Rapid Analysis of Curcuminoids in Turmeric Extract Using the Agilent 1290 Infinity LC and STM Columns

Application Note

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Abstract

A method has been developed for the separation of curcuminoids in turmeric using an Agilent 1290 Infinity LC and STM column. This method is proven to provide rapid cycle times with good resolution, over the traditional USP method.

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Introduction

Turmeric is derived from the plant *Curcuma Longa*, a member of the ginger family. Curcumin is a natural extract from the Turmeric spice, and has been used for many centuries in India and Asia because of its numerous potential benefits. Curcumin has been used since ancient times to treat disorders such as arthritis, digestive problems, and urinary issues. It can also be used to treat low energy and a variety of skin and wound conditions. Some researchers believe curcumin can be helpful in treating Alzheimer's disease, diabetes, allergies, and arthritis [1].

Separation and quantification of curcuminoids can be accomplished with liquid chromatography. Advancements in LC instrumentation and column design have drastically increased the efficiency of this technology. The instrumental quantification of curcuminoids can be completed 30 times faster than the current USP method using an Agilent 1290 Infinity LC with a sub-2 µm (STM) column, and an alternative instrument method developed at Schwabe North America. This method also reduces solvent consumption by 95%.

Experimental

Sample preparation

A 25-mg amount of sample extract was dissolved in 50 mL of reagent alcohol by sonication. The sample was further diluted 1:25 in reagent alcohol.

The sample extract was obtained from Natural Remedies in Karnataka, India.

The sample was run with an Agilent 1290 Infinity LC System, using the method parameters listed in Table 1. These results were compared to those of the same analysis, using the USP method parameters listed in Table 2 [2].

Table 1. Instrument Parameters for New Method for Curcuminoid Separation

Temperature	35 °C
Injection amount	1.0 µL
Detection	UV, 425 nm
Flow rate	1.0 mL/minute
Mobile phase A	0.1% *o*-phosphoric acid in water
Mobile phase B	Acetonitrile
Mobile phase composition	Isocratic 60/40, A/B

Table 2. Instrument Parameters for USP Method for Curcuminoid Separation

Temperature	40 °C
Injection amount	20.0 µL
Detection	UV, 420 nm
Flow rate	1.0 mL/minute
Mobile phase (4:6)	Tetrahydrofuran: 1 mg/mL citric acid in water

Results and Discussion

Figure 1 shows the separation of three curcuminoids, using the new method developed with a 1290 Infinity LC System. As the figure illustrates, the method achieved good separation of all compounds, in less than 2 minutes. This is drastically faster than the USP analysis, which takes more than 60 minutes (Figure 2). The USP analysis uses an Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm column, while this method uses a ZORBAX Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm column.

Table 3 lists the results of the analysis of a reference standard, using the new method parameters with the 1290 Infinity LC System. Separation of the compounds is illustrated by the chromatogram shown in Figure 3. The results of this analysis indicate that the calibration curve was linear, with a correlation factor of 0.99998. The method shows good resolution, with factors of 2.51 for curcumin and 2.23 for demethoxycurcumin.

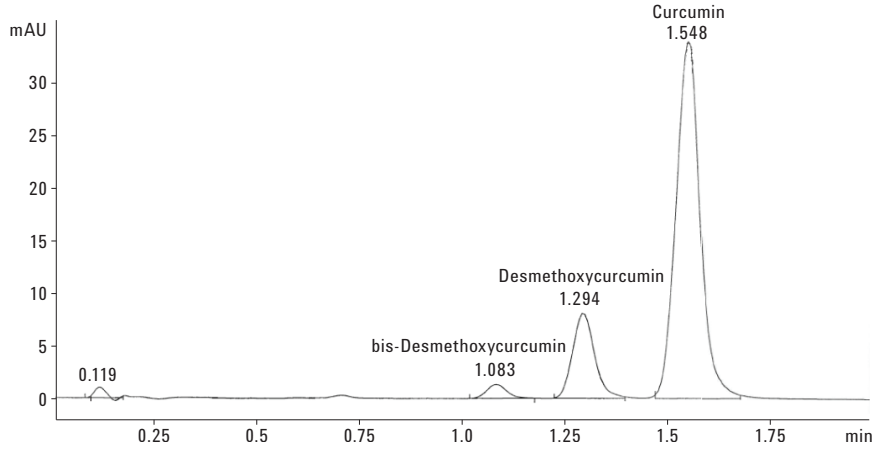

Figure 1. Curcuminoids in turmeric extract using an Agilent 1290 Infinity LC System with an Agilent ZORBAX Eclipse Plus C18 2.1 × 50 mm, 1.8 µm column.

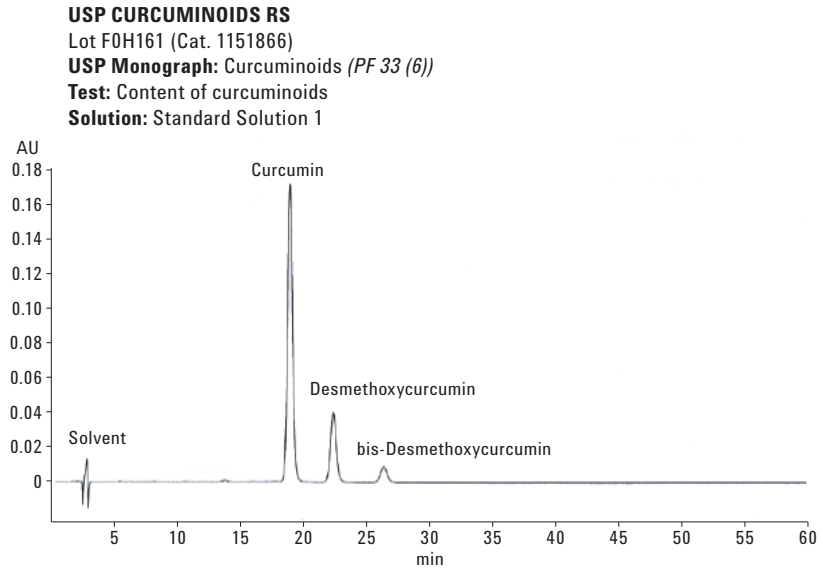

Figure 2. Curcuminoids in turmeric extract using USP method with an an Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5.0 µm column.

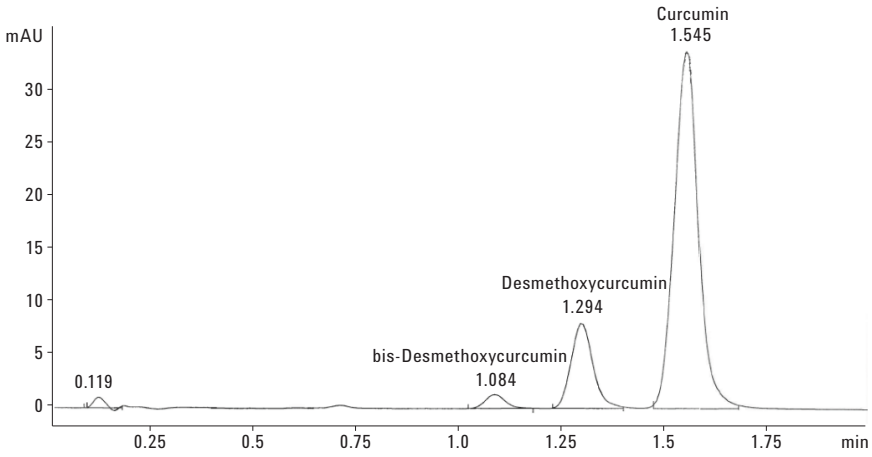

Figure 3. Chromatogram of reference standard analysis.

Table 3 . Reference Standard Analysis

Curcuminoids reference standard	Sigma-Aldrich #C7727 – Lot #SLBB7593V
Calibration range	4.72–94.4 µg/mL
Curve correlation	0.99998
Resolution factor demethoxycurcumin	2.23
Resolution factor curcumin	2.51

Conclusions

The Agilent 1290 Infinity LC System coupled with an STM column reduces analysis time when separating complex botanical extracts. This results in significant cost savings in labor and solvent use, over the traditional USP analysis method for curcumins. This application note illustrates that this can be accomplished without any loss in resolution.

References

1. K. Rybicki "A Look at the Positive Effects and Health Benefits of Curcumin". http://neovitin.com/curcumin.aspx

2. "Powdered Turmeric Extract Monograph" *United States Pharmacopeia* USP36, NF31 p.1612-3.

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Printed in the USA
November 11, 2013
5991-3340EN

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