Direct Plasma Analysis of Drug Compounds Using Onyx Monolithic Columns

Scott Waite, Michael McGinley
Phenomenex, Inc., Torrance, CA, USA

Introduction

The tandem use of liquid chromatography and mass spectrometry has become the principal mode of pharmaceutical analysis, especially for high throughput analysis employed in DMPK studies. Rapid analytical data generation is pivotal during these early stages of drug discovery. Analytical chemists have to deal with matrices such as plasma, serum, urine, or even whole blood, requiring time consuming sample preparation/clean-up prior to LC/MS analysis meant to eliminate matrix components which cause rapid decline in HPLC column performance and have deleterious effects on quantitative analysis. Solid phase extraction (SPE) and liquid extraction (LLE) are the preferred sample pretreatment techniques since they are amenable to automation, more efficient, and less labor intensive compared to other sample preparation methods. However, the constant push for faster analysis times has stimulated investigation in alternative, more rapid approaches to sample preparation. A viable and increasingly popular alternative to off-line SPE is to perform the sample clean up on-line, coupled to an LC/MS that facilitates complete automation of the whole operation of pretreatment and analysis. Such on-line extraction methods allow for the direct injection of biological samples for LC/MS analysis, improves automation, and maintains HPLC column performance.

In this study, we present an evaluation of using Onyx Monolithic columns for direct injections of serum samples spiked with a mix of 11 different pharmaceuticals. We demonstrate consistent and reproducible performance with over one hundred direct serum injections.

Materials and Methods

Analyses were performed using an Agilent 1100 HPLC system, Bruker Esquire 2000 Ion-Trap MS analyzer, and a Rheodyne LabPro 10 port, 2 position-switching valve. The HPLC column used was an Onyx Monolith C18, 50 x 4.6 mm. All standards used were purchased from Sigma Chemicals (St. Louis, Missouri). Solvents were purchased from Fisher Scientific (Fairlawn, New Jersey). Column temperature was maintained at 30 °C.

Table 1.

	HPLC Gradient			
Time	Mobile Phase A	Mobile Phase B	Flow Rate (mL/min)	Valve Position*
0.00	95	5	4.0	2
0.75	95	5	4.0	2
0.76	95	5	1.0	1
5.00	15	85	1.0	1
5.01	95	5	4.0	2

*Valve position 2, HPLC flow is diverted to waste; valve position 1, HPLC flow is diverted to mass spectrometer.

Mobile Phase A: 0.1 % Formic acid in Water

Mobile Phase B: 0.1 % Formic acid in Acetonitrile

Serum samples were diluted 2:1 with 0.1 % formic acid and spiked with 11 pharmaceutical drug compounds at a concentration of 200 ng/mL. Aliquots of 10 µL of diluted serum samples were directly injected into the Onyx Monolithic C18 for MS analysis. The flow rate for loading sample was set at 4 mL/min for 0.75 minutes with the switching valve diverting flow to waste. The valve is then switched to position 1 for the mass spectrometer, flow adjusted to 1 mL/min and a gradient of 5 % - 85 % mobile phase B initiated to elute and separate the analytes. The valve is switched back to position 2 for equilibration of initial starting conditions.

MS Conditions:

- ESI in positive ion mode
- Nebulizer set at 60 psi
- Dry Gas set at 12 L/min
- Dry Temp set at 365 °C

Results and Discussion

This work examined the use of Onyx Monolithic columns for the direct injection of diluted serum samples for LC/MS analysis. The dual role of the monolithic column is both to remove the matrix macromolecules (as a trapping column) and to maintain chromatographic performance for the elution and separation of analytes (as an analytical column). Compared to a traditional particle packed silica column, the monolithic column generates a much flatter Van Deemter plots at high flow rates due to the better mass-transfer properties allowing for faster HPLC separations without a noticeable effect on chromatographic resolution. However, running high flow rates directly into the MS source will result in lower signals. To compensate for this, either a post-column split can be used or the flow rate can be adjusted during the elution step for optimal conditions for detection as is summarized in ***Table 1***.

Reproducibility and ruggedness was demonstrated by sequential injections of 100 serum samples with apparent recoveries greater than 90 %. ***Figure 1*** shows overlay of five sequential injections of drug mix in serum. ***Figure 2*** shows individual chromatograms of test compounds in serum samples.

Figure 1.
Overlay of 5 sequential injections demonstrating reproducibility.

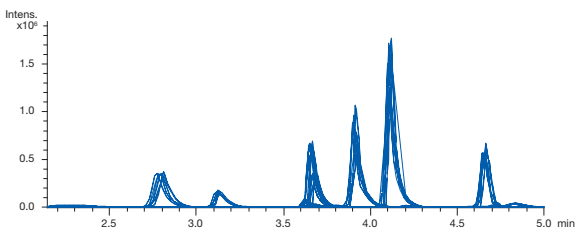

Figure 1: Overlay of 5 sample injections. Pharmaceutical compounds are spiked into a plasma sample and selected ions are monitored by MS. Results indicate that repetitive dilute-and-shoot injections show little effect on the chromatography and recovery of key analytes.

