

# Analysis of Catechins Using a Thermo Scientific Accucore XL C8 4 $\mu$ m HPLC Column

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## Key Words

Accucore XL C8, solid core, superficially porous, catechins, large solid core particle

## Abstract

This application note compares the performance of a solid core Accucore™ XL C8 4  $\mu$ m HPLC column with that of a fully porous 5  $\mu$ m traditional HPLC column for the analysis of catechins under gradient mobile phase conditions.

## Introduction

Based on Core Enhanced Technology™ using 4  $\mu$ m solid core particles, Accucore XL HPLC columns allow users of conventional HPLC methods to enjoy performance far beyond that of columns packed with 5  $\mu$ m or even 3  $\mu$ m fully porous particles. Very high separation efficiencies using standard HPLC instruments and conditions provide increased peak resolution and lower limits of detection. An ultra-stable packed bed results in exceptionally robust columns that demonstrate excellent retention and response reproducibility.

Catechins are found in many food products, particularly in wine and tea. They act as antioxidants, have anti-inflammatory, anti-carcinogenic and anti-mutagenic properties.<sup>1,2</sup> Some catechins exhibit antimicrobial characteristics and prove beneficial in applications such as prevention and treatment of infections and the promotion of oral and digestive health.<sup>3,4</sup>

Since catechins are structurally similar, their analysis relies on high peak resolution. The method described in this application note demonstrates improved chromatographic performance in the separation of seven catechins on an Accucore XL column in relation to a fully porous traditional HPLC column, under the same experimental conditions.



## Experimental Details

| Consumables                                                                                                                 | Part Number  |
|-----------------------------------------------------------------------------------------------------------------------------|--------------|
| Fisher Scientific formic acid (AR grade)                                                                                    | F/1900/PB08  |
| Fisher Scientific methanol (HPLC grade)                                                                                     | M/4056/17    |
| Fisher Scientific water (HPLC grade)                                                                                        | W/0106/17    |
| Thermo Scientific Finnpiptette F2 pipettor kit                                                                              | PMP-020-220F |
| Thermo Scientific Finn tip pipette tips, 200 $\mu$ L                                                                        | PMP-107-600F |
| Thermo Scientific Finn tip pipette tips, 1000 $\mu$ L                                                                       | PMP-103-206K |
| Thermo Scientific Borosilicate glass vials (2 mL, 12 mm x 32 mm) with 8 mm black screw cap fitted with a silicone/PTFE seal | 60180-600    |

## Sample Preparation

A mixed working standard for epigallocatechin catechin (200 µg/mL), epicatechin (100 µg/mL), epigallocatechin gallate (100 µg/mL), epicatechin(100 µg/mL), galocatechin gallate (100 µg/mL), epicatechin gallate (200 µg/mL) and catechin gallate (100 µg/mL) was prepared in 1:1 v/v methanol and water.

## Separation Conditions

## Part Number

|                     |                                                                                                 |              |
|---------------------|-------------------------------------------------------------------------------------------------|--------------|
| Instrumentation:    | Thermo Scientific UHPLC Accela 600 system equipped with a PDA detector and CTC open autosampler |              |
| Columns:            | Thermo Scientific Accucore XL C8 4 µm, 150 x 4.6 mm<br>Fully porous C8 5 µm, 150 x 4.6 mm       | 74204-154630 |
| Mobile phase:       | A: water + 0.1% formic acid<br>B: methanol + 0.1% formic acid                                   |              |
| Gradient:           | Time (min)                                                                                      | % B          |
|                     | 0.0                                                                                             | 20           |
|                     | 15.00                                                                                           | 50           |
|                     | 15.10                                                                                           | 20           |
|                     | 20.00                                                                                           | 20           |
| Flow rate:          | 1 mL/min                                                                                        |              |
| Column temperature: | 25 °C                                                                                           |              |
| Detection:          | UV at 280 nm                                                                                    |              |
| Injection volume:   | 5 µL                                                                                            |              |

## Data Processing

Data acquired and processed using Thermo Scientific ChromQuest 4.2.3 software

## Results

Under the conditions adopted for this analysis, resolution of greater than 2.6 was achieved for seven catechins on the Accucore XL C8 4 µm HPLC column (Figure 1). On the fully porous C8, resolution between the critical pair (peaks 4 and 5) was 1.17, this improved by 129% with the 4 µm Accucore XL C8 column.

