

# Simultaneous Quantitative Determination of Cinnamaldehyde and Methyl Eugenol From Stem Bark of *Cinnamomum zeylanicum* Blume Using RP-HPLC

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## Abstract

A simple, sensitive, and precise reversed-phase high performance liquid chromatographic (HPLC) method has been developed, validated and used for simultaneous quantitative determination of cinnamaldehyde and methyl eugenol from the methanolic extract of dried bark powder of *Cinnamomum zeylanicum* Blume (family Lauraceae). The ultrasonic extraction method was used for the extraction of these compounds. The reversed-phase HPLC analysis was carried out using a Intersil ODS-3V-C<sub>18</sub> (150 mm × 4.6 mm, 5 μm) column and a mobile phase comprising of methanol–acetonitrile–water in the volume ratio of 35:20:45, delivered at a flow rate of 1.0 cm<sup>3</sup>/min. The detection and quantitation of both the compounds was carried out at 221 nm.

## Introduction

*Cinnamomum zeylanicum* Blume (cinnamon) is a hardy plant, which can grow on any soil under a wide variety of tropical conditions. Stem bark of *Cinnamomum zeylanicum* Blume contains cinnamaldehyde (60–75%) along with other compounds like methyl eugenol and phellandrene (1). Cinnamaldehyde is present as a major constituent in *Cinnamomum zeylanicum* Blume. Methyl eugenol is reported to be human carcinogenic agent (2). As *Cinnamomum zeylanicum* is used as a spice and flavor, a need was felt to quantitate methyl eugenol from *Cinnamomum zeylanicum*. Thus in the present research work, simultaneous quantitation of cinnamaldehyde and methyl eugenol from *Cinnamomum zeylanicum* Blume has been carried out.

High-performance liquid chromatography (HPLC) methods have been reported in literature for determination of cinnamaldehyde, eugenol, and piperine in pepper-contaminated cinnamon (3). Another, HPLC method reported for determination of cinnamaldehyde, coumarin, and cinnamyl alcohol from *Cinnamomum zeylanicum* Blume (4). Also, determination of

trans-cinnamaldehyde from *Cinnamomum zeylanicum* Blume and from rodent plasma was reported in literature (5,6). But no HPLC method has been reported for simultaneous quantitation of cinnamaldehyde and methyl eugenol from stem bark of *Cinnamomum zeylanicum* Blume.

## Experimental Conditions

### Chemical reagents, standards, and plant material

Methanol (purity 99%) and acetonitrile (purity 99%) used were HPLC-grade and were obtained from Qualigens Fine Chemicals (Mumbai, India). All solvents were filtered through 0.5-μm (Millipore, Billerica, MA) membrane and degassed in an ultrasonic bath. Distilled water was prepared by using Milli-Q water purifying system (Millipore).

Reference standards methyl eugenol and cinnamaldehyde both with 99% purity were procured from Fluka and Sigma Aldrich (Albuch, Germany), respectively.

Stem barks of *Cinnamomum zeylanicum* Blume were collected from wild plants found in Keshav Shrusti, Bhayandar, Mumbai, India, and its herbarium was prepared and authenticated from National Botanical Research Institute (Lucknow, India). The stem barks were cleaned, shade dried, and powdered. The powder was sieved (BSS mesh number 85 sieve) and was placed in an air-tight labeled container at room temperature (28 ± 2°C).

### Preparation of stock solutions of cinnamaldehyde and methyl eugenol

The stock solution of cinnamaldehyde (1000 μg/mL) was prepared by dissolving about 100 mg cinnamaldehyde in 25 cm<sup>3</sup> of methanol and then diluting to 100 cm<sup>3</sup> with methanol in 100 cm<sup>3</sup> standard volumetric flask.

Similarly, the stock solution of methyl eugenol (1000 μg/mL) was prepared by dissolving about 100 mg of methyl eugenol in 25 cm<sup>3</sup> of methanol and by diluting to 100 cm<sup>3</sup> with methanol in 100 cm<sup>3</sup> standard volumetric flask.

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### Preparation of working standard solutions of cinnamaldehyde and methyl eugenol

Working standard solutions of cinnamaldehyde in concentration range of 1–200  $\mu\text{g}/\text{mL}$  were prepared by diluting aliquots of 0.1–20  $\text{cm}^3$  of stock solution of cinnamaldehyde (1000  $\mu\text{g}/\text{mL}$ ) to 100  $\text{cm}^3$  with mobile phase.

Working standard solutions of methyl eugenol in concentration range of 0.30–12  $\mu\text{g}/\text{mL}$  were prepared by diluting aliquots of 0.03–1.2  $\text{cm}^3$  of stock solution of methyl eugenol (1000  $\mu\text{g}/\text{mL}$ ) to 100  $\text{cm}^3$  with mobile phase.

