Separation of Morphine and Glucuronated Metabolites on a Bonded Zwitterionic Stationary Phase

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Introduction

Hydrophilic interaction liquid chromatography (HILIC) is an ideal technique for polar and hydrophilic compounds that are poorly retained on a reversed-phase column. In this separation mode, an eluent containing a high content of water-miscible organic solvent (e.g., acetonitrile) is used to promote the hydrophilic interactions between the analyte and a hydrophilic stationary phase.

Consequently, analytes that elute in or close to the void volume and that may be affected by wettability problems on a reversed-phase column are those that are the most retained on a HILIC column. In addition, HILIC is a versatile alternative to straight phase separations with respect to the superior analyte solubility in the mobile phase and matrix compatibility.

This application note provides an example of an isocratic HILIC separation of morphine and its glucuronated metabolites morphine-3-glucuronide and morphine-6-glucuronide that are known for their analgesic effects.

Figure 1: The ZIC®-HILIC stationary phase.

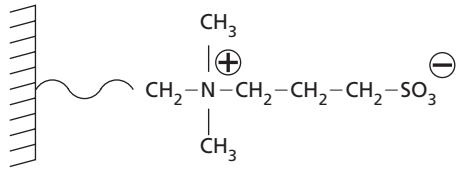
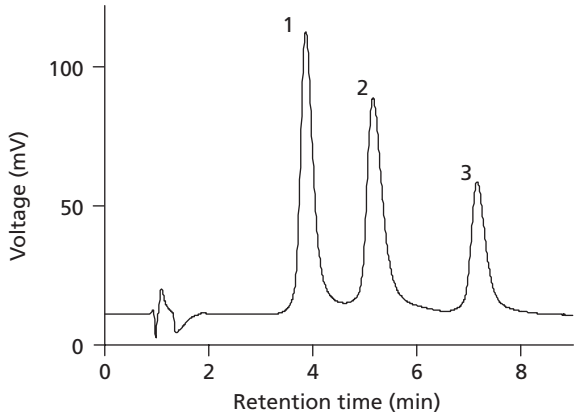


Figure 2: Separation of morphine and two of its metabolites.



Peaks: 1= morphine-6-glucuronide, 2= morphine-3-glucuronide, 3= morphine.

The ZIC®-HILIC column retains and resolves the metabolites, in the same way as it previously successfully have been used for glucosylated compounds and sugars.

ZIC®-HILIC Columns

The ZIC®-HILIC columns have a sulfobetaine type zwitterionic stationary phase covalently attached to 200 Å pore size and 5 µm particle size silica (see Figure 1). The permanently charged, yet overall neutral, highly polar zwitterionic column provides a unique environment particularly capable of solvating polar and charged compounds, which enables high-performance HILIC separations.

The column can provide a selectivity benefiting from both hydrophilic and weak electrostatic interactions, while maintaining a low eluent ionic strength, making the column an ideal choice for LC/MS analysis.

Experimental Conditions

Column:	ZIC®-HILIC 50 × 4.6 mm, 5 µm
Mobile phase:	acetonitrile/5 mM ammonium acetate 70/30 (v/v)
Flow-rate:	0.5 mL/min
Detector:	UV @ 212 nm
Injection volume:	2 µL
Sample:	In elution order; morphine-6-glucuronide, morphine-3-glucuronide and morphine all dissolved in mobile phase.

Results

Excellent resolution for morphine and two of its metabolites can be achieved using a straightforward isocratic elution protocol, as seen in Figure 2. This illustrates that the ZIC®-HILIC column retains and resolves the metabolites, in the same way as it previously successfully have been used for glucosylated compounds and sugars.

The metabolites are, because of their additional sugar moiety, expected to be more hydrophilic than the native morphine. However, beside hydrophilicity the retention of the metabolites are also influenced by the weak electrostatic effects between the zwitterionic functional group and the glucuronide acid, controlled by the ion strength and operational pH of the mobile phase.

Conclusion

The ZIC®-HILIC column is suitable for HILIC mode separation of morphine and its metabolites. By taking advantage of the weak electrostatic interactions between the analytes and the overall neutral zwitterionic stationary phase, the column exhibits a novel and unique selectivity in the analysis of a wide range of compounds.

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