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Research Article

HPLC Method Development and Validation of Mangiferin

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ABSTRACT

Mangiferin is a naturally occurring antioxidant that belongs to glucoxyl xanthone and has potential therapeutic activity with less side effect it is mostly available from the leaves and stems of the Mangifera indica (Mango Tree). To aquire, track and analyse the output chromatogram data, the analytical method was created using the Schimadzu HPLC system equipped with a SPD-20A detector and the Lab solution system control software.C18 column used as a stationary phase to separate the drug. Isocratic elution was obtained with Acetic acid and Methanol mixture as a mobile phase in the ratio of 60:40% v/v with the flow rate of 1ml/min at 254nm, which appears to be fair based reported run time. The calibration plot for Mangiferin in the range between 30ppm to 330ppm showed good linearity with 0.998R2 value and the validation completed. The linearity and accuracy testing followed the acceptance criteria.

INTRODUCTION

Analytical methods development and validation play important roles in the discovery, development and manufacture of pharmaceuticals. Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need analytical method development and validation by combining the therapeutic effects of two or more drugs in one product. These combination products can present during challenges to the analytical chemist responsible for the development and validation of analytical methods. Identification and quantification of impurities is a crucial task in pharmaceutical process development for quality and safety. Related components are the impurities in pharmaceuticals which are unwanted chemicals that remain with the active pharmaceutical ingredients (API) or develop during stability testing or develop during formulation or upon aging of both API and formulated API to medicines. The presence of these unwanted

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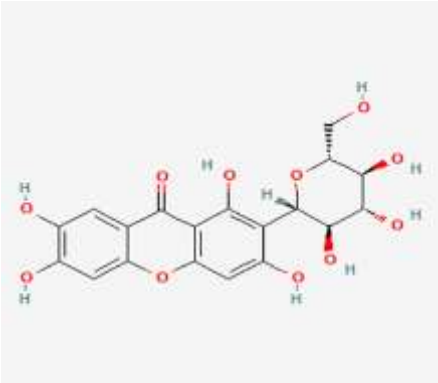
chemicals even in small amounts may influence the efficacy and safety of pharmaceuticals products. Various analytical methodologies are employed for the determination of related components in pharmaceuticals. There is great need for development of new analytical methods for quality evaluation of new emerging drugs. Basic Criteria for new method development of drugs and their metabolites in biological fluids and creates a specific procedure to enable unite of interest to be identify and at the same time to be quantified in a matrix. A coalesce is measured by several procedure

Chemistry of Mangiferin:



Mangiferin is a naturally occurring polyphenolic compound mainly found in the leaves, bark, and fruit of the mango tree (Mangifera indica). It belongs to the class of xanthone glycosides and is known for strong antioxidant and pharmacological activities.

Mangiferin is a naturally occurring C-glucosyl xanthone predominantly obtained from the leaves, bark, and stems of Mangifera indica. Chemically, it is known as 1, 3, 6, 7-tetrahydroxyxanthone-C2-β-D-glucoside with a molecular formula of $C_{19}H_{18}O_{11}$ and a molecular weight of 422.34 g/mol. The structure of mangiferin consists of a xanthone nucleus (dibenzo-γ-pyrone system) attached to a glucose moiety through a stable C–C glycosidic bond, which distinguishes it from typical O-glycosides. It contains multiple phenolic hydroxyl (–OH) groups and a carbonyl (C=O) group, making it a polyphenolic compound with high polarity and good water solubility. These hydroxyl groups contribute significantly to its strong antioxidant properties. Mangiferin also exhibits characteristic UV absorption, which is useful in analytical methods such as UV spectroscopy and HPLC. Chemically, it is stable under normal conditions but may degrade under extreme pH or environmental stress.

1. Chemical Structure

- **IUPAC Name:** 1,3,6,7-Tetrahydroxyxanthone-C2-β-D-glucoside
- **Molecular Formula**: $C_{19}H_{18}O_{11}$
- **Molecular Weight**: ~422.34 g/mol

➢ **Key Structural Features**:

- **Xanthone nucleus** → tricyclic aromatic backbone
- **Four hydroxyl (-OH) groups** → responsible for antioxidant activity
- **C-glycosidic linkage** → glucose is attached directly to carbon (not oxygen)

➢ This C-glycoside bond makes mangiferin:

- More stable than O-glycosides
- Resistant to acid hydrolysis

2. Chemical Classification

- Polyphenol
- Xanthone derivative
- C-glucoside

3. Functional Groups

Mangiferin contains:

- **Phenolic –OH groups** → antioxidant property
- **Aromatic rings** → stability and UV absorption
- **Ether linkage (C–O–C)** in xanthone core
- **Sugar moiety (glucose)** → increases solubility

4. Physicochemical Properties

- **Appearance**: Yellow crystalline powder
- **Solubility:**
  - Slightly soluble in water
  - Soluble in methanol and ethanol
- **Melting point:** ~270°C (decomposes)
- **UV absorption**: Strong peak around 254nm & 316 nm

