

STABILITY INDICATING HPTLC METHOD FOR ESTIMATION OF MANGIFERIN IN BULK AND DOSAGE FORM

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ABSTRACT

Introduction - There are so many promising plant based chemical constituents are present which act as alternative therapy for the control of diabetes. But due to lack of its proper quality control parameters they are not widely used. Mangiferin is a xanthone glucoside and active phytochemical principle which is present *Mangifera Indica*. Studies suggest that mangiferin, xanthone compound possesses anti diabetic activity. **Objective** - In the present study a novel stability-indicating high-performance thin-layer chromatography (HPTLC) method for quantitative determination of Mangiferin in bulk drug and formulation has been developed and validated as per ICH guideline Q2(R1) for global acceptance of standardized Herbal formulations. **Methodology** – HPTLC method is developed and validated using solvent Ethyl acetate: Ethanol: Formic acid (10:1.5:1v/v/v) (R_f of Mangiferin 0.66 ± 0.03) in the absorbance mode at 340 nm. Various forced degradation conditions were used to check degradation of drug. **Results** - The method showed a good linear relationship ($r^2 = 0.9965$) in the concentration range 200-1000 ng per spot. It was found to be linear, accurate, precise and specific. **Conclusion** - It can be applied for quality control as well as for stability testing of different dosage forms containing mangiferin. The developed method is validated as per ICH guideline Q2(R1) for global acceptance of standardized herbal formulations.

KEY WORDS

Mangiferin, stability-indicating HPTLC method, ICH guidelines, quality control

INTRODUCTION:

Herbal medicinal technology is used for exploring medicinal plant materials as medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. The chromatographic techniques and marker compounds are used to standardize herbal formulations.¹

The stability indicating assays are important to determine the shelf life of the products. It also helps to determine the storage conditions by knowing the process of degradation. It is carried out by performing forced degradation studies. These types of studies using sophisticated techniques are important for global acceptance of herbal products.²

Mango (*Mangifera indica* L.), the most important fruit in Anacardiaceae family, is a tropical fruit with high

nutritional and medicinal value. It originated from the Asia Indo-Burmese region approximately 4000 years ago, and is now commercially grown in more than 87 countries.³

Mangiferin is a xanthone glucoside and active phytochemical principle which is present *Mangifera Indica*. Studies suggest that mangiferin, xanthone compound possesses anti diabetic activity.⁴

There are some reports on the application of Spectrophotometry, Spectro fluorimetry, Thin layer chromatography, Liquid chromatography–Mass spectrometry (LC–MS) and High-performance liquid chromatography (HPLC) methods. ICH guidelines have given guidance for the estimation of degradation during storage. Hence, it was considered worthwhile to develop precise, cost effective and stability indicating HPTLC method for determination of Mangiferin in bulk

drug and dosage form. The HPTLC analytical methods are advantageous over other analytical methods for analysis of botanicals, plant-derived drugs and biomarkers. Several stability-indicating HPTLC methods have been published and being utilized for plant origin biomarkers like curcumin and forskolin. The method developed in the present investigation was validated as per the International Conference on Harmonization (ICH) guideline (ICH, 2005). The drug was analyzed under different stress conditions to explain the inherent stability characteristics of the active substance and to develop the validated stability-indicating HPTLC assay method (ICH, Q2(R1)).⁷⁻¹³

EXPERIMENTAL

Chemicals & Reagents

Methanol, Acetonitrile, Toluene, Ethyl acetate, Formic acid & Acetic acid of AR Grade were purchased from Astron, Ahmedabad. Reference standard of Mangiferin (MF) is purchased from Sigma Aldrich.

Chromatographic Conditions

Stationary phase was Precoated Silica gel G60 F₂₅₄ aluminum Sheets 10×10 cm², layer thickness 0.2 mm. Activated the TLC plates by prewashing with methanol & activated in Oven at 50°C for 5 minutes. The Optimized Mobile phase was Ethyl acetate: Ethanol: Formic acid (10:1.5:1, %v/v/v). Chamber saturation time was 20 minutes at ambient temperature & migration distance was 75mm. The detection was done at 340 nm.

Preparation of Solutions

Preparation of Mangiferin (MF) standard stock solution (1000µg/ml)

Accurately weighed 10mg of Mangiferin was transferred into 10ml volumetric flask, dissolved and diluted up to the mark with methanol to get stock solution having concentration 1000µg/ml.

