# Analysis of Cholesterol-Lowering Drugs (Statins) Using Dried Matrix Spot Technology

## Application Note

Small Molecule Pharmaceuticals and Generics


## Authors

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## Introduction

Dried blood spot (DBS) technology, combined with the analytical capability of modern mass spectrometers (LC/MS/MS), is an important method for the quantitative bioanalysis of small molecules. It is increasingly being looked at as a microsampling approach for preclinical and clinical pharmacokinetic/toxicokinetic (PK/TK) studies [1]. The primary advantage of DBS is the significant reduction in blood volume requirements, leading to cost and ethical benefits (3Rs implications - reduction, refinement, and replacement) for animal use, facilitating pediatric studies, and offering simplified sample collection [2]. It also facilitates reduction in processing, sample shipping, and storage costs under ambient conditions. An unexpected benefit of this technology is the on-card metabolite stability, specifically for those metabolites known to be very labile and susceptible to degradation.

Four statins - atorvastatin, simvastatin, pravastatin, and lovastatin - were analyzed on noncellulose-based Agilent Bond Elut Dried Matrix Spotting (DMS) cards. Except for atorvastatin, these compounds, being acidic in nature, are challenging in terms of achieving lower detection limits.


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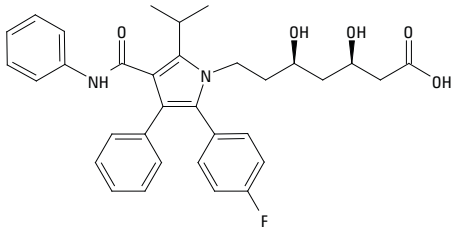
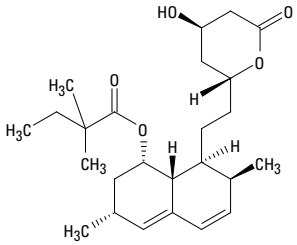
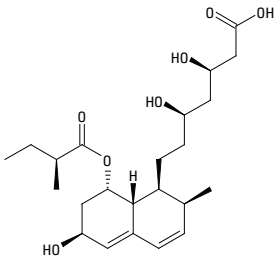
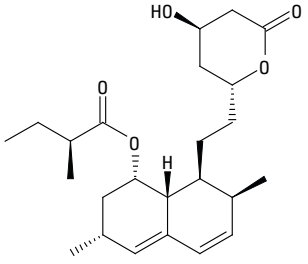
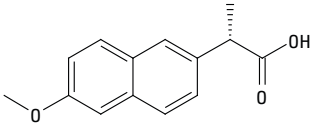
## Experimental

The structures, log P, and pKa information of the four statins, along with naproxen (internal standard), screened on DMS cards are given in Table 1.

*Table 1. Statins Investigated – Structures, and General Information*

| Compound | Structure | Log P | pKa |
|---|---|---|---|
| Atorvastatin |  | 6.36 | 6.36 |
| Simvastatin |  | 4.68 | N/A |
| Pravastatin |  | 2.18 | 4.70 |
| Lovastatin |  | 4.26 | N/A |
| Naproxen (IS) |  | 3.18 | 4.15 |

## DMS Procedure

Fresh human whole blood (from Biochemed) was spiked with a mix of four statins at a concentration range of 20 to 2000 ng/mL for generating calibration curves. Specifically, 990 µL human blood was spiked with 10 µL of each 100x concentrated working standard to create a calibration curve of 20, 50, 100, 200, 500 and 2,000 ng/mL. After vortexing, 15 µL of each concentration of spiked blood was spotted on Agilent Bond Elut DMS cards (p/n A400150), which are noncellulose 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. Three-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 ~ two hours, samples were then removed and put in conical vials, followed by evaporation to dryness. Samples were reconstituted in 100 µL mobile phase (70% 5 mM ammonium formate: 30% $CH_3CN$), vortexed and subjected to LC/MS/MS analysis.

