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High performance thin layer chromatographic method for the determination of cinnamaldehyde in *Cinnamomum zeylanicum* bark powder

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Abstract

Objective: To develop a simple, precise and quantitative High Performance Thin Layer Chromatographic (HPTLC) method for the determination of cinnamaldehyde in *Cinnnamomum zeylanicum* bark powder. Materials and Method: Cinnamaldehyde content of bark powder of *Cinnnamomum zeylanicum*, were determined using mobile phase, Toluene: Ethyl acetate: Formic acid, 19:1:0.1 (v/v). Instrument with Camag Linomat V and Camag TLC Scanner 3 was used. Results: HPTLC method was developed for the determination of cinnamaldehyde by scanning the plates at 295nm. Conclusion: Proposed HPTLC method was simple, accurate, precise, economic and can be utilized for the routine analysis and quantitative determination of cinnamaldehyde from *C. zeylanicum*.

Key words: *Cinnnamomum zeylanicum*, Cinnamaldehyde, Camag TLC Scanner 3, Camag Linomat V.

1. Introduction

Cinnamomum zeylanicum B. (family: Lauraceae), commonly known as cinnamon is mainly used for antiallergic, antiulcerogenic, antipyretic, anaesthetic and antimicrobial activities [1-3]. The bark of the plant has medicinal properties like vasodilatory, antitumor, antifungal, cytotoxic and antimutagenic properties have been reported [4-6]. Literature survey reveals some methods for the determination of cinnamaldehyde based on TLC, microscopic studies, HPLC and GC techniques [7-10]. HPTLC has emerged as an efficient tool for the quantitative evaluation of herbal drugs, due to its simplicity and minimum sample clean-up requirement. HPTLC method has not been found reported in literature for the quantitation of cinnamaldehyde in the bark and therefore this method has been developed for its identification as well as quantification of the constituents in the bark.

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Table 1. Validation parameters for estimation of cinnamaldehyde

Parameters	Results
1. Precision (% CV)	< 2%
2. Range	31.50 – 157.50 ng/spot
3. Limit of Detection	3 µg/ml
4. Limit of Quantification	9.9 µg/ml
5. Accuracy	98.39 – 100.40 %
6. Specificity	Specific

Table 2. Recovery studies for estimation of cinnamaldehyde

Level of addition	Standard cinnamal-dehyde added	Cinnamaldehyde found by the proposed method (mg/g of cinnamon)				Standard deviation (±)	Coefficient of variation %	Recovery %
		1	2	3	Average			
0	0	2.62	2.41	2.29	2.44	0.17	0.69	98.39
1	2 µl	2.64	2.44	2.32	2.47	0.16	0.65	99.59
2	4 µl	2.65	2.44	2.40	2.49	0.14	0.54	100.4
						0.16	0.63	99.46

2. Materials and Methods

2.1 Plant material

The *C. zeylanicum* bark was collected from authentic sources and authenticated by Dr. R. P. Pandey, Dept. of Botany, M.M.H. College, Ghaziabad, U.P. India. and dried in shade, crushed into powder passed through 80-mesh sieve and stored in an airtight container for use.

2.2 Chemicals

Standard cinnamaldehyde (99%) (Acros Organics), chloroform, toluene, ethyl acetate and formic acid (AR grade) were used in the study.

2.3 Preparation of plant extract

Accurately weighed 2 g bark powder was placed in 20 ml of n-hexane: chloroform 1:1(v/v) mixture overnight at room temperature with occasional shaking, the extract was filtered through Whatman No. 41, the filtrate was collected and evaporated to dryness. The residue was dissolved in 10 ml of methanol and shaken well.

2.4 Standard preparation

Accurately weighed 26.25 mg (99% v/v) cinnamaldehyde was diluted with methanol to get a final concentration of 20 µg/ml in methanol.

2.5 Chromatographic conditions

Instrument: Camag Linomat V equipped with Camag TLC Scanner 3 and Software version win CATS-3.

Stationary Phase: Precoated HPTLC plate of silica gel GF_{254} (E. Merck).

Mobile Phase: Toluene: Ethyl acetate: Formic acid, 19: 1: 0.1(v/v).

Spotting parameter: Calibration curve: 2µl of each of the standard solutions of cinnamaldehyde (3 µg/ml to 10.5 µg/ml).

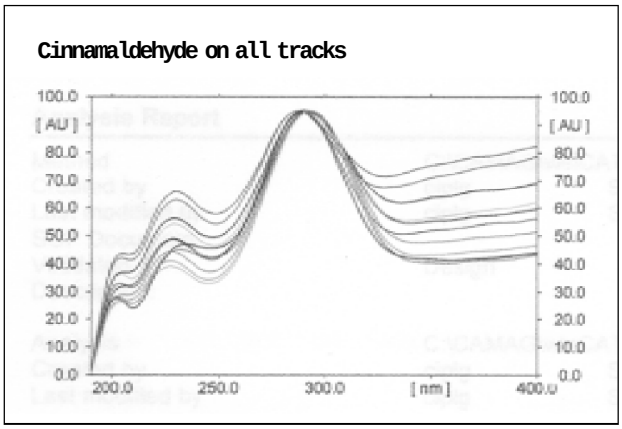

Fig.1. Overlaying UV Absorption Spectra of different bands of Standard Cinnamaldehyde with Sample showing λmax= 295 nm

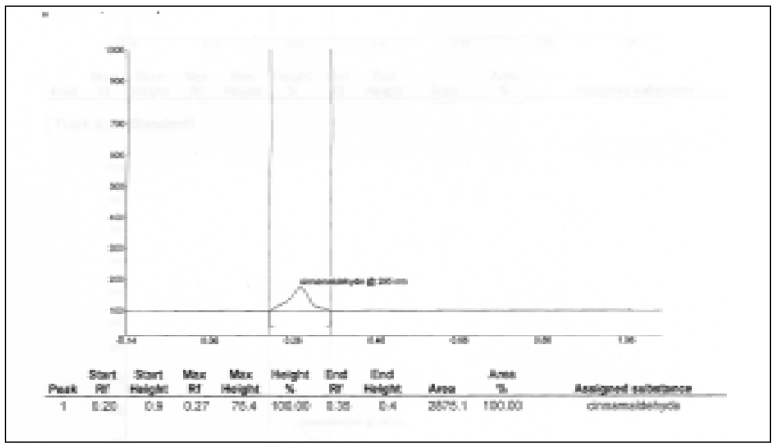

Fig. 2. Typical Chromatogram of Standard

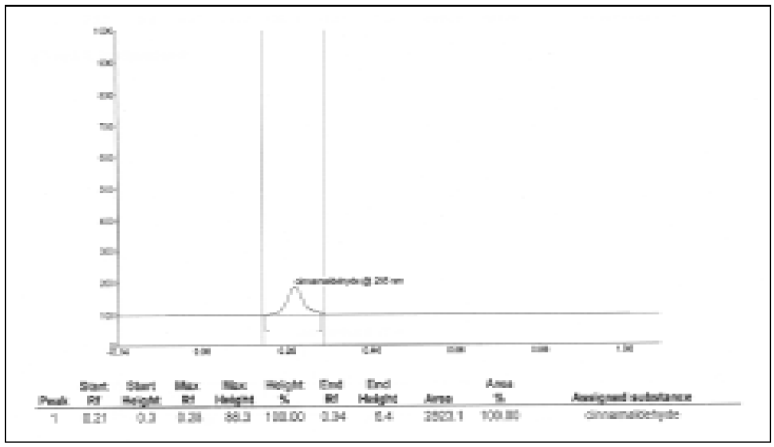

Fig.3. Typical Chromatogram of extract of *Cinnamomum zeylanicum*.

Test sample: 2 µl

Temperature: 25 ± 2°C

Migration Distance: 80 mm

Detection: 295 nm

2.6 Experimental

Accurately 2µl of each of Standard and sample solutions were spotted on Pre-washed HPTLC plate (E. Merck) by Camag Linomat V applicator.

The plate was developed in the mobile phase, toluene: ethyl acetate: formic acid (19: 1: 0.1). After development, the plate was dried in air and scanned at 295 nm. The calibration curve was plotted from the peak area obtained from standard solutions of cinnamaldehyde in the range from 31.5 ng to 157.5 ng per spot.

3. Results and discussion

The proposed method utilizes silica gel GF_{254} HPTLC plate as stationary phase and toluene: ethyl acetate: formic acid (19: 1: 0.1) as mobile phase which gives good separation of cinnamaldehyde (R_f = 0.27) from the other components. The identity of the band of cinnamaldehyde in the sample extract was confirmed by overlaying the UV absorption spectra of sample with that of reference standard showing λmax = 295 nm Fig. 1. The cinnamaldehyde content was found to be 0.248% w/w.The chromatograms of standard cinnamaldehyde and the extract of *Cinnamomum zeylanicum* are shown in Fig. 2 and Fig. 3.

The aim of this study was to develop a simple, precise and quantitative High Performance Thin Layer Chromatographic (HPTLC) method for the determination of cinnamaldehyde in *C. zeylanicum* bark which has been achieved by validation parameters like linearity, accuracy, precision, repeatability and reproducibility which is shown in Table 1.

A simple, accurate, precise and economic method was developed, which can be utilized for the routine analysis and quantitative determination of cinnamaldehyde from *C. zeylanicum* bark.

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