Preparation of working standard solution (100µg/ml)

1 ml of standard stock solution of MF was transferred to 10 ml volumetric flask and diluted to up to the mark with methanol to get working standard solution having 100µg/ml.

Preparation of solution for calibration curve

To obtain calibration curve, working standard solutions ranging from 2, 3, 4, 5, 6, 7µl was applied by Hamilton syringe with the help of Linomat V applicator on TLC plate having concentration 200-700ng/spot.

Isolation of Mangiferin from *Mangifera Indica*:⁶

The leaves of *Mangifera indica* L. var Alphonso (Anacardiaceae) were collected, shade dried and powdered. The powdered plant materials (100g) were defatted with petroleum ether (60-80°C). Defatted powdered leaves were extracted by soxhelt with required quantity of methanol for 21h and concentrated under reduced pressure to yield semisolid mass. The concentrated mass were resuspended in 50ml of 50% methanol then partitioned with 100ml dichloromethane for 4 times. The aqueous methanolic phase was hydrolysed by reflux with 2N Sulphuric acid at pH 3 for an hour with continuous stirring. After cooled to room temperature, it was partitioned with 100 ml ethyl acetate for 3 times. Subsequently, the combined ethyl acetate layer was dried at 40°C using a vacuum rotary evaporator. The dried ethyl acetate fraction was dissolved in ethanol and left in a refrigerator (4-8°C) over night. After that the precipitate came out and was isolated by filtration. For crystallization, the precipitate was dissolved in 70% aqueous ethanolic solution and left in a refrigerator (4-8°C) over night. Lastly, the pale-yellow needle-shaped crystals of mangiferin were isolated and dried. The isolated compound was further characterized using TLC, melting point, UV/VIS spectroscopy compared with reference standard mangiferin.

Analysis of MF in Marketed Formulation

Capsule powder equivalent to 100 mg *mangifera indica* extract was transferred to 100 ml volumetric flask containing 10 ml methanol, sonicated for 15 min and diluted to mark with methanol to obtain 1 mg/ml of extract solution. The resulting solution was filtered using Whatman filter paper. From the above solution 1ml was transferred into 10 ml volumetric flask and diluted to mark with same solvent. So, resultant solution 20 µl was injected and amount of MF (446 ng/spot) per 100 µg/ml of extract solution is calculated.

Forced degradation studies

Stress studies were carried out under the acidic, basic, thermal and oxidation conditions as mentioned in ICH Q1A (R2).

Preparation of standard stock solution for forced degradation studies:

Accurately weighed 10mg of MF transferred to 10 ml volumetric flask and diluted up to the mark with methanol to produce 1000 µg/ml.

Preparation of Control:

2.5 ml aliquot of standard stock solution was transferred to 25 ml volumetric flask and diluted up to the mark with methanol to produce mixture of 100 µg/ml of MF which was used as control. 4.5 µl of resultant solution of MF (450 ng/spot) was applied on TLC plate and developed and scanned as per optimized chromatographic conditions.

Degradation under acid catalysed hydrolytic condition:

2.5 ml of standard stock solution of MF was mixed with 1 ml of 1 N HCl. The solution was diluted to 25 ml with methanol and kept for 4 hours at room temperature. 4.5 µl of resultant solution of MF (450 ng/spot) was applied on TLC plate and developed and scanned as per optimized chromatographic conditions.

Degradation under alkali catalysed hydrolytic condition

2.5 ml of standard stock solution of MF was mixed with 1 ml of 1 N NaOH. The solution was diluted to 10 ml with methanol and kept for 1 hour at room temperature. 4.5 µl of resultant solution of MF (450 ng/spot) was applied on TLC plate and developed and scanned as per optimized chromatographic conditions.

Oxidative degradation

2.5 ml of standard stock solution of MF was mixed with 5 ml of 2% H₂O₂. The solution was diluted to 25 ml with methanol and refluxed for 1 hour. 4.5 µl of resultant solution (450 ng/spot of MF) was applied on TLC plate and developed and scanned as per optimized chromatographic conditions.

Thermal degradation

2.5 ml aliquot of standard stock solution was transferred to 25 ml volumetric flask and diluted up to the mark with methanol to produce 25 µg/ml. The resulting solution was subjected to 100 °C for 30 minutes. 4.5 µl of resultant solution (450ng/spot of MF) was applied on TLC plate and developed and scanned as per optimized chromatographic conditions.

