Biosciences, Biotechnology Research Asia Vol. **5**(1), 383-388 (2008)

Development of new reversed-phase HPLC method for the determination of Mangiferin in *Mangifera indica* Linn.

PRADEEP KUMAR*, VISHAL SRIVASTAVA and LALIT KUMAR[1]

*Department of Pharmaceutical Analysis, PES College of Pharmacy, Bangalore, Karnataka (India)
[1]Department of Pharmaceutical Chemistry,
Manipal College of Pharmaceutical Sciences, Karnataka (India)

(Received: May 10, 2008; Accepted: June 02, 2008)

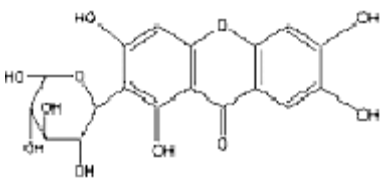
ABSTRACT

Mangiferin is the active constituent obtained from the dried parts such as leaves and barks of the Mango tree (*Mangifera indica* L.) which belongs to the family Anacardiaceae. Analytical method is developed and validated for the determination of Mangiferin in *Mangifera indica* by reversed-phase HPLC method. The HPLC method is described for determination of Mangiferin from leaves and barks Extract. The separation of the Mangiferin was achieved on a Lichrospher ® 100; RP- 18e (5 µm) column using mobile phase Acetonitrile: Buffer (Potassium dihydrogen orthophosphate (1.36 gm) in 900 ml HPLC grade water, adjust the P^H to 2.5-2.8 with orthophosphoric acid and detection was carried at 254 nm (Isocratic Elution). The Lichrospher ® 100; RP-18e (5 µm) shows most favorable chromatographic parameters for analysis of Mangiferin in *Mangifera indica*. The linear range for Mangiferin was found to be 15.0-1000 µg mL^{-1}. The Method has been successfully validated for linearity, Specificity, Precision, Accuracy, range and with good recoveries. Hence the present RP-HPLC method is suitable for assay of the Mangiferin in raw materials, extracts in quality control laboratories.

Key words: Mangiferin, leaves, bark, RP-HPLC, isocratic elution and alcoholic extracts.

INTRODUCTION

Mangiferin obtained from the dried stem bark and leaves of *Mangifera indica Linn.* and having a molecular formula $C_{19}H_{18}O_{11}$ and molecular weight 422 . The major bioactive constituent of *Mangifera indica* is a xanthone c-glucoside mangiferin. The other constituents are triterpenen viz. mangiferolic acid, isomangiferolic acid and several cycloartenod derivatives a flavonoide amantoflavone.



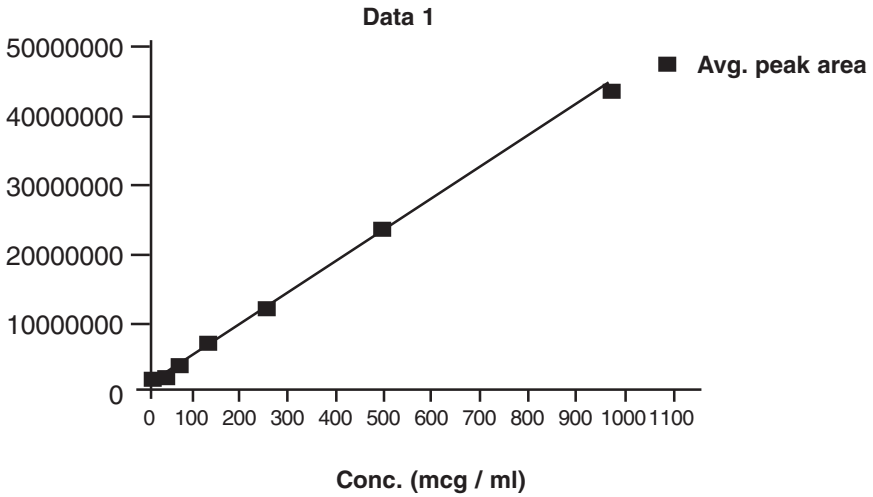
Mangiferin
(1,3,6,7 tetrahydroxy C2 beta-D-glucoside)

Dried mango flowers, containing 15% tannin, serve as astringents in cases of diarrhea, chronic dysentery, catarrh of the bladder and chronic urethritis resulting from gonorrhea. The bark contains Mangiferin and is astringent and employed against rheumatism and diphtheria in India. The resinous gum from the trunk is applied on cracks in the skin of the feet and on scabies, and is believed helpful in cases of syphilis[1-10].

