Increasing LC-MS/MS Sensitivity with Luna HILIC

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Introduction

The analysis of polar compounds in support of clinical and pre-clinical pharmacokinetic studies requires an analytical methodology capable of achieving ultra-low detection and quantification limits. The high sensitivity afforded by coupling HPLC with tandem mass spectrometry (MS/MS) has made it the technique of choice in this environment, but it is subject to the following limitations when reversed phase liquid chromatography (RPLC) is used:

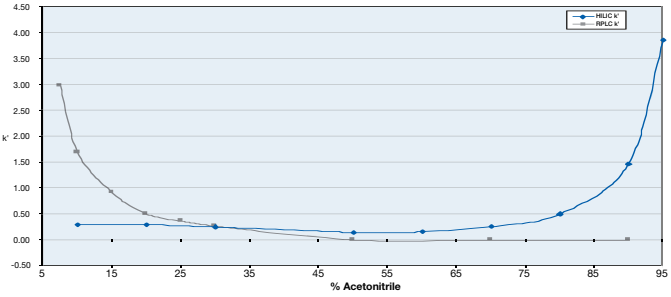
1. **Polar compound elution in a highly aqueous mobile phase:** Gas phase ion generation is facilitated with more volatile solvents (1); therefore, the high water content used for RPLC retention of polar compounds decreases desolvation efficiency. In addition the highly aqueous mobile phase required for retention of polar compounds increases solvent surface tension, which decreases ESI spray stability (2).

2. **Polar compound elution in the primary ion suppression region:** Poorly retained compounds in RPLC increase the likelihood of analyte compounds eluting with the many endogenous compounds present in bioanalytical samples. These endogenous compounds compete for charge in the source and can suppress analyte ionization, reducing sensitivity and degrading LOD and LOQ. It is critical that a robust bioanalytical method separate analytes from the ion suppression region (3).

Luna HILIC columns allow for improved sensitivity in the analysis of complex bioanalytical samples by creating more favorable conditions for polar compound retention and ionization.

Results

Figure 1. Atenolol is a polar drug requiring low organic for retention in RPLC. In HILIC mode, atenolol retention increases with increasing % Acetonitrile. All analyses were performed in isocratic mode. Atenolol, log P = 0.16, pK_a = 9

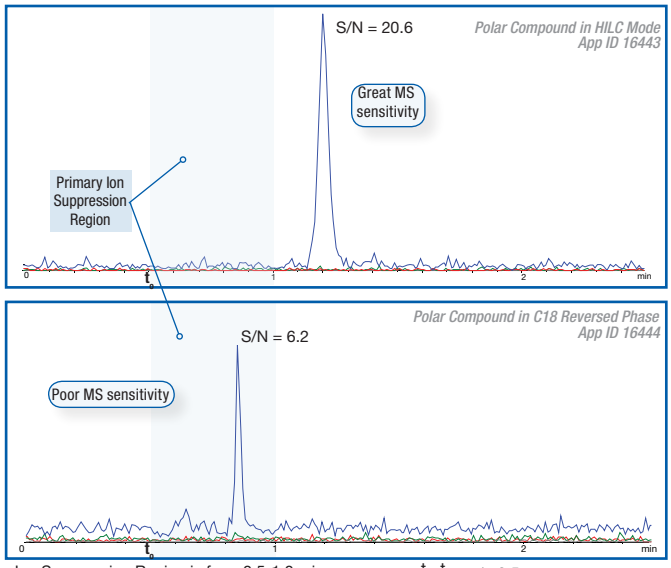


On the Luna HILIC column, atenolol elutes in a high organic volatile mobile phase, which facilitates analyte desolvation and results in enhanced LC-MS/MS sensitivity.

Polar metabolites are weakly retained under RPLC conditions and elute near the solvent front, often in the “primary ion suppression region”.

Under HILIC chromatographic conditions, where polar analytes are retained using high organic mobile phase, Luna HILIC strongly retains polar compounds that are otherwise weakly retained in RPLC.

Figure 2. Chromatographic separation of the analytes away from the solvent front is an effective way to eliminate or minimize ion suppression caused by endogenous sample components.



Ion Suppression Region is from 0.5-1.0 min

$$k'1 = \frac{t_R - t_o}{t_o} = \frac{1 - 0.5}{0.5}$$

App ID 16443 | Polar Compound in HILC Mode

Column:	Luna 3 µm HILIC
Dimension:	100 x 2.0 mm
Part No.:	00D-4449-B0
Mobile Phase:	Acetonitrile / 100 mM Ammonium Formate, pH 3.2 (90:10)
Flow Rate:	0.4 mL/min
Detection:	Mass Spectrometer (MS)
Temperature:	Ambient
Sample:	Bamethan

App ID 16444 | Polar Compound in C18 Reversed Phase

Column:	Gemini 3 µm C18
Dimension:	100 x 2.0 mm
Part No.:	00D-4435-B0
Mobile Phase:	0.1 % Formic Acid / Acetonitrile (97:3)
Flow Rate:	0.4 mL/min
Detection:	Mass Spectrometer (MS)
Temperature:	Ambient
Sample:	Bamethan

Conclusion

The Luna HILIC column retains and elutes hydrophilic/polar compounds in highly organic mobile phase conditions, which improve analyte desolvation efficiency and ESI spray stability. The combination of these two improvements in the MS interface will often provide improved polar analyte detection and quantitation.

In HILIC mode, the analyte elution in the primary ion suppression region is avoided as a result of the mode's increased retention of hydrophilic compounds. This increased retention for polar compounds regularly results in less interference from endogenous sample compounds, improved sensitivity and accuracy, and reduced LOD and LOQ. The Luna HILIC column meets the requirements of a robust bioanalytical method by eluting analytes outside the primary ion suppression region.

It has been shown here that Luna HILIC columns can improve LC-MS/MS sensitivity of polar compounds in biological matrices in support of clinical and preclinical pharmacokinetic studies where ultra-low detection and quantification limits are highly desirable.

References:

1) Cech, N.B.; Enke, C.G. Mass Spectrom. Rev. 2001, 20, 362-387.
2) Kerbarle, P. J. Mass. Spectrom. 2000, 35, 804-817.
3) Matuszewski B.K.; Constanzer M.L.; Chavez-Eng C.M., Anal. Chem. 2003, 75, 3019-3030

Ordering Information

Luna 3µm HILIC 200 Å

Part No.	Dimensions
00B-4449-B0-TN	50 x 2.0 mm
00B-4449-Y0-TN	50 x 3.0 mm
00D-4449-B0-TN	100 x 2.0 mm
00D-4449-Y0-TN	100 x 3.0 mm
00D-4449-E0-TN	100 x 4.6 mm
00F-4449-B0-TN	150 x 2.0 mm
00F-4449-Y0-TN	150 x 3.0 mm
00F-4449-E0-TN	150 x 4.6 mm