Rapid Development and Validation of Improved Reversed-Phase High-performance Liquid Chromatography Method for the Quantification of Mangiferin, a Polyphenol Xanthone Glycoside in Mangifera indica

P. Naveen¹, H. B. Lingaraju¹,², K. Shyam Prasad²

¹Analytical Research and Development, Vidya Herbs Pvt. Ltd., ²Phytochemistry Lab, Vidya Herbs Pvt. Ltd, Bengaluru, Karnataka, India

ABSTRACT
Mangiferin, a polyphenolic xanthone glycoside from Mangifera indica, is used as traditional medicine for the treatment of numerous diseases. The present study was aimed to develop and validate a reversed-phase high-performance liquid chromatography (RP-HPLC) method for the quantification of mangiferin from the bark extract of M. indica. RP-HPLC analysis was performed by isocratic elution with a low-pressure gradient using 0.1% formic acid: acetonitrile (87:13) as a mobile phase with a flow rate of 1.5 ml/min. The separation was done at 26°C using a Kinetex XB-C18 column as stationary phase and the detection wavelength at 256 nm. The proposed method was validated for linearity, precision, accuracy, limit of detection, limit of quantification, and robustness by the International Conference on Harmonisation guidelines. In linearity, the excellent correlation coefficient more than 0.999 indicated good fitting of the curve and also good linearity. The intra- and inter-day precision showed <1% of relative standard deviation of peak area indicated high reliability and reproducibility of the method. The recovery values at three different levels (50%, 100%, and 150%) of spiked samples were found to be 100.47, 100.89, and 100.99, respectively, and low standard deviation value <1% shows high accuracy of the method. In robustness, the results remain unaffected by small variation in the analytical parameters, which shows the robustness of the method. Liquid chromatography–mass spectrometry analysis confirmed the presence of mangiferin with M/Z value of 421. The assay developed by HPLC method is a simple, rapid, and reliable for the determination of mangiferin from M. indica.

Key words: Liquid chromatography–mass spectrometry, Mangifera indica, mangiferin, reversed-phase high-performance liquid chromatography, validation

SUMMARY
The present study was intended to develop and validate an RP-HPLC method for the quantification of mangiferin from the bark extract of M. indica. The developed method was validated for linearity, precision, accuracy, limit of detection, limit of quantification and robustness by International Conference on Harmonisation guidelines. This study proved that the developed assay by HPLC method is a simple, rapid and reliable for the quantification of the mangiferin from M. indica.

INTRODUCTION
Plant-based medicaments had served from the onset of human civilization as the most important therapeutic weapon available to fight various human and animal diseases. In the last few years, there has been an exponential growth in the field of herbal medicine, and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects.[1] Standardization and identification of phytochemicals are always very challenging era in polyherbal formulations because of complex nature and inherent variability of the chemical constituents of plant-based drugs. Quantitative determination of individual phytochemicals in the polyherbal preparation required optimal separation technique by which each phytoconstituents are separated with the highest resolution and the least interferences from each other. The World Health Organization has emphasized the need to ensure the quality of medicinal plant products using modern controlled technique and applying suitable standards.[3]

Mangifera indica (L.), belonging to the family Anacardiaceae, is one of the second largest tropical fruit crops in the world.[3] M. indica, also known as mango, has been an important herb in the ayurvedic and indigenous medical systems for over 4000 years. Phytochemical studies on different parts of M. indica have demonstrated that it contains polyphenols, flavonoids, phytosterols,[4,5] and mangiferin. Mangiferin, a polyphenolic antioxidant and stable a xanthone glycoside, is reported to be present in stem bark, heartwood, leaves, and dried roots of M. indica.[6,7] Mangiferin has been reported to possess antioxidant[3,8,10] and antitumor[11,12] properties.

Abbreviations Used: M. indica: Mangifera indica, RP-HPLC: Reversed-phase high-performance liquid chromatography, M/Z: Mass to charge ratio, ICH: International conference on harmonization, % RSD: Percentage of relative standard deviation, ppm: Parts per million, LOD: Limit of detection, LOQ: Limit of quantification.

