APPLICATION NOTE

Liquid Chromatography

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UHPLC-PDA Analysis of Curcuma Using a Quasar SPP Column

Introduction

Turmeric is the powdered dry rhizome of the plant Curcuma longa L., and is used as a coloring agent to give a yellow-orange color to food dishes. It has been found to be a rich source of phenolic compounds, namely, three curcuminoids: curcumin (C), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC).[1] Commercially available curcumin consists of a mixture of these naturally occurring curcuminoids, with curcumin as the main (≈77%) constituent, followed by demethoxycurcumin (≈13%), and bisdemethoxycurcumin (≈3%).[2]

The structures for curcumin, demethoxycurcumin, and bisdemethoxycurcumin are shown in Figure 1.

Research to date suggests that turmeric, in addition to having an immunomodulatory role, is also of use in preventing oxidative stress that can lead to inflammation, cancer, and arthritis. Curcumin is now recognized as being responsible for most of the therapeutic effects associated with consuming tumeric.[3] In recent times, there has been great interest in transforming curcumin into a drug candidate with prospective multipotent therapeutic applications.[4]

This application note describes the sample preparation and analytical method for the chromatographic separation and quantitative monitoring of the three curcuminoids in commercially available turmeric spices and roots by UHPLC, using photodiode array (PDA) detection.

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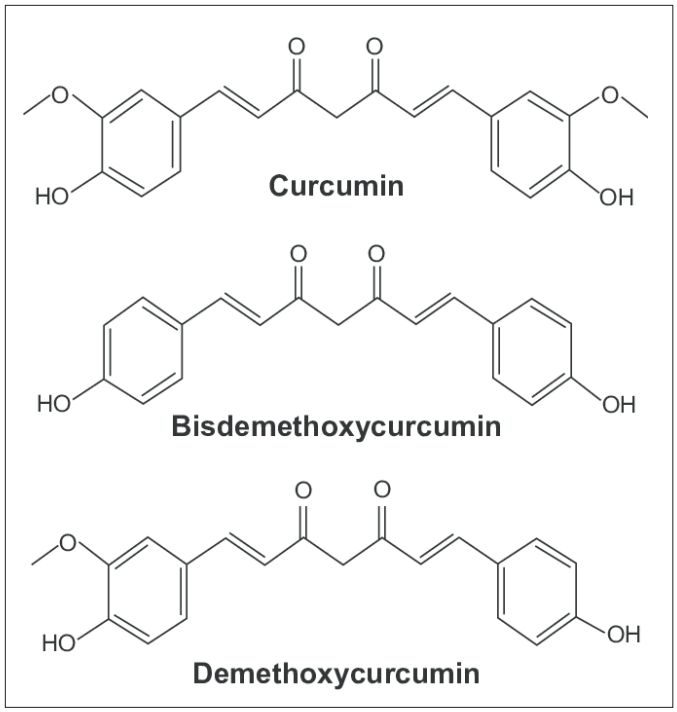

Figure 1. Chemical structure of curcumin, bisdemethoxycurcumin and demethoxycurcumin.

Experimental

Hardware/Software

A PerkinElmer Flexar™ UHPLC system was utilized in this work, configured with a binary pump, autosampler with Peltier cooling, column heater, and PDA Plus (photodiode array) detector with 10-mm and 50-mm flow cells. A PerkinElmer Quasar™ SPP C18, 2.7 µm, 4.6 x 100mm column was used for all analyses (PerkinElmer, Shelton, CT, USA). All instrument control, data analysis and processing was performed using PerkinElmer Chromera™ CDS software.

Method Parameters

All UHPLC method parameters are shown in Table 1.

Solvents and Standards

The solvents and diluents used in this work were HPLC grade. All standard and sample extract dilutions were prepared using 50:50 acetonitrile/water. A standard mix containing all three curcuminoids was prepared in acetonitrile. This standard mix contained 64 µg/mL (ppm) curcumin, 19.2 µg/mL demethoxycurcumin and 6.4 µg/mL bisdemethoxycurcumin. For calibrants, the mix was serially diluted to six concentration levels between 1 - 32 ppm for curcumin, 0.3 - 9.6 ppm for demethoxycurcumin and 0.1 - 3.2 ppm for bisdemethoxycurcumin to reflect the varying natural distribution of curcuminoids.

