

STANDARDIZATION OF *CALENDULA OFFICINALIS* LINN WITH REFERENCE TO QUERCETIN BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

Kuppusamy Sujatha, Krishnan Chitra*, Haritha Poliseti, Sarika Karri, Sahitya Nimmalapudi and C. Umamaheswara reddy

Department of pharmaceutical chemistry, Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai, Tamil Nadu, India.

*Corresponding Author: chitrasrmc@rediffmail.com

ABSTRACT

A simple and reproducible HPTLC method for the determination of quercetin in *Calendula officinalis* was developed and is described. The HPTLC method involves separation of components by TLC on precoated silica gel 60 F 254 plate with a solvent system of chloroform : glacial acetic acid : methanol: water (6.4: 3.2: 1.2: 0.8) and detection at 254 nm in absorbance mode. The sensitivity of HPTLC method was found to be 0.15 µg and the linearity was observed in the range of 0.15 to 4 µg. The quercetin content of 8.72% was observed in test sample. The proposed method being precise and sensitive can be used for detection, monitoring and quantification of quercetin in *Calendula officinalis*.

Keywords: *Calendula officinalis*. HPTLC. quercetin.

INTRODUCTION

The use of plant materials in the modern medicine is increasing with the extensive research on process in phytochemistry. Several pharmacopoeias containing monographs on plant materials describe only the physiochemical parameters of such plant materials, are lacking in identification and quantification of active compounds. Hence modern methods describing the identification and quantification of active compound in plant may be useful for proper standardization of herbs and their formulation. In this work, a suitable, sensitive and reliable quantitative HPTLC method has been

developed for quality control determination of quercetin from *Calendula officinalis*.

Calendula officinalis (Linn), Family- Compositae, commonly known as marigold is being used for a variety of disorders in indigenous system of medicine. Anti-inflammatory activity of terpenoids was substantial and comparable to that of indomethacin¹⁻⁴. Tests demonstrate that *Calendula* constituents activate tissue regeneration and epithelial tissue development⁵. The extract was found to have anti-bacterial activity⁶. Principles isolated from *calendula* showed significant anti- tumor activity⁷. Anti-oxidant activity by free radical scavenging mechanism was studied⁸. Anti-viral and

immunostimulating activity was reported and it was found to exert anti-HIV activity by inhibiting immunodeficiency virus type 1⁹. Uterotonic activity was studied¹⁰ and significant protective property was found to exist¹¹.

MATERIALS AND METHODS

Extraction

The flowers of *Calendula officinalis* were procured from the local market during Sep-Oct 2009 were used in this investigation and were

identified in our pharmacognosy Department where voucher specimen is maintained. Coarsely powdered flower parts (1.5 kg) were extracted with hexane, chloroform and methanol successively in an aspirator bottle by cold percolation method (48hrs). The flower was extracted with each solvent twice. Nearly 80% of solvent from each extract was removed by distillation over a water bath at atmospheric pressure and last traces of solvent were removed under pressure.

Chromatographic condition

Instrument	: A camag HPTLC system equipped with a sample applicator Linomat IV, twin through plate development, chamber, TLC Scanner III and Integration soft ware CATS 4.05.
Absorbent	: TLC Aluminium plate precoated with silica gel 60 F 254 (E.Merck) CAT NO 5554.
Solvent system	: Chloroform : Glacial acetic acid : Methanol: water.
Solvent run up to	: 80 mm.
Scanning wavelength	: 254 nm.
Standard preparation	: A 0.1 mg/ml solution of quercetin standard was prepared in methanol.

Procedure

2 and 5 µl of methanol extract were applied as bands of silica gel 60 F 254 precoated aluminium plates (Merck) along with authentic sample of standard quercetin using Linomat IV sample applicator. The speed of applicator was maintained at 10 µl /sec. The width of the band was kept at 6 mm. The chromatogram was developed up to 80 mm under chamber saturation condition. The plate was dried and scanned at 254 nm in absorbance mode. The amount of quercetin was determined using the calibration curve plotted between concentration and area of standard quercetin.

Method validation and recovery

The equation for quercetin was found to be $Y=10628 X + 628.3$ with a correlation coefficient of 0.987 where Y is the response in peak area and X is the concentration in mg/ml. A varying known amount of quercetin was added to about 1g of methanol extracts in which the contents of quercetin had been estimated previously by proposed method. The sample were extracted and analysed separately as per the procedure

mentioned above. The contents of quercetin were quantified using proposed method and percent recovery was calculated.

RESULTS AND DISCUSSION

Sample preparation and development of suitable mobile phase of solvent system are the two important steps in developing the analytical procedure, which becomes more significant for herbal drugs because of the complexity of chemical compounds and their affinity towards various solvents. By trying different composition of mobile phase, the desired resolution of quercetin with symmetrical and reproducible peaks was achieved by using chloroform: glacial acetic acid: methanol: water (6.4: 3.2: 1.2: 0.8) using the proposed HPTLC method. The R_f value of quercetin was about 0.6. The HPTLC chromatogram of standard quercetin and test sample are shown in fig I and II respectively.

