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Determination Of Quercetin In *Hypericum Mysorense* By RP-HPLC Method

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ABSTRACT

A simple, selective, rapid and precise reverse phase HPLC method has been developed for the standardization of *hypericum mysorense* (Family: Guttiferae) using quercetin as an analytical marker. The method was carried out on a PrincetonSPHER C_{18} (150 x 4.6 mm i.d. 5 µ) column with a mobile phase consisting of acetonitrile: phosphate Buffer (pH, 7.5 adjusted with orthophosphoric acid) (30:70 v/v) at a flow rate of 1 ml/min. Detection was carried out at 406 nm. Retention time of quercetin was found to be 3.189 min. The calibration curve was linear in the range of 2 µg/ml to 25 µg/ml of quercetin and the correlation coefficient was 0.995, indicating good linear dependence of peak area on concentration. The developed method was validated in terms of accuracy, precision, linearity, limit of detection and limit of quantitation. The proposed method can be used for the standardization of quercetin in *hypericum mysorense* extract

KEYWORDS

Standardization;
Hypericum mysorense;
Quercetin;
RP-HPLC.

INTRODUCTION

Hypericum (Family: Guttiferae) is a large genus of herbs or shrubs comprising more than 1000 species[1]. Hypericum is used as traditional medicinal plants in different parts of the world[2]. About 20 different species occur in India, including a few cultivated in gardens[3]. In recent years, the antidepressant activity of *hypericum perforatum* L., known as St. John's wort, has caused wide spread interest in the study of hypericum genus[4]. Flavonoids isolated from hypericum genus have shown several biological ac-

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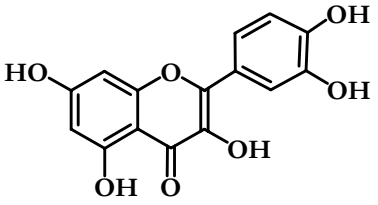

Figure 1: Structure of Quercetin

tivities such as antidepressant[5], nitric oxide synthase inhibition[6], antiproliferative activity[7] and antioxidant activity[8].

Hypericum mysorense is an ornamental bush found in the Konkan and Palani hills at 900-1500 m. Earlier studies in our laboratory have shown significant antiviral[9], cytotoxicity and antitumour[10], antimicrobial[11] and wound healing[12] properties of this plant. Triterpenes, hypercorin, hyperenone A and B, mysorenone A, B, C and xanthone have been isolated from the aerial parts of *H.mysorense*[13, 14]. Because of its widespread use in various geographic regions and to detect its adulteration with other materials, it is important to standardize the different parts of *hypericum mysorense*. No method of standardization of this potentially bioactive plant has been reported so far. We have therefore, developed an RP-HPLC method for the standardization of its extract using quercetin (Figure 1) as marker compound. The method was validated as per the ICH guidelines[15, 16].

EXPERIMENTAL

Plant material

Hypericum mysorense was collected from in and around Ootacamund, a famous hill station in southern India, belonging to the district Nilgiris of Tamil Nadu state. The plant was identified and authenticated by Medicinal Plants Survey and Collection Unit, Ootacamund, Tamil Nadu, India.

Extraction procedure

The aerial parts, flowering tops, leaves, root and stem were separated and shade dried. The dried plant material was powdered and passed through sieve no. 20 and extracted separately using methanol by soxhlation. The extracts were concentrated to dryness under reduced pressure and controlled temperature 7.26 ± 0.05, 10.33 ± 0.29, 9.25 ± 0.11, 4.21 ± 0.06 and 4.1 ± 0.02 % respectively for aerial parts (HMA), flowering tops (HMF), leaves (HML), root (HMR) and stem (HMS) of Hypericum mysorense. All extracts were preserved in refrigerated condition till further use.

Chemicals and reagents

Acetonitrile HPLC grade was procured from E.merck (India) Ltd, Mumbai. Water HPLC grade was obtained from a Milli-QRO (0.2 μ) water purification system. All other chemicals used were of analytical grade.

Analytical marker

Quercetin was isolated from the methanol extract of flowering tops of *hypericum mysorense* and was characterized by spectral studies[17].

