

## Application Note

# Increasing Sensitivity and Throughput for LC-MS/MS-based Bioanalytical Assays Using UPLC

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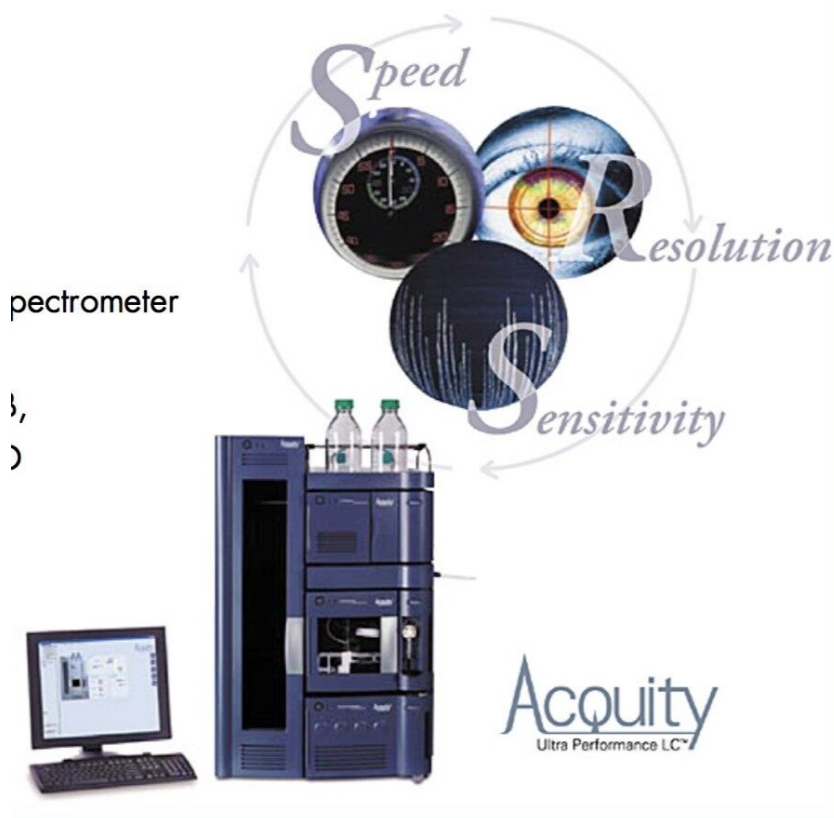
## Abstract

In this application note, we compare the sensitivity and throughput of a bioanalytical assay of an atherosclerosis-targeting candidate and its associated metabolites in human plasma by traditional HPLC/MRM and UPLC/MRM.

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## Introduction

The current approaches within drug discovery and development generate greater numbers of new chemical entities (NCE's) with increased potency and duration of action. This results in more compounds entering into clinical programs. Thus, there are increased sensitivity and throughput demands placed upon today's drug and metabolite screening assays in early Phase I clinical trials. In order to monitor circulating metabolites at low concentration levels, a highly sensitive LC/MRM analytical method is required. Ultra Performance LC (UPLC) takes advantage of the increased chromatographic performance generated by sub-2  $\mu\text{m}$  particle stationary phases operated at high mobile phase linear velocities. This results in increased resolution, peak capacity, and more sensitive high throughput assays. In this application note, we compare the sensitivity and throughput of a bioanalytical assay of an atherosclerosis-targeting candidate and its associated metabolites in human plasma by traditional HPLC/MRM and UPLC/MRM.



## Experimental

Human plasma samples were collected from healthy subjects who were orally administered with the target NCE. The pooled plasma extract was then vortexed mixed, sonicated, and centrifuged at approximately 3,000  $g_{av}$  at room temperature for approximately five minutes. The resulting supernatant was then analyzed by LC/MS/MS.

### LC Conditions

LC system:

Waters ACQUITY UPLC System

Column:

Phenomenex Luna C<sub>18</sub> Column, 4.6 x 250 mm, 5  $\mu$ m (HPLC)

|                   |                                                                                                                                                                        |
|-------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                   | ACQUITY UPLC BEH C <sub>18</sub> Column, 2.1 x 50 mm,<br>1.7 µm (UPLC)                                                                                                 |
| Mobile phase A:   | Aqueous ammonium acetate, 50 mM                                                                                                                                        |
| Mobile phase B:   | Acetonitrile                                                                                                                                                           |
| Gradient:         | 0 min. A=72%, 50 min. A=40%, 51 min. A=5%,<br>62 min. A=5%, 63 min. A=72% (HPLC) 0 min.<br>A=90%, 18 min. A=50%, 19 min. A=5%, 20 min.<br>A=5%, 20.5 min. A=90% (UPLC) |
| Flow rate:        | 1 mL/min. (HPLC), 0.6 mL/min. (UPLC)                                                                                                                                   |
| Injection volume: | 45 µL                                                                                                                                                                  |
| Sample temp.:     | 10 °C                                                                                                                                                                  |
| Column temp.:     | 40 °C                                                                                                                                                                  |

