

Separation of Curcuminoids from Turmeric – Comparison of Polar Embedded and C18 Solid HPLC Core Columns

Mark Tracy, Thermo Fisher Scientific, Sunnyvale, CA, USA

Key Words

Accucore Polar Premium, turmeric, curcumin, solid core, Core Enhanced Technology

Abstract

Turmeric is a popular spice, coloring agent, and ingredient in traditional Ayurvedic medicine made from the root of *Curcuma Longa*. Its chief bioactive components are the brilliant yellow pigments curcumin, desmethoxycurcumin, and bis-desmethoxycurcumin, along with other minor curcuminoids. The Thermo Scientific™ Accucore™ Polar Premium column has a polar-embedded C18 surface on a superficially porous silica substrate. This chemistry provides a selectivity that resolves the major and minor components under simple isocratic conditions in about three minutes, while C18 columns fail to separate these components.

Introduction

Turmeric, the ground dried root of *Curcuma Longa*, is a popular seasoning and coloring for foods. It is used in traditional Ayurvedic medicine, and various health benefits have been claimed for it [1]. Turmeric is sometimes used to adulterate the much more expensive saffron [2]. Curcumin (Figure 1) and its de-methoxylated congeners are the primary bio-active principles and the markers used for assay. Purified curcumin is also used as an analytical reagent for borate [3]. Therefore, there are several situations where analysis for curcuminoids is needed. In many cases, HPLC analysis using a C18 column is the default for starting method development. While C18 is satisfactory for many cases, the alternate selectivity of polar-embedded stationary phases such as the Accucore Polar Premium phase may provide a superior solution. Solid-core particle technology is the state of the art in modern high-performance columns.



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 instead have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. The tightly controlled 2.6 μm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 μm materials. This work will compare a competitor's solid-core C18 column against the Accucore Polar Premium column to demonstrate the use of alternate selectivity to solve separation problems.

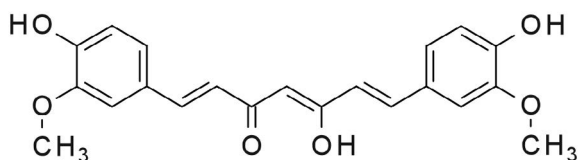


Figure 1: Structure of curcumin

Experimental Details

Consumables	Part Number
Methanol, HPLC grade, Fisher Scientific™	A454
Acetonitrile, Optima™ LC/MS grade, Fisher Scientific	A996
Ethanol, denatured 3A	
Phosphoric acid, 85%, HPLC grade	
Formic acid, 98%	
Water, HPLC grade	

Sample Handling Equipment	Part Number
Thermo Scientific membrane filters: 0.2 µm nylon, 17 mm dia. syringe-tip filter	60183-212

Sample Preparation	
Compound(s):	Curcumin; desmethoxycurcumin; bis-desmethoxycurcumin
Matrix:	Ground turmeric obtained from a local food market
	Extract 100 mg ground, dried turmeric root with 20 mL of 70% ethanol in a screw-cap vial at 80 °C until all color is extracted from the matrix. Centrifuge and filter an aliquot through a membrane filter. Dilute a filtered aliquot 10x with methanol.

Separation Conditions	Part Number
Instrumentation:	Thermo Scientific™ Dionex™ UltiMate™ 3000RS HPLC system consisting of LPG-3400RS pump, WPS-3000RS split-loop sampler, TCC-3100RS column oven, and DAD-3000RS diode-array detector
Columns:	Accucore Polar Premium, 2.6 µm, 100 × 3.0 mm 28026-103030 Competitor's solid-core C18, 2.7 µm, 100 × 3.0 mm
Mobile phase A:	Methanol
Mobile phase B:	10 mM phosphoric acid
Method:	Isocratic 80:20 A/B (v/v)
Flow rate:	0.80 mL/min
Run time:	3.0 min
Column temperature:	40 °C
Injection details:	6 µL
UV/Vis detector wavelength:	428 nm; Diode array scan 200–500 nm; 20 Hz data rate; 0.2 s time constant

Data Processing	
Software:	Thermo Scientific™ Chromeleon™ 6.8

Results

While developing this method, two columns, a C18 and the Accucore Polar Premium, and two solvents, methanol and acetonitrile, were compared. The Accucore Polar Premium column resolved the major and minor curcuminoids completely in the same elution order using either 80% methanol or 70% acetonitrile. The C18 column could provide partial resolution of the major curcuminoids using 60% methanol or 50% acetonitrile. Typical chromatograms are shown in Figure 2. Notice the different elution orders. Using the C18 column under the conditions optimized for the Accucore Polar Premium column, the three components co-elute. The efficiency of the curcuminoid peaks was unexpectedly low for both columns, possibly due to keto-enol tautomerism. These peaks had acceptable symmetry ($A_s = 1.15$) for the Accucore Polar Premium column, but tailing was severe ($A_s > 1.8$) for the C18 column. These data emphasize the importance of column selectivity in this application.

Methanol and acetonitrile both produced good resolution and peak shape with the Accucore Polar Premium column, but methanol was selected for economy. While formic and phosphoric acids are both effective, phosphoric is cheaper to use; on the other hand, formic acid is compatible with LC/MS interfaces. Figure 3 shows the major and minor curcuminoids resolved under optimized conditions using the Accucore Polar Premium column.