Correspondence: Dr. P. Naveen, Analytical Research and Development, Vidya Herbs Pvt. Ltd., Jigani, Anekal Taluk, Bengaluru, Karnataka, India. E-mail: naveen2384@gmail.com

DOI: 10.4103/0974-8490.204652

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Naveen P, Lingaraju HB, Prasad KS. Rapid development and validation of improved reversed-phase high-performance liquid chromatography method for the quantification of mangiferin, a polyphenol xanthone glycoside in Mangifera indica. Phcog Res 2017;9:215-9.
immunomodulatory, ant-HIV, antiviral, anti-inflammation, cardioprotective, antipyretic activity, anticancer, anti-diabetic, radioprotection, neuroprotective, antimicrobial, hepatoprotective, and hypocholesterol activity. However, there have been limited studies on the quantification of mangiferin from M. indica. In the present investigation, we have developed a simple, optimized, and validated reversed-phase high-performance liquid chromatography (RP-HPLC) method for quantitative determinations of mangiferin, a major bioactive polyphenol in the stem bark of M. indica. The developed method was validated on the basis of its linearity, repeatability, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and robustness as per the International Conference on Harmonisation (ICH) guidelines.

MATERIALS AND METHODS

Materials

Mangiferin standard (98%) was obtained from Sigma-Aldrich (India). All reagents and solvents were analytical and HPLC grade. The powdered bark material of M. indica was procured from Ess Kay Herbs, Lucknow, India.

Preparation of the extract

Hydroalcoholic extract of mangiferin was prepared by Soxhlet extraction of 100 g of powdered bark material of M. indica using 70% ethanol at 75°C for about 3 h in three successive batches. The first batch extraction by adding 800 ml solvent for 2 h and further two successive extractions were done by adding 500 ml per batch for 1 h. After completion of extraction, the cooled liquid extract was concentrated by evaporating its liquid contents in rotary evaporator, with an approximate yield of 12% w/w.

Instrumentation and chromatographic conditions

HPLC was performed on a Shimadzu LC-2030 C Prominance-i (Japan) system equipped with a quaternary low-pressure gradient Solvent Delivery LC-2030 pump with high-pressure switching valves, online LC-2030 degasser unit, a high sensitivity LC-2030 ultraviolet (UV)-detector, high-speed drive LC-2030 autosampler with a 100 µl loop, and it accommodates 216 samples at a time with direct access rack system and large capacity column oven. The system controlled and data analyzed by LabSolutions software. A separation was carried out in Kinetex XB-C18 column (100 A, 250 mm × 4.6 mm, 5 µm pore size). The mobile phase consists of isocratic elution with a low-pressure gradient using 0.1% formic acid: Acetonitrile (87:13) with a flow rate of 1.5 ml/min and the injection volume of 10 µl. All solutions were degassed and filtered through 0.45 µm pore size filter. The column was maintained at 26°C throughout analysis, and the UV detector was set at 254 nm. Nearly 70% methanol used as a diluent for assay by HPLC analysis and the total liquid chromatography (LC) run time was 15 min. The instrument was calibrated and qualified before the analysis. Using these chromatographic conditions, it was possible to confirm the retention time of mangiferin by injection of corresponding standard separately. The hydroalcoholic extract of M. indica was also analyzed by LC–mass spectrometry (LC-MS) (Shimadzu LC-MS 8040) and identified by comparison of their M/Z value with the reference standard mangiferin.

Preparation of standard solution

Accurately weighed known weight of mangiferin reference standard to 100 ml volumetric flask and was dissolved in 70% methanol to obtain a stock solution of 500 ppm. Working standard solutions were obtained by diluting the 5.0 ml standard stock solution to 25 ml volumetric flask and made up with 70% methanol to achieve the final concentration of 100 ppm. Before analysis, the solutions were filtered through 0.20 µm nylon membrane filters.

Preparation of sample

The dried hydroalcoholic bark extract of M. indica was prepared in 70% methanol to achieve the final concentration of 100 ppm. Before analysis, the solutions were filtered through 0.20 µm nylon membrane filters.

Preparation of spiked sample

Three different volumes (2.5, 5.0, and 7.5 ml) of standard stock solution were added to the sample solution (100 ppm) separately. The standard stock solution was spiked into the samples to determine recovery. Before analysis, the solutions were filtered through 0.20 µm nylon membrane filters.