## MS Conditions

|                   |                                                                                   |
|-------------------|-----------------------------------------------------------------------------------|
| MS system:        | Waters Micromass Quattro Premier Mass<br>Spectrometer                             |
| Ionization mode:  | ESI Positive Ion                                                                  |
| MRM transitions:  | 736>142 compound A, 722>128 compound B,<br>678>235 compound C, 578>186 compound D |
| Cone voltage:     | 100 V                                                                             |
| Collision energy: | 25 eV                                                                             |
| Dwell:            | 20 ms                                                                             |

Collision gas:

Argon

## Results and Discussion

The MRM chromatograms in Figures 1 and 2 clearly show the increased sensitivity achieved by using UPLC (bottom) over HPLC (top). The measured peak widths were 0.13 min. and 0.60 min. and resulting peak volumes were 0.078 mL and 0.600 mL for UPLC and HPLC, respectively. The sensitivity advantage expected from column geometry and gradient duration [5.2x] multiplied by column efficiency improvements a [1.7x] yields an overall 7.8 fold increase. The measured sensitivity increase of 7.7 fold was achieved with UPLC-MS/MS. Thus, the actual measured increase agrees extremely well with the predicted increase in sensitivity.

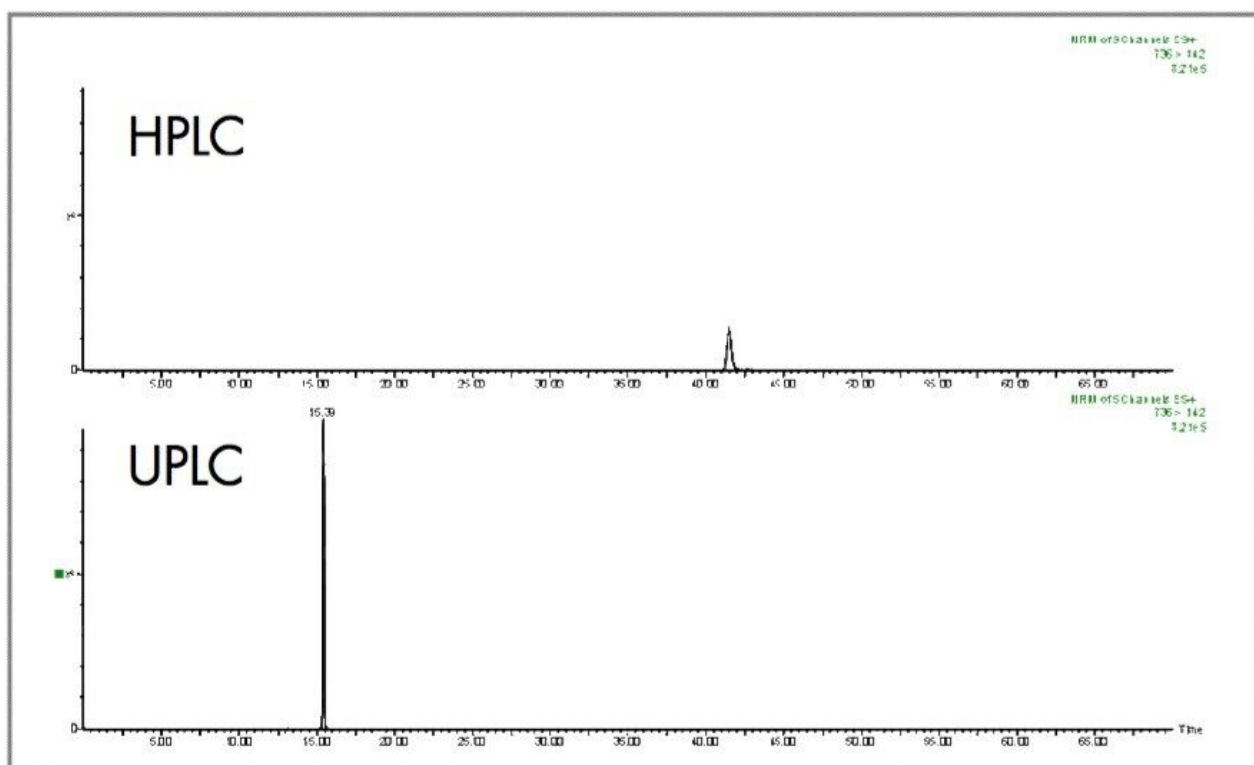


Figure 1. LC-MS/MS with MRM transition 736 > 142.

