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HPLC Estimation of Mangiferin in *Salacia chinensis* Linn.

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A selective and reliable quantitative high performance liquid chromatographic method has been developed and validated for the determination of mangiferin from *Salacia chinensis* Linn. The detection and quantitation limits were 2.55 and 7.55 μg mL^{-1}, respectively, while the linear range of detection was between 7.55 to 255 μg mL^{-1}.

Key Words: HPLC, Mangiferin, *Salacia chinensis* Linn.

INTRODUCTION

Herbal drug technology is used for exploring botanical materials as medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. The use of chromatographic techniques and marker compounds to standardize botanical preparations has limitations because of their variable sources and chemical complexity[1].

In herbal medicinal products the entire herbal drug or a herbal drug preparation is regarded as the active pharmaceutical ingredient (API), regardless of whether or not constituents with defined therapeutic activity are known. In quality control and stability testing of herbal medicinal products fingerprint chromatograms are used as powerful tool to evaluate and compare the composition of compounds in such products. In order to fulfill the ICH and GMP based regulatory requirements in pharmaceutical quality control chromatographic fingerprint analysis needs to be validated. Validation parameters addressed include stability of the analyte, selectivity, robustness testing and method reproducibility.

Marker compounds are characteristic phytochemicals found in a plant. They are often chosen to represent the standard for a standardized extract. Active constituents are phytochemicals which are important for a given therapeutic effect of an herbal extract. This is a highly complex issue, but one proposition is simple and clear: marker compounds are not necessarily active compounds[2].

EXPERIMENTAL

The HPLC system consisted of a 2695 separation module, a 2996 photo-diode array (PDA) detector/2487 dual λ absorbance detector; data were acquired and

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processed using a Empower software ver. 5.00 (all from Waters, Milford, USA). The chromatographic separations were carried out on Ascentis Express (Supelco) C_{18} columns (100 mm × 4.6 mm i.d., with a particle size of 2.7 μm)[3].

Mangiferin was purchased from Sigma-Aldrich. HPLC grade triethylamine was purchased from Spectrochem Pvt. Ltd., Mumbai. Ortho phosphoric acid analytical grade was purchased from Qualigens Fine Chem, Mumbai. HPLC grade acetonitrile was purchased from Merck Specialities Pvt. Ltd., Mumbai.

Standard preparation of mangiferin: 15 mg of reference standard mangiferin was taken in 100 mL volumetric flask dissolved in 15 mL of N,N-dimethylformamide, warmed on steam water bath for 5 min, cooled and the volume was made upto 100 mL with water to yield solution of 150 μg mL^{-1} concentration.

Sample preparation: 1 g of root powder were taken in 100 mL volumetric flask and extracted in 20 mL of N,N-dimethylformamide, warmed on steam water bath for 5 min, cooled and the volume was made upto 100 mL with water, extract was filtered through Whatmann no. 42 filter paper. The mangiferin contents were analyzed after subjecting to HPLC.

Chromatographic conditions: The chromatographic column used was a 100 × 4.6 mm ascentis express (Supelco) with 2.7 μm particles. The mobile phase A was 0.2 % triethylamine pH adjusted to 4.0 with orthophosphoric acid and mobile phase B is acetonitrile in following gradient program:

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-10	98 → 65	2 → 35	Linear gradient
10-11	65 → 98	35 → 2	Linear gradient
11-15	98	2	Re-equilibrium

The flow rate of the mobile phase was 1.0 mL/min. The column temperature was maintained at 25 °C and the eluant was monitored at a wavelength of 254 nm. The injection volume was 5 μL.

Method validation: The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines Q2A and Q2B[4]. Recommend validation characteristics depend on the type of analytical procedure. An important part of method validation is system suitability test (SST), details of which are usually given in pharmacopoeias.