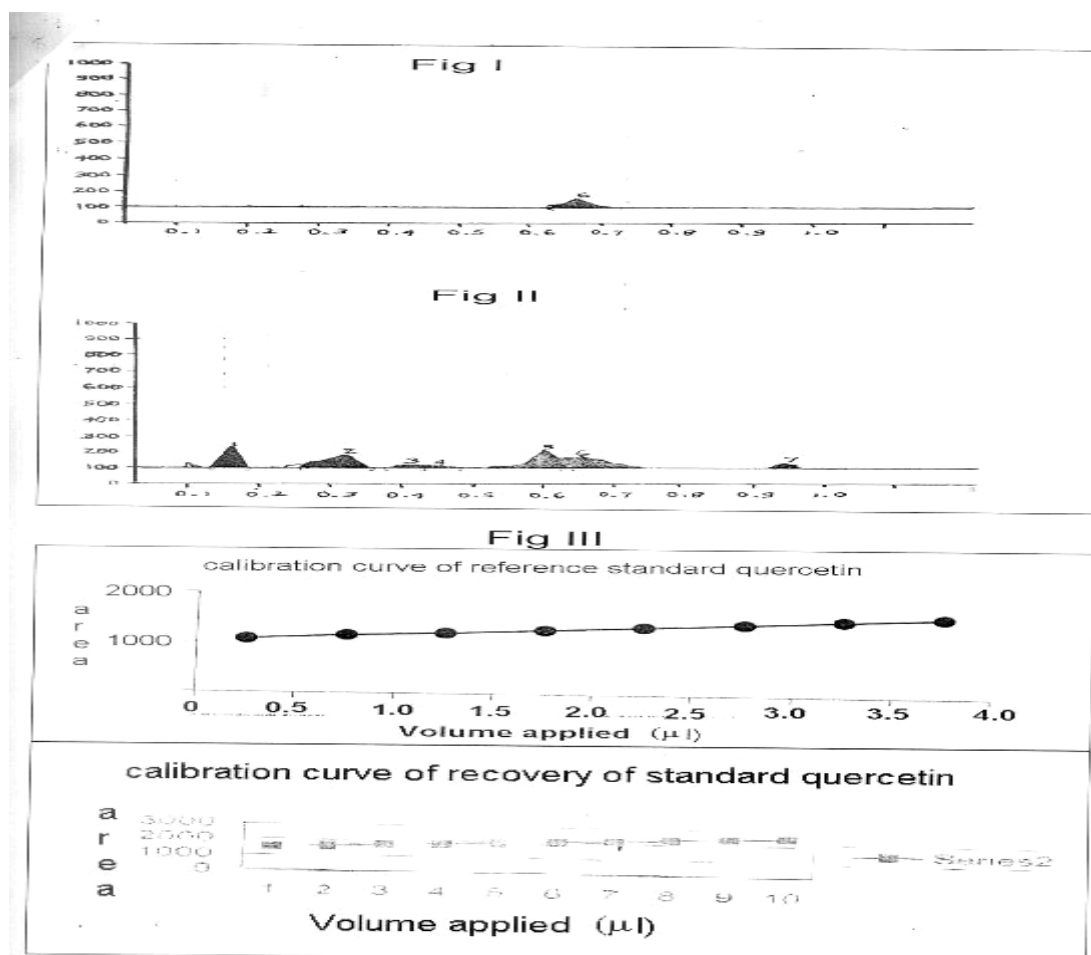
The calibration curve was linear in the range of 0.15 to 0.4 µg for quercetin. Further a correlation coefficient of 0.9 indicates good linearity between concentration and area. The method

allows reliable quantification of quercetin and provides good resolution and separation of quercetin from other constituents of *Calendula officinalis*. To ascertain the purity of peak in test sample, its insitu reflectance spectrum was compared with standard quercetin which

provides clear super impossibility indicating the purity of peaks (fig III). Further, recovery values of 99.81 % to 99.97 % (99.70 ± 1.50) were obtained showing the excellent reliability and reproducibility of proposed method.

Table 1: Method validation and recovery of quercetin

S. No.	Sample	Amount of extract taken (mg)	Amount of Quercetin in A (mg)	Amount of Quercetin In A (mg)	Amount of Quercetin Taken B + C (mg)	Total Quercetin present	% Recovery E/Dx100
		A	B	C	D	E	
1	Methanol Extract <i>Calend-ula officin-alis</i>	1080	94.17	2.00	96.17	99.82	96.00
2	Methanol Extract of <i>Calendula officinalis</i>	1020	88.74	2.00	90.37	99.97	90.72
3	Methanol Extract of <i>Calendula officenalis</i>	1010	88.37	2.00	90.37	99.81	90.20



CONCLUSION

A suitable, sensitive and reliable quantitative HPTLC method has been developed. The proposed HPTLC method is rapid, simple and accurate for quantitative monitoring of quercetin in *Calendula officinalis* and can be used for routine quality testing.

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