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LC-MS Quantification of Mangiferin inhydroalcoholic extract of *Salacia oblonga*, *Salacia roxburghii* and polyherbal formulation

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Abstract

Mangiferin is the one of the major active constituents in the tubers of the *Salacia* species, which is one of the valuable species in Ayurveda with antidiabetic, antihypertensive, hepatoprotective, anticaries and anticancer potentials. A simple and rapid liquid chromatography coupled mass spectrometry analysis was developed for the quantification of Mangiferin in two species of *Salacia* and also in polyherbal formulation. This method allowed the direct coupling of an electron spray mass selective detector for the LC system with photo diode array detector, Phenomenex C_{18} column, acetonitrile and aqueous formic acid (0.5%) as mobile phase. Under these conditions, Mangiferin was well separated from the mixture of components present in the extract and detected with mass spectrometry (mass selective detector). Liquid chromatography coupled with electron spray mass was used in positive ion mode and detected at m/z 423$(M+1)^+$. The proposed method is more accurate and sensitive can be used for the routine quantification of the Mangiferin in the herbal extracts as well as polyherbal formulations.

Keywords: *Salacia oblonga*, *Salacia roxburghii*, Mangiferin, Polyherbal formulation

1. Introduction

Indian System of Medicine (ISM) is one of the most ancient system of traditional medicine and it has been practiced in Indian peninsula since 5000BC to offer natural ways to treat diseases and to promote healthcare[1]. Single active components, herbal extracts and herbal combinations have played a significant role in the prevention and management of diseases, especially in complicated chronic conditions.

Salacia species are one of the most important species in the traditional system medicine with antidiabetic, antihypertensive, hepatoprotective, anticaries and anticancer activities. It has been officially listed in the Ayurvedic Pharmacopeia[2]. Literature survey revealed that, *Salacia* species plays an important role in the management of diabetes by various mechanisms like inhibition of protein kinase, activation of PPAR γ, inhibition of the α-glucosidase enzyme[3,4]. Mangiferin is one of the major active components in the tubers of the *Salacia* species. It can also be isolated from the other medicinal plants such as *Mangifera indica*, *Folium pyrrosiae*, *Swetia chirata* and *Rhizoma anemmarrhenae*[5]. Chemically Mangiferin is a (1,3,6,7-tetrahydroxyxanthone-C-2-β-D glycoside having variety of pharmacological effects including anti-oxidative[6], anti-diabetic[7], neuroprotective[8], gastro protective[9], immunomodulatory activities[10]. In the present study liquid chromatography coupled with mass spectroscopy employed for quantification of Mangiferin in the extracts of *Salacia oblonga*, *Salacia roxburghii* and its polyherbal formulated capsules with other plants.

2. Materials and methods

Polyherbal formulation was obtained from M/s Varanasi Bio Research Pvt. Ltd, Varanasi, India. Mangiferin (99.0% purity) was obtained from M/s Sigma Aldrich. Acetonitrile of HPLC grade was purchased from the Merck co. (India). Milli-Q water from Millipore (Massachusetts) was used throughout the study and Formic acid (Rankem, India).

2.1 LC-MS Instrumentation and Analytical Conditions: LC analytical procedure was performed using a system consisting of a LC10ADVP pump and a single quadruple mass spectrometer with electron spray ionization (ESI) source (LCMS-2010) (Shimadzu, Japan). Data acquisition and processing software Lab solutions version 7.0 was from Shimadzu.

Chromatographic separations were carried out using Phenomenex C_{18} analytical (5µm, 250 x 4.6mm, i.d.) (Phenomenex, USA). The analysis was achieved by gradient elution using (A) Water (containing 0.5%formic acid) and (B) Acetonitrile as the mobile phase. The flow rate was 0.5ml/min. The diode array detector was set at 280nm and column was maintained at ambient temperature.

The ESI was performed using nitrogen gas to assist nebulization (the flow rate was set at 1.5L/min). Selective ion monitoring (SIM) with positive mode, capillary voltage at 1.6 kV and temperatures of Curved Desolvation Line (CDL) and heat block at 250ºC and 300ºC were used. Target ion was measured at m/z 423$(M+1)^+$ for Mangiferin.

2.2 Preparation of Mangiferin calibration standards: The standard stock solution was prepared by dissolving 1.5mg of Mangiferinin 10ml of acetonitrile and water (1:1) to furnish a concentration of 150µg/ml. Then this solution was sonicated for 5min. Calibration standards at six levels of concentrations were prepared by serial dilution of the stock solution with HPLC grade acetonitrile and water (1:1) from 2µg/ml to 128µg/ml. Injection of 20µL in triplicate were made from each concentration and chromatographed under the specified conditions described above.

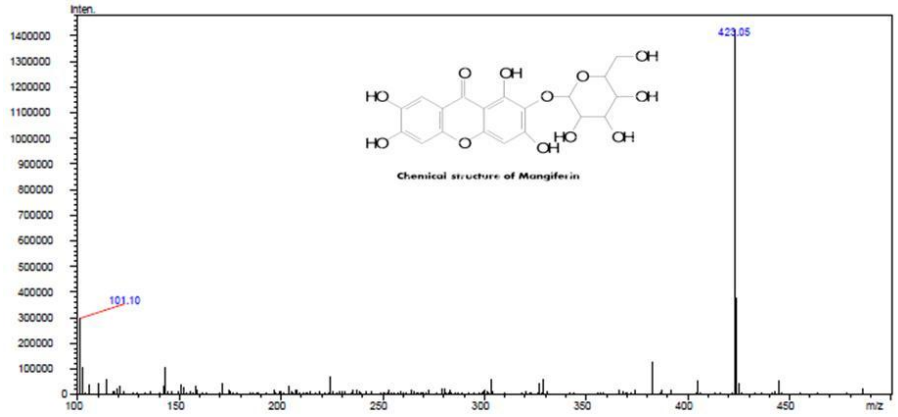
2.3 Sample preparation: *Salacia oblonga* and *Salaciaroxburghii* extracts were prepared by dissolving 200mg of the extract powder in 10ml of Acetonitrile and Water (1:1). The polyherbal formulation capsule powder equivalent to 100mg of the *Salacia oblonga* was weighed accurately in 10ml of acetonitrile and water (1:1). All the solutions were sonicated cooled and then made up to the volume and filtered through 0.22µ membrane filter for further analysis.

2.4 Optimization of the LC and MS conditions: On the basis of the structure, solubility and acid-base properties of Mangiferin, the method was developed with a C_{18} column and the mobile phase containing acetonitrile and aqueous formic acid. The first mobile phase was 10:90 binary mixtures of acetonitrile and buffer. When the same binary mixture in different proportions (20:80, 15:85and 10:90) was tested, it was found that, as the proportions of acetonitrile in the mobile phase was reduced the retention time of the compound gradually increased. As a result, the mobile phase with gradient program was developed with Acetonitrile and water (0.5% Formic acid) was finally selected to achieve good resolution with short retention time.

The detection of Mangiferin was set at wavelength 280nm taking above things to in consideration. With these chromatographic conditions baseline resolution was achieved with reasonable retention time. Typical chromatograms were obtained from a standard, extracts and herbal formulation.

To select an appropriate ionization mode in LC-MS analysis, the mass spectra were measured in ESI and APCI positive and negative mode using Mangiferin standard solution. In both ionization modes, the base peak intensities of the positive ion were higher than that of negative ion, and efficiencies of the ionization in the ESI were higher than APCI. Thus, selective ion monitoring (SIM) mode at 423 $(M + 1)^+$ was used for quantitative analysis of Mangiferin. SIM mode mass spectrum of Mangiferin was shown in **Fig. 1**

Fig. 1: SIM mode mass spectrum of Mangiferin



Mass spectrometer conditions were optimized by direct infusion of the standard and SIM acquisition mode was used for analysis, in order to detect only specific mass ions during analysis.

2.5 Application of the method: The validated LC-MS method can be used for routine quantification of Mangiferin in *Salacia oblonga*, *Salaciarox burghii* and its polyherbal formulation. Results obtained are listed in **Table 5**. The 3D graph representation of the *Salacia* species and polyherbal formulation was shown in **Fig.6**, **Fig.7** and **Fig.8**.

3. Results and discussion

3.1 Method validation

3.1.1 Specificity: Typical chromatograms of standard Mangiferin, *Salacia* species extract and polyherbal formulation containing *Salacia* species are shown in the **Fig.2**, **Fig.3**, **Fig.4** and **Fig.5**. The retention time for Mangiferin was found to be at 6.7min with total run time of 30min. A good separation of the Mangiferin peak from other constituent was obtained under developed chromatographic conditions. Other constituents from different plants do not interfere with Mangiferin.

3.1.2 Calibration curve: Linearity was determined by constructing calibration plot. Five different concentrations of the standard solutions were prepared, chromatographed and linearity was obtained by calibration plot. Peak area (A) and concentration (C) for each compound were subjected to regression analysis to calculate the calibration equations and correlation coefficients. The regression equations obtained for Mangiferin was 2.19e-005 X-0.436.The linearity range was 1.25 to 20µg/ml. The result shows that there was a first-rate correlation between peak area and concentration for compound. The correlation coefficient, slope, intercept and % relative standard deviation (%RSD) are suitable as general acceptable criterion to the linearity performance of an analytical procedure.

3.1.3 Quantification limits (LOD & LOQ): The quantification limit is the lowest concentrations of the compound, that can be accurately and precisely quantified, which is ten times than noise level. The LOQ of the compound was determined experimentally by performing six injections of each concentration and it was found to be 9.16ng for Mangiferin.

3.1.4 Accuracy: Accuracy was determined by developed method and spiking extracts with known amount of the Mangiferin standards and compared the measured value with true values. Triplicates injections were made with all spiked samples. **Table1** summarizes the accuracy results, expressed as recovery percentage. The method has shown 98.12 ± 1.70 % recovery of sample from extract.

Table 1. **Recovery of Mangiferin from the extract of *Salacia oblonga* (n=3)**

Sl. No	Amount of Mangiferin added (µg/ml)	Amount Found (Mean±SD)	% Recovery	% R.S.D
1	160.46	158.21±1.59	98.61	1.04
2	239.86	235.55±1.36	98.20	0.58
3	361.24	352.46±2.79	97.57	0.79

Each value is mean of three observations

3.1.5 Precision: The precision of the method as repeatability intra-day assay precision [%CV] was assessed by performing six independent analysis of sample and qualified reference standards together at 100% of the test concentration. Inter-day precision was determined by repeating the analysis of the same concentration by repeating the studies by three different analysts on three different days over a period of 1 week also expressed in terms of %CV in **Table 2**.

Table 2.Summary of intra-day and inter-day method precision

Amount (µg/ml)	Intra-day precision by peak area			Inter-day precision by peak area		
	Average peak area	S.D of peak area	CV[%]	Average peak area	S.D of peak area	CV[%]
1.25	65872.33	647.32	0.98	66382.00	1140.10	1.21
5.0	218537.30	2588.59	1.18	218267.30	2449.14	1.12
20.0	904410.30	1381.25	0.15	906981.30	15946.00	1.55
-	-	-	0.77	-	-	1.29

System precision is a measure of the method variability that can be expected if a given analyst performs the analysis at three different concentrations. It was determined by performing three replicate analysis of each standard solution at three different concentrations. The RSD values for Mangiferin were shown in **Table 3**.

Table 3.System precision for Mangiferin at different concentrations (n=5)

Mangiferin			
Concentration (µg/ml)	2.5	10	20
RSD (%)	1.1	0.97	0.49

The method precision was determined by replicate sample solutions and RSD values obtained were 1.1%, 0.97% and 0.47% for 2.5µg/ml, 10µg/ml and 20µg/ml of Mangiferin respectively. The summary of the validated method and results are depicted in **Table 4**.

Table 4.Summary of method validation parameters

Parameter	Mangiferin
Linearity range (µg/ml)	1.25-20
Correlation coefficient	0.9986
Limit of detection(µg)	3.02
Limit of quantification(µg)	9.16
Recovery (mean ± SD)	98.12±1.70
Precision (CV)	
Intra-day (n=6)	0.77
Inter-day (n=3)	1.53

Table 5. **Percentage of Mangiferin content in the *Salacia* species and its Polyherbal formulation**

S. No	Sample name	Mangiferin content (%w/w)
1	*Salacia oblonga*	0.174%
2	*Salaciaroxburghii*	0.319%
3	Polyherbal formulation	0.617%

