Application Note: ANCCSCETSULPH

# Analysis of Sulphonamides Using a Core Enhanced Technology Accucore HPLC Column

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**Key Words**

- Sulphonamides
- Peak capacity
- Accucore C18
- Fused core
- Superficially porous
- Core Enhanced Technology

## Abstract

This application note will demonstrate the use of the Thermo Scientific Accucore C18 HPLC column by the separation of five sulphonamides in less than 2 minutes and compare the similarity of the peak capacity achieved to that obtained with a Thermo Scientific Hypersil GOLD 1.9 µm.

## 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 optimised phase bonding creates a series of high coverage, robust phases. The carbon loading of Accucore C18 provides high retention of non-polar analytes via a predominantly hydrophobic interaction mechanism. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

Peak capacity is a measure of the number of peaks that can be successfully resolved within a chromatogram, assuming that each peak is separated by 4σ. It is essentially of theoretical interest, however in this application note we will examine the peak capacity obtained on a fused core product with that obtained using a fully porous sub-2 µm column.

## Results

The analysis was carried out on an Accucore C18 2.6 µm 50 x 2.1 mm column. As shown on Figure 1, sulfamethizole, sulfamonemethoxide, sulfaquinoxaline, sulfamerazine, and sulfathiazole are eluted in less than 2 minutes.

Demonstrated in table 1, there is a 55% reduction in backpressure using Accucore C18 in comparison to Hypersil GOLD® 1.9µm and significantly the average peak capacity for Accucore C18 is comparable to that obtained on Hypersil GOLD 1.9 µm.

The following equation was used to calculate peak capacity: Peak capacity = 1 + ( gradient time in minutes / average peak width)

**Sample Preparation**

Primary standard of sulfamethizole and sulfamerazine at a concentration of 1 mg/mL in acetonitrile.

Primary standard of sulfamethizole and sulfathiazole at a concentration of 1 mg/mL in methanol.

Primary standard of sulfamonemethoxide at a concentration of 2 mg/mL in methanol.

Working standard contained 100 µg/mL of each sulphonamide in 50:50 organic / water

| Thermo Scientific Column | Part Number |
|---|---|
| Accucore C18 2.6 µm 50 x 2.1mm, | 17126-052130 |
| Hypersil GOLDTM 1.9 µm, 50 x 2.1 mm | 25002-052130 |
| Measured pressure: 100 bar | |

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| | |
|---|---|
| Column temperature | 45 °C |
| Injection volume | 1 µL |
| Flow rate | 0.6 mL/min |
| UV detection | 260 nm |

**Mobile Phase**

Mobile phase A: 0.1% formic acid in water

Mobile phase B: 0.1% formic acid in acetonitrile

Gradient: 5-60%B in 2.3 minutes

| Consumables | Part Number |
|---|---|
| Fisher Scientific HPLC grade water | W/0106/17 |
| Fisher Scientific HPLC grade acetonitrile | A/0626/17 |
| Fisher Scientific Analytical grade formic acid | F/1900/PB08 |
| NSC Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap | MSCERT4000-34W |

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## Conclusions

The use of Accucore C18 column allowed to successfully separate five sulphonamides in less than 2 minutes, with comparable peak capacity to Hypersil GOLD 1.9 µm and at a significantly lower back pressure. Accucore C18 columns are therefore an excellent choice for fast analysis allowing high sample throughput.

| | Hypersil GOLD 1.9 µm | Accucore C18 2.6 µm |
|---|---|---|
| Average peak width at 10% | 0.03 | 0.04 |
| Gradient time | 2.33 | 2.33 |
| PEAK CAPACITY | 71 | 67 |
| Back pressure (bar) | 220 | 100 |

Table 1. Results obtained from Accucore C18 and Hypersil GOLD 1.9µm.

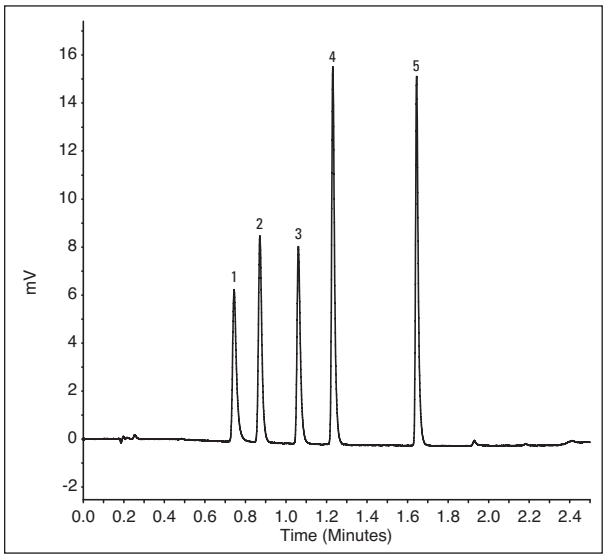


Figure 1: Chromatogram for
1. sulfamethizole,
2. sulfamonemethoxide,
3. sulfaquinoxaline,
4. sulfamerazine and
5. sulfathiazole separated on an Accucore C18 2.6 µm 50 x 2.1 mm column

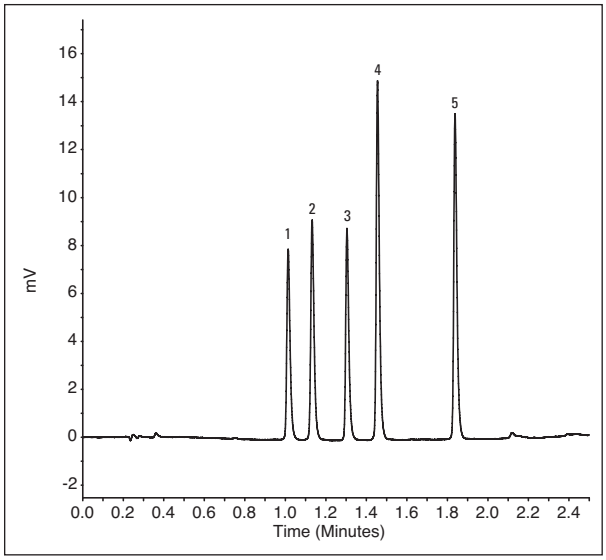


Figure 2: Chromatogram for
1. sulfamethizole,
2. sulfamonemethoxide,
3. sulfaquinoxaline,
4. sulfamerazine, and
5. sulfathiazole separated on an Hypersil GOLD 1.9 µm 50 x 2.1 mm column

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