## Results and Discussion

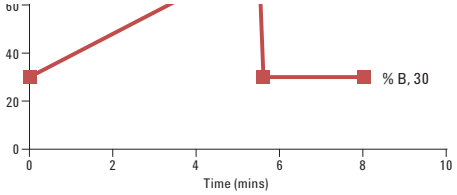
Figure 1 is an example of 50 ng/mL spiked blood after work-up with DMS cards. The column used for the analysis is an Agilent Poroshell 120 EC-C8 2.7 µm column based on a superficially porous microparticulate column packing. This particle technology is designed to generate high efficiency separations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

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

### 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%<br>$t_{5.0}$ A: 25%, B: 75%<br>$t_{5.5}$ A: 25%, B: 75%<br>$t_{5.6}$ A: 70%, B: 30%<br>$t_{8.0}$ A: 70%, B: 30% |

Mobile phase gradient




| | |
|---|---|
| Column temp | 30 °C |
| Back pressure | 394 bar |
| Run time | 8:00 min |
| Instrument | Agilent 1260 Infinity LC/6460 Triple Quadrupole LC/MS |
| 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 |

*Table 2. MS/MS Transition Parameters of Statins*

| 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.

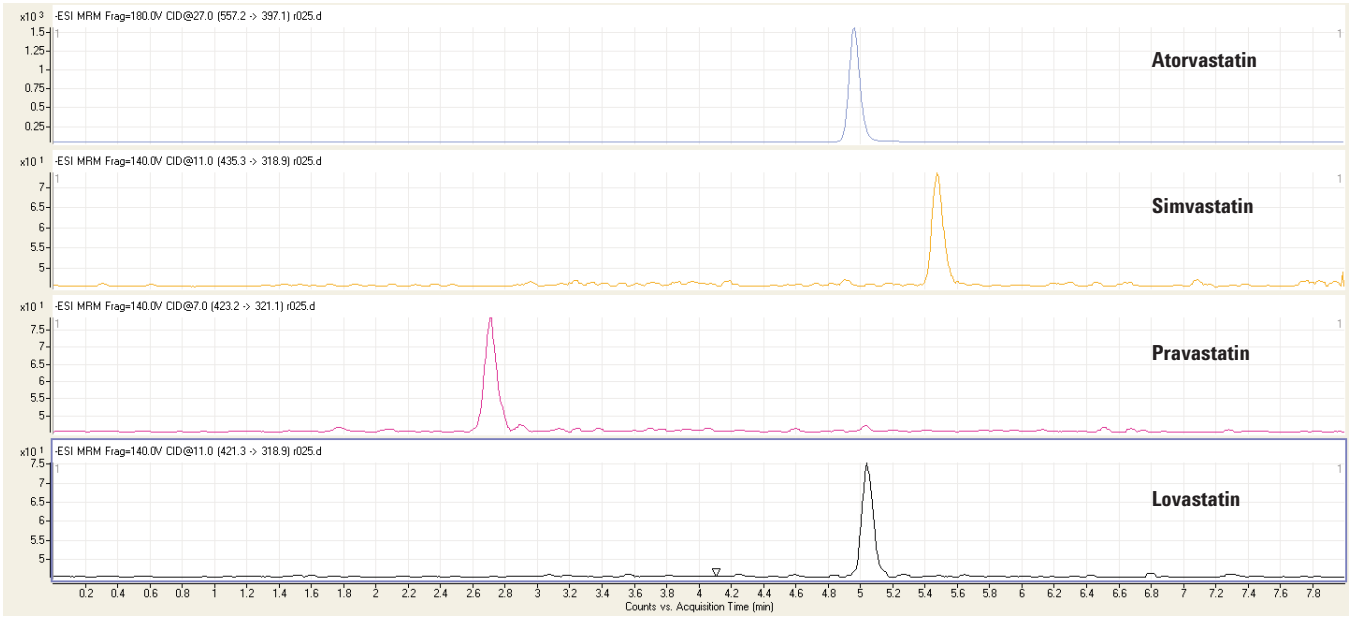



*Figure 1. LC/MS/MS chromatogram of 50 ng/mL blood spiked with statins after DMS work-up.*