EXPERIMENTAL

Chromatographic condition

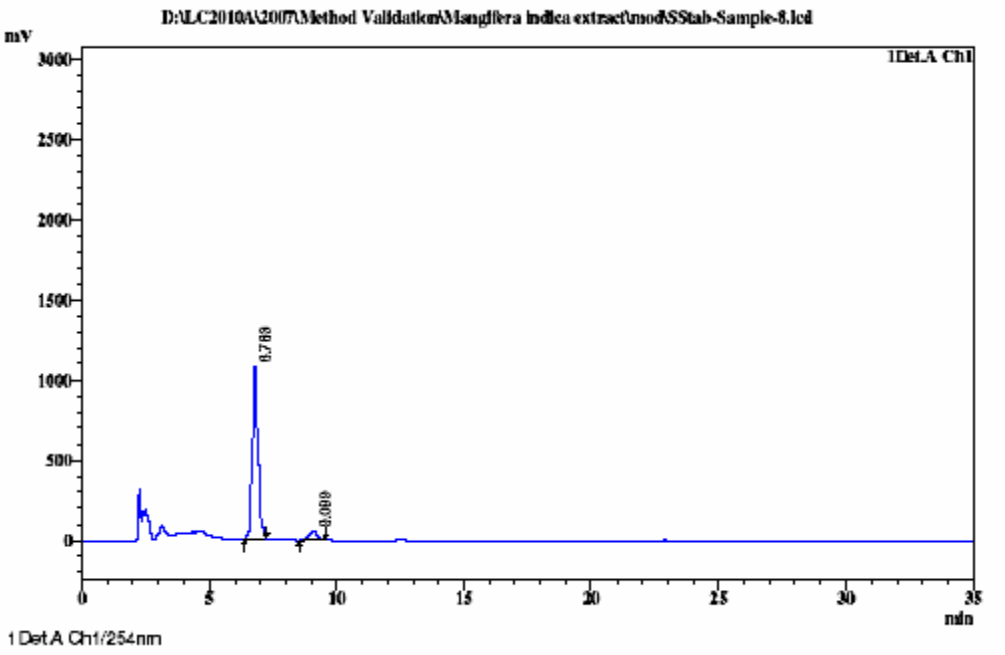
The following HPLC condition was maintained through out the experiment.



linear graph pzf layout 1-Mon Apr 30 13:35:57 2007
Mangiferin

Slope	44570 ± 495.6
Y-intercept when x=0.0	496800±210600
X-intercept when x=0.0	-11.14
1/slope	0.00002243
95% confidence intervals	
Slope	43300 to 45850
Y-intercept when x=0.0	-44630 to 1038000
X-intercept when x=0.0	-23.74 to 0.9829
Goodness of Fit	
r^2	0.9994
Sy-X	423600
Is slope significantly non-zero ?	
F	8089
DFn, DFd	1.000, 5.000
P value	<0.0001
Devitation from zero	Significant
Data	
Number of X values	7
Maximum number of Y replicates	1
Total number of values	7
Number of missing values	0

Fig. 1: Linear graph of mangiferin



Detector Ch1 254 nm

Peak#	Ret. Time	Area	Height	Area%	Name
1	6.783	17055367	1070209	92.967	Mangiferin
2	9.009	1290260	60421	7.033	RT 9.099
Total		18345626	1130630	100.000	