Sample Prepration

Four samples, including two commercially available spices, one purified powder sample and a finely ground dried rhizome, were prepared by weighing 30 mg of each sample, and dissolving it in 50 ml of acetonitrile to yield a concentration of approximately 0.6 mg/mL. Samples were then shaken for 1 min, followed by ultrasonication for 15 min. All prepared samples were subsequently filtered through 0.45 µm filters to remove any insoluble parts. Thereupon, the resulting sample extracts were further diluted, where necessary, and injected.

Table 1. UHPLC Method Parameters.

Column	PerkinElmer Quasar SPP C18, 2.7 µm, 4.6 x 100 mm (Part# N9306880)
Mobile Phase	Solvent A: Water with 2% acetic acid Solvent B: Acetonitrile Solvent Program:

Step	Step Time (min.)	Flow Rate (mL/min.)	%A	%B
0 (Equil.)	5	1.0	70.0	30.0
1	13	1.0	30.0	70.0
2	2	1.0	30.0	70.0

Analysis Time	15 min., equilibration time: 5.0 min.
Flow Rate	1.0 mL/min
Oven Temperature	30 °C
PDA Detection	Wavelength: 425 nm
Injection Volume	3 µL
Sampling (Data) Rate	5 pts./sec
Diluent	50:50 water/acetonitrile
Analytes	1. Curcumin 2. Bisdemethoxycurcumin 3. Demethoxycurcumin

Results

Figure 2 shows the chromatogram of the separation of the three curcuminoids, all well resolved from each other, as well as the analysis of individual standards for identification purposes. Chromatographic repeatability was found to be excellent, with RSD values for six replicates of the L3 standard (8 µg/mL C, 1.6 µg/mL DMC, 0.4 µg/mL BDMC) between 2-4%.

Six-level calibration fits were determined for all three curcuminoids. Representative linearity plots for BDMC, DMC and C are shown in Figure 3. The results reflect the averaged triplicate injections for all calibrants. The R^2 values for all three curcuminoids were above 0.999 (origin included and 1/x weighing factor).

LOQ (limit of quantitation) levels were established for each curcuminoid, based upon their averaged L1 calibration standard response (representative L1 chromatogram is shown in Figure 4). The calculated LOQs (≥10 S/N) are 0.04 µg/mL for all three curcuminoids.

