

# Analysis of $\beta$ -agonists Using a Core Enhanced Technology Accucore Phenyl-Hexyl HPLC Column

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## Abstract

This application note demonstrates the use of the Thermo Scientific Accucore PhenylHexyl HPLC column for the fast chromatographic resolution of a mixture of six  $\beta$ -agonists, using LC/MS.

## Introduction

Accucore™ HPLC columns use Core Enhanced Technology to facilitate fast and high efficiency separations. The 2.6  $\mu$ m diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimised phase bonding creates a series of high coverage, robust phases. The C6 chain in Accucore Phenyl-Hexyl exhibits classical RP retention and selectivity, while the phenyl ring can add enhanced selectivity by interacting with polar groups within the solutes. This results in a mixed-mode separation mechanism. The tightly controlled 2.6  $\mu$ m diameter of Accucore particles provides in much lower backpressures than typically seen with sub-2  $\mu$ m materials.

$\beta$ -adrenergic agonists ( $\beta$ -agonists) are synthetic compounds used therapeutically in human and veterinary medicine to treat pulmonary diseases such as asthma and bronchitis.  $\beta$ -agonists are orally active and when fed at around ten times the therapeutic dose, they are also effective growth promoters, increasing protein deposition and decreasing fat mass. There is a risk that residues of  $\beta$ -agonists may be present in meat products, which may be harmful to the consumer. The use of  $\beta$ -agonists as growth promoters has been banned in most countries. Consequently, it is important that abuse of these compounds is monitored and therefore regulatory agencies require specific and robust methods.

In this application note, a LC/MS method for the separation of the six  $\beta$ -agonists in 6 minutes is demonstrated.



## Sample Preparation

A standard mixture of the six  $\beta$ -agonists in methanol /water (95:5, v/v) at 10  $\mu$ g/mL was diluted ten fold with water.

Thermo Scientific Column	Part Number
Accucore Phenyl-Hexyl 2.6 $\mu$ m 100 x 2.1 mm	17926-102130
Measured pressure: 120 bar (at $t_0$ )	

## Thermo Scientific Accela UHPLC system

Column temperature	40 °C
Injection volume	1.0 $\mu$ L
Flow rate	0.25 mL/min
Mobile phase:	A – Ammonium acetate 5 mM, pH 4; B – ACN
Gradient:	
Time (min)	%B
0	5
1	5
10	100
10.01	5
13	5

## Thermo Scientific MSQ MS system

Detection: +ESI (probe temperature 450 °C, electrospray voltage 4.5 kV, cone voltage 60 V, full scan m/z 150 - 350)

Consumables	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17
Fisher Scientific Optima Acetic acid	A/0415/07
NSC Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap	MSCERT4000-34W

## Key Words

- Accucore Phenyl-Hexyl
- Fused core
- Superficially porous
- $\beta$ -agonists

## Results

Figure 1 shows the extracted mass chromatograms for the six compounds: terbutaline, cimaterol, salbutamol, ractopamine, clorprenaline, and clenbuterol. Good separation is achieved for these compounds although without full baseline resolution. Methodologies for the analysis of residues of these analytes in food products requires MS detection to achieve established LOQs, therefore full chromatographic resolution is not essential.

The C6 chain in the stationary phase provides hydrophobic interactions with the analytes and the electron-rich  $\pi$  bond in the phenyl ring provides interactions with electron deficient polar groups in the analytes.

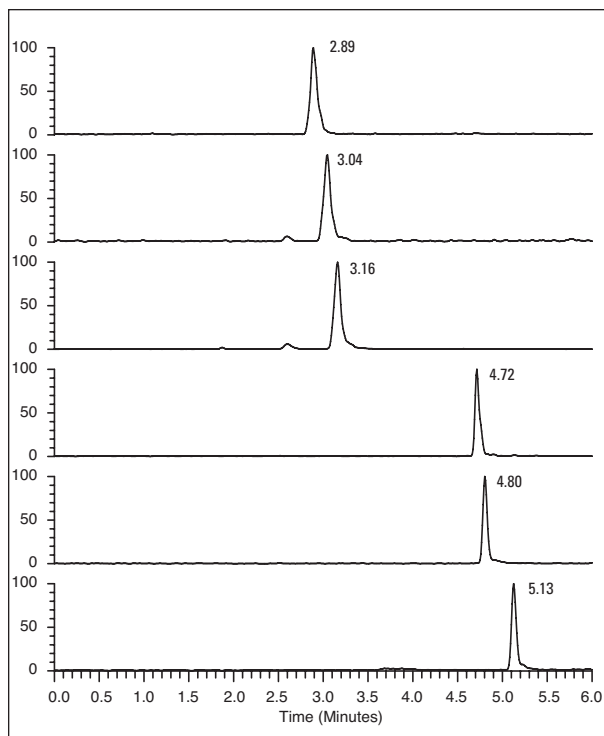


Figure 1: Chromatogram for the six  $\beta$ -agonists separated on the Accucore Phenyl-Hexyl 2.6  $\mu$ m 100 x 2.1 mm column

## Conclusions

The chromatographic separation of six  $\beta$ -agonists in 6 minutes has been achieved using an Accucore Phenyl-Hexyl HPLC column.

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