Validation of the method

The validation of the developed method was done according to the ICH guidelines. The method is validated for linearity, precision, accuracy, LOD, LOQ, and robustness.

Linearity

Linearity was determined by different known concentrations of mangiferin standard (20–140 ppm) solution in triplicate by diluting the standard stock solution. The standard solutions were injected, and the peak area was measured. Calibration curve was constructed for mangiferin by plotting peak areas against concentration and linear regression equations. The correlation coefficient was also computed.

Precision

Precision is a measure of the reproducibility of the whole analytical method. It is determined using the method to assay a sample for a sufficient number of times to obtain statistically valid results. Precision was determined by studying the intra- (repeatability) and inter-day precision. The intra- and inter-day precision was determined at three different concentration levels of mangiferin standards 60, 80, and 100 ppm and the sample solution (100 ppm). The intra-day precision was examined by 6 times within 1 day, whereas the inter-day precision was examined for 3 consecutive days. The precision was expressed as percentage relative standard deviation (% RSD).

Accuracy

Accuracy is a measure of closeness of test results obtained by a method to the true value. The accuracy of the method was tested by performing the recovery studies at three different levels of standard stock solution added to the samples. The standard stock solution was spiked into the samples to determine recovery. Three different volumes (2.5, 5.0, and 7.5 ml) of standard stock solution (500 ppm) were added to the sample solution (100 ppm). Triplicate injections were made with all the spiked samples.

% of Recovery= \( \frac{(b - a)}{c} \times 100 \)

where, 'a' is the amount of drug found in the sample before addition of standard drug

'b' is the amount of drug found after addition of standard drug

'c' is the amount of standard drug added

Limit of detection and limit of quantification

Detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated under the stated experimental conditions. Quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with 216 Pharmacognosy Research, Volume 9, Issue 2, April-June, 2017
accuracy. LOD and LOQ were determined based on the signal-to-noise ratio response. For LOD, it should not be not <3. For LOQ, it should not be not <10.

Robustness
The robustness is evaluated by varying the analytical parameters. The robustness is evaluated by varying the analytical parameters such as changing the wavelength parameter from 254 to 256 nm and by changing the mobile phase concentration of formic acid from 0.1% to 0.08%. The robustness of the method was done at three different standard concentration levels 60, 80, and 100 ppm and sample concentration of 100 ppm at triplicate injections. The corresponding peak areas were recorded and expressed in terms of % RSD.

Statistical analysis
Results were expressed as mean ± standard deviation. The data were submitted to statistical analysis using Excel software.

RESULTS AND DISCUSSIONS
An RP-HPLC method was developed and validated for the determination of mangiferin in M. indica. The chromatographic conditions were optimized to provide a good performance of the assay. During optimization of the method, different mobile phases, different stationary phases, and organic modifiers were tried to get a best separation of peaks. The Kinetex XB-C18 column (100 Å, 250 mm × 4.6 mm, 5 µm pore size) provided better separations of mangiferin in the extract using combination of acetonitrile and 0.1% formic acid. The maximum absorption of mangiferin was detected at 254 nm and this wavelength is chosen for the assay method. The retention time of mangiferin standard (100 ppm) and sample (100 ppm) solution was at 6.129 and 6.151, respectively [Figure 1]. The chromatographic parameters such as column efficiency and peak symmetry were done to mangiferin standard according to the ICH guidelines.[27] The theoretical plates and tailing factor were 11763 and 1.307, respectively, which indicated column efficiency is satisfactory. LC-MS showed M/Z value of 421 [M – H]− for hydroalcoholic extract of M. indica and reference standard mangiferin. In literature, same M/Z value observed for mangiferin.[28]

Linearity was evaluated by the peak area against concentrations of mangiferin standard in the range of 20–140 ppm, and the calibration plots were linear are summarized in Figure 2. The correlation coefficient (r) of mangiferin is 0.999 (n = 7). This indicates good fitting of the curve and the method is good linearity corresponds to peak area on concentrations. The minimum acceptable correlation coefficient is 0.990.[28,29] The calibration curve was represented by the linear equation y = 28477x – 10771 for mangiferin (where y is the response as peak area and x is the concentration).