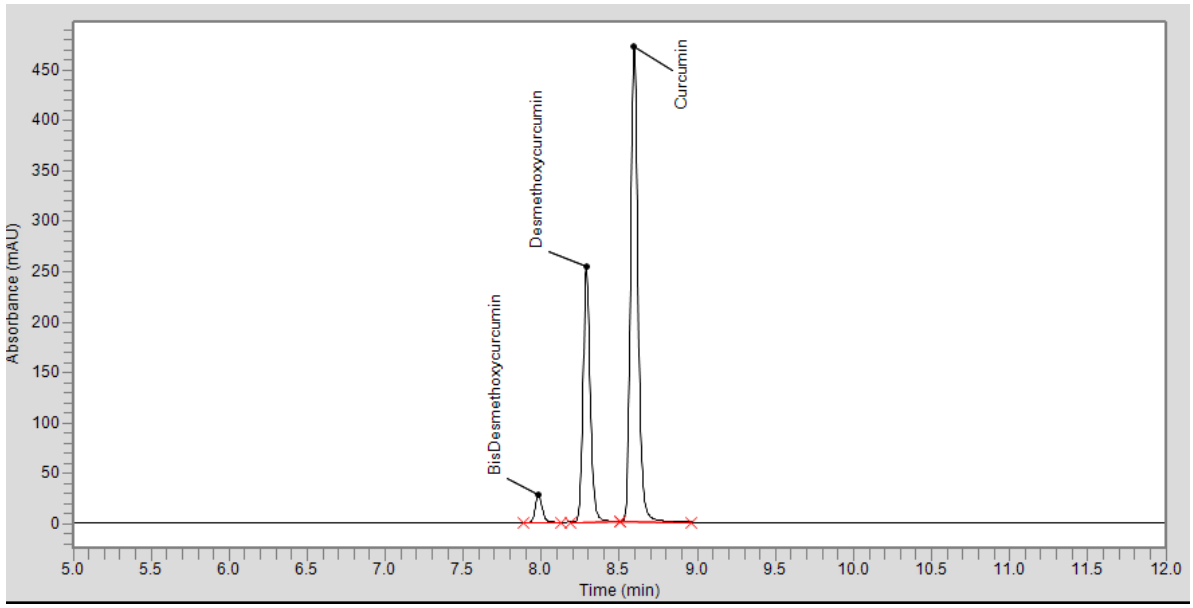

Figure 2. HPLC chromatogram showing the separation of standard mixture Bisdemethoxycurcumin (BDMC), Demethoxycurcumin (DMC) and Curcumin (C), as well as analysis of individual standards; λ = 425 nm.

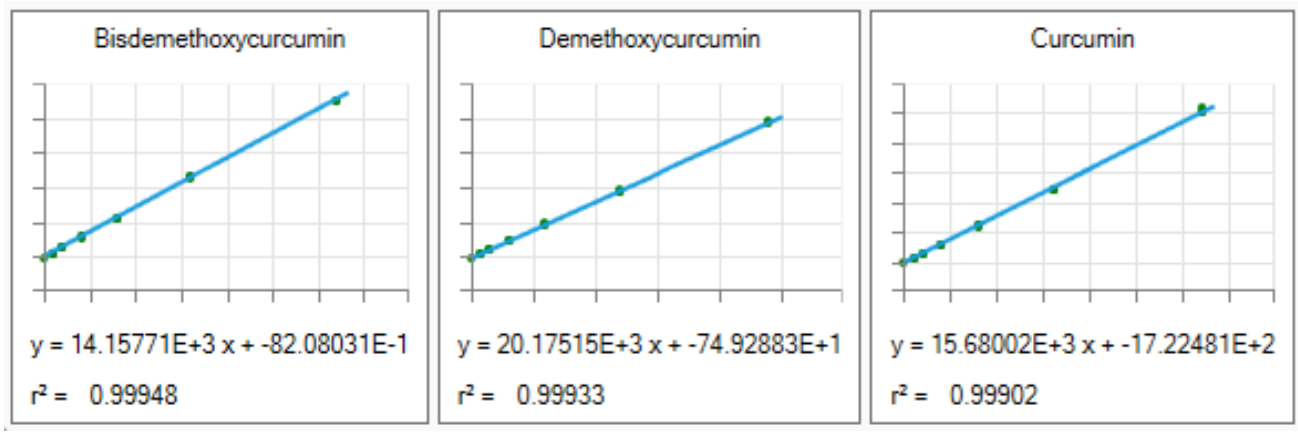

Figure 3. Linearity plots for Bisdemethoxycurcumin, Demethoxycurcumin and Curcumin, analyzed in triplicate, concentration range: 0.1 - 3.2 µg/mL for bisdemethoxycurcumin, 0.3 - 9.6 µg/mL for demethoxycurcumin and 1 - 32 µg/mL for curcumin.