Method validation characteristics were tested in accordance with ICH guidelines[4] and pharmacopoeial requirements. Method accuracy (% recovery and % RSD of individual measurements) and method precision (% RSD) using 6 samples in three replicates have been established. Linearity (correlation coefficient) was tested in the range LOQ-150 % for mangiferin (7.5-225 μg mL^{-1}). Limits of detection and quantitation were provided for mangiferin. Calculation was made by means of the standard deviation of the response and the slope method. Also, method robustness with regard to mobile phase pH and flow was studied.

Linearity and range: A stock solution of the drug (1500 mg mL^{-1}) was prepared. This stock solution was diluted to prepare solutions containing 7.5-225 μg mL^{-1} of

the drug. The solutions were injected in triplicate into the HPLC column, using the mobile phase and keeping the injection volume constant (5 µL).

Precision: Six injections, of concentrations (150 µg mL^{-1}), were given on the same day and the values of relative standard deviation were calculated to determine intra-day precision. Six sample preparations are also injected for result precision.

Limit of detection and limit of quantitation: Limit of detection and limit of quantitation was determined based on the standard deviation of the response and the slope method.

Accuracy: Accuracy was evaluated by adding standard solutions with four known concentrations of mangiferin in sample. The recovery of added mangiferin was determined.

Specificity and selectivity: The specificity of the method towards the drug was established through study of resolution factor of the drug peak from the nearest resolving peak. Overall selectivity was established through determination of purity for mangiferin peak using PDA detector.

Robustness: To determine the robustness of the method experimental conditions were purposely altered. The flow rate of the mobile phase was 1.0 mL/min, to study the effect of flow rate; it was changed by 0.1 units from 0.9 to 1.1 mL/min. The pH of the mobile phase was 4.0 which also changed by 0.2 units from 3.8 to 4.2.

RESULTS AND DISCUSSION

Accuracy: Percentage recovery was calculated from differences between the peak areas obtained for spiked and standard solutions. As shown from the data in Table-1, excellent recoveries were made at each added concentration, despite the fact that the mangiferin was added to a sample that contained mangiferin as well as the other ingredients of plants.

TABLE-1
RECOVERY STUDIES

Actual concentration (µg mL^{-1})	Calculated concentration (µg mL^{-1})	SD	RSD (%)	Recovery (%)
7.53	6.69	3.52	3.96	88.84
120.40	126.78	0.41	0.39	105.30
150.50	138.87	0.81	0.88	92.27
180.60	176.97	3.71	3.79	97.99

(n = 3).

Linearity and range: The response for the mangiferin was strictly linear in the concentration range between 7.5-225 µg mL^{-1}. The values of slope, intercept and correlation coefficient were 18497, 12757 and 1.0000, respectively (Table-2).

Precision: The RSD value for intra-day precision study is 0.08 %. The RSD value for result precision is 0.40 % (Table-3), which confirms that the method is sufficiently precise.

TABLE-2
LINEARITY RANGE, CORRELATION COEFFICIENT, SLOPE AND INTERCEPT

Parameters	Values
Linear range	7.5-225 μg mL^{-1}
Correlation coefficient	1.0000
Slope	18497
Intercept	12757

TABLE-3
PERCENTAGE OF MANGIFERIN CONTENT IN SIX DIFFERENT WEIGHING OF *Salacia chinensis* Linn. ROOT POWDER

Weighing	% w/w mangiferin	Weighing	% w/w mangiferin
1st weighing	1.57	4th weighing	1.56
2nd weighing	1.57	5th weighing	1.58
3rd weighing	1.57	6th weighing	1.57

Limit of detection and limit of quantitation: Limit of detection was found to be 2.55 μg mL^{-1} and limit of quantitation was 7.55 μg mL^{-1}.

Specificity and selectivity: The present method is sufficiently specific to the mangiferin. The mangiferin peak is well resolved from adjacent peaks in sample preparation. The method is also selective to mangiferin as the peak is pure, which was proved through PDA purity studies.

Conclusion

This study is a typical example of development of method for estimation of mangiferin in *Salacia chinensis* Linn., established following the recommendations of ICH guidelines. The developed method is simple, accurate, precise, specific, selective and robust. It is proposed for analysis of mangiferin in *Salacia chinensis* Linn.

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