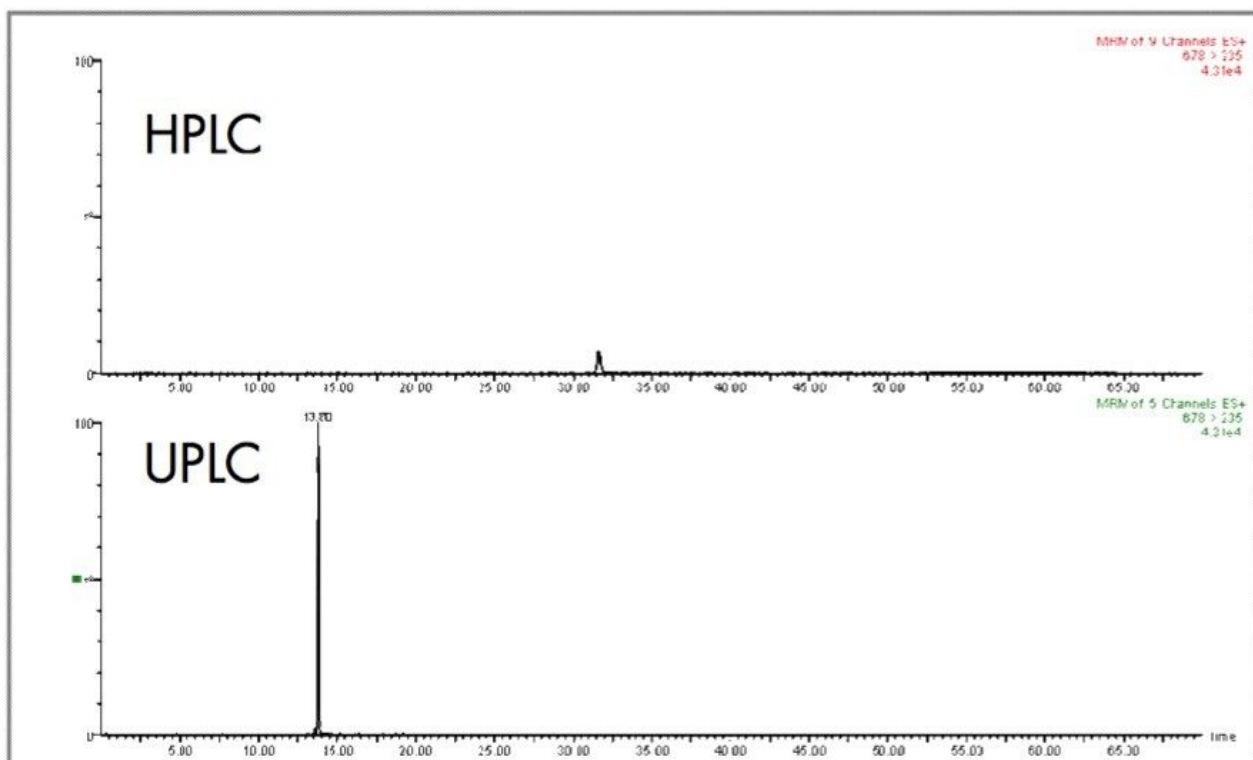


Figure 2. LC-MS/MS with MRM transition 678 > 235.

This illustrates the benefits that can be gained when transitioning this HPLC-based gradient method (~63 minutes) to a higher throughput UPLC-based gradient method (~20 minutes). In this particular application, the original HPLC method was developed to resolve several critical metabolites detected in the preclinical drug metabolism studies. Obviously, if UPLC was implemented earlier in the drug development process, a more time-efficient method could be realized.

## Conclusion

By employing UPLC over traditional HPLC, the sensitivity of this LC-MS/MS assay was improved by a factor of 7.7, and the analysis time reduced by a factor of 2.3. The dramatically increased sensitivity and throughput demonstrated by UPLC makes it an invaluable tool for bioanalysis assays.

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ACQUITY UPLC System <<https://www.waters.com/514207>>

720001122, February 2005

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