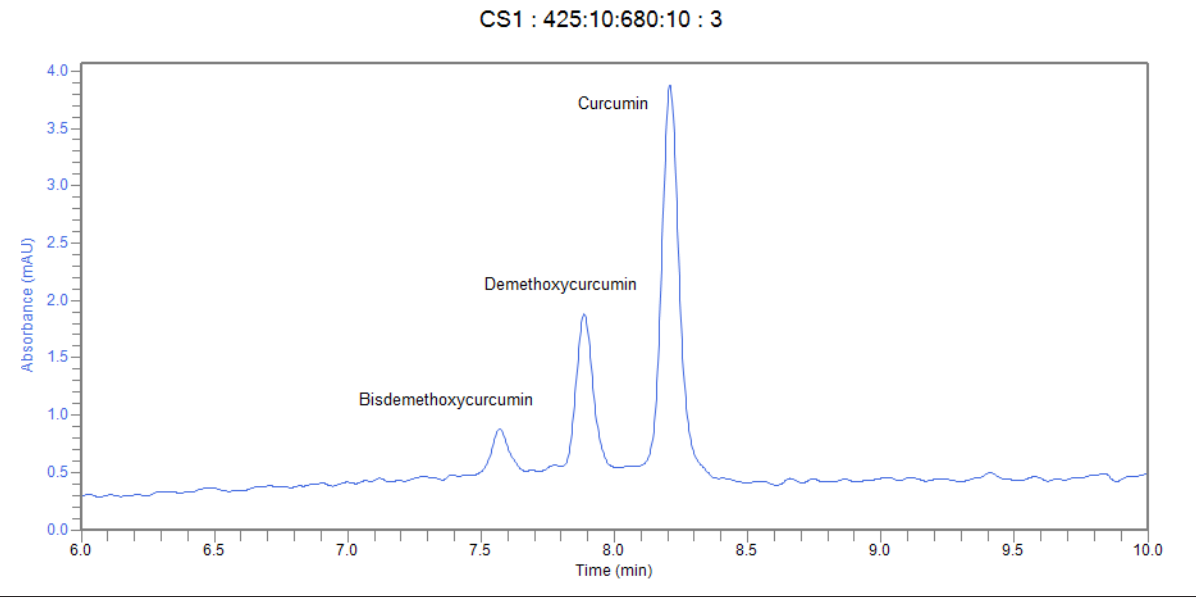

Figure 4. Chromatogram of the L1 standard (1 µg/mL C, 0.3 µg/mL DMC, 0.1 µg/mL BDMC).

It should be noted that, moving forward, if even lower LOQs are required, the Flexar PDA Plus Detector's optional 50-mm flow cell would allow for this, as presented in Figure 5. The 50 mm flow cell provides up to five times more sensitivity than the 10 mm path length flow cell. Thus, both samples with lower levels of analyte concentration and relatively high analyte amounts can be measured with the same detector by simply exchanging the flow cell in a virtually effortless single motion.

Further, the use of a PDA detector gives confidence in results, and allows for the examination of the measured spectra post-analysis to confirm the chosen wavelength, as illustrated in Figure 6.

To check for possible analyte carryover or background interference, a 50:50 water/acetonitrile "blank" was run, both after the calibration set, and after the samples. In all cases, no carryover was observed for any of the analytes.

No discernable peaks were found within the region in which the curcuminoids eluted.

Results of the commercially available samples are summarized in Table 2, while Figure 7 and 8 represent the chromatograms of two analyzed samples, one containing all three curcuminoids, while the other only curcumin, most likely due to a purification step.

Table 2. Summary of results of Bisdemethoxycurcumin (BDMC), Demethoxycurcumin (DMC) and Curcumin (C).

Sample	BDMC (µg/g)	DMC (µg/g)	C (µg/g)	Total Curcuminoids (µg/g)
Tumeric Powder 1	0.41	0.40	1.43	2.24
Tumeric Powder 2	0.33	0.37	1.64	2.34
Dried rhizome	0.32	0.25	0.81	1.38
Purified Powder (Diluted 1:100)	-	-	102.48	102.48

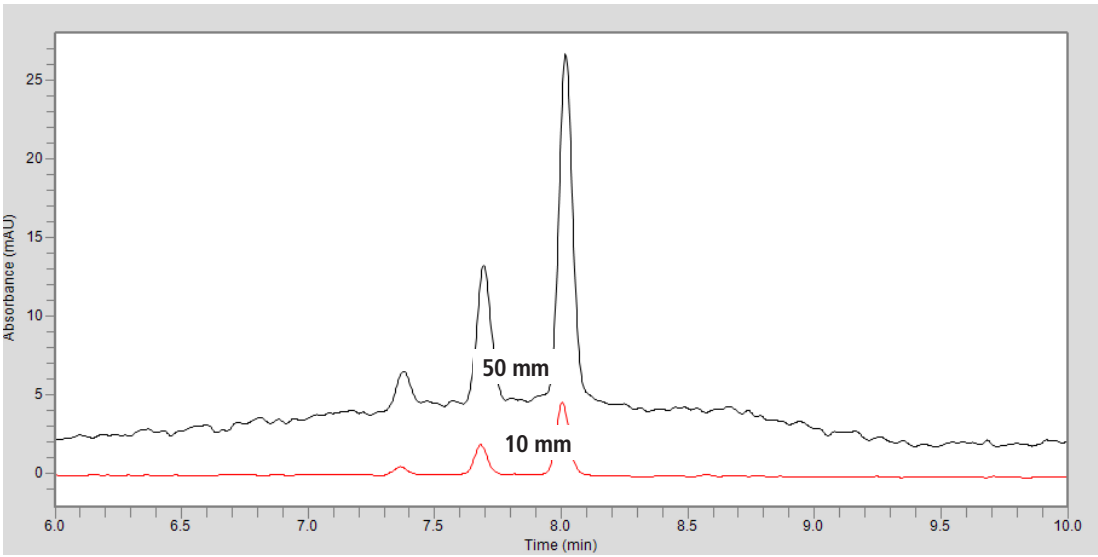

Figure 5. Comparison of L1 chromatogram for the curcuminoids of interest, measured with the standard 10-mm flow cell and optional 50-mm flow cell for increased sensitivity (around factor 5).