5. Chemical Behavior

a) Antioxidant Activity

Due to phenolic OH groups:

- Donates hydrogen atoms
- Neutralizes free radicals

b) Metal Chelation

- Forms complexes with Fe^{2+} and Cu^{2+}
- Prevents oxidative damage

c) Stability

- Stable under mild acidic conditions
- Degrades under strong alkaline or oxidative conditions

6. Biosynthesis (Brief)

Mangiferin is synthesized via:

- **Shikimate pathway** → formation of aromatic rings
- Followed by **polyketide pathway** → xanthone formation
- **Glycosylation** → addition of glucose

7. Chemical Reactions

Mangiferin can undergo:

- **Oxidation** → quinone formation
- **Hydrolysis (limited)** due to strong C-glycosidic bond
- **Complex formation** with metals

8. Importance in Analysis (HPLC relevance)

- Strong UV absorbance helps in **HPLC detection**
- Typically analyzed using:
  - Reverse phase column (C18)
  - Mobile phase: methanol + water (acidified)
- Peak area corresponds to concentration

9. Summary

Mangiferin is a stable, polyphenolic xanthone glycoside with:

- Strong antioxidant capacity
- Unique C-glycosidic linkage

Important role in pharmaceutical and analytical chemistry.

MATERIALS AND METHODS

Mangiferin was purchased from India Mart. Chromatogaphy-grade Acetic acid and Methanol was purchased from Sahyadri Scientific Suppliers while the 0.45µ nylon filter membrane was obtained from Sahyadri Scientific Suppliers. Also the HPLC grade water were obtained from Sahyadri Scientific Suppliers.

INSTRUMENTS AND SOFTWARE:

The analytical method for Mangiferin separation was done by using HPLC system of Shimadzu , Degassing unit (DGU-20A_{5R}), (SPD-20A)detector, auto sampler SIL-20AC $_{HT}$ The software used for the process of chromatographic output data is lab solution(UFLC), The stationary phase utilized C18 column. Standard substances are preciously weighed using the analytical balance Shimadzu (AY220). After filtering the Methanol and Acetic acid through a 0.45µ filter membrane using a vaccum filtration unit, an ultrasonic bath sonicator Citizen(Digital Ultrasonic Cleaner) used for degassing. The absorbance of mangiferin was checked by UV-Spectrophotometer (UV-1800) Shimadzu.

Condition for Chromatography:

Mangiferin is effectively separated and detected at a wavelength of 254nm by employing an isocratic elution method using a mixture of Acetic acid and Methanol in a volumetric ratio 60:40. The injection volume for mangiferin was set as 20µl, while the total run time is10 Minutes, maintaining flow rate of 10ml/min. The auto sampler temperature is controlled at 22, and the room temperature is regulated within a range of 26^{+}–2°C.

Standard and Sample Dilution Preparations:

The normal stock of 0.05 gm of Mangiferin were precisely weighed and dissolved in 50ml methanol. The mixture was then sonified for 8min at room temperature. Then the sample of 30ppm, 90ppm, 150ppm, 210ppm, 270ppm, 330ppm was prepared by diluting with methanol to check the linearity.

Mobile Phase Preparation:

The 0.5%acetic acid was prepared by adding 1.5ml Acetic acid in 300ml HPLC grade water and HPLC grade methanol of quantity 300ml was taken and both are sonified for 16 minutes respectively.

Analytical Method Validation

The ICH rules were followed for determining the validity of the optimized approach. The subsequent validation criteria were assessed:

Linearity:

If an analytical method has the capability to produce test results directly proporational to the concentration of the tested components within the sample, particularly within a specific range, it is deemed to be linear. A linearity plot was generated by adding mangiferin in concentration ranging from 30ppm to 330ppm. Plotting the substance amounts over the relevant peak areas was the first step in creating the regression equation.

Precision:

To assess the method's ability to produce consistent results under specific conditions, three replicates were injected into HPLC at a concentration of 150ppm. This was done for both intra- and inter-day precision testing. The areas were used to compute the % relative standard deviation.

Accuracy

Three replicates of quality control samples containing mangiferin were injected into the High-Performance Liquid Chromatography (HPLC) system at concentration of 150ppm, 210ppm and 270ppm respectively. This was conducted as a part of an accuracy assessment aimed at evaluating the agreement between the obtained values and established reference values for determining the accuracy of analysis. The calculated recovery percentage was assessed against predefined acceptance criteria to validate the accuracy of the results.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

Adhering to the directives established by the International Council for Harmonization (ICH), LOD and LOQ for mangiferin were established by utilizing the formula: LOD = 3.3(SD)/S. Similarly, LOQ= 10(SD)/S. Here, SD denotes the standard deviation of response, while S represents the Slope of the regression line.

Standard Deviation

In the context of HPLC, Standard Deviation is not just a statistical abstract; it is the fundamental measure of peak width. It tells you how much sample "spreads out" as it travels through the column.