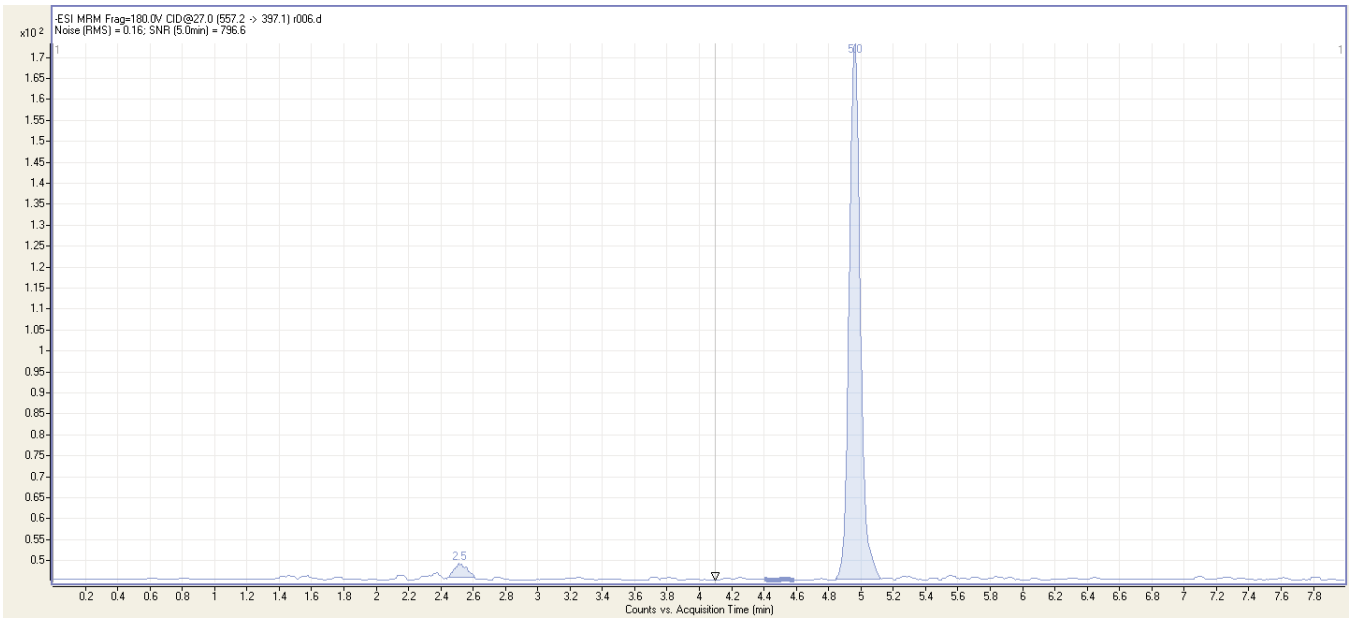



*Figure 2. LC/MS/MS chromatogram of atorvastatin at 1 ng/mL spiked blood.*

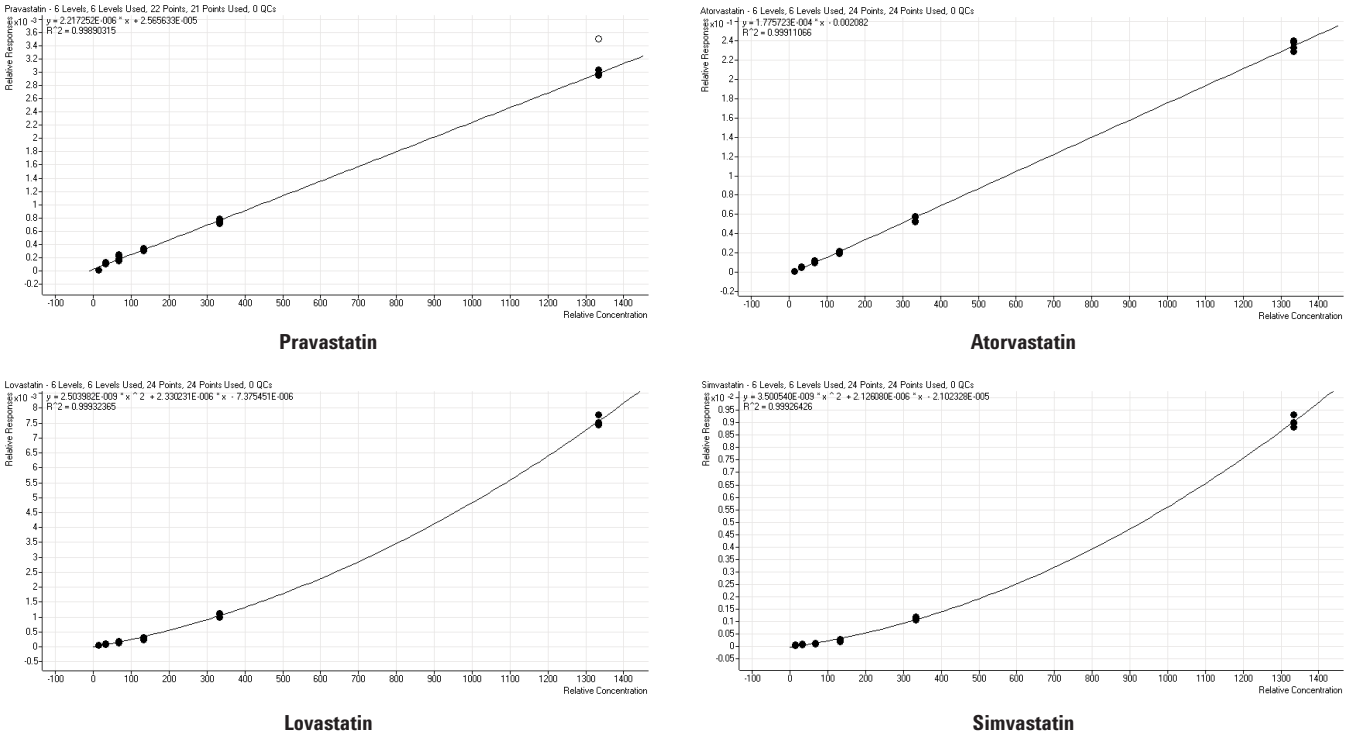



*Figure 3. Calibration curves of statins in spiked blood.*

Table 3 lists relative recoveries of statins from blood after DMS desorption from Bond Elut DMS cards and competitive cards. Pravastatin could not be detected at 20 ng/mL on either card. Even at 500 ng/mL, recoveries were artificially high. Being the most polar statin amongst the four investigated, it may be suffering from ion enhancement. Further method development could involve looking at additional sample cleanliness via LLE, PPT, SPE, or other sample cleanup technique. For the rest of the series, recoveries on Bond Elut DMS cards are within 16% of the true value, and those on the cellulose-based cards are within 65% of the true value. RSD values on the noncellulose Bond Elut cards are within 17%, while those on the cellulose-based cards are within 9%. Even though the RSDs on the noncellulose-based cards are somewhat higher for simvastatin at 20 ng/mL, and some others marginally, the recoveries are unrealistic on the competitive product (165% versus 111% on Bond Elut DMS). The same is true for lovastatin at 500 ng/mL (162% versus 116% on Bond Elut DMS) This indicates that there is ion enhancement occurring with blood constituents when the cellulose product is used, resulting in artificially high recoveries. These data support our contention that the noncellulose product exhibits better quality data for desorption compared to traditional cellulose cards.

*Table 3. Recoveries of Statins from Agilent Bond Elut DMS Cards and Competitive Cards (n = 3)*