Situations similar to that described here often occur in HPLC analysis of natural products where related components co-elute. Analysis using a column with alternate selectivity increases the confidence that the separation is complete and quantitative.

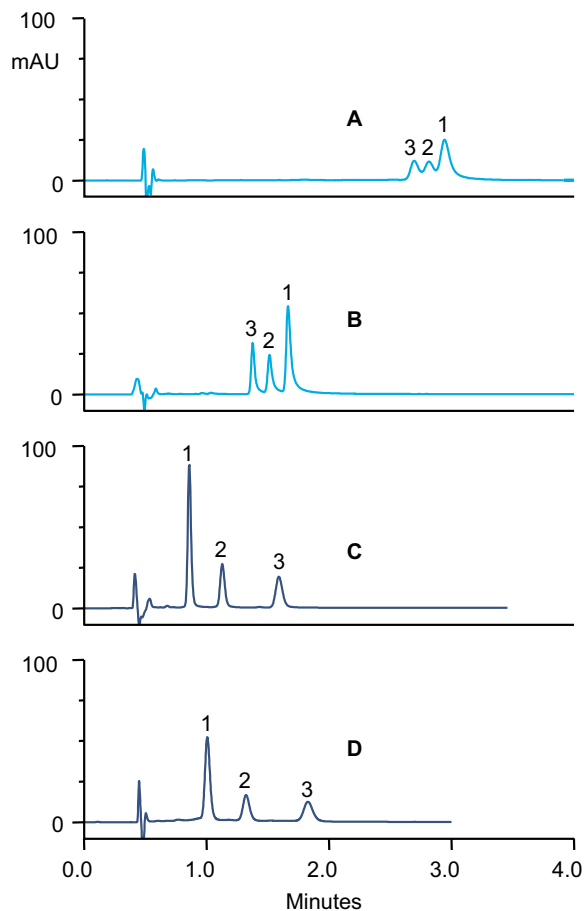


Figure 2: Comparison of C18 column versus Accucore Polar Premium column, and methanol versus acetonitrile
Peaks: 1. Curcumin; 2. Desmethoxycurcumin;
3. Bis-desmethoxycurcumin

A: C18 column; Methanol / water / 100 mM formic acid (60:20:20 v/v/v)
B: C18 column; Acetonitrile / water / 100 mM formic acid (50:30:20 v/v/v)
C: Polar Premium column; Acetonitrile / water / 100 mM formic acid (70:10:20 v/v/v)
D: Polar Premium column; Methanol / water / 100 mM formic acid (80:0:20 v/v/v)

Other conditions as described above

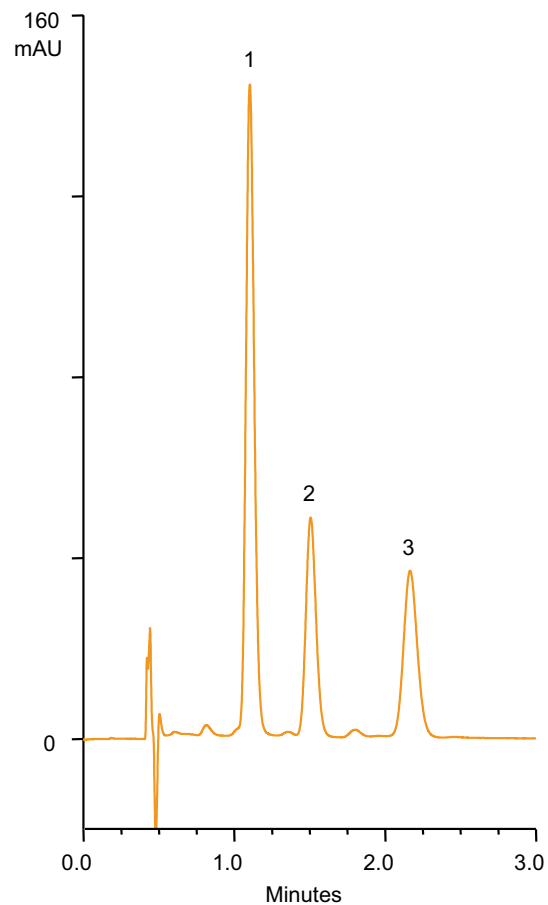


Figure 3: Turmeric extract analyzed using optimized conditions
Peaks: 1. Curcumin; 2. Desmethoxycurcumin;
3. Bis-desmethoxycurcumin

Column: Accucore Polar Premium, 2.6 μ m, 100 x 3.0 mm
Mobile phase: Methanol / 10 mM phosphoric acid (80:20 v/v) at 0.80 mL/min

Other conditions as described above

Conclusion

- Even high-performance C18 columns do not adequately separate the natural curcuminoids.
- Accucore Polar Premium columns have the right selectivity for a simple isocratic separation of major and minor curcuminoids found in turmeric.

References

[1] <http://en.wikipedia.org/wiki/Turmeric>, Retrieved 2-Apr-2013.

[2] <http://homecooking.about.com/od/spices/f/turmericfaq.htm?p=1>, Retrieved 2-Apr-2013.

[3] <http://en.wikipedia.org/wiki/Curcumin>, Retrieved 2-Apr-2013.

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