The data [Table 1] pertaining to precision test revealed that in intraday, the % RSD of peak area of mangiferin standard (60, 80, and 100 ppm) and sample (100 ppm) concentration was found to be 0.05, 0.05, 0.06, and 0.10, respectively. In interday, the % RSD of peak area of mangiferin standard (60, 80, and 100 ppm) and sample concentration (100 ppm) was found to be 0.42, 0.52, 0.30, and 0.83, respectively. The method developed in the present study shows the acceptable intra- and inter-precision with RSD value <1.0%. This indicated the method was found to be precise and reproducible.

The recovery study was determined by the method of standard addition. The excellent recovery values at three different levels (50%, 100%, and 150%) of spiked samples were found to be 100.47%, 100.89%, and 100.99%, respectively, and low standard deviation value <1% shows the high accuracy of the method. Therefore, this HPLC method can be regarded as selective, accurative, and precise. The results of recovery study are summarized in Table 2.

LODs at signal-to-noise ratio of (3:1) the smallest concentration of mangiferin standard that gives a measurable response was found to be 0.48 ppm, and the LOQs, at signal-to-noise ratio of (10:1), was found to be 1.95 ppm for mangiferin. The robustness of an analytical procedure is its ability to remain unaffected by small variation in the analytical parameters. This indicates the proposed method was stable, precise, and reproducible.

CONCLUSION
The developed assay by HPLC method is a simple, rapid, and reliable for the determination of mangiferin from the bark extract of M. indica. This method was proved to be useful for the determination of the purity of the drug available from various extracts by detecting the related impurities in quality control laboratories. It has advantages over all available methods in the literature because it is faster and a mobile phase composition is simpler. With the growing demand for herbal drugs, the development of standardization tool will help in maintaining the quality control and herbal preparations of M. indica. Further explorations are needed to investigate the standardization of individual phytoconstituents of M. indica.
Acknowledgement

