Increased Sample Throughput with Luna® HILIC

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Introduction

Luna HILIC columns can lead to a more efficient interface between sample cleanup/extraction and LC–MS–MS chromatographic methods. Rational method development today includes optimization of chromatographic conditions, detector conditions and sample preparation design. Because many samples are unsuitable for direct analysis; extraction and concentration are important sample preparation techniques. The commonly used elution solvents for SPE and protein precipitation are stronger solvents than the mobile phase used in reversed-phase gradient methods. This disparity will generally lead to significant band broadening and loss of sensitivity in subsequent chromatographic analysis. To prevent this peak deterioration eluent evaporation followed by reconstitution in weak mobile-phase is necessary. The elimination of these time consuming steps would greatly enhance sample throughput in the high volume analysis lab. In this application note it is shown that Luna HILIC improves the interface between sample preparation and HPLC. Luna HILIC allows for good retention and resolution of polar metabolites with direct injection following protein precipitation.

Experimental Conditions

Nicotine and metabolites (Cotinine and Nornicotine) spiked into human plasma at 20 ng/mL was used to investigate protein precipitation as sample preparation for Luna HILIC. Plasma proteins were precipitated by adding 5 mL acetonitrile to 1 mL spiked plasma. Samples were centrifuged then filtered prior to injection.

Instrumentation

HPLC System: HP 1100 series (www.agilent.com)
MS Detector: API 3000 LC/MS/MS (www.appliedbiosystems.com)

Chromatographic Conditions

Column: Luna 3 µm HILIC, 100 x 2.0 mm
Flow Rate: 0.4 mL/min
Mobile Phase: 90/10 Acetonitrile/100 mM Ammonium formate, pH 3.2
Injection Volume: 10 µL
Temperature: Ambient

Results

Following standard procedures for protein precipitation, a 10x concentration was achieved by removing a 2.5 mL aliquot of supernatant and evaporating to dryness under a stream of nitrogen. The residue was reconstituted in 250 µL of 95/5 Acetonitrile/Water and 10 µL injected ***(Figure 1)***. Following this procedure the total sample preparation time was >2 hours. Under HILIC chromatographic conditions these polar metabolites were retained and resolved with good signal-to-noise ratios — but was all of this work necessary? Rather than following the tedious and time-consuming concentration procedure, 10 µL of the protein-precipitated supernatant was simply filtered and injected onto the column. As shown in ***Figure 2*** excellent peak shape and retention was obtained. This example demonstrates that simple protein precipitation and HILIC chromatography provides adequate signal strength and good peak symmetry without the time-consuming evaporation and reconstitution steps. Signal-to-noise ratios (S/N) for nicotine and metabolites on Luna HILIC are at acceptable levels for LOD and LLOQ and have been achieved in a time that better suits fast analysis requirements.

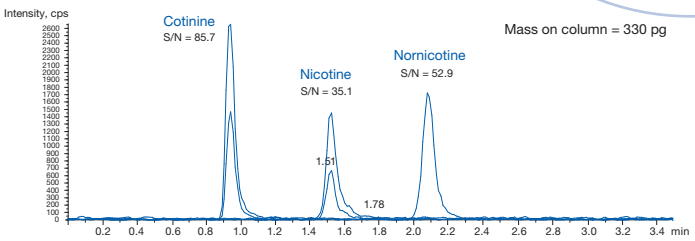

Figure 1: *Protein Precipitated Plasma (20 ng/mL), 10 x concentration, 10 µL injected.*

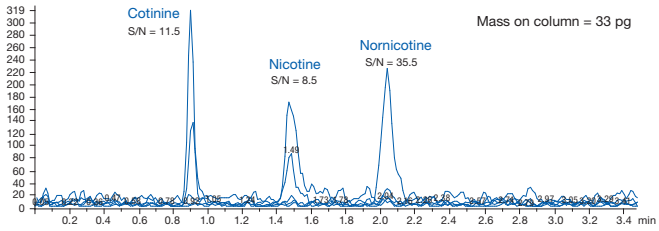

Figure 2: *Protein Precipitated Plasma (20 ng/mL), direct inject, 10 µL injected.*

Conclusion

Luna HILIC HPLC columns increase laboratory throughput by allowing for the direct injection of the high organic containing elution solvents of both SPE and protein precipitation. In HILIC these are weaker elution solvents than the mobile phase and thus do not contribute to band broadening. Elimination of the final evaporation and reconstitution steps can significantly decrease (up to 50 %) the total time spent on sample preparation. Luna HILIC columns offer an effective and cooperative interface between sample preparation and LC–MS–MS chromatographic methodology.

Ordering Information

Part No.	Description
00D-4449-B0-TN	Luna 3 µm HILIC, 100 x 2.0 mm