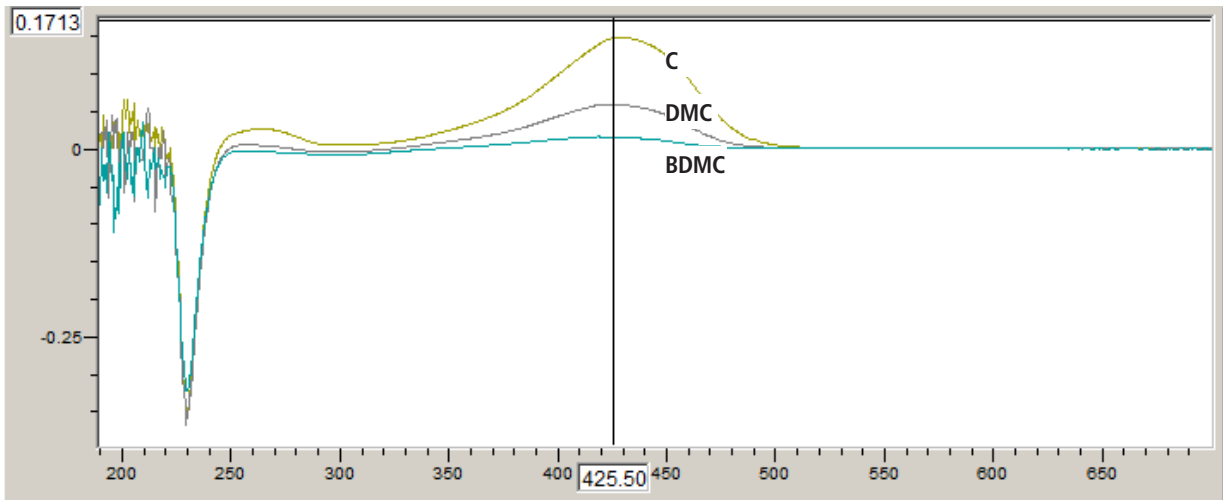

Figure 6. Comparison of spectrum for the three curcuminoids, with an optimal wavelength of around 425 nm for all three compounds