Method Validation

Linearity For the calibration curve the 2-7 µl from the working standard solution having 100ng/µl concentration of MF was injected. So, linearity responses for MF were assessed in the concentration range of 200-700ng/spot of working standard solutions. Precision:

Instrument Repeatability- The precision was checked by repeated injecting, scanning and measuring the area

of MF (400 ng/spot) solutions (n=5) without changing the parameters.

A) Intra-day precision- The intra-day precision of the proposed method was determined by analysis of corresponding responses in triplicate on the same day for 3 different concentrations of standard solution of MF (300, 400 and 500 ng/spot).

B) Inter-day precision: The inter-day precisions of the proposed method was determined by analysis of corresponding responses in triplicate on 3 different days over a period of 1 week for 3 different concentrations of standard solution of MF (300, 400 and 500 ng/spot). Results were reported in terms of % RSD.

Accuracy The accuracy of the method was carried out at three levels in the range of 50-150% of the working concentration of sample. Calculated amount of standard was added in placebo containing volumetric flask to prepare 100 mg/ml.

2, 4, 6 µl (400 ng of MF) of sample solution was spotted on TLC plate on succeeding spots to obtain final concentration range of 200 - 600 ng/spot. Amount found, %Recovery and mean recovery was calculated at each level and recorded. The plate was developed, dried and photometrically analyzed. The same procedure repeated three times.

Sensitivity: The sensitivity of measurement of MF by the use of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated by standard formula.

Specificity: The specificity of an analytical method is ability to measure accurately an analyte in presence of interferences like synthetic precursor, excipients, degradants or matrix component. Purity of spectra was determined at three different levels, at starting, middle & end. Correlation between the spectra of standard & spectra of drug in sample track was considered for determination of peak purity. The specificity was also determined by checking whether the sample matrix, solvent or mobile phase interfered in the analysis.

RESULT AND DISCUSSION:**Selection of Detection Wavelength**

The sensitivity of HPTLC method with UV detection depends upon proper selection of detection wavelength. An ideal wavelength was the one that give good response for the drug to be detected. The TLC

plate was scanned between 200 – 400nm. MF showed maximum absorption at 340 nm. Thus, it was selected as detection wavelength for HPTLC analysis (Figure No 1)

Mobile Phase Optimization

The solvent system was developed and optimized using trial and error method. Various proportions of different solvents were tried to get resolution of the compound. The optimized mobile phase was Ethyl acetate: Ethanol: Formic acid (10:1.5:1 v/v/v). The optimized mobile phase could resolve the compound and the band obtained was compact too. Various mobile phases were tried. Optimized chromatograph is showed in Figure No 2.

The R_f value of standard MF was shown in Table No.1, below.

Results from various stress condition for standard MF are shown in Table No 3 below: Forced degradation shows that MF is most degraded by alkali conditions.

Method Validation

Linearity: linearity responses for MF were assessed in the concentration range of 200-700ng/spot of working standard solutions. Table No 3 shows the data of calibration curve and Figure No 3 shows the calibration curve (200-700ng/spot). The linear equation for the calibration plot was $y = 16.90x + 2483$ with correlation coefficient r^2 0.9965 (Figure No 4).

Precision

Method Repeatability-

The % RSD for peak area value of MF was found to be 1.64, as given in Table No 4.

a) Intra-day precision It was expressed as relative standard deviation (RSD %). The %R.S.D. values for intra-day precision study were between 0.92 to 1.44 %. The %RSD value (Table No 8) was <2.0%, confirming that the method was sufficiently precise.

b) Inter-day precision-The %RSD value for inter-day study was found to be between 1.50 to 1.98 % which is shown in Table no IV indicated the method was precise.

Accuracy

The amount of drug was calculated by employing corresponding calibration curve equations. The recovery was found to be between 99.97 -101.50% (Table no.5). The closeness of the result nearly to 100 % assured the accuracy of the developed method.

Sensitivity

Based on the standard deviation of the response and the slope, the values obtained are 11.22 ng/spot and 34.98 ng/spot respectively for LOD and LOQ.

Specificity

The peak purity of MF was assessed by comparing their respective spectra at peak start, apex and peak end positions of the spot i.e., $r(S, M) = 0.9991$ and $r(M, E) = 0.9990$. Good match was obtained between standard and sample spectra of MF.

ANALYSIS OF MF IN MARKETED FORMULATION

The Marketed preparation of Brand A and Extract both are analyzed for %w/w of Mangiferin present. Table no 6 suggest that 44.6 % w/w Mangiferin availed in capsule while extract of Mangiferin content is 22.23% w/w.

