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Applikationsbericht

Direct Injection of Polar Compounds in Highly Organic Protein Precipitated Plasma Using UPC^2-MS/MS

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This work demonstrates direct-inject separation of highly polar compounds in highly organic protein precipitated plasma for bioanalysis.

Benefits

By changing MS inlet technology from RPLC to UPC^2, it is possible to directly inject polar compounds from highly organic samples for separation without additional sample preparation steps such as evaporation and reconstitution.

Introduction

A majority of bioanalytical methods utilize protein precipitation (PPT) extraction methods due to the simplicity, speed, and low cost of the technique. A typical PPT utilizes a 3:1 ratio of organic solvent to biological sample, producing an extract approximately 75% organic. Traditionally, samples are analyzed using reversed-phase liquid chromatography (RPLC).

For compounds that are highly polar, direct injection of the highly organic extract onto the RPLC system is not possible due to strong solvent effects that produce poor chromatographic peak shape, most notably peak fronting and/or peak splitting. Therefore, additional sample manipulation including evaporation and reconstitution or dilution with water is required prior to injection onto the chromatographic system.

Results and Discussion

The use of UltraPerformance Convergence Chromatography (UPC^2), which utilizes supercritical carbon dioxide as the major mobile phase, allows for the direct injection of highly organic extracts due to differences in retention mechanisms compared to RPLC.

In this example, four relatively polar compounds were extracted from rat plasma with a 3:1 PPT with acetonitrile, and directly injected onto an ACQUITY UPC^2 System as well as a traditional RPLC system for

comparison. The UPC^2 analysis was performed on an ACQUITY UPC^2 BEH Column using methanol modified with ammonium hydroxide as the co-solvent (mobile phase B). The RPLC analysis was performed on an ACQUITY UPLC System with an ACQUITY UPLC BEH C_{18} Column, employing water and acetonitrile modified with ammonium hydroxide as the mobile phases.

Figure 1 compares the direct injection of a 1-µL injection and a 3-µL injection of a PPT extract containing caffeine on the ACQUITY UPLC System to a 7-µL injection of the same extract on the ACQUITY UPC^2 System. The 1-µL injection on RPLC shows adequate peak shape, but at 3-µL injection volume the peak shape is distorted, as can be observed by peak fronting and splitting. Conversely, the injection performed using UPC^2 still shows adequate peak shape at the 7-µL injection volume.

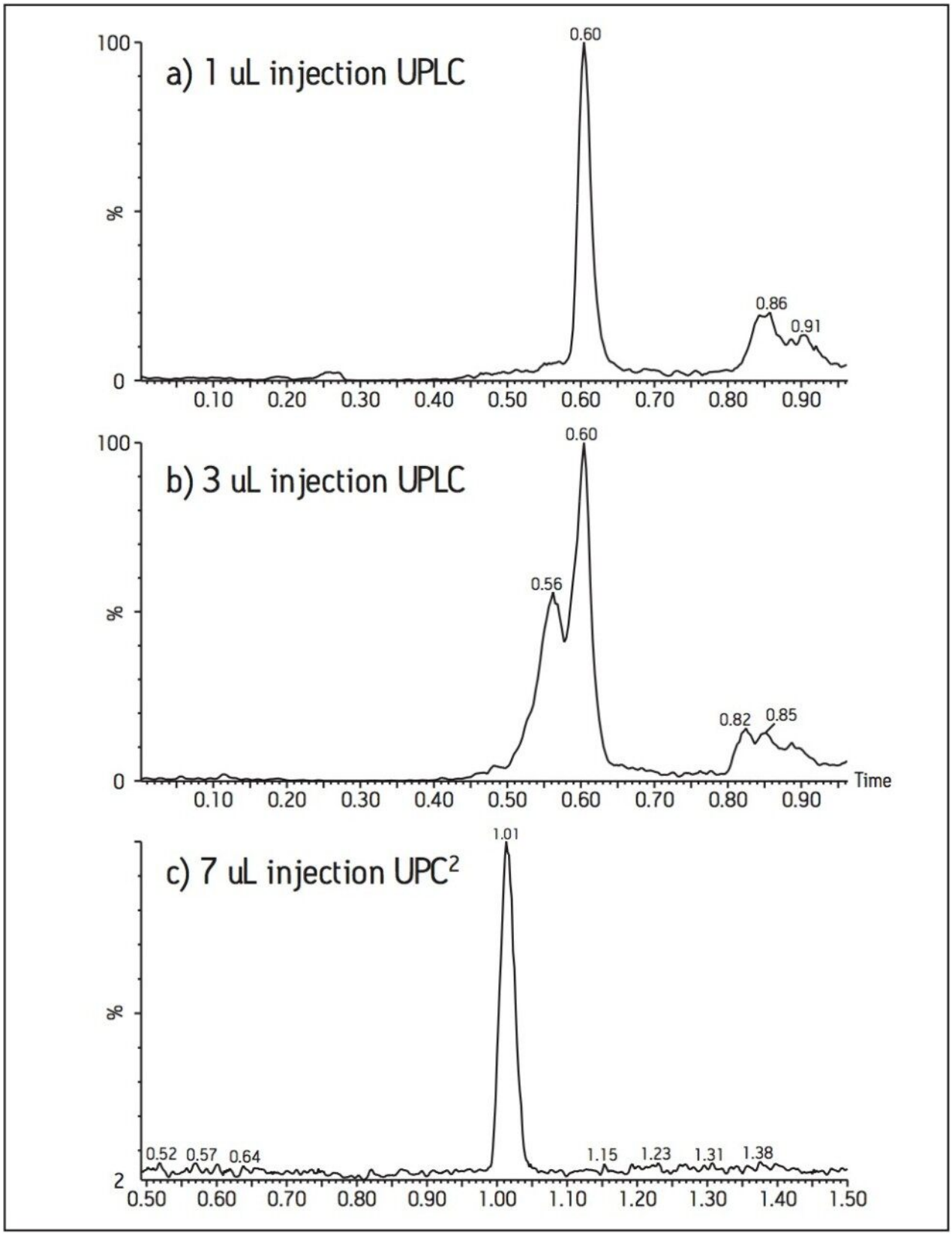

Figure 1. Example chromatograms of a caffeine PPT extract obtained from (a) 1-µL injection, (b) 3-µL

injection on a UPLC operating in reversedphase mode, and (c) 7-µL injection of the same caffeine PPT extract on the ACQUITY UPC² System.

Similar results were observed for other polar molecules tested in the same manner (Table 1). Table 1 also shows the maximum injection volume for all analytes tested in the PPT extract using both RPLC and UPC².

	Caffeine	Ranitidine	Fluconazole	Acetaminophen
UPLC	1 µL	NA	3 µL	NA
UPC²	7 µL	10 µL	5 µL	7 µL

Table 1. Maximum injection volume determined for UPLC and UPC². NA = peak shape was not adequate for integration at 1 µL.

These data clearly demonstrate the benefit of analyzing polar compounds in highly organic extracts using the ACQUITY UPC² System without further sample preparation that would be required to inject the same sample onto an RPLC system, thus simplifying the workflow.

Conclusion

By changing the MS inlet technology from standard RPLC to UPC², it is possible to directly inject polar compounds from highly organic samples onto the ACQUITY UPC² System without additional sample preparation steps such as evaporation and reconstitution. The addition of UPC² in a bioanalytical laboratory provides a simplified methodology for the analysis of polar compounds in highly organic sample preparations.

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