Relative Standard Deviation (%RSD)

In HPLC, Percent Relative Standard Deviation is the gold standard for measuring precision. %RSD tells you how large that spread is relative to the average.

RESULT AND DISCUSSION

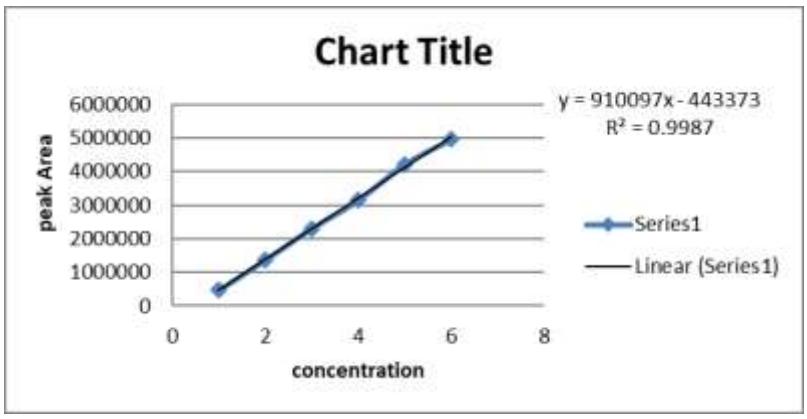
Analytical method validation

For determining the validity of the optimized approach, ICH rules were followed. The subsequent validation criteria were assessed.

Linearity and Range:

The ability of an analytical process to generate test outcomes that correspond accurately to the quantity of the test substance in the sample is termed as linearity. To assess linearity, known concentrations of Mangiferin were spikes at levels ranging from 30ppm to 330ppm. The relationship between peak are and drug concentrations was plotted to form a regression equation, demonstrating the correlation between these variables.

Method	Linearity
Equation	91009x-44337
Correlation coefficient(R^2)	0.998

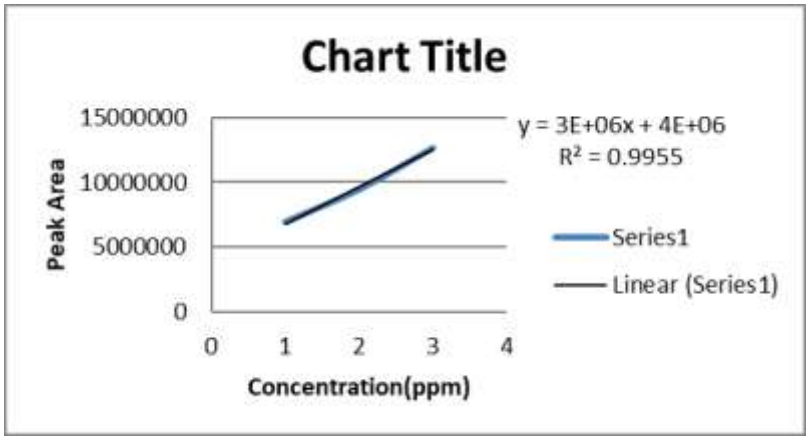


The LOD for mangiferin was established at 15.02 ng/ml, while the LOQ was determined to be 45.50 ng/ml respectively.

Accuracy:

As a part of accuracy assessment, triplicate injections of three different mangiferin quality control samples (at concentration of 150ppm, 210ppm and 270ppm) were carried out using HPLC. This aimed to gauge the agreement between the obtained values and established reference values. The calculated recovery percentages fell within the acceptance criteria, ranging from

concentration	peak area
150	6924600
210	9457200
270	12660300



Standard Deviation:

In HPLC context, it is the fundamental measure of peak width. The standard deviation for magiferin was established at 1703754.537.

CONCLUSION

In this research work, a robust and reliable HPLC analytical method was successfully developed and validated for the estimation of mangiferin. The method demonstrated excellent performance characteristics in terms of simplicity, accuracy, precision, specificity, and linearity, making it highly suitable for analytical applications. The chromatographic conditions, including the selection of stationary phase and optimization of mobile phase composition, were carefully adjusted to achieve sharp peak shape, good resolution, and

appropriate retention time for mangiferin. The developed method showed a strong linear relationship over the selected concentration range, indicating its suitability for quantitative analysis.

Furthermore, the validation of the method was carried out in accordance with ICH guidelines, confirming its reliability and reproducibility. Parameters such as accuracy, precision, linearity, and system suitability were found to be within acceptable limits, ensuring consistent analytical performance. The method also exhibited good stability and sensitivity, allowing for accurate detection even under varied chromatographic conditions.

Overall, the developed HPLC method is simple, cost-effective, and efficient, and it holds significant potential for routine quality control analysis of mangiferin in pharmaceutical formulations as well as herbal products. This method can be effectively applied in research laboratories and industrial settings for ensuring the quality, safety, and efficacy of mangiferin-containing formulations.

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