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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(4): 2614-2626. **OPEN ACCESS**

A new UPLC method for the analysis of Mangiferin in *Mangifera indica, Swertia chirayita* and *Canscora decussate* by using Fractional Factorial Design and Its DPPH Scavenging Activity

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Mangiferin is a very valuable bio active natural compound that is chemically a xanthonoid and has more significance in pharmacy industry. The main aim of this study was to identify and quantify of mangiferin in *Mangifera indica*, *Swertia chirayita* and *Canscora decussate* and to develop a novel ultra-performance liquid chromatography (UPLC) method. Drug samples were collected from the local market of Dammam, KSA. The mobile phase was used in this study [acetonitrile (ACN): water (50:50 *v/v*) with isocratic elution with a flow rate of 0.300 mL min-1]. Retention and total run times were 0.680 min and 2.0 min, respectively, with injection volume of 10 µL at 380 nm. The method was validated for linearity (r2 ≥ 0.997 ± 0.0976), accuracy 98.52-102.95%), and RSD of precision (0.7449736-2.284584) with a calibration curve range of 100.00–1200.00 ng/mL. The limits of detection and quantification were found to be 24.68 ng/mL and 74.75 ng/mL, respectively. The fractional factorial design expertwas applied for the validation of robustness and method was found to be robust. The proposed method was found to be accurate, precise and specific and can be applicable for the determination of mangiferin in plant extracts as well as related formulations.

Keywords: *Swertia chirayita, Mangifera indica*, *Canscora decussate,* Ultra Performance Liquid Chromatography, Mangiferin

INTRODUCTION

Herbal products that have developed the concept of health care worldwide since the earliest days of the widely utilized human area region, and have considerable importance in intercontinental trade. Identification of their pharmaceutical research, clinical trials and financial importance is still growing, while this differs widely across countries (Jayasuriya, 1990). *Mangifera indica* Linn. also known as mango, is very significant plant of family Anacardiaceae. It has a sub-tropical fruit, containing of best dietary and therapeutic importance. It is stated in the literature that mango was first used in the province of Indo-Bermese around 4000 years ago, but it is currently grown for commercial purposes nearly 90 nations (Tharanathan et al. 2006). *M. indica* is one of the very valuable and abundant

fruit on the earth and India supplies to the most country of the global mango production (Stoilova et al. 2005). Various part of plant is used as an antiseptic, astringent, anti-inflammatory (Latha et al. 2012), antioxidant (Pitchaon, 2011), anti allergic (Garcia et al. 2003) and hepatoprotective activity (Saruth et al. 2009). Different parts of *M. indica* plant contain various bio active constituents such as stigmasterol, friedelin, gallic acid, lupeol, quercetin, catechin, mangiferin and benzoic acid (Ponce et al. 2012; Rodeiro et al. 2007). *Swertia chirayita* Roxb. is a very popular herb of family Gentianaceae. *S. chirayita* normally called as Chirata. It is an annual or biennial herb near about 1.5 m tall. It is found in Himalaya and Meghalaya region at aheight of 1200-1300 m. *S. chirayita* is used in traditional medicine to cure various types of health issues, including cancer, constipation, asthma, diarrhea, dyspepsia, liver ailments (Shah, 1999; Duke et al. 2002), epilepsy, ulcer, mental disorders (Chatterjee and Parkrashi, 1995). The therapeutic importance of this herbal drug as antidiabetic (Chandrasekar et al. 1990), anti-inflammatory (Chodhary et al. 1995), hepatoprotective (Karan et al. 1999) and antioxidant action has been reported (Khanom et al. 2000). Xanthone derivatives, flavonoids, iridoid glycosides, triterpenes and dimeric xanthones are main secondary metabolites of *S. chirayita* (Patil et al. 2013). The other compounds such as chiratin, ophelic acid, palmatic acid, chiratanin are also found in *S. chirayita* (Patil et al. 2013). *Canscora decussate* Schult. is popularly herbal drug known as Shankhpushp of Gentianaceae and grown all over India, up to a height of 1400 m. *C. decussate plant* is also found in Myanmar and Sri Lanka. The plant is grown as ornamental herb in parks and gardens as ornamental flower. This plant has many activecompounds such as xanthones, triterpenes and alkaloids (Kokate et al. 2002). Pentaoxygenated, hexaoxygenated and dimeric xanthones are also isolated from this natural herb (Peres and Nagem, 1997). *C. decussate,* an important herb of indigenous system of medicine is reputed as a nerve tonic, alternative and laxative, also recommended for sexual and seminal debilities (Shah et al. 2000; Chunekar et al. 1969). Mangiferin (Figure 1) is a very important natural biologically active compound that is chemically xanthonoid and has greater importance in the pharmaceutical and other similar industries (Sato et al. 1992). Mangiferin has antiviral activity, antioxidant action and antitumor activity (Guha et al. 1996; Sanchez et al. 2000; Zheng and Lu, 1990).

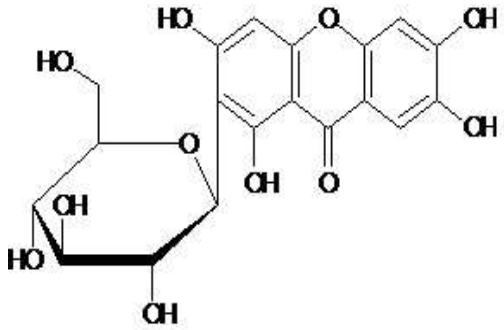

Figure 1: Structure of Mangiferin

Mangiferin has also been documented to exhibit vascular protective (Beltran et al. 2004), hepatoprotective and analgesic activities (Roome et al. 2005). Mangiