ISSN : 0974 - 7508 Volume 12 Issue 1

Natural Products

An Indian Journal

Full Paper

NPAIJ, 12(1), 2016 [026-030]

A simple binary reverse phase high performance liquid chromatographic method for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin

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ABSTRACT

A simple binary reverse phase high performance liquid chromatographic method has been developed for the determination of curcumin and its derivatives Demethoxycurcumin, and Bisdemethoxycurcumin. The purity of the curcuminoids and its derivatives was analyzed by an improved, simple HPLC method. The advantages of the proposed method over then previously reported ones are the use of a DAD detector which is widely available in the ordinary laboratories. This method can be said to be more economical as compared to other methods reported in literature.

KEYWORDS

Curcumin;
Curcuminoids;
Demethoxycurcumin;
Bisdemethoxycurcumin.

INTRODUCTION

Curcumin [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3, 5-dione] is the major yellow pigment extracted from turmeric, a commonly used spice, derived from the rhizome of the herb Curcuma longa Linn[1]. It is a naturallyoccurring polyphenolic phytochemical currently being examined in preclinical trials for cancer chemoprotective drug development, with pharmacological actions that including antioxidant[1],[2], anti-inflammatory[3],[4], and cancer chemopreventive actions[5],[6],[7]

Different HPLC methods have been described for the analysis of Curcumin, such as HPLC methods with fluorescence detector which have been used for its quantification in biological samples.[8]HPLC-MS methods have been used for the assay of Curcumin in plasma[9]. Commercially available curcumin, a bright orange-yellow color pigment of turmeric, consists of a mixture of three curcuminoids, namely, curcumin enol form(1), Keto form (2), demethoxycurcumin (3), and bisdemethoxycurcumin (4).

The objective of this study to develop method for the determination of Curcumin and its derivatives with short run time. This analytical which can also be used for its structurally related compounds analysis.

EXPERIMENTAL

Materials and methods

Solvents and organic modifiers used in the HPLC mobile phases were Acetonitrile HPLC grade purchased from SIGMA ALDRICH and Ortho phosphoric acid HPLC Grade (assaye”88%) purchased from RANKEM. The HPLC grade water used in analysis

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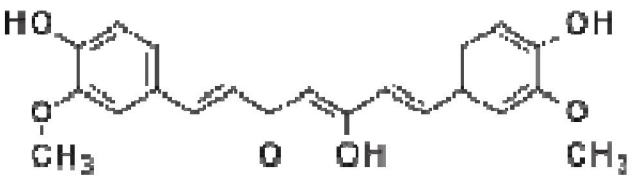
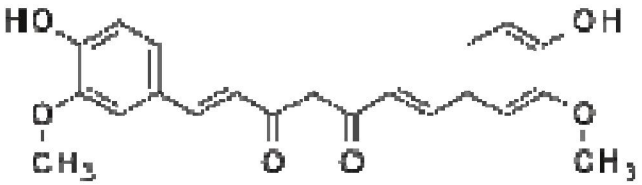
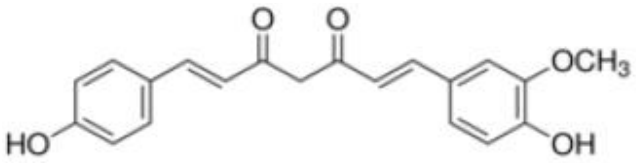


Curcumin enol form (1)



Curcumin keto form (2)



Demethoxycurcumin (3)

Bisdemethoxycurcumin (4)

was purchased from RANKEM. The reference standards Curcumine" 95% were purchased from SIGMA ALDRICH.

Instruments

We have used two HPLC systems for our research work; conventional binary pump HPLC system 1525 made by Waters equipped with an UV-VIS detector 2489,and a shimadzhu HPLC system(model: LC-2010CHT) equipped with an UV-VIS detector and an auto injection with a 20 µl loop, consisted of a computer controlled system with VP 3.20 software, AnalyticalBalance. Make Essay model #GR202.(5 digit balance and having resolution 0.01mg.)

HPLC analysis

Chromatographic conditions

The chromatographic separation was achieved on a C-18(ODS-3, 250mm x 4.6mm,5µ) reversed-phase column. Column temperature 25±2°C, The analytes were analyzed at single wavelength420 nm for total curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin), Injection volume 20 µl,

Gradient flow program

The mobile phase was pumped through the column at an isocratic flow rate of 1.0 mL/min, for a run time of 25min. The output signal was monitored and processed using a EMPOWER System Software (Version 2.0). Peak areas were integrated and final concentrations were calculated in comparison with a known standard response.

Determination of total curcuminoids by HPLC

Preparation of mobile phase

Mix well 500ml of 0.1% w/v Orthophosphoric acid (H_3PO_4) HPLC grade (assaye"88%) and 500ml of Acetonitrile. Filter through 0.45µmmembrane filter paper and degassed by sonication.

Diluent

HPLC gradeMethanol.

Standard solution preparation

Weigh accurately 0.01g of Curcumin, working standard and transfer to a 100ml volumetric flask. Dissolve with 20ml of diluent and sonicate for 2-3min. Cool the sample and make up to the volume with diluent. Filter through 0.45µm Nylon syringe filter.

Standard solution preparation-2

Weigh accurately 0.01g of Curcumin Reference standard and transfer into 100ml volumetric flask. Dissolve in 20ml of diluent and sonicate for 2-3 min. Cool the sample and make up to the volume with diluent. Filter through 0.45µm Nylon syringe filter.

Test solution preparation

Weigh accurately 0.01g of sample and transfer to a 100ml volumetric flask. Dissolve with 20ml of diluent and sonicate for 2-3min. Cool the sample and make up to the volume with diluent. Filter through 0.45µm Nylon syringe filter.

Quantification of areas for standardization

The quantification of catechins and caffeine were

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performed by quantifying the areas of standardization, where [Sample] g.mL–1 = Area standard × [default g.mL–1] / sample area. The results obtained in g.mL–1 were expressed in%.

RESULTS AND DISCUSSION

Development of HPLC method

Different compositions of the mobile phase were tested, and the desired resolution of Curcumin, Demethoxycurcumin, and Bisdemethoxycurcuminwith symmetrical and reproducible peaks was achieved by using the mobile phase of 0.1% Ortho phosphoric acid–Acetonitrile (1:1 v/v). We have injected the standard of Curcumin (Figure 1) and our finalized chromatogram of curcumin extract was shown in Figure 2. Initially methanol and water were tried in various ratios forTotal Curcuminoids. Curcuminoids showed splitting peaknature and was unable to show three sepa-

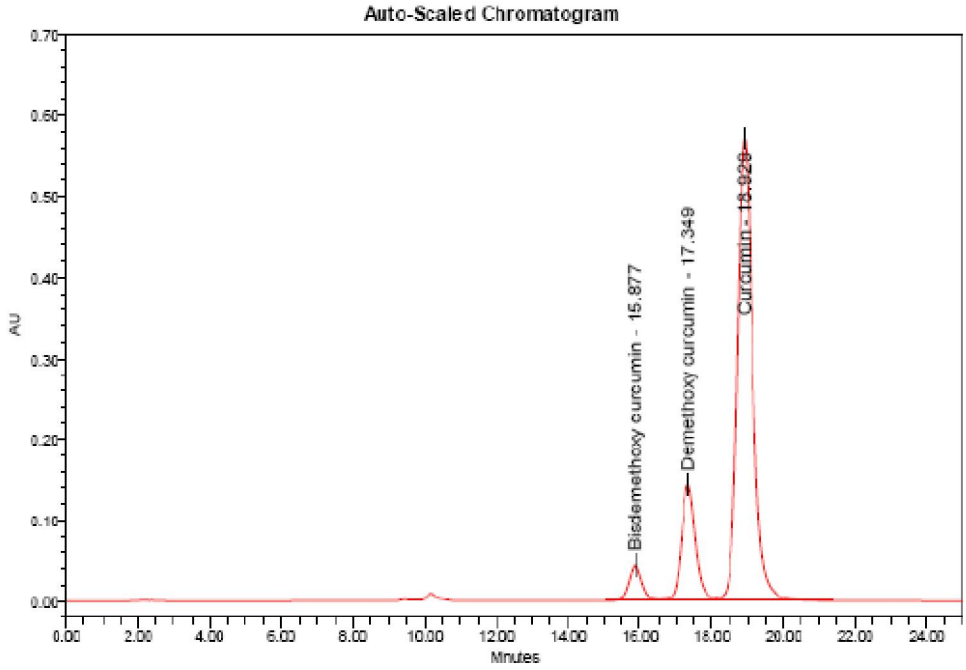

Figure 1 : HPLC chromatogram of total curcuminoids trial 1

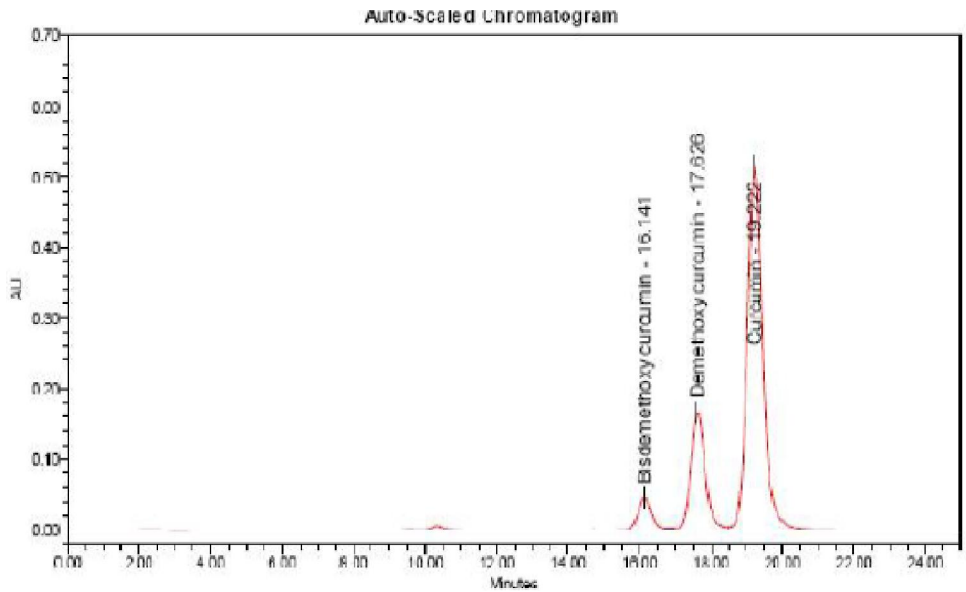

Figure 2 : HPLC chromatogram of total curcuminoids trial 2

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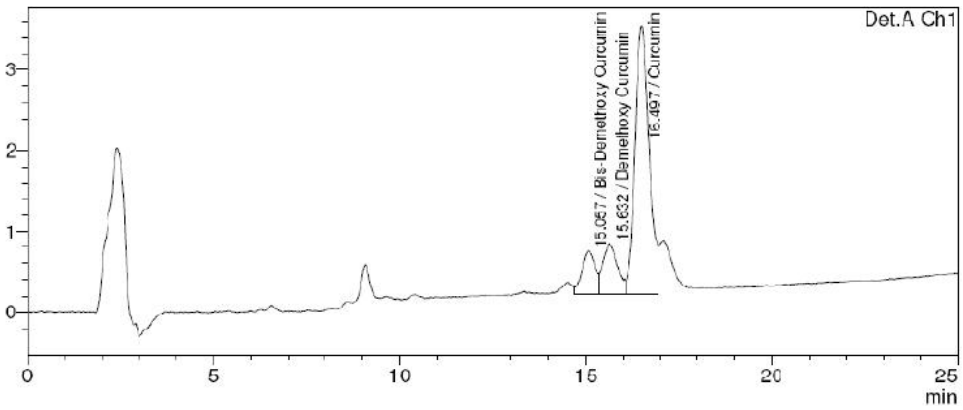

Figure 3 : HPLC Chromatogram that showed split peaks

rate peaks. Then both the drugs were tried with combination of methanol and water(1% acetic acid; adjusted to pH of 3.0 using 50% triethanolamine) at various ratios, still curcuminoids were unable to separate into three peaks.(Figure 3)Therefore, methanol was completely replaced with acetonitrile: water(0.1% phosphoric acid) in the ratio 1:1 (v/v) which exhibited good peak nature and peaks were found to be symmetrical at 420 nm. Tailing factor for Curcumin was less than 2% with good resolution.

Selectivity experiment showed that there is no interface or overlapping of the peak either due to excipients or diluents with main peaks of Curcumin, the assay was linear over a conc of 50mcg-150mcg/ml of Curcumin. Accuracy and precision % RSD of the method was found to be less than 1%. The ruggedness and robustness % RSD were found to be well within limits.

Solution stability

The short term stability studies were carried out at ambient laboratory temperature protected from sun light (22-25 °C) and at refrigerated temperature (2-8 °C) for 8hours in solvent. % RSD for solution of curcumin concentrations during solution stability experiments was within 1%.

No significant changes were observed for the chromatograms of standard solution and the experimental solution. Further, absence of degradation peaks confirmed that the sample is stable in solvent used during the assay for 8 hours.

Limit of quantification & limit of detection

The LOD and LOQ were determined at a signal-to-noise (S/N) ratio of 3 and 10 respectively. Linearity was established over the range of 25-300 ng/mL using the weight least square regression analysis. Accuracy of the method was determined by recovery experiments. 25 ng/mL, 50 ng/mL, and 100 ng/mL of the curcumin were added to the Nano suspension of 50 ng/mL to become 75 ng/mL, 100 ng/mL and 150 ng/mL. The average recovery obtained from six injections was reported as percentage nominal of the analyzed concentration.

CONCLUSION

A gradient HPLC method has been developed for the simultaneous determination of Curcumin and its derivatives. The advantages of the proposed method over then previously reported ones is the use of a DAD detector which is widely available in the ordinary laboratories, with no need for the more sophisticated mass or fluorescence detectors. Another noted advantage is its stability-indicating power and itsability to selectively analyze the studied drugs in the presence of their forced degradation products. This method can be said to be more economical as compared to other methods reported in literature. The proposed RP-HPLC method may be utilized for other curcuminoids and it is subject of further studies.

ACKNOWLEDGEMENT

The authors would like to thank Mr. Anirudh Ranga, Managing Director of Natural& Essential Oils Pvt. Ltd for his constant encouragement and support.

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