LC-MS-MS. The method was demonstrated to be accurate, precise and robust. Linearity was demonstrated for pravastatin and atorvastatin using a linear regression with correlation coefficients better than 0.998 and good recoveries were seen for all analytes except pravastatin. These cards offered better desorption properties compared to the cellulose-based competitive product. A Poroshell 120 EC-C8, an excellent column choice for bioanalytical applications, yielded good peak shapes and enabled fast analysis with decreased back pressures. Dried blood spots should be considered as a sample collection technique when developing and validating quantitative bioanalytical methods for the analysis of drugs in pre-clinical and clinical studies.

Table 2: Recoveries of statins from Bond Elut DMS cards and competitive cards (n = 3).

	Concentration (ng/mL)	Bond Elut DMS cards (non-cellulose based)		Competitive cards (cellulose based)	
		% Recovery	RSD	% Recovery	RSD
Atorvastatin	20.0	103%	3%	105%	1%
	500.0	98%	1%	103%	1%
Simvastatin	20.0	111%	17%	165%	9%
	500.0	98%	5%	119%	6%
Pravastatin	500.0	150%	4%	198%	11%
Lovastatin	20.0	104%	11%	112%	8%
	500.0	116%	5%	162%	2%