



Fast Methods for Structurally Similar Compounds, Carbon HPLC Columns

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Dwight Stoll, Clayton V. McNeff, Peter W. Carr
ZirChrom Separations, Inc.

Method development can be challenging for structurally similar compounds in RPLC. A new carbon-clad zirconia phase offers dramatically different chromatographic selectivity for these types of compounds when compared to traditional silica-based bonded phases. This note shows baseline separation of 6 structurally similar sulfate-steroid conjugates using a zirconia-based ZirChrom-CARB HPLC column.

Introduction

Method development in reversed-phase liquid chromatography (RPLC) has traditionally been difficult for molecules which are geometric isomers or structurally very similar. In bonded-phase silicas, the partition mechanism responsible for retention in RPLC often does not offer adequate chemical selectivity for such compounds. On the other hand, carbon-based phases, provide retention in RPLC through an adsorption mechanism which often times increases the chromatographic selectivity for these types of compounds and dramatically increases the chances of resolving pairs of these analytes. In addition to this enhanced chromatographic selectivity, carbon-based phases also offer increased chemical and stability of the stationary phase.

Carbon-clad zirconia

ZirChrom Separations, Inc. has developed new materials using zirconia as a stationary phase support, and patented chemical vapor deposition technology to produce a carbon-clad zirconia particle suitable for use in reversed-phase liquid chromatography. The surface bond is C-C, which is extremely resistant to chemical and thermal attack. The authors have run mobile phases at very high pH (1M NaOH), very low pH (0.5M HNO₃), and at elevated temperature (up to 150 °C), and have not observed loss of stationary phase.

Experimental

A mixture of sulfate-steroid conjugates was separated at elevated temperature using a ZirChrom®-CARB column. The separation conditions were as follows:

Column: 4.6 mm x 100 mm ZirChrom-CARB
 Mobile Phase: Gradient elution from 55/5/40 to 90/5/5 A/B/C from 0 to 4.5 minutes.
 A: Acetonitrile
 B: Tetrahydrofuran
 C: 25mM Ammonium fluoride, 10mM Ammonium acetate, pH 5.6
 Temperature: 80 °C
 Injection Vol.: 10 µl
 Flow rate: 3.0 ml/min.
 Pressure Drop: 195 bar
 Detection: UV at 270 nm

Figure 1 – Structures of steroid-sulfate Conjugates

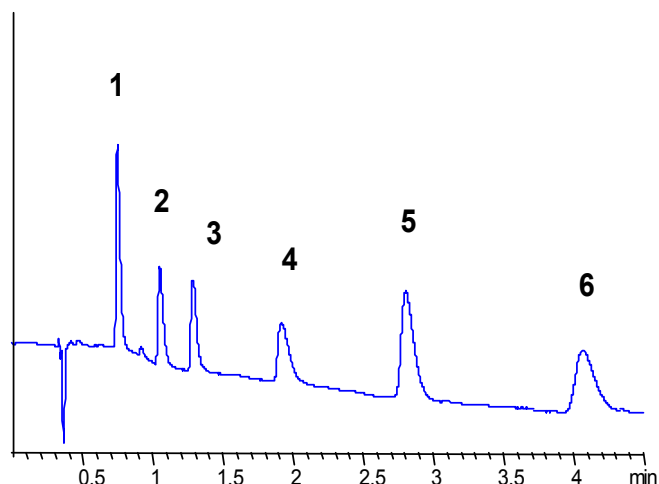
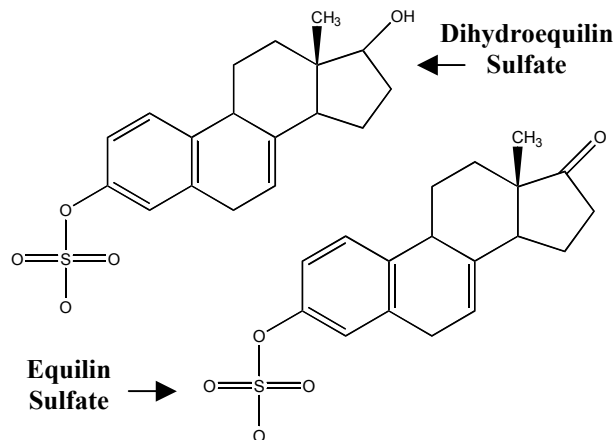


Figure 2: Steroid conjugate selectivity

1=Dihydroestradiol sulfate, 2=Dihydroequilin sulfate, 3=Equilin sulfate, 4=Estrone sulfate, 5=Equilenin sulfate, 6=Dihydroequilenin sulfate

ZirChrom Separations, Inc.
 617 Pierce Street, Anoka, MN 55303
 1-866-STABLE-1
 support@zirchrom.com

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