The authors are grateful to the all members of analytical research and development at Vidya Herbs Pvt. Ltd., Bengaluru, for their cooperation and technical help.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TP. Indian herbs and herbal drugs used for the treatment of diabetes. J Clin Biochem Nutr 2007;40:163-73.
2. World Health Organization. Quality Control Methods for Medical Plants Materials. Geneva: World Health Organization; 1998. p. 1-15.
3. Barreto JC, Trevisan MT, Hull WE, Erben G, de Brito ES, Pfundstein B, et al. Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peel of mango (Mangifera indica L.). J Agri Food Chem 2005;53:5986-91.
4. Singh UP, Singh DP, Singh M, Maurya S, Srivastava JS, Singh RB, et al. Characterization of phenolic compounds in some Indian mango cultivars. Int J Food Sci Nutr 2004;55:163-9.
5. Núñez Sellés AJ, Vélez Castro HT, Agüero-Agüero J, González-González J, Naddeo F, De Simone F, et al. Isolation and quantitative analysis of phenolic antioxidants, free sugars, and polyols from mango (Mangifera indica L.) stem bark aqueous decoction used in Cuba as a nutritional supplement. J Agric Food Chem 2002;50:762-6.
6. Ramanathan JD, Seshadri TR. Constitution of mangiferin. Curr Sci 1960;29:131-2.
7. Nott PE, Roberts JC. The structure of mangiferin. Phytochemistry 1967;6:741-7.
8. Shah KA, Patel MB, Patel RJ, Parmar PK. Mangifera indica (mango). Pharmacogn Rev 2010;4:42-8.
9. Hiraganahalli BD, Chinnampudur VC, Dethé S, Mundkinajeddu D, Pandre MK, Balachandran J, et al. Hepatoprotective and antioxidant activity of standardized herbal extracts. Pharmacogn Mag 2012;8:116-23.
10. Dar A, Faizi S, Naqvi S, Roome T, Zikru-Rehman S, Ali M, et al. Analgesic and antioxidant activity of mangiferin and its derivatives: The structure activity relationship. Biol Pharm Bull 2005;28:596-600.
11. Guha S, Ghosal S, Chattopadhyay U. Antitumor, immunomodulatory and anti-HIV effect of mangiferin, a naturally occurring glucosylxanthone. Chemotherapy 1996;42:443-51.
12. Noratto GD, Bertoldi MC, Krenk K, Talcott ST, Stringheta PC, Mertens-Talcott SU. Anticarcinogenic effects of polyphenolics from mango (Mangifera indica) varieties. J Agric Food Chem 2010;58:4104-12.
13. Sarkar A, Sreenivasan Y, Ramesh GT, Manna SK. Beta-D-glucoside suppresses tumor necrosis factor-induced activation of nuclear transcription factor kappa-B but potentiates apoptosis. J Biol Chem 2004;79:33788-91.
14. Lai L, Lin LC, Lin JH, Tsai TH. Pharmacokinetic study of free mangiferin in rats by microdialysis coupled with microbore high-performance liquid chromatography and tandem mass spectrometry. J Chromatogr 2003;987:367-74.
15. Carvalho RR, Pelilizzon CH, Justulin L Jr., Ferreira SP, Velgaz W, Bruni F, et al. Effect of mangiferin on the development of periodontal disease: Involvement of lipoxygen A4, anti-chemotactic activation in leukocyte rolling. Chem Biol Interc 2010;179:344-50.
16. Nair PS, Shyamala Devi CS. Efficacy of mangiferin on serum and heart tissue lipids in rats subjected to isoproterenol induced cardiotoxicity. Toxicology 2006;233:135-9.
17. Awe SO, Olajide OA, Oladiran OO, Makinde JM. Antiplasmodial and antipyretic screening of Mangifera indica extract. Phytother Res 1998;12:437-8.
18. Munaza DN, Euler KL, Williams L, Newman DJ. Screening for antitumor and anti-HIV activities of nine medicinal plants from Zaire. Int J Pharmocol 1995;33:98-106.
19. Yoshimi N, Matsunaga K, Katayama M, Yamada Y, Kuno T, Qiao Z, et al. The inhibitory effects of mangiferin, a naturally occurring glucosyxanthone, in bowel carcinogenesis of male F344 rats. Cancer Lett 2001;163:163-70.
20. Muruganandan S, Srinivasan K, Gupta S, Gupta PK, Lai J. Effect of mangiferin on hyperglycemia and atherogenicity in streptozotocin diabetic rats. J Ethnopharmacol 2005;97:497-501.
21. Kumar BD, Krishnakumar K, Jaganathan SK, Mandal M. Effect of mangiferin and mahonibine on glucose utilization in 3T3-L1 cells. Pharmacogn Mag 2013;9:72-5.
22. Ganesh CJ, Venkatasubbaiah AV. Mangiferin protects human peripheral blood lymphocytes against γ-radiation-induced DNA strand breaks: A fluorescence analysis of DNA unwinding assay. Nutr Res 2006;26:303-11.
23. Gottlieb M, Leal-Campanario R, Campos-Esparza MR, Sánchez-Gómez MV, Alberdi E, Arranz A, et al. Neuroprotection by two polyphenols following excitotoxicity and experimental ischemia. Neurobiol Dis 2006;23:374-86.
24. Singh M, Khatoon S, Singh S, Kumar V, Rawat AK, Mehrotra S. Antimicrobial screening of ethnobotanically important stem bark of medicinal plants. Pharmacognosy Res 2010;2:254-7.
25. Gururaja GM, Mundkinajeddu D, Dethe SM, Sangli GK, Abhilash K, Agarwal A. Cholesterol esterase inhibitory activity of bioactives from leaves of Mangifera indica L. Pharmacognosy Res 2014;7:358-62.
26. International Conference on Harmonization. ICH (Q2B) Note for Guidance on Validation of Analytical Procedures: Methodology. Geneva, Switzerland: IFPMA; 1996.
27. International Conference on Harmonization. ICH (Q2A) Validation of Analytical Procedures: Text and Methodology. Geneva: International Conference on Harmonization; 2005.
28. Luo F, Lv Q, Zhao Y, Hu G, Huang G, Zhang J, et al. Quantification and purification of mangiferin from Chinese mango (Mangifera indica L.) cultivars and its protective effect on human umbilical vein endothelial cells under H2O2-induced stress. Int J Mol Sci 2012;13:11260-74.