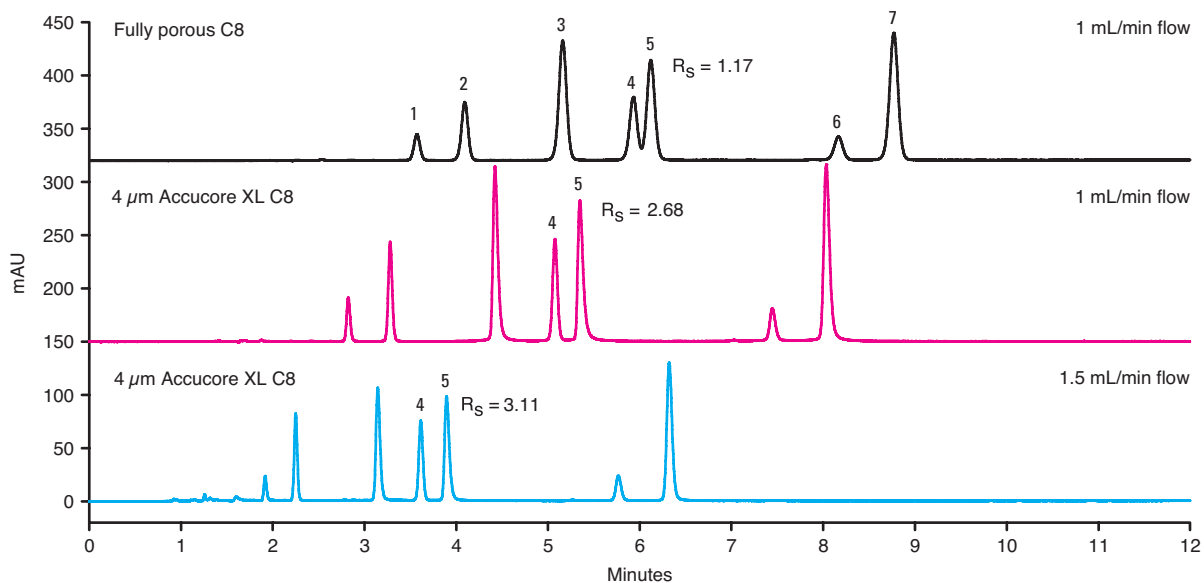


Figure 1: Chromatograms for the seven catechins analyzed on a fully porous C8 and Accucore XL C8 4 µm HPLC column

At higher flow rate the Accucore XL C8 4  $\mu\text{m}$  HPLC column gave even better resolution for the critical pair (3.11) and a reduced analysis time (10 minutes on the fully porous column at 1 mL/min as compared to 7 minutes on the Accucore XL C8 4  $\mu\text{m}$  HPLC column at 1.5 mL/min). Peak widths for catechins improved by 34% (based on average peak width) when changing from the 5  $\mu\text{m}$  fully porous column to the Accucore XL C8 4  $\mu\text{m}$  HPLC column (Table 1).

| Compound                           | Peak Width         |                                            |                      |
|------------------------------------|--------------------|--------------------------------------------|----------------------|
|                                    | Fully Porous       | Accucore XL C8 4 $\mu\text{m}$ HPLC column |                      |
|                                    | 1 mL/min flow rate | 1 mL/min flow rate                         | 1.5 mL/min flow rate |
| <b>1. Epigallocatechin</b>         | 0.157              | 0.094                                      | 0.075                |
| <b>2. Catechin</b>                 | 0.173              | 0.101                                      | 0.080                |
| <b>3. Epigallocatechin gallate</b> | 0.201              | 0.132                                      | 0.108                |
| <b>4. Epicatechin</b>              | Partial co-elution | 0.124                                      | 0.106                |
| <b>5. Gallic acid</b>              |                    | 0.149                                      | 0.124                |
| <b>6. Epicatechin gallate</b>      | 0.228              | 0.151                                      | 0.136                |
| <b>7. Catechin gallate</b>         | 0.227              | 0.158                                      | 0.139                |

Table 1: Peak width at 5% height for the seven catechins

The backpressure for Accucore XL C8 4  $\mu\text{m}$  HPLC column was measured at 241 bar and the 5  $\mu\text{m}$  fully porous column backpressure was measured at 182 bar at 1 mL/min flow rate with 1:1 v/v methanol/water mobile phase. Increasing the flow rate to 1.5 mL/min the backpressure was measured at 325 bar on an Accucore XL C8 4  $\mu\text{m}$  HPLC column. The increase in performance is gained with an increase in backpressure which is still within the operating limits of a conventional HPLC system.

In addition six replicate injections illustrate excellent retention time reproducibility for all seven catechins (Table 2).

| Compound                           | $T_R$ /min (%RSD n=6) |            |                       |            |                       |            |
|------------------------------------|-----------------------|------------|-----------------------|------------|-----------------------|------------|
|                                    | 1 mL/min Flow Rate    |            |                       |            | 1.5 mL/min Flow Rate  |            |
|                                    | Fully Porous          | %RSD (n=6) | Accucore XL LC Column | %RSD (n=6) | Accucore XL LC Column | %RSD (n=6) |
| <b>1. Epigallocatechin</b>         | 3.57                  | 0.33       | 2.82                  | 0.23       | 1.91                  | 0.30       |
| <b>2. Catechin</b>                 | 4.09                  | 0.28       | 3.28                  | 0.22       | 2.24                  | 0.25       |
| <b>3. Epigallocatechin gallate</b> | 5.16                  | 0.28       | 4.42                  | 0.10       | 3.14                  | 0.12       |
| <b>4. Epicatechin</b>              | 5.93                  | 0.20       | 5.08                  | 0.09       | 3.62                  | 0.20       |
| <b>5. Gallic acid</b>              | 6.12                  | 0.20       | 5.35                  | 0.11       | 3.90                  | 0.25       |
| <b>6. Epicatechin gallate</b>      | 8.17                  | 0.16       | 7.44                  | 0.10       | 5.75                  | 0.19       |
| <b>7. Catechin gallate</b>         | 8.77                  | 0.18       | 8.02                  | 0.10       | 6.30                  | 0.19       |

Table 2: Retention time and precision data from six replicate injections

## Conclusion

The use of an Accucore XL C8 4  $\mu\text{m}$  HPLC column gave significant performance improvement over a conventional 5  $\mu\text{m}$  fully porous column under the same chromatographic conditions with no changes in system configuration. Resolution of the critical pair improved by 129% and peak width was reduced by 34% (based on average peak width). Further reduction in run time and an improvement in resolution were observed when flow rate was increased by 50%. Data on the Accucore XL C8 4  $\mu\text{m}$  HPLC column shows excellent retention time reproducibility at both 1 mL/min and 1.5 mL/min.

## References

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