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Reducing the Effect of Ion Suppression in Mass Spectrometry Using ACQUITY UPLC

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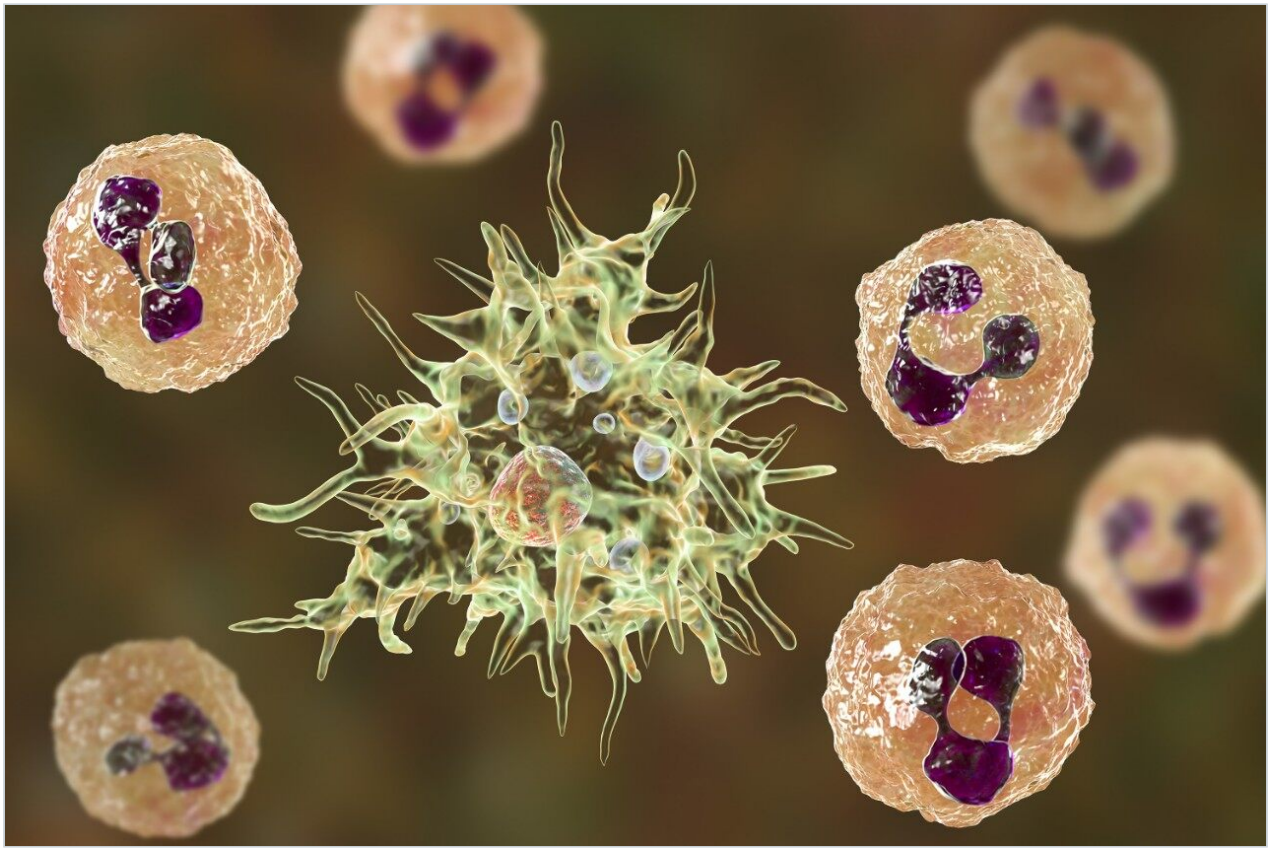

Abstract

The aim of this study was to demonstrate how this increased resolution afforded by UPLC (vs. HPLC) reduces ion suppression in the mass spectrometer.

Introduction

The assay sensitivity for the quantification of small molecules in biological fluids is often limited or compromised by ion suppression caused by the coelution of endogenous components from the sample. These components are often large molecules such as phospholipids, which elute in the middle of the chromatogram. This ion suppression effect of these endogenous molecules can often be mitigated by SPE or longer chromatographic separations. UltraPerformance LC (UPLC) has been shown to significantly increase chromatographic performance and give rise to increased MS sensitivity. It has been proposed that this increase in sensitivity is due to increased resolution of the target analytes from the endogenous material. The aim of this study was to demonstrate how this increased resolution afforded by UPLC (vs. HPLC) reduces ion suppression in the mass spectrometer.

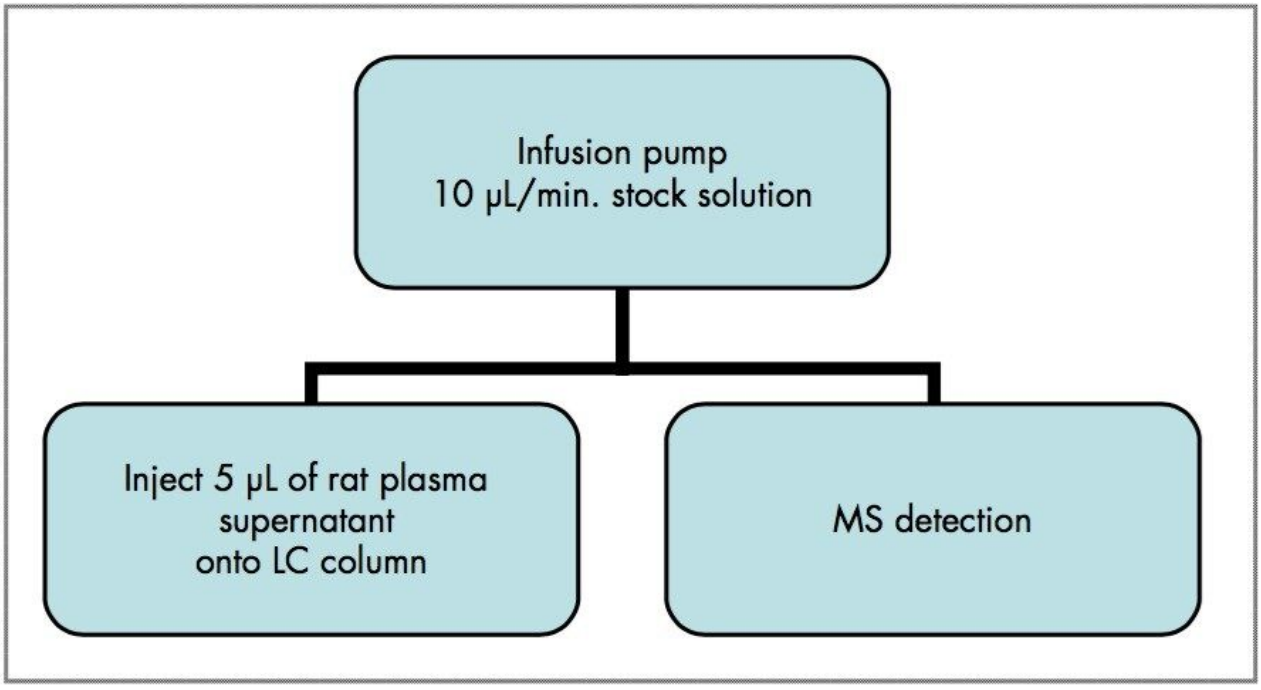

Figure 1. Schematic of the experimental setup.

The LC-MS system was configured such that the column eluent was mixed with a stream containing three

reference compounds: amitriptyline, doxepin, and verapamil at a concentration of 100 pg/µL in methanol. An injection of acetonitrile-precipitated rat plasma (2:1) was made onto a 2.1 x 50 mm HPLC (3.5 µm) or UPLC (1.7 µm) column. The column was eluted with a gradient of 5–95% acetonitrile over 5 minutes for both separations.