Standard and sample solutions

10 mg of quercetin was dissolved in 5 ml of methanol in a 10 ml volumetric flask and the volume was made up to 10 ml with the same solvent (stock solution). Various concentrations were prepared from the stock solution. 10 mg each of different extracts of *hypericum mysorense* was dissolved in 5 ml of methanol in a 10 ml volumetric flask and the volume was made up to 10 ml with the same solvent (stock solution). Various concentrations were prepared from the stock solution and used for HPLC analysis and stored between 2 - 8°C until use.

Apparatus and chromatographic conditions

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-10AT-vp solvent delivery system (pump), SPD M-10AVP photo diode array detector and Rheodyne 7725i injector with 20 μl loop volume. Class-VP 6.01 data station was applied for data collecting and processing (Shimadzu, Japan). A Princeton SPHER C_{18} (150 × 4.6 mm i.d. 5 μ) was used for the separation. Mobile phase of a mixture of acetonitrile and phosphate buffer pH (7.5 adjusted with orthophosphoric acid) (30:70 v/v) was delivered at a flow rate of 1 ml/min with detection at 406 nm. Retention time of quercetin was found to be 3.189 min. The mobile phase was filtered through

a 0.2 µ membrane filter and degassed. The injection volume was 20 µl. Analysis was performed at ambient temperature.

RESULTS AND DISCUSSION

Chromatography

Standardization of quercetin in different parts of *hypericum mysorense* by RP-HPLC method was carried out using the optimized chromatographic conditions. Typical chromatogram of quercetin (Figure 2.) and methanol extract of HMA, HMF, HML, HMS and HMR are shown in figures 3, 4, 5, 6 and 7 respectively. Detection was done at 406 nm. The peak area ratios of standard and sample solutions were calculated. The assay procedure was repeated six times and the mean peak area and mean peak area ratio of standard were calculated. The results are given in TABLE 1. The low relative standard deviation (RSD) values are indicative of the high accuracy and precision of the method.