### Preparation of sample solution

Dried bark powder (100 mg) of *Cinnamomum zeylanicum* Blume was accurately weighed and added to a stoppered tube. To this, 10  $\text{cm}^3$  of methanol was added. The contents of the tube were sonicated in an ultrasonic bath for 15 min at room temperature ( $28 \pm 2^\circ\text{C}$ ). The contents of the tube were filtered through Whatmann filter paper no. 41, and the filtrate was used as the sample solution.

### Chromatographic conditions

Chromatographic separation was performed on a Jasco HPLC system, having PU 980 isocratic pump, AS-1555-10 autosampler, and 20  $\mu\text{L}$  loop (Essex, UK). The instrument was equipped with a PU-970 UV-visible detector. Borwin Chromatography software 1.21 was used for data acquisition. A reversed-phase  $\text{C}_{18}$  column Intersil ODS-3V- $\text{C}_{18}$  (150 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ ) was used. The mobile phase comprising a mixture of methanol–acetonitrile–water in volume ratio of 35:20:45 was delivered at a

flow rate of 1.0  $\text{cm}^3/\text{min}$ , and the detection of both cinnamaldehyde and methyl eugenol was done at 221 nm. A TRANS-SONIC ultrasonic bath set at frequency 50 Hz (Pawan Trading Corporation, Mumbai, India) was used for the extraction of phytochemicals.

## Method Validation

### Linearity

Six working standard solutions of cinnamaldehyde in concentration range 1–200  $\mu\text{g}/\text{mL}$  and methyl eugenol in concentration range of 0.3–12  $\mu\text{g}/\text{mL}$  were prepared. Each solution was injected in triplicate in the chromatographic system under optimized conditions (7,8). The calibration plot for each standard was obtained by plotting a graph of mean peak areas of that standard against its injected concentration. The results are listed in Table I.

### Limits of detection and limit of quantitation

The limits of detection (LOD) and limit of quantitation (LOQ) for both cinnamaldehyde and methyl eugenol were established at signal-to-noise ratios of 3:1 and 10:1, respectively (7,8). The results for both the standards are represented in Table I.

### Precision

Precision is determined in terms of instrumental precision, intra-assay precision, and inter-assay precision. The instrumental precision for both cinnamaldehyde and methyl eugenol was studied by separate, repetitive injections ( $n = 10$ ) of standard solutions of cinnamaldehyde (50.00  $\mu\text{g}/\text{mL}$ ) and methyl eugenol (5.00  $\mu\text{g}/\text{mL}$ ).

The intra-assay precision was performed by analysis of replicate injections of sample solutions of three different concentrations on the same day. The intermediate precision was evaluated by replicate analysis of sample solutions of three different concentrations on three different days. The values of percent relative standard deviation of peak areas of cinnamaldehyde and methyl eugenol for instrumental, intra-assay, and intermediate precision were determined, and results are tabulated in Table I.

### System suitability

The system suitability test was carried out by injecting stan-

	Cinnamaldehyde	Methyl eugenol
Linearity ( $\mu\text{g}/\text{cm}^3$ ) ( $n = 6$ )	1.00 to 200.00	0.30 to 12.00
Correlation coefficient ( $r$ )	0.9999	0.9998
LOD ( $\mu\text{g}/\text{cm}^3$ )	0.50	0.10
LOQ ( $\mu\text{g}/\text{cm}^3$ )	1.00	0.30
Instrumental precision % RSD ( $n = 10$ )	0.59	0.41
Intra-assay precision % RSD ( $n = 6$ )	1.10	1.02
Intermediate precision mean ( $n = 6$ )	1.10	1.25

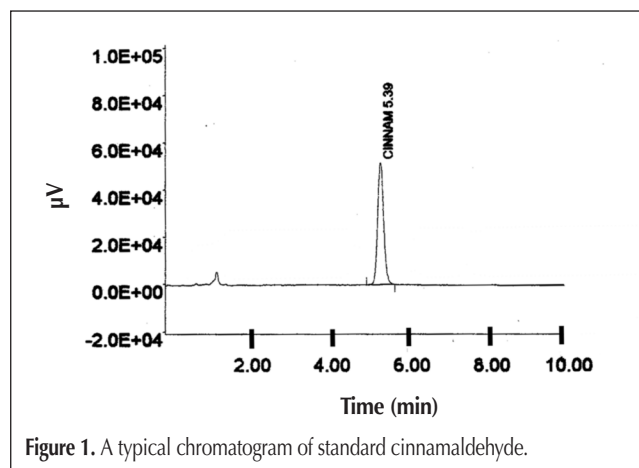


Figure 1. A typical chromatogram of standard cinnamaldehyde.

