

Analysis of Catecholamines Using a Core Enhanced Technology Accucore HPLC Column

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Key Words

- Accucore HILIC
- Fused core
- Superficially porous
- Catecholamines
- Hormones
- Neurotransmitters

Abstract

This application note demonstrates the use of the Thermo Scientific Accucore HILIC HPLC column for the fast analysis of seven catecholamine related molecules with excellent peak shapes

Introduction

Accucore™ HPLC columns use core enhanced technology to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The low hydrophobicity of the surface has been optimised for HILIC separations, where an aqueous rich layer of the mobile phase interacts with the surface and the analytes partition between this layer and the organic rich layer of the mobile phase. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

Catecholamines are naturally occurring hormones and neurotransmitters produced by the adrenal glands in response to stress. Their concentration in blood influences heart rate, blood pressure, blood glucose levels and the sympathetic nervous system. High levels are associated with stress, and extremely high levels with trauma, neuroendocrine tumors, amphetamine drugs and neurotransmitter degradation enzyme deficiency. They are derivatives of the amino acid tyrosine. Catecholamine testing can be used in the diagnosis of medical conditions and for monitoring the effectiveness of treatment.

The separation of seven of these compounds in a fast analysis with good peak shape at standard HPLC backpressures is demonstrated in this application.



Sample Preparation

Standard solutions containing 5000 µg/mL of catechol, 5-HIAA, DOPAC, serotonin, and dopamine were each prepared in mobile phase, 2500 µg/mL of L-DOPA was prepared in 1:1 water:mobile phase and a 1000 µg/mL standard solution of L-tyrosine in 4:1 5 % formic acid: mobile phase. These solutions were then mixed and diluted in mobile phase to give a final concentration of 30 µg/mL each.

Thermo Scientific Column	Part Number
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Accucore HILIC 2.6 µm 150 x 4.6 mm	17526-154630
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Measured pressure: 157 bar

Thermo Scientific HPLC system

Column temperature	40 °C
Injection volume	5.0 µL
Flow rate	2.0 mL/min
UV detection	280 nm

Mobile Phase

85:15 Acetonitrile:100mM ammonium formate, pH 3.2

Consumables	Part Number
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Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17
Fisher Scientific ammonium formate	A/5040/53
Fisher Scientific formic acid	F/1900/PB08
Thermo Scientific Premium vial	60180-600

Results

The analysis was carried out on an Accucore HILIC 2.6 μm 150 x 4.6 mm HPLC column. As shown on Figure 1, all seven catecholamine related molecules eluted between 0.74 and 3.20 minutes. Over 6 replicate injections there was no deviation in retention time for any of these molecules and the peak shapes are excellent.

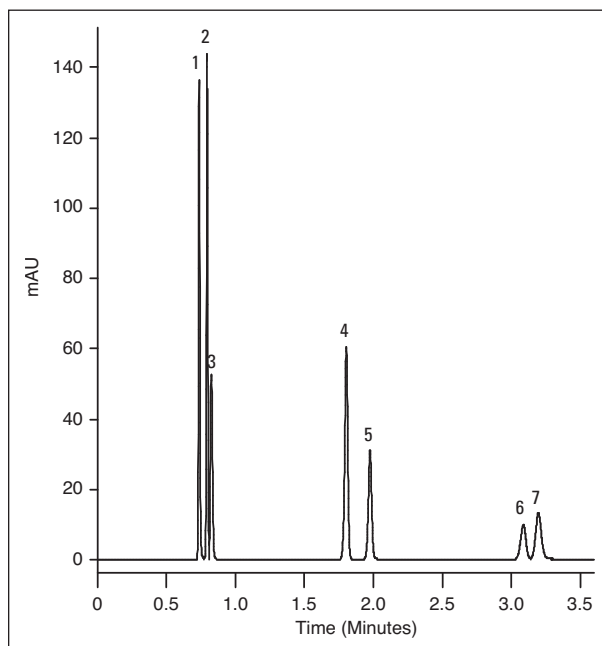


Figure 1: Chromatogram for seven catecholamine related molecules (1. catechol, 2. 5-HIAA, 3. DOPAC, 4. serotonin, 5. L-tyrosine, 6. dopamine, 7. L-DOPA) separated on an Accucore HILIC 2.6 μm 150 x 4.6 mm column

Conclusions

The use of an Accucore HILIC column allowed the separation of seven catecholamine related molecules in less than four minutes and at standard HPLC backpressures. Accucore HILIC columns are therefore an excellent choice for the fast analysis of catecholamines, allowing high sample throughput and analysis on conventional HPLC systems.

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