Fig. 2: Graph showing retention time of Mangiferin

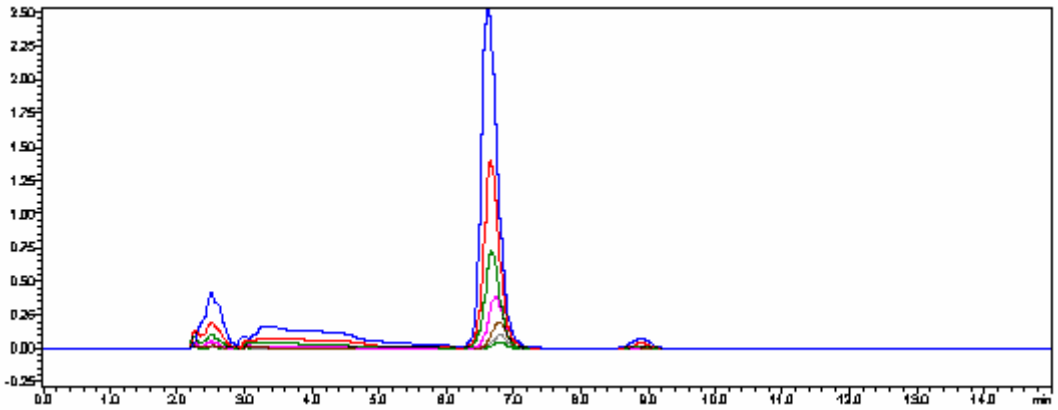


Fig. 3: Overlay spectra of Mangiferin by using different concentration

Table 1: Correlation coefficient (r2) for Mangiferin was found to be 0.9994 indicating that the method is linear between the concentration 15.212 to 973.589 µg mL-1.

S. no	Dilution	Analyte	Concentration (mcg/ml)
1.	1	Mangiferin	15.212
2.	2	Mangiferin	30.425
3.	3	Mangiferin	60.849
4.	4	Mangiferin	121.699
5.	5	Mangiferin	243.397
6.	6	Mangiferin	486.794
7.	7	Mangiferin	973.589

Mobile Phase

(Buffer 85% Acetonitrile 15%)

Buffer

Weigh accurately 1.36 gm. of Potassium dihydrogen orthophosphate in 900 ml of HPLC grade water; adjust the P^H to 2.5 to 2.8 with dilute orthophosphoric acid than make up the volume to 1000ml. (Solvent A)

Acetonitrile (solvent B)

2. Detector: UV Detector.
3. Wavelength: 254 nm.
4. Flow rate: 1.0ml/min
5. Injection volume: 20µl
6. Run Time: For Standard: - 15 min; For

Table 2: For peak area of mangiferin

Replicate	Dilution I	Dilution II	Dilution III	Dilution IV	Dilution V	Dilution VI	Dilution VII
1	720827	1635258	3017575	6417442	1164290 6	22801006	43270923
2	720289	1640056	3070415	6412587	11553605	22456240	43943343
3	723671	1635065	3075631	6417073	11322855	22812351	43488394
Average	721596	1636793	3054540	6415701	11506455	22689866	43567553
SD	1817.310	2827.488	32118.975	2702.819	165152.89	202405.26	343127.98
R. Factor	47434.95	53798.269	50198.462	52717.966	47274.409	46610.792	44749.454
RSD%	0.252	0.173	1.052	0.042	1.435	0.892	0.788

Table 3: For retention time of mangiferin

Replicate	Dilution I	Dilution II	Dilution III	Dilution IV	Dilution V	Dilution VI	Dilution VII
1	6.801	6.737	6.789	6.769	6.684	6.657	6.614
2	6.787	6.798	6.780	6.733	6.676	6.658	6.611
3	6.789	6.800	6.786	6.707	6.679	6.654	6.611
Average	6.792	6.778	6.785	6.736	6.680	6.656	6.612
SD	0.008	0.036	0.005	0.031	0.004	0.002	0.002
RSD%	0.111	0.528	0.068	0.462	0.061	0.031	0.026

Average of retention time = 6.7204 minutes

Sample: - 15min

7. Data acquisition: Using suitable software CFR-21 Part 11 Compliant
8. Record the Chromatogram: Record the chromatogram with the following Details Retention time, Peak area, Peak height, % Area, Assigning the name to the Peaks.
9. Integration parameters: Integrate peaks base to base.
10. For calculating concentration, Mean, Standard Deviation, Relative Standard Deviation, Relative retention time, Response factor, Theoretical plate,Resolution, Asymmetry, Tailing factor by EXCELL or any other suitable Software can be used.
11. Plotting the Graph: EXCELL and PRISUM software.