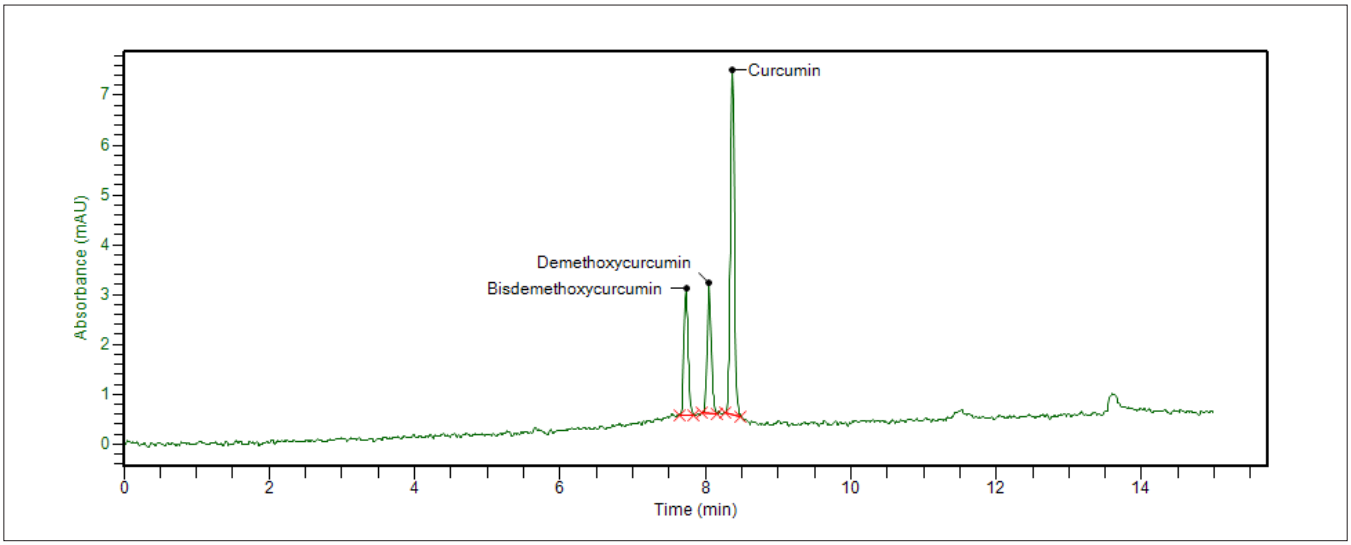

Figure 7. Example of turmeric powder 2, containing all three curcuminoids at a total amount of 2.34 µg/g (0.33 µg/g BDMC; 0.37 µg/g DMC and 1.64 µg/g C).

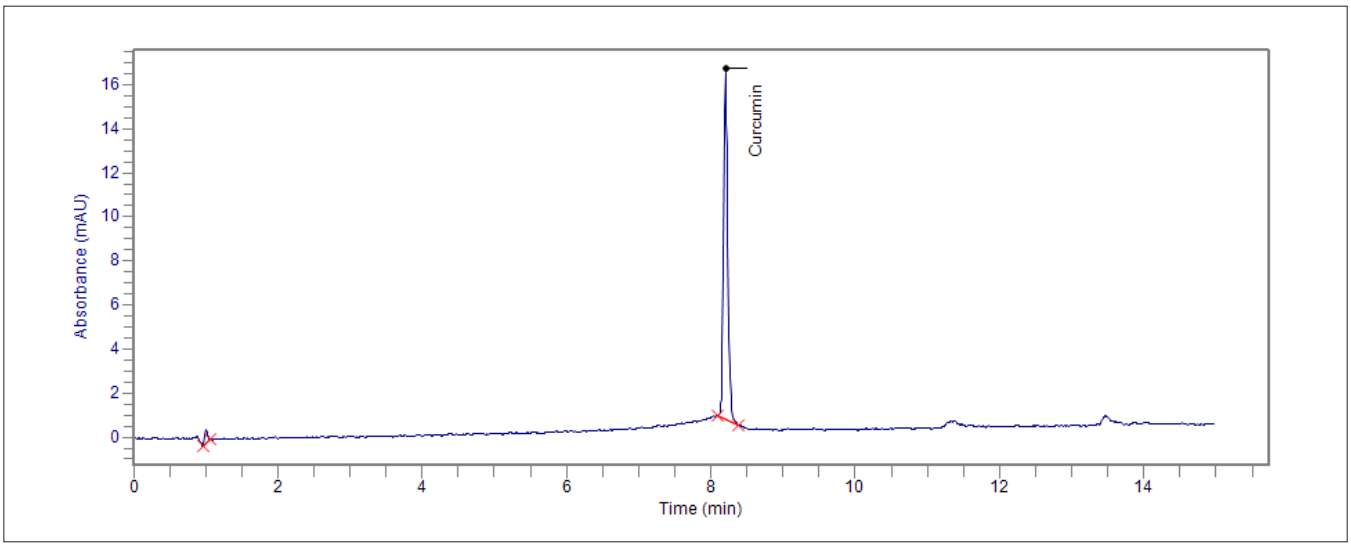

Figure 8. Example of a purified turmeric powder extract, containing only curcumin (diluted 1:100) at 102.48 µg/g.

Conclusions

- The Flexar PDA Plus Detector gives confidence in results, and measures at a high data acquisition rate (up to 200 Hz) with a linear and highly reproducible response.
- The modular flow cell design allows for easy exchange of the flow cell, and measurement of a wide range of concentrations in samples, exhibiting superior sensitivity with the 50 mm flow cell, and omitting the use of different types of detectors.
- The Quasar SPP HPLC phase offers highly efficient separation of the natural curcuminoid compounds (curcumin, bisdemethoxycurcumin and demethoxycurcumin).

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References

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