Experimental

Chromatography Conditions

HPLC system:	Alliance HT System
UPLC system:	ACQUITY UPLC System
Mobile phase A:	0.1 % Formic acid
Mobile phase B:	0.1 % Formic acid/MeCN
HPLC column:	BEH C_{18}, 3.5 µm 2.1 x 50 mm
UPLC column:	ACQUITY UPLC BEH C_{18}, 1.7 µm 2.1 x 50 mm
Column temp.:	40.0 °C (HPLC and UPLC)
Injection volume:	5 µL (HPLC and UPLC)
Gradient:	5–95% B over 5 min. (HPLC and UPLC)
Flow rate:	270 µL/min. (HPLC) 550 µL/min. (UPLC)

MS Conditions

MS system:	Waters Micromass LCT Premier Mass Spectrometer

Ionization mode:	ES+
Capillary voltage:	3200 V
Cone voltage:	75 V
Source temp.:	120 °C
Desolvation temp.:	300 °C
Cone gas:	10 L/hr
Desolvation gas:	600 L/hr
Acquisition range:	100–1000 *m/z*
Scan Duration:	0.3 sec
Inter-Scan Delay:	0.02 sec

Results and Discussion

The data in Figure 2 shows the chromatograms obtained from both the UPLC (top) and HPLC (bottom) separation of the precipitated rat plasma (data has been normalized to account for the gradient delay of both instruments). We can see from the extracted ion chromatogram (*m/z* = 455), the signal intensity relating to the verapamil ion. The presence of eluting endogenous components is indicated by negative peaks in the baseline. The UPLC separation produced sharper peaks, and hence improved resolutionof the endogenous components. This point is highlighted by the triplet of eluting peaks between 3.40 and 3.75 minutes, and the two endogenous peaks eluting between 4.15 and 4.60 minutes in the UPLC trace. In the HPLC trace, these are only partially resolved, whereas with UPLC, the peaks are fully baseline resolved. With UPLC, any analytes of interest have less potential for coelution, thus reducing the possibility of ion suppression.

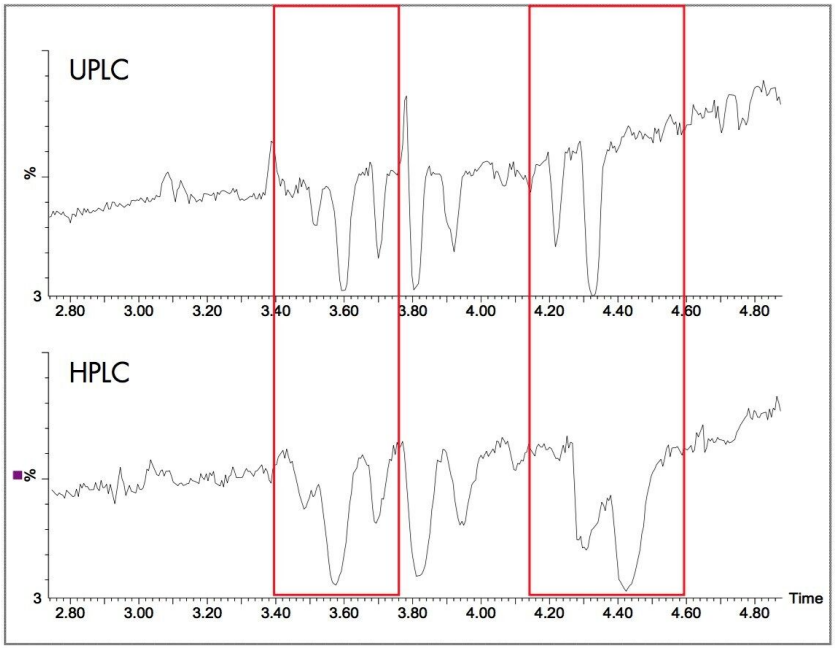

Figure 2. XIC for m/z = 455 for the UPLC (top) and HPLC (bottom) separation of protein-precipitated rat plasma.

The extra chromatographic resolution of the ACQUITY UPLC System, while not eliminating the need for good sample preparation, significantly reduces the ion suppression of samples in complex biological fluids extracts.

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