| | | Bond Elut DMS cards (noncellulose-based) | | Competitive cards (cellulose-based) | |
|---|---|---|---|---|---|
| | **Concentration (ng/mL)** | **% 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 |

## Conclusions

A simple and rapid method was developed for the analysis of an acidic group of compounds such as statins in human DMS samples by LC/MS/MS. The method was accurate, precise, and robust. Linearity was demonstrated for pravastatin and atorvastatin using linear regression with correlation coefficients better than 0.998. Atorvastatin displayed much lower sensitivity compared to the other statins screened and had good signal-to-noise ratios at 1 ng/mL. Relative recoveries on the noncellulose-based Bond Elut DMS cards were within 16% of the true value and RSDs within 17%; however, atorvastatin delivered RSDs of up to 5%. These cards offered better desorption properties compared to the cellulose-based competitive product. A Poroshell 120 EC-C8 column yielded good peak shapes and decreased back pressures for all the analyses. Dried blood spots should be considered as a sample collection technique.

## References


1. N. Spooner, R. Lad, M. Barfield. *Anal. Chem.* **81**, 1557 (2009).
2. M. Barfield, N. Spooner, R. Lad, S. Parry, S. Fowles. *J. Chromatogr. B.* **870**, 32 (2008)..


## For More Information

Agilent Bond Elut DMS cards are intended for use in DMPK/ADME research applications only. They should not be used in diagnostic procedures. For more information on our products and services, visit our Web site at www.agilent.com/chem.

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