Levels	Wt of sample (mg)	Amounts added ( $\mu\text{g}$ ) to plant powder		Mean amounts found ( $\mu\text{g}$ ) with % RSD*			
		Cin <sup>†</sup>	ME <sup>‡</sup>	Cin.		ME	
				Mean <sup>§</sup>	% RSD	Mean	% RSD
0	100.2	0	0	860.7	1.35	46.22	1.61
1	100.5	500	25	1292.4	1.52	68.62	1.43
2	100.3	750	50	1535.1	1.63	94.27	1.43
3	100.4	1000	75	1872.0	1.45	120.34	1.52

\*  $n = 7$ .

† ME = Methyl eugenol.

‡ Cin. = Cinnamaldehyde.

§ Mean = Mean amount found.

standard solutions of cinnamaldehyde (50 µg/mL) and methyl eugenol (5 µg/mL) five times in the chromatographic system under optimized chromatographic conditions (9). The peak areas values and retention times of cinnamaldehyde and methyl eugenol were noted for each injected concentration of both the standards. As the values of percent relative standard deviations for peak areas and retention times of both the standards were found to be less than 2%, the system was found to be suitable.

### Accuracy

The accuracy of the method was established by performing a recovery experiment using standard addition method. For zero level, only sample solution was analyzed by HPLC in seven replicates. To about 100 mg of sample, pure standards of cinnamaldehyde concentration (500 µg/mL, 750 µg/mL, and 1000 µg/mL, respectively) were added. Similarly, pure standards of methyl eugenol concentration (25 µg/mL, 50 µg/mL, and 75 µg/mL, respectively) were added to same sample. The solutions were prepared as described earlier and were analyzed by HPLC ( $n = 7$ ) for each level, and mean amounts of cinnamaldehyde and methyl eugenol present in each level of sample solution were determined. The average values of percent recoveries of cinnamaldehyde and methyl eugenol were determined and were found to be 99.09 and 99.20, respectively. The results are given in Table II.

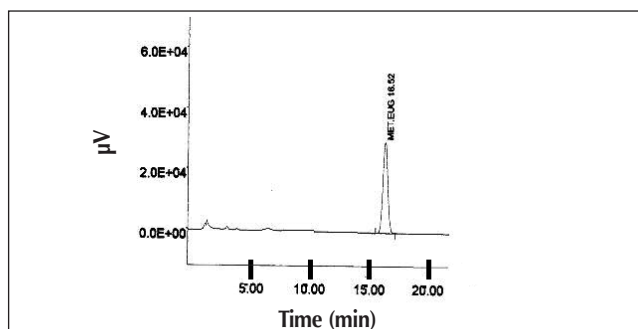


Figure 2. A typical chromatogram of standard methyl eugenol.

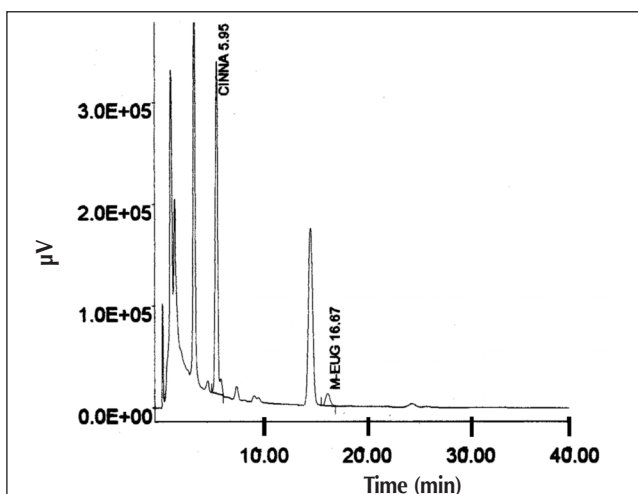


Figure 3. HPLC chromatographic pattern for simultaneous determination of cinnamaldehyde and methyl eugenol from extract of *Cinnamomum zeylanicum* Blume bark powder.

### Quantitation of cinnamaldehyde and methyl eugenol from bark of *Cinnamomum zeylanicum* Blume

Twenty microliters of the sample solution was injected into the chromatographic system ( $n = 7$ ) under optimized chromatographic conditions. The identities of peaks of cinnamaldehyde and methyl eugenol were determined by comparing the chromatogram of sample solutions with those of standard cinnamaldehyde and standard methyl eugenol. The mean amounts of cinnamaldehyde and methyl eugenol present in the sample were calculated. The results are listed in Table III. The typical chromatograms of standards cinnamaldehyde and methyl eugenol are shown in Figure 1 and 2. The chromatograms of sample solution are shown in Figure 3.

