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Note d'application

Comparison of the Resolution and Sensitivity of UPLC and Monolithic Columns for Metabolite Profiling

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Abstract

In this application note, to demonstrate and compare the attributes, we evaluate the resolution and sensitivity for the analysis of the *in-vitro* metabolites of verapamil, a calcium antagonist.

Benefits

The ACQUITY UPLC System provides excellent resolution and throughput, allowing all of the major and minor metabolites of verapamil to be resolved and detected in just oneshort ten-minute separation.

Introduction

The rapid profiling of drug metabolites of candidate pharmaceuticals is an essential part of the drug discovery process, and is usually achieved by LC-MS analysis. In this task, there is a likely trade-off between throughput

and the comprehensive analysis of the sample. While sample throughput can be increased, it is usually at the expense of chromatographic resolution, thus resulting in the failure to detect all of the metabolites. An alternative to traditional HPLC are high throughput chromatographic techniques such as monolithic chromatography or UltraPerformance LC (UPLC). Both techniques promise higher throughput with no significant loss of resolution. To demonstrate and compare the attributes of each, we evaluate the resolution and sensitivity for the analysis of the in-vitro metabolites of verapamil, a calcium antagonist.

Experimental

A 50 µmol solution of verapamil was incubated with rat liver microsomes for 30 minutes at 37 °C. The reaction was stopped by the addition of 2 volumes of cold acetonitrile. The sample was then centrifuged and the resulting supernatant layer was removed for analysis by LC-MS. Prior to analysis, the sample was diluted 1:5 with distilled water.

LC Conditions

LC system:	Waters ACQUITY UPLC System
Column:	Chromolith SpeedRODRP-18e HPLC Column (Merck), 4.6 x 50 mm (monolith analysis) ACQUITY UPLC BEH C_{18} Column, 1.7 µm, 2.1 x 50 mm (UPLC analysis)
Mobile phase A:	0.1% aqueous formic acid
Mobile phase B:	Acetonitrile with 0.1% formic acid
Flow rate:	2 mL/min. split 100 µL/min. to MS (monolith analysis) 500 µL/min., no split (UPLC analysis)

Gradient:	15–90 %B over 10 min.
Injection volume:	10 µL (monolith analysis) 2 µL (UPLC analysis)
Sample temp.:	10 °C
Column temp.:	40 °C

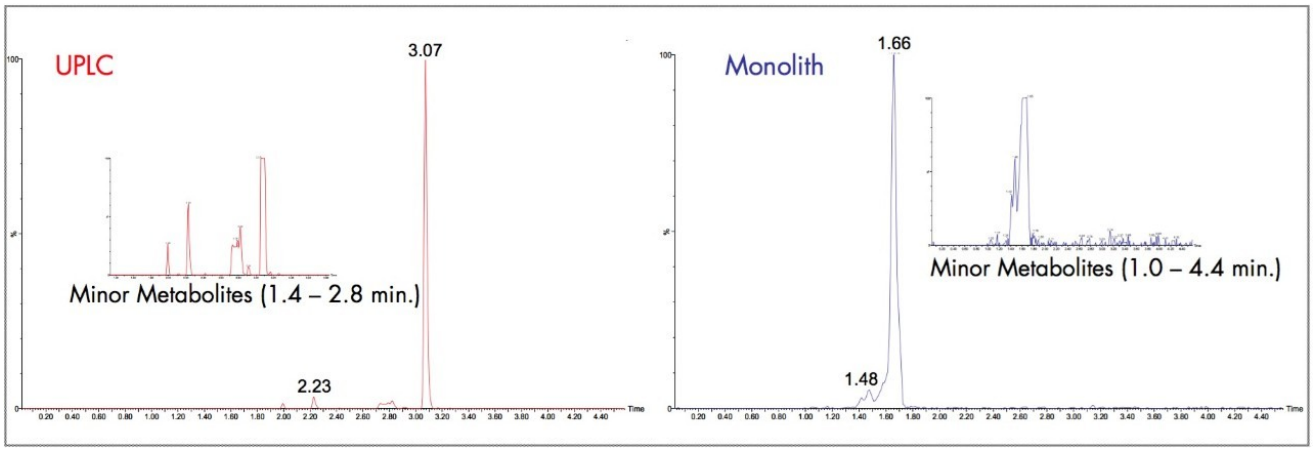
MS Conditions

MS system:	Waters Micromass Q-Tof micro Mass Spectrometer
Ionization mode:	ESI+
Source gas:	300 L/hr. at 250 °C
Acquisition mass range:	100–800 *m/z*
Cone voltage:	35 V
Collision energy:	10 eV
Dwell:	0.1 s
Collision gas:	Argon

Results and Discussion

The verapamil molecule can undergo metabolic dealkylationat seven different sites, resulting in seven different

isobaric metabolites of verapamil, *m/z*=441.2753. The data shown in the figure below represents the extracted ion chromatograms (*m/z*=441.2753) from the monolithic analysis (right) and the UPLC analysis (left). As can be seen from this data, the UPLC analysis produces one major and four minor (inset, left) distinct dealkylated metabolites of verapamil, whereas the monolithic analysis results in the minor dealkylated metabolites eluting as one unresolved peak (inset, right), just before the major metabolite peak.



UPLC (left) versus monolithic (right) analysis of the in vitro metabolites of verapamil.

Conclusion

The ACQUITY UPLC System provides excellent resolution and throughput, allowing all of the major and minor metabolites of verapamil to be resolved and detected in just oneshort ten-minute separation. The extra resolution and sensitivity afforded by ACQUITY UPLC makes it theideal choice for such complex mixture analysis over monolithic HPLC.

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