

## The Metabolism of Acetaminophen: Harnessing the Power of UPLC-MS

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### Abstract

This application note demonstrate the dramatic improvement that a new technology, UltraPerformance LC (UPLC), brings to pharmaceutical science of metabolite ID by exploring the metabolites of acetaminophen in human urine.

### Benefits

- Enhanced resolution resulting in the detection of more components
- Increased sensitivity improves the discovery of low-level metabolites
- Higher throughput allows for faster decision making in a discovery environment

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### Introduction

Many of us are familiar with the popular over-the-counter headache medicine acetaminophen, but have you ever

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wondered what happens to this compound after it enters the humanbody? In simple terms, most drugs are converted into other compounds, metabolites, by enzymatic action in the body. These metabolites may or may not have an adverse effect on one's health, and it is therefore crucial to study of the metabolic fate of drugs. For example, an overdose of acetaminophen may generate toxic metabolites that could lead to adverse effects. The prediction of these toxicological events is an ever-present challenge in the pharmaceutical industry. These toxic events are normally characterized by the presence of certain metabolites such as glutathione. High Performance Liquid Chromatography/Mass Spectrometry (HPLC-MS) has been routinely used for metabolite identification. We will demonstrate the dramatic improvement that a new technology, UltraPerformance LC (UPLC), brings to pharmaceutical science of metabolite ID by exploring the metabolites of acetaminophen in human urine.

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## Experimental

A side-by-side comparison of two instrumental configurations operating under similar conditions will be made for the investigation of acetaminophen metabolites in human urine. The first system will use HPLC-MS for the analysis, and the second will employ UPLC-MS. A representative result from each of these systems is shown.

### LC Conditions

LC system:	Waters ACQUITY UPLC System (UPLC) Waters Alliance HT System (HPLC)
Column:	2.1 x 100 mm, 1.7 $\mu$ m ACQUITY UPLC BEH C <sub>18</sub> Column (UPLC) 2.1 x 100 mm, 3.5 $\mu$ m Symmetry C <sub>18</sub> Column (HPLC)
Mobile phase:	A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile
Flow rate:	500 $\mu$ L/min.

Gradient:	0–40% B in 10 min. (UPLC) 0–20% B in 30 min. (HPLC)
Injection volume:	5 µL
Sample temp.:	10 °C
Column temp.:	40 °C

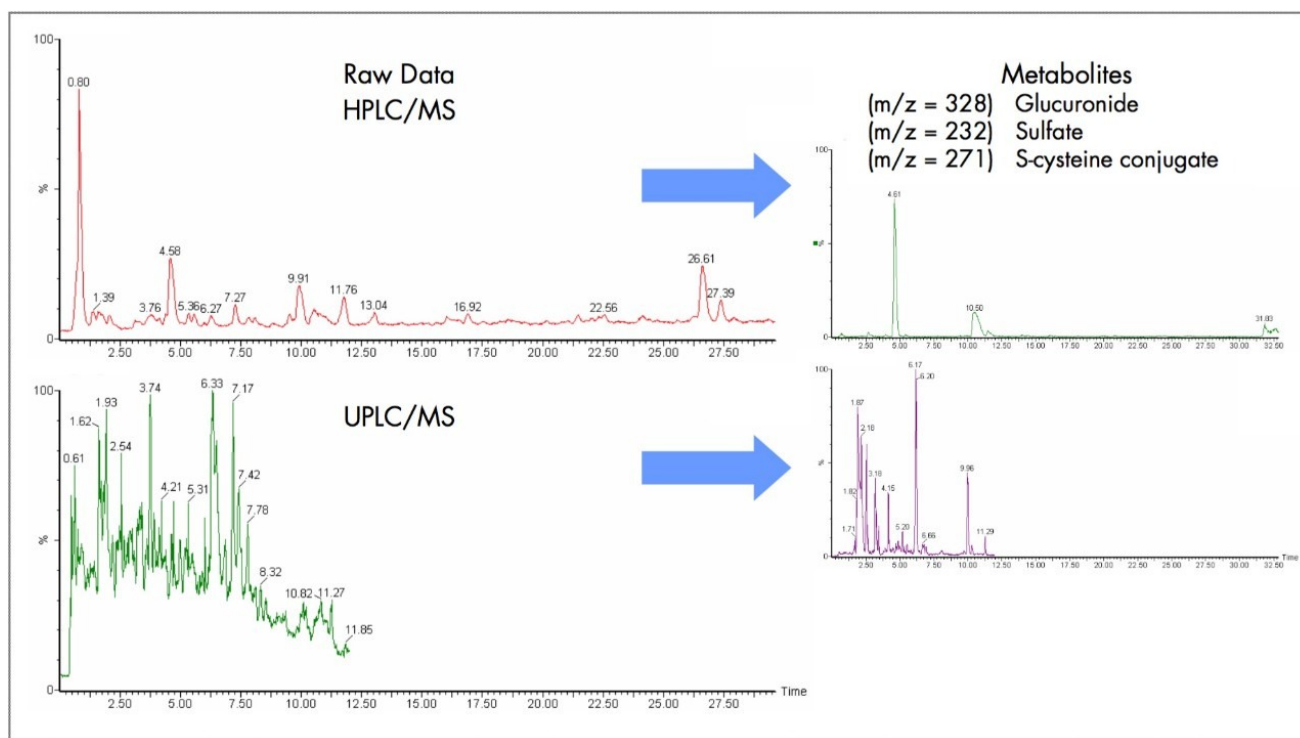
## MS Conditions

MS system:	Waters Micromass LCT Premier Mass Spectrometer
Ionization mode:	ESI+
Acquisition range:	80–800 <i>m/z</i> with Woptics resolution
Cone voltage:	45 V
Capillary voltage:	3000 V
Scan time:	0.3 s
Interscan delay:	0.05 s
LockSpray:	25 fmol/µL Leucine enkephalin at 30 µL/min.

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## Results and Discussion

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Analyzing acetaminophen metabolites in human urine by HPLC-MS (top) and UPLC-MS (bottom).

## Conclusion

As demonstrated in this simple exercise, UPLC-MS analysis offers several advantages over conventional HPLC-MS for metabolite identification, namely:

- Enhanced resolution resulting in the detection of more components
- Increased sensitivity improves the discovery of low-level metabolites
- Higher throughput allows for faster decision making in a discovery environment

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