SUMMARY OF VALIDATION PARAMETERS FOR HPTLC METHOD

The HPTLC method for determination of MF was developed and validated. The results for each validation parameters confirmed linearity, accuracy, precision and selectivity of the developed analytical method. The method showed good linearity over the selected linearity range. The summary validation parameters for the developed analytical method are shown in Table no 7.

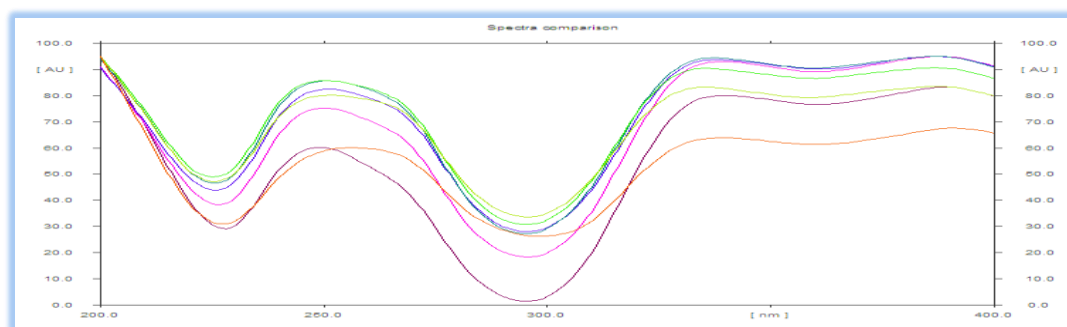


Figure No. 1 Wavelength selection for MF

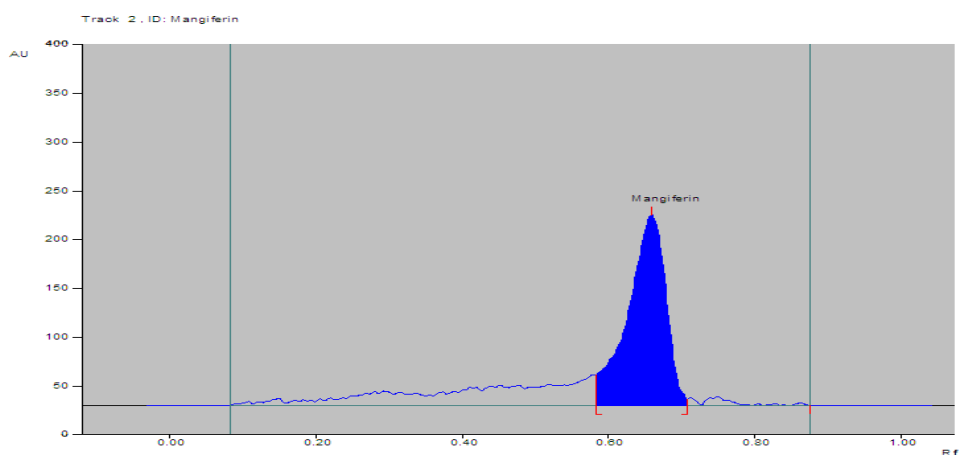


Figure No. 2 Standard Chromatograph of MF (400 ng/spot)

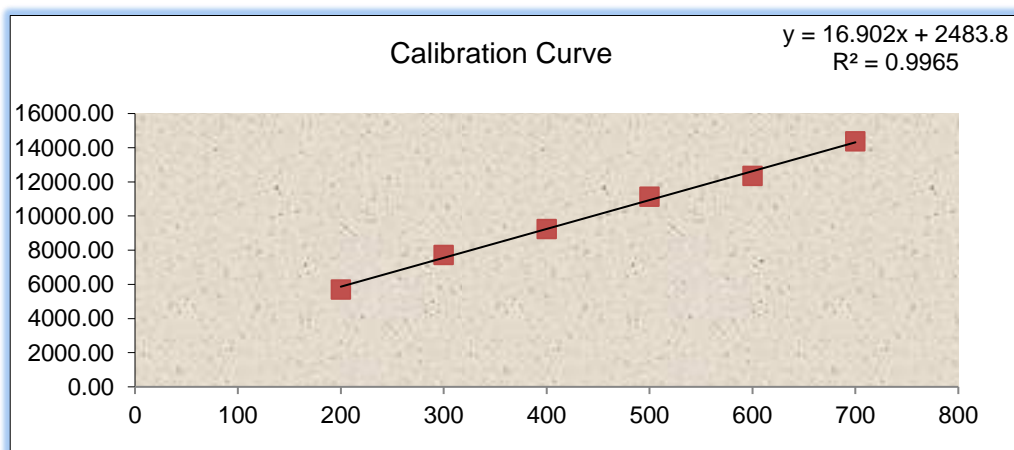


Figure No.3 Calibration curve of MF (200-700ng/spot)