### Results and Discussion

The use of mobile phase comprising methanol–acetonitrile–water in the present research work shows a better resolution of different components present in *Cinnamomum zeylanicum* Blume. Methanol and acetonitrile used in the mobile phase are water miscible, have low viscosity, low surface tension, and are readily available in pure form. The wavelength selected for analysis is 210 nm, which yields better sensitivity for both the standards.

As the values of percent relative standard deviation for instrumental precision, intra-assay precision, and intermediate precision (Table I) are less than 2% for both the standards, method was found to be precise.

The values of percent recoveries for cinnamaldehyde and methyl eugenol were 99.09 and 99.20, respectively, indicating that there is no interference of other constituents present in *Cinnamomum zeylanicum* Blume on peaks of cinnamaldehyde and methyl eugenol.

The HPLC method reported in literature for simultaneous determination of cinnamaldehyde, eugenol, and piperine in pepper-contaminated cinnamon used a combination of UV and electrochemical detector (3). The method uses gradient mode with retention time of cinnamaldehyde as 10.2 min while the present developed method uses isocratic mode with retention time of 5.95 min for cinnamaldehyde. The reported method uses trifluoroacetic acid in mobile phase, which can damage the column while mobile phase used in present research work will have not have an effect on the column.

A HPLC method for the quantitation of trans-cinnamaldehyde from cinnamon has been reported (5), which uses a mixture of chloroform and *n*-heptane as mobile phase at a flow rate of 2.0

Table III. Amounts of Cinnamaldehyde and Methyl Eugenol Present in Stem Bark Powder of *Cinnamomum zeylanicum* Blume

	Mean amounts in bark powder (mg/g)*	% RSD
Cinnamaldehyde	8.76	1.39
Methyl eugenol	0.45	0.99
* $n = 7$ .		

cm<sup>3</sup>/min. Chloroform as a solvent may cause CNS depression. Flow rate of 2 cm<sup>3</sup>/min can also lead to consumption of more mobile phase, setting up high backpressure in the column.

A HPLC method has been reported in literature for determination of cinnamaldehyde, coumarin and cinnamyl alcohol from *Cinnamomum zeylanicum* Blume (4). The column used in the reported method is Chromosorb RP-8 reversed-phase column (250 mm × 7 mm, 5 μm), which elutes cinnamaldehyde at 9.3–9.5 min due to larger column length. The column used in the present research work is Inertsil ODS-3V C<sub>18</sub> (150 mm × 4.6 mm, 5 μm), which being shorter in length, elutes cinnamaldehyde at 5.95 min, making this method faster and economical.

Several chromatographic methods have been reported in literature for the determination of cinnamaldehyde alone or in combination with other components in cinnamon species or its formulations likewise.

Determination of cinnamaldehyde by carrying out its derivatization with 4,5-dimethyl-o-phenylene diamine in acidic medium, followed by reversed-phase HPLC analysis (10) has been reported in literature. Detection was done spectrofluorimetrically in this method.

The literature for estimation of cinnamaldehyde in Guilong Kechuaning granules, a Chinese medicine was reported (11). Simultaneous HPLC determination of puerarin, daidzin, paeoniflorin, liquiritin, cinnamaldehyde, and glycyrrhizin in kampo medicines is reported using a gradient elution with a mobile phase 0.01% phosphoric acid–acetonitrile (12). Also, HPLC method was reported for separation and detection of cinnamaldehyde and eugenol from cinnamon oils (13).

Gas chromatography (GC) methods have also been reported in literature for determination of cinnamaldehyde in essential oil of *Ramulus cinnamomi* (14).

A non-aqueous capillary electrophoresis method along with UV detection has been found for determination of cinnamaldehyde and cinnamic acid from *Cinnamomum cassia* (15).

However, no method has been developed for simultaneous quantitation of cinnamaldehyde and methyl eugenol from *Cinnamomum zeylanicum* Blume. Methyl eugenol is anticipated to be human carcinogen, and thus the need was felt to determine its amount in *Cinnamomum zeylanicum* Blume barks as they are used mainly as food product (2). Hence, it is necessary to quantitate methyl eugenol, along with cinnamaldehyde as bark of *Cinnamomum zeylanicum* is commonly used as a spice.

## Conclusion

In the present research work, a simple, precise HPLC method has been developed for simultaneous quantitation of cinnamaldehyde and methyl eugenol from *Cinnamomum zeylanicum* Blume stem bark. The method can be used as a quality control method.

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Manuscript received January 24, 2008;

Revision received June 5, 2008.