

Comparison of Epinephrine and Norepinephrine Analysis Using Core Enhanced Technology Accucore HPLC Columns with HILIC and aQ Stationary Phases

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Abstract

This application note demonstrates the use of the Thermo Scientific Accucore aQ and HILIC HPLC columns for the analysis of epinephrine and norepinephrine. While the Accucore™ aQ column shows marginal retention and separation, the Accucore™ HILIC column allows increased retention and full baseline resolution between the two analytes.

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.

Epinephrine (adrenaline) and norepinephrine (noradrenaline) 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 and are important members of the catecholamine family. Catecholamine testing can be used in the diagnosis of medical conditions and for monitoring the effectiveness of treatment.

The separation of epinephrine and norepinephrine in a fast analysis at standard HPLC backpressures is demonstrated in this application and the performance of the Accucore aQ and Accucore HILIC columns are compared.

Sample Preparation

A 500 µg/mL of epinephrine and a 500 µg/mL of norepinephrine standard solutions were prepared in 50:50 methanol:water; these solutions were then mixed and diluted in mobile phase to give a final concentration of 50 µg/mL each.



Key Words

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

Thermo Scientific Column	Part Number
Accucore HILIC 2.6 µm 150 x 4.6 mm	17526-154630
Accucore aQ 2.6 µm 100 x 2.1 mm	17326-102130

Measured pressure: 250 bar (HILIC)
326 bar (aQ)

Thermo Scientific HPLC system

HILIC

Column temperature	40 °C
Injection volume	10.0 µL
Flow rate	3.5 mL/min
UV detection	230 nm
Mobile phase	92:8 acetonitrile:100mM ammonium formate, pH 3.2

aQ

Column temperature	30 °C
Injection volume	2.0 µL
Flow rate	0.6 mL/min
UV detection	230 nm
Mobile phase	5 mM ammonium formate, pH 3.2

Consumables	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17
Fisher Scientific HPLC grade methanol	M/4056/17
Fisher Scientific ammonium formate	A/5080/53
Fisher Scientific formic acid	F/1900/PB08
Thermo Scientific Premium vial	60180-600

Results

Both Accucore aQ and Accucore HILIC HPLC columns separate epinephrine and norepinephrine to different extents in a fast analysis due to their different selectivities. In the case of Accucore HILIC the analytes were retained and fully baseline resolved (epinephrine and norepinephrine eluted at 4.01 min and 4.60 min respectively) while with the Accucore aQ phase the analytes were only marginally separated.

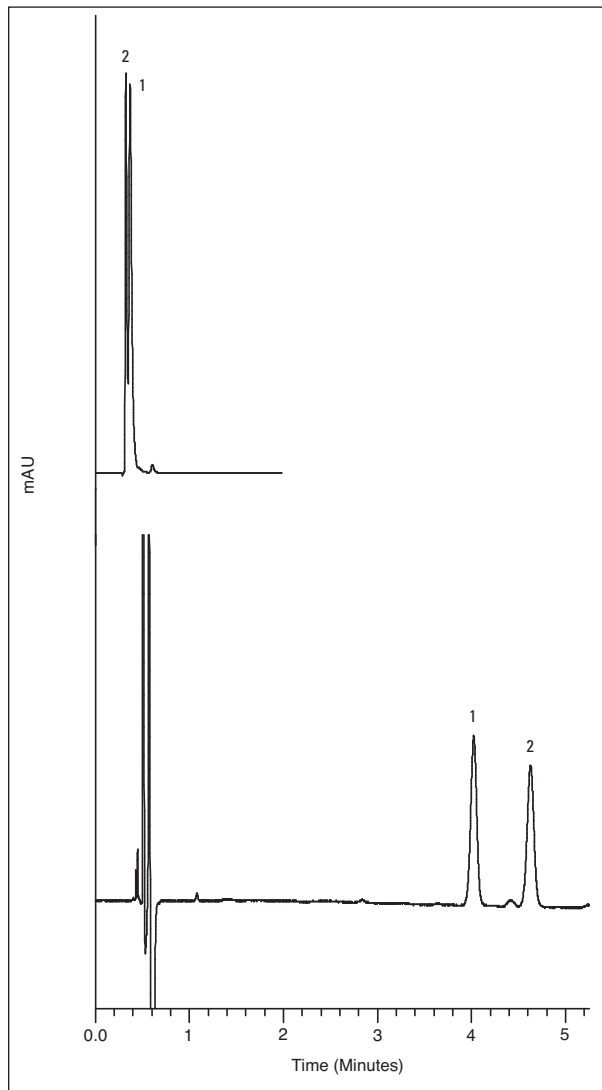


Figure 1: Chromatograms for 1. epinephrine and 2. norepinephrine separated on an Accucore aQ 2.6 μm 100 x 2.1 mm column (upper chromatogram) and an Accucore HILIC 2.6 μm 150 x 4.6 mm column (lower chromatogram)

Conclusions

The partial separation of epinephrine and norepinephrine can be achieved using an Accucore aQ HPLC column, while full baseline resolution was achieved in less than 5 minutes using an Accucore HILIC HPLC column. The back pressure is suitable for use on a conventional HPLC system.

	Epinephrine		Norepinephrine	
Accucore phase	aQ	HILIC	aQ	HILIC
Retention time (min)	0.37	4.01	0.33	4.80

Table 1: Retention times for epinephrine and norepinephrine on Accucore aQ and HILIC columns

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