Standard solution

Weigh accurately 10.089 mg of Mangiferin in the 10ml volumetric flask; add 1ml of D.M.F and make up the volume 10 ml by using methanol i.e. dilution I, Dilution II, III, IV, V, VI, VII were made consecutively by dissolving 5ml to 10ml in sufficient quantity of methanol in a standard flask. The Peak area & Retention time for individual standard are tabulated in the Table 2-3 respectively.

The concentration of analyte in 10 ml is;

$$\text{Mangiferin} = \frac{10.089}{100} x 96.5 = 9.7358 \text{ mg}$$

Sample solution :(for raw material)

The sample solution was prepared by weighing the required quantity of sample (coarse powder) and transferred to a 100 ml beaker. Extract with 10 ml of DMF (Dimethyl form amide) and 80 ml of HPLC grade methanol sonicate for 5 minutes, by warming on water bath for about 20 min. discard the supernatant liquid (extract) to a 250 ml beaker. Repeat the procedure 4-5 times till the raw material was completely extracted or till the extract is colorless. Collected all the extract in a 250ml beaker and concentrated it to below 100ml volumetric flask and made up the volume to 100ml with methanol, mixed well and filtered through 0.45µl membrane.

Procedure

Take required quantity of *Mangifera indica* extract or raw material such as leaves or bark of Mangifera indica,(After extraction) weighed and transfer into 100ml volumetric flask, dissolved in 10ml dimethyl form amide and 25ml of methanol of HPLC grade, sonicated for 5 minutes. Warmed on steam water bath for 5 minutes, cool and make up the volume to 100ml with Methanol. Inject 20µLof sample and record the chromatogram. Inject 3 times and calculated the results.

RESULTS AND DISCUSSION

The main constituents present in *Mangifera indica* is mangiferin. Quantitative determination of mangiferin from Raw material and extracts of *Mangifera indica* was done.

The Present method was done on HPLC, using Isocratic pump, C_{18} column with UV detector. As mangiferin is a polar molecule, its analysis was carried out using reverse phase HPLC method. The column used for separation of mangiferin was Lichrospher® C_{18} with a particle size 5 µl. The mobile phase used was (Buffer 85% Acetonitrile 15%). The mangiferin have Absorption maxima at 254 nm. Therefore, the wavelength was chosen as 254 nm. From the typical chromatograms the Retention time for mangiferin was found to be 6.7204 min. The method is validated in terms of Linearity, precision, repeatability, accuracy, range, Quantification and specificity. A good linear relationship was observed for mangiferin with correlation coefficient (r^2) of more than 0.9994 the chromatograms indicating that the method is free from interference. The repeatability and reproducibility indicated that the proposed method is precise and reproducible. The percentage recovery of different batches of extract and raw material were found to be in between 95-110%. This demonstrates that the developed HPLC method is simple, precise and accurate

ACKNOWLEDGEMENTS

The authors are grateful to Natural Remedies Private Limited, Bangalore for providing gift samples of Extract and Raw materials and for providing laboratory facility and constant encouragement.

REFERENCES

1. The Ayurvedic Pharmacopoeia of India, by government of india Ministry of health and family welfare department of ISM and H, 1st Edition, Part -1, Vol. III, 9.
2. Molecular formula and molecular weight of mangiferin. (Source: http://www.made-in-china.com/showroom/byro 2000/product-list/Sap-Extract-1.html).
3. Clasificación of Mangifera indica. (Source :http://en.wikipedia.org/wiki/Mango)
4. Parts used for the estimation of mangiferin in Mangifera indica. (Source: http://www.rain-tree.com/mango.htm)
5. Ivan Ross A, Medicinal plants of the world, Published by Humana Press Totowa, New Jersey, 197.
6. Rastogi RP, Mehrota BN, Compendium of Indian Medicinal Plants, Central Drug Research Institute Lucknow; India, 265-6 (1991).
7. Rastogi RP, Mehrota BN, Compendium of indian Medicinal Plants, Central Drug Research Institute Lucknow; India, 407-9 (1993).
8. Rastogi RP, Mehrota BN, Compendium of indian Medicinal Plants, Central Drug Research Institute Lucknow; India, 456 (1995).
9. Rastogi RP, Mehrota BN Compendium of indian Medicinal Plants, Central Drug Research Institute Lucknow; India, 524 (1998).
10. Trease and Evans, A Textbook of Pharmacognosy, 14th Edition, 249.