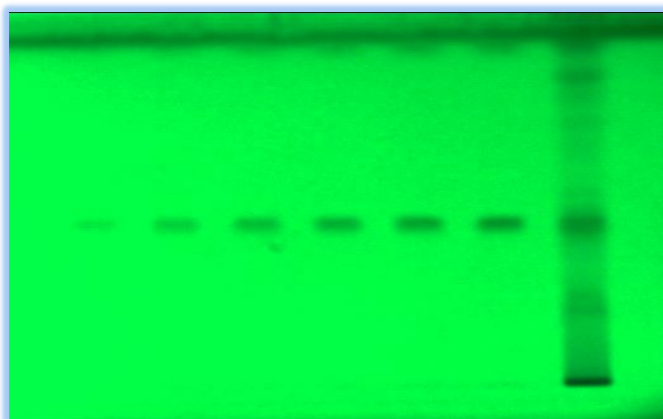


Figure No. 4: Photograph of developed TLC plate of MF (200 - 700ng/spot)

Table No 1: Results of R_f of standard MF

Name of Drug	R _f
MF	0.66 ± 0.03

Table No 2: Results of degradation

Concentration (ng/spot)	Area Mean \pm SD	%RSD
200	5701.83 \pm 52.94	0.93
300	7728.67 \pm 153.53	1.99
400	9238 \pm 124.33	1.35
500	11137 \pm 102.56	0.92
600	12354.50 \pm 220.93	1.79
700	14377.83 \pm 237.01	1.65

Table No3: Result of Calibration curve for MF

Stress Condition	% degradants formed Standard
Alkali	39.41 %
Acidic	23.20%
H ₂ O ₂	14.56 %
Thermal degradation	21.92%

Table No 4: Intra-Day, Inter-Day and Repeatability study of MF

Concentration (ng/spot)	Intra-Day Area Mean (n=3) \pm SD	%RSD	Inter-Day Area Mean (n=3) \pm SD	%RSD	Concentration (ng/spot)	Repeatability Area Mean (n=6) \pm SD	%RSD
300	7730.83 \pm 144.08	1.44	7728.66 \pm 153.52	1.98			
400	9238 \pm 124.33	1.34	9242.16 \pm 144.08	1.55	400	9231.33 \pm 1.64	1.64
500	11232 \pm 0.92	0.92	11180.83 \pm 167.75	1.50			

Table No 5: Determination of Accuracy for MF

Level	Amount of standard Ng/spot	Amount obtained	Average assay (%) Recovery	Assay % (RSD)
Level I (50%)	200	213	101.510 \pm 0.81	0.81
Level II (100%)	400	367	100.41 \pm 1.79	1.78
Level III (150%)	600	610	99.97 \pm 1.22	1.23

Table No 6: Assay Result of Formulation

Formulation	Labelled claim	Avg. Amt. Recovered % w/w MF
Capsule	18 mg <i>Mangifera indica</i> extract per capsule	44.6
Extract	-	22.23

Table No 7: Summary of validation parameter

Parameters	HPTLC method
Linearity (ng/spot)	200 – 700
LOD (ng/spot)	11.22
LOQ (ng/spot)	34.98
%Recovery	99.97 -101.50%
Repeatability (%RSD, n = 5)	
Precision (%RSD),	
Inter-day (n = 3)	0.92 to 1.44 %.
Intra-day (n = 3)	1.50 to 1.98 %

CONCLUSION

The developed method is validated as per ICH guideline Q2(R1) for global acceptance of standardized herbal formulations. The method showed linearity in the concentration range of 200-700ng/spot with coefficient of correlation, $r^2 = 0.9965$ at 340 nm. The result of the analysis by the proposed method was found to be highly reproducible and reliable. So, the developed HPTLC method is simple, precise and accurate and can be used for determination of MF in pharmaceutical dosage forms. It can estimate MF in presence of degradation too. The method can be applied for the routine analysis in quality control lab.

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