Figure 2. Individual chromatograms of test compounds.

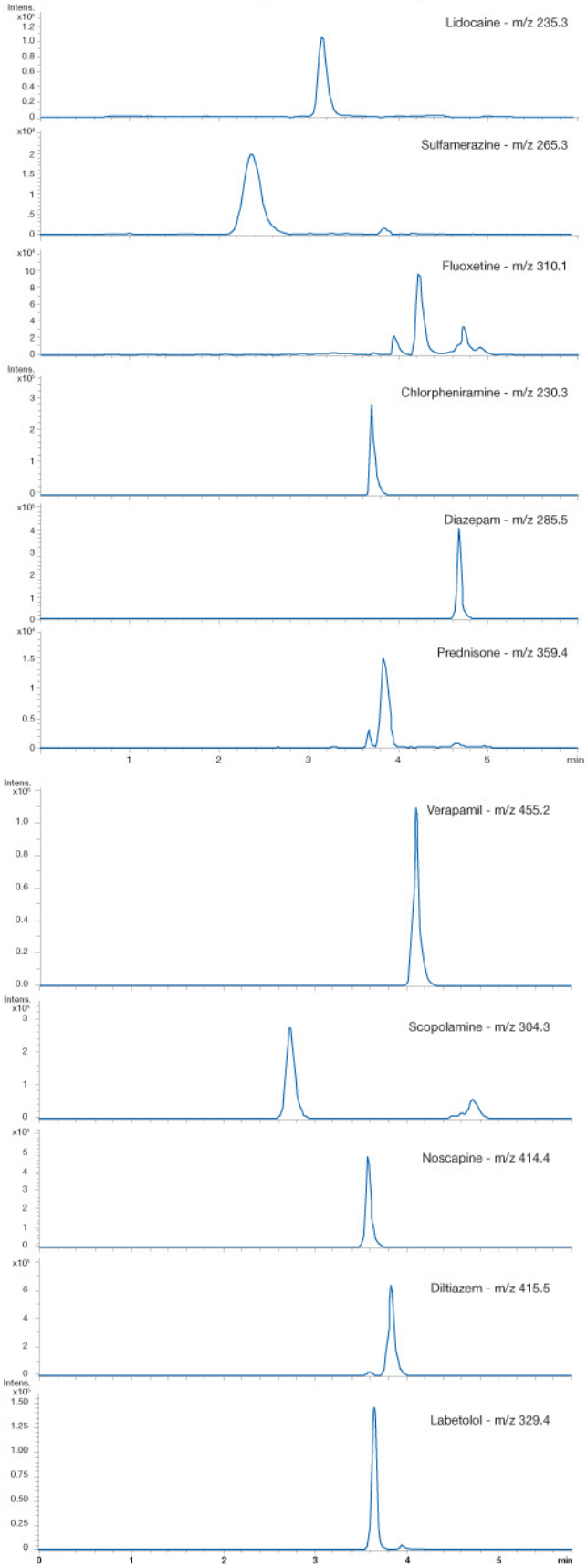

Figure 2: No sample preparation was performed on the serum samples except dilution in formic acid. As seen in the individual chromatograms of the 11 pharmaceutical compounds, there is virtually no peak interference.

Conclusion

An online bioanalytical method using Onyx Monolithic columns for the direct analysis of serum samples for LC/MS analysis has been demonstrated. The online extraction procedure using Onyx Monolithic columns was designed for the removal of unwanted biological components that can cause MS ion suppression effects, while retaining the analytes of interest. When directly injecting serum samples, the entire process of conditioning, loading, washing, and elution of the analyte directly into the mass spectrometer can be achieved. The "dilute-and-shoot" methodology allows for direct injections of large volumes of plasma/serum samples into the LC/MS system. This application eliminates time consuming sample preparation from the analysis process, resulting in significant improvement in sample throughput.

If you would like more information on these columns, or any of the applications listed, please contact Phenomenex.

ORDERING INFORMATION

Part Number	Description
CH0-8158-TN	Onyx Monolithic C18 100 x 3.0 mm
CH0-7645-TN	Onyx Monolithic C18 25 x 4.6 mm
CH0-7644-TN	Onyx Monolithic C18 50 x 4.6 mm
CH0-7643-TN	Onyx Monolithic C18 100 x 4.6 mm
NEW CH0-8373-TN	Onyx Monolithic C18 50 x 2.0 mm

ONYX™ Monolithic HPLC Columns

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