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On-Line Sample Clean-Up for the LC/MS Analysis of Small Molecules in Serum

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Drug discovery/detection commonly focuses on analytes which are present in biological matrices. Direct injection of such samples onto LC and LC/MS systems is problematic as analytes of interest can be 'lost' in the concentrated matrix peak, plus instrument and column contamination readily occurs. Traditionally, solid phase extraction (SPE) has been used for sample pre-treatment and clean-up, but this can be time consuming and adds an additional stage to the analytical method. The ability to automate biological assays, minimize the number of steps and reduce overall analysis time is becoming increasingly important. On-line sample clean-up utilizing size exclusion chromatography (SEC) fulfills these criteria. On-line SEC is a two-dimensional approach in which serum is injected directly onto a small pore size exclusion column and then the analytes, once separated from the matrix, are eluted onto an analytical column. On-line SEC reduces sample preparation time, minimizes potential analyte loss and results in increased productivity and higher sample throughput (1).

Experimental Conditions

For sample clean-up, 2 μ L of serum was injected onto a 150 x 2.1 mm Thermo Scientific BioBasic™ SEC 60 size exclusion column (5 μ m particle size) held at ambient temperature and separation was performed using a 50 x 2.1 mm Thermo Scientific Hypersil GOLD™ analytical column (3 μ m particle size) held at 45 °C. LC/MS analysis was performed using a Thermo Scientific Surveyor™ HPLC coupled to a Thermo Scientific LCQ™ Deca ion trap mass spectrometer with the flow rate maintained at 500 μ L/min. The gradient (mobile phase A comprised 10 mM ammonium formate, pH3, and mobile phase B comprised methanol) is summarized below:

Time (min)	%B	
0	0	eluent diverted to waste
0.75	0	eluent diverted to waste
0.80	13	
7.0	13	
7.10	100	
10.10	100	
10.20	13	
13.20	13	

The SEC column was equilibrated with 100% buffer for 2 minutes prior to the start of the gradient, with the eluent diverted to waste.

Results and Discussion

To determine the time to divert the flow to waste after sample injection, a comparison of the injections of serum alone and serum dosed with the analyte was performed. Figure 1a shows

the resultant UV trace for blank serum. Lamivudine (the least retained of the analytes) was found to elute at approximately 0.8 minutes, therefore, the switch valve was set to direct flow to the analytical column at 0.75 minutes. The major serum peak elutes earlier at approximately 0.5 minutes and, therefore, is prevented from contaminating the analytical column. The separation of the drugs from serum with on-line SEC teamed with isocratic analytical separation is depicted in Figure 1b. The percentage recoveries achieved were greater than 100, 89 and 79% for abacavir, lamivudine and zidovudine, respectively. During the course of experimentation, over 110 runs were performed with no significant deterioration in column performance observed, illustrating that the methodology is robust and reproducible.

References

- (1) C.E. Blythe et al, Poster presented at HPLC 2007, Ghent, Belgium.

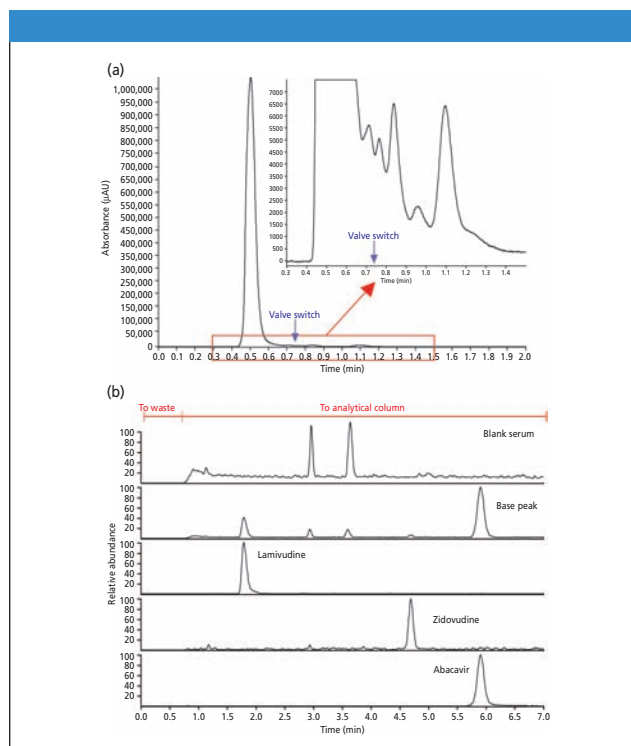


Figure 1: (a) Blank serum SEC extraction to determine valve switch time. (b) On-Line SEC Extraction of Anti-Retrovirals from Serum

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