Fig. 2: Chromatogram of Mangiferin standard

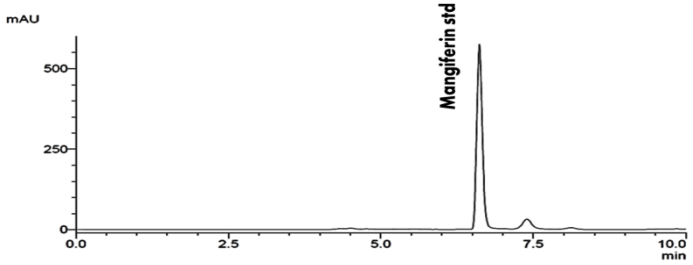


Fig. 3: Chromatogram of Mangiferin in *Salaciarox burghii*

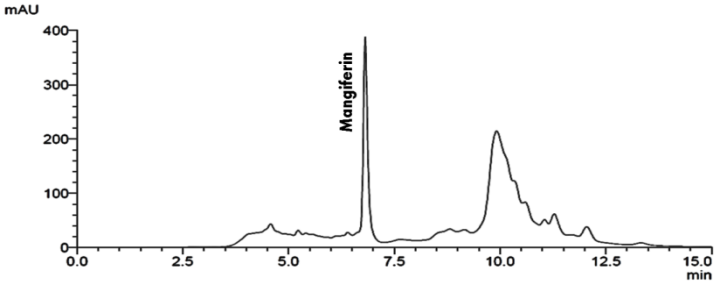


Fig. 4: Chromatogram of Mangiferin in *Salacia oblonga*

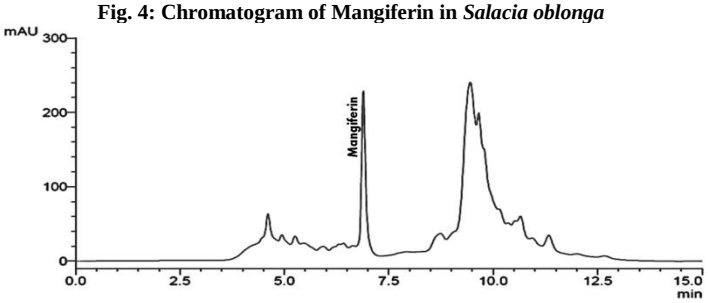


Fig. 5: Chromatogram of Mangiferin in polyherbal formulation containing Salacia species

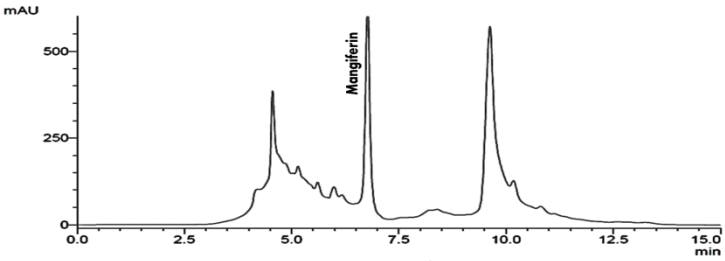


Fig. 6: 3D Graph representation of the *Salacia oblonga*

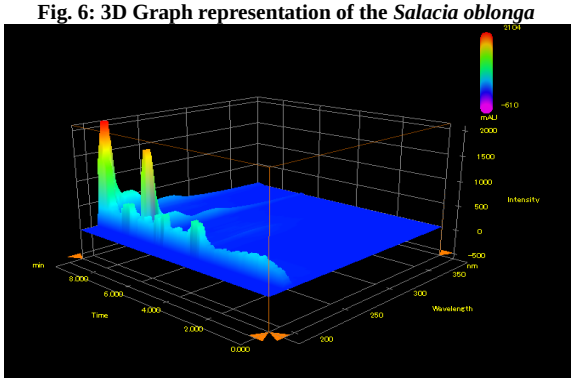


Fig. 7: 3D Graph representation of the *Salacia burghii*

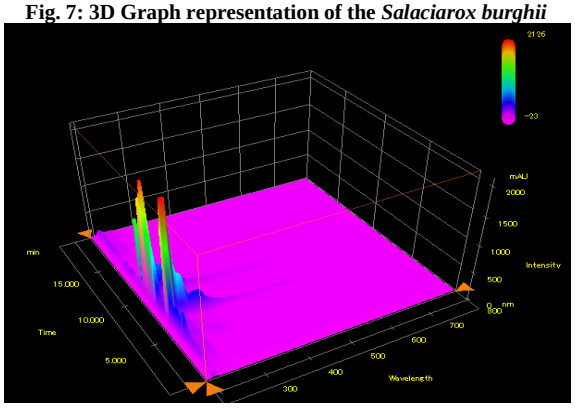
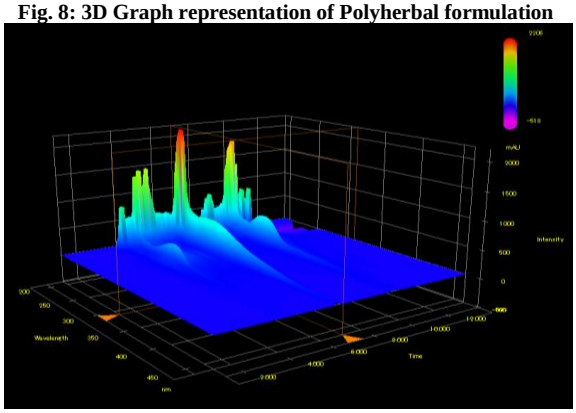


Fig. 8: 3D Graph representation of Polyherbal formulation



4. Conclusion

The results described above showed that the developed LC- MS method was highly suitable for rapid determination of standard Mangiferin, individual extracts and or with a combination of other extracts in the form of formulations. This work also showed that LCMS was a powerful technique for the quantification of Mangiferin in the complex extracts obtained from the medicinal plants and also in the form of polyherbal formulations.

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