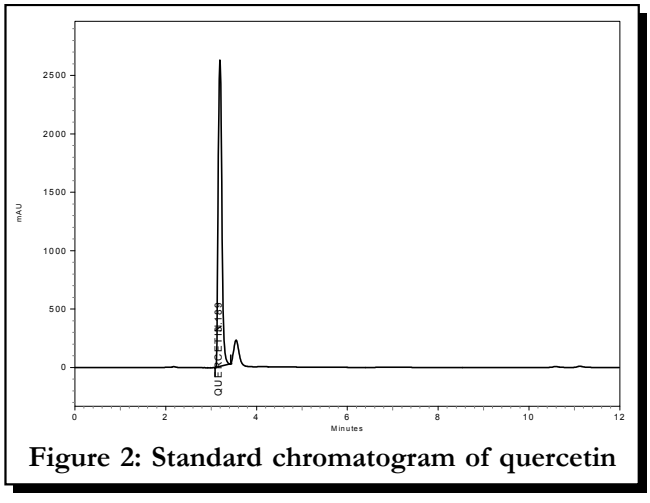

Figure 2: Standard chromatogram of quercetin

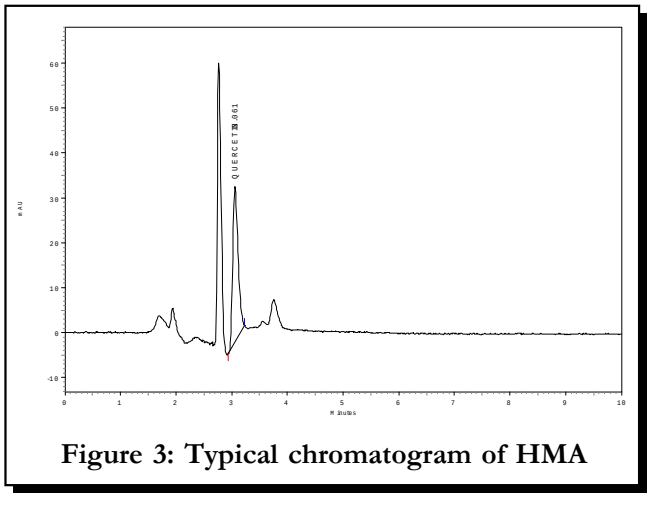

Figure 3: Typical chromatogram of HMA

Method validation

1.Accuracy and precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and

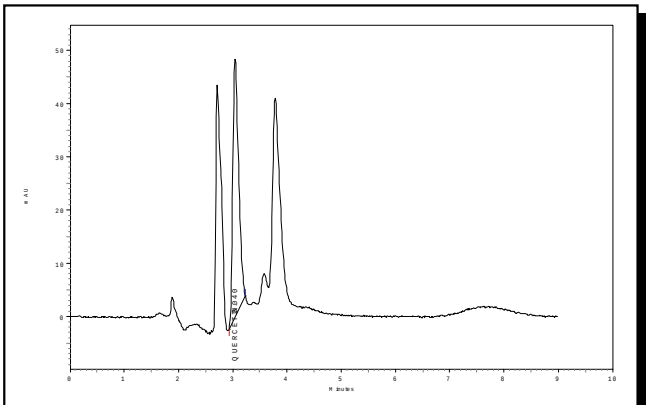

Figure 4: Typical chromatogram of HMF

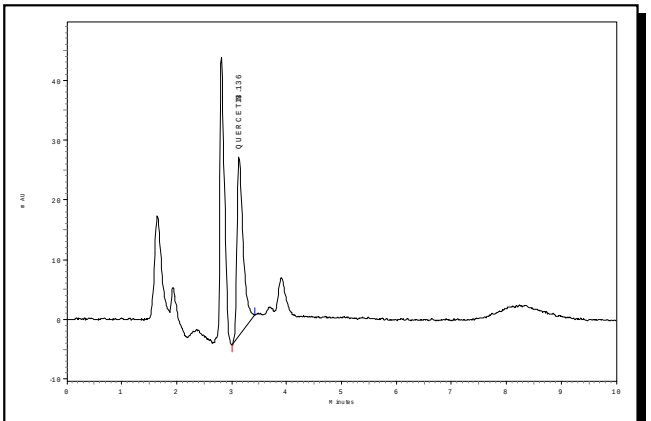

Figure 5: Typical chromatogram of HML

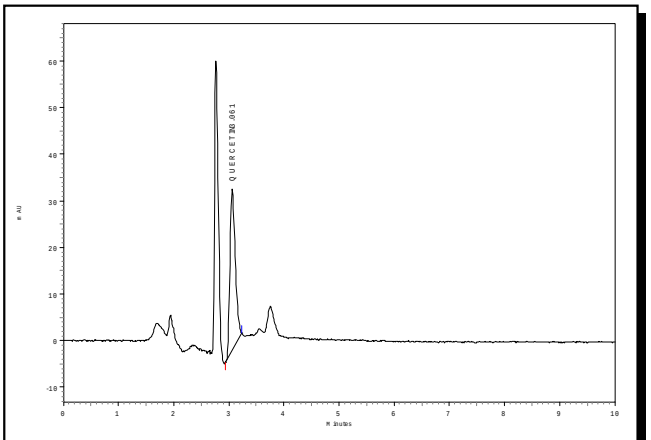

Figure 6: Typical chromatogram of HMS

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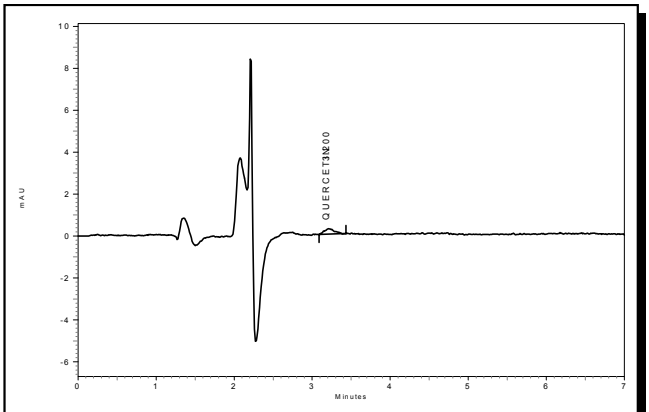

Figure 7: Typical chromatogram of HMR

TABLE 1: Estimation of quercetin content in different parts of *hypericum mysorense*

Extract	Amount of quercetin [% w/w]	RSD (%) (n=6)
HMA	0.805	2.73
HMF	2.109	4.91
HML	1.672	3.14
HMS	0.658	2.01
HMR	0.198	1.04

TABLE 2: Results of recovery analysis of quercetin in HMF

Extract	Amount of Quercetin present in (ng) A	Amount of Quercetin Added to A (ng) B	Total Quercetin Taken (A+B) (ng) C	Total Quercetin found (ng) D	% Recovery (D/C) x 100 (Mean)
HMF	500.0	250.0	750.0	741.0	98.80

TABLE 3: Results of intra and inter-day variability during validation

Concentration (µg/ml)	Intra-day (n =6)		Inter-day (n =6)	
	Accuracy (%)	Precision (CV)	Accuracy (%)	Precision (CV)
5.0	97.05	5.01	96.71	5.19
15.0	98.65	1.64	98.87	1.03
25.0	99.56	0.36	98.23	1.24

standard deviation of the percentage recovery were calculated and is presented in TABLE 2. From the data obtained, added recoveries of standard drugs were found to be accurate.

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra day studies, six repeated injections of validation samples (5, 15 and 25 µg/ml) were made and the peak area and percentage coefficient of variation (CV) were calculated and is presented in TABLE 3. In the inter day variation studies, six repeated injections of validation samples (5, 15 and 25 µg/ml) were made for three consecutive days and peak area and percentage CV were calculated and is presented in TABLE 3. From the data obtained, the developed RP-HPLC method was found to be precise.

2. Linearity and range

The linearity of the method was determined at seven concentration levels ranging from 2 to 25 µg/ml. The calibration curve was constructed by plotting peak area against concentration of drugs. The slope and intercept value for calibration curve was y = 1260617 × -19868 (R^2 = 0.9955). The results show an excellent correlation between peak area and concentration of quercetin within the concentration range indicated above. The calibration curve is shown in figure 8.

3. Limit of detection and limit of quantification

The limit of detection (LOD) (the smallest concentration of the analyte that gives a measurable response, signal to noise ratio of 3) and limit of quantification (LOQ) (the smallest concentration of the analyte, which gives response that can be accurately quantified, signal to noise ratio of 10) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD and LOQ were found to be 50 ng/ml and 150 ng/ml, respectively (TABLE 4).

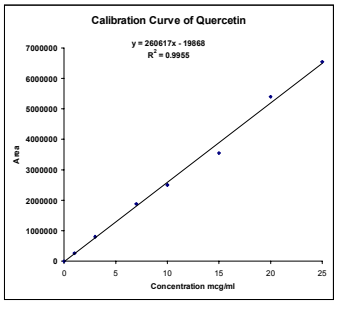

Figure 8: Calibration curve of Quercetin

TABLE 4: System suitability studies

S.No	Parameters	Quercetin
1	Theoretical Plate	3500
2	Asymmetric factor	0.71
3	LOD (ng/ml)	50
4	LOQ (ng/ml)	150
5.	Tailing factor	1.2

4. Ruggedness and robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC-10AT), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Hypersil C_{18}, Phenomenex LUNA C_{18} and Hichrom C_{18}. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there are no marked changes in the chromatograms thus demonstrating that the developed RP-HPLC method is rugged and robust.

5. System suitability studies

The column efficiency and peak asymmetry were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of quercetin in different extracts of *hypericum mysorense*. System suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method (TABLE 4).

CONCLUSION

The proposed RP-HPLC method for standardization of quercetin in different extracts *hypericum mysorense* is simple, rapid, accurate, precise, linear, rugged and robust. Hence the present RP-HPLC method is suitable for the standardization of quercetin in *hypericum mysorense*.

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