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QUANTIFICATION OF ρ (pera) - METHOXY CINNAMIC ACID ETHYL ESTER (PMCAEE) FROM *HEDYCHIUM SPICATUM* BY HPTLC

ARORA RITU* AND JAIN SHIPRA

Noida Institute of Engineering & Technology
Dept. of Pharmaceutical Sciences
19, knowledge park,Phase 2
Greater Noida, U.P. (INDIA)

**** Corresponding author*** ritu.wadhwa84@gmail.com

ABSTRACT

Hedychium spicatum Ham (Zingiberaceae), is an aromatic annual herb, which is used in Traditional System of Medicine to treat various diseases like like antimicrobial, antidiabetic, pediculicidal and antioxidant. The reported constituents are flavonoids, glycosides and saponins. From literature it was found that the quantification work was not carried out. It is considered to contain ρ -methoxy cinnamic acid ethyl ester (PMCAEE) confirmed by TLC and by qualitative test. Thus it was quantified using HPTLC a sensitive method for development of marker compounds. The method was carried out on precoated TLC aluminum plates with silica gel 60 F254 as stationary phase using solvent system as Hexane: Acetone(80:20 v/v). with Rf value of 0.43. Quantitative analysis was carried out in the absorbance at 310 nm. The linearity regression for the calibration showed r = 0.6548 and 0.999 with respect to height and area in a range of 0.5-5.0 g per spot. Thus HPTLC method provides a faster and cost effective quality control for the routine analysis of PMCAEE in extracts containing *Hedychium spicatum*..

KEYWORDS

Hedychium spicatum, PMCAEE, HPTLC analysis, Marker compound.

INTRODUCTION

Hedychium spicatum Ham(Zingiberaceae) commonly known as shati is a perennial rhizomatous herb which is used in traditional system of medicine to treat various diseases.The reported main constituents are essential oil, starch, resins, organic acids, Ethyl ester of *p*-methoxy cinnamic acid, d-sabirene, sesquiterpenes.[1] The plant grows

throughout the plains and in subtropical Himalayan regions up to a height of 1000 m in India as is used as carminative, emmenagogue, expectorant, stimulant, stomachic & tonic. This plant is used because of its varied sources of biological activities like antimicrobial, antidiabetic, pediculicidal and antioxidant. The rhizomes are used as insect repellant & also in the treatment of snake bite.[2] So far no reports have been studied for its quantification of marker compounds present in the extracts. High Performance thin layer chromatography is an important tool that can be used qualitatively as well as quantitatively for checking the purity and identity of crude drug and also for quality control of finished product.[3] However recent reviews shows that the thin layer chromatography and HPTLC techniques can be used to rectify many qualitative and quantitative analytical problems in a wide range of fields including medicines, pharmaceutical, chemistry, biochemistry and toxicology[4,5].Thus in the present investigation an attempt was made to quantify the marker compound by using HPTLC. High Performance Thin Layer Chromatography is an important tool which can be used for both qualitative and quantitative analysis, which includes purity and identification of compounds.

MATERIALS AND METHODS

Reagents and Chemicals

All solvents used were of AR-grade and were obtained from Merck, Mumbai (India).
Standard PMCAEE was obtained from Sigma chemicals.

Collection of raw material

The raw materials of *Hedychium spicatum* were received from the Taxonomist, Ranbaxy Research Laboratories, supplied by the Supplier (S.S.Herbal, 485/2, Katra Ishwar Bhavan, Khari Baoli, Delhi, 11006. Phone: 011-23964221). As per the information given by Supplier, the raw material has been collected from the Delhi. The Taxonomist, Dr.Gyanesh Shukla, Ranbaxy Research Laboratories, confirmed the botanical identity of raw material of *Hedychium spicatum*

Chromatographic conditions

Instrument: – Camag HPTLC system, consisting of Linomat V spotting device and scanner III with Win Cats 4 software
Stationary phase: – TLC aluminum sheets silica gel 60 F254 pre coated layer (20 cm X 10 cm), thickness 0.2 mm., no. of tracks : 18, band length : 6 mm.
Mobile phase: – Hexane: Acetone (80:20 v/v).
Development chamber: – Twin through chamber (20 X 10)
Distance run: – 80 mm
Scanning wavelength: – 310 nm
Experimental conditions: Temperature 25± 2 0C, relative humidity 40%

Standard preparation

Accurately weighed about 5.95 mg of standard PMCAEE of purity 95% w/w was dissolved in 25ml of methanol.

Calibration curve for Standard

The standard solution of PMCAEE (1 µg to 5µg per respective spot) was applied in triplicate on TLC plate. Quantitative evaluation of the plate was performed in absorption / reflection mode at 310 nm using a slit width of 6.0 □0.30 mm, scanning speed 20 mm/s with a computerized CAMAG, TLC scanner-3 integrated with CATS – III software. The plate was developed and scanned as per the chromatographic conditions and the peak areas were recorded.

Preparation of Extract

About 10g of the drug was weighed and refluxed with 25 ml 60% ethanol four times,

then combined and filtered through Whatman filter paper. The filtrate was concentrated to dryness in a rotary vacuum evaporator.

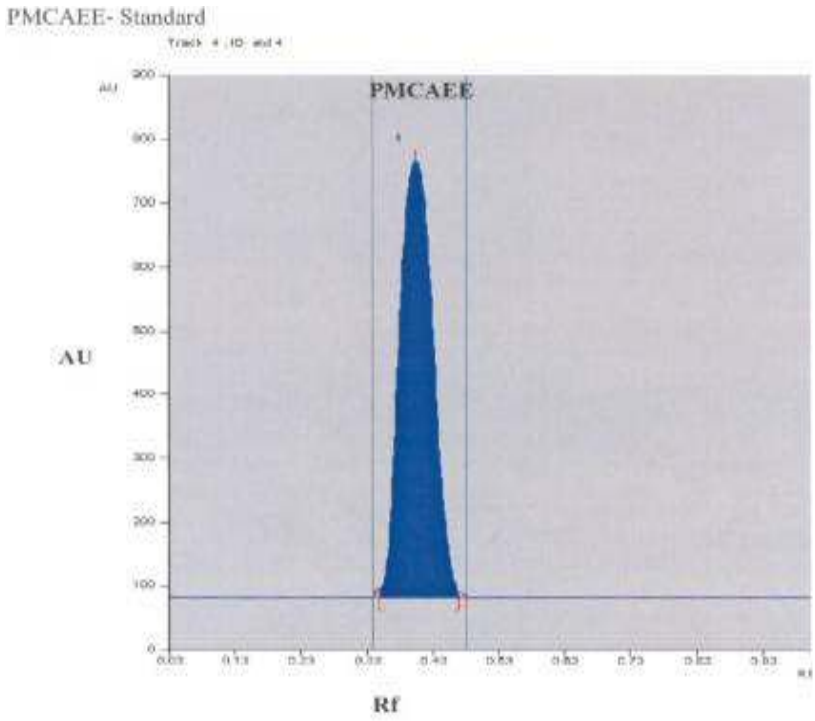
HPTLC Quantification in Test Sample

Accurately weighed 500mg of extract as prepared above was dissolved in 25 ml of Methanol by sonication. Cooled at room temperature and reconstitute the volume upto 10 ml. Filtered and used the resulting solution as test solution. 20 µL per spot of these solutions were applied on to a precoated silica gel plates (in triplicates). The plates were developed by ascending mode to a distance of 80 cm and scanned as per the conditions mentioned above. The PMCAEE content of extract was determined by comparing the area of the chromatogram with the calibration curve of working standard. The Rf value of the standard PMCAEE (0.43) was compared with the Rf value of the extracts. The content of PMCAEE was expressed as %w/w of extract.

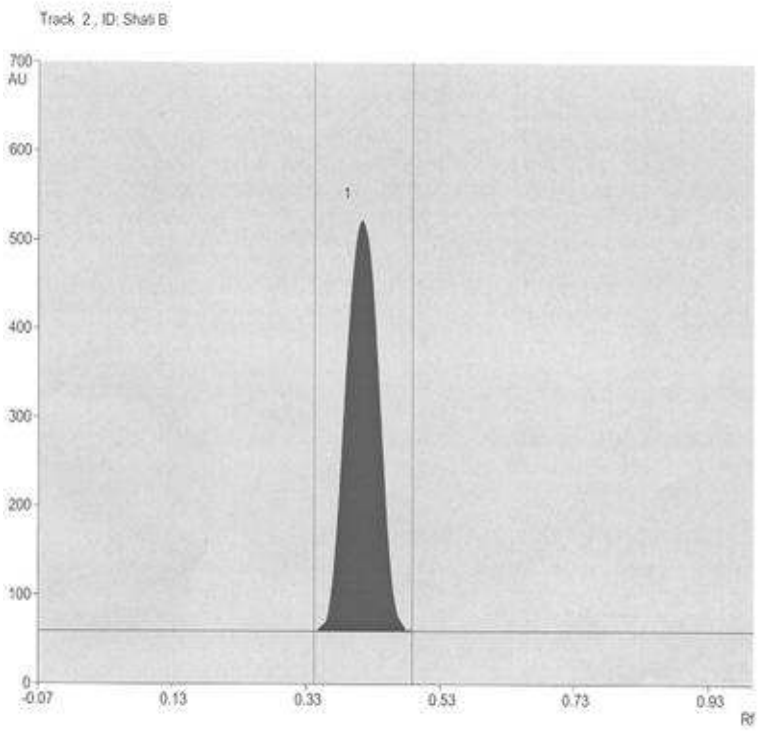
RESULTS AND DISCUSSIONS

Different compositions of the mobile phase were tested and the desired resolution was achieved by hexane: acetone (8:2) Spectral characteristics of the peak of standard and that of the extracts were compared for identification of PMCAEE. Calibration curve of PMCAEE was obtained by plotting peak areas verses concentration applied. It was found to be linear in the range of 1 µg to 5 µg per spot. Equation of the calibration curve is y = 822.1x + 22916. The correlation coefficient was found to be 0.9813 and thus exhibits good linearity between concentration and area. The amount of PMCAEE in the extract was found to be 0.81% w/w of alcoholic extract.

FIGURES



Chromatogram of Standard PMCAEE (R_f =0.43)



Chromatogram of *Hedychium spicatum* Extract. Peak belongs to PMCAEE (R_f =0.43) present in the Extracts. Mobile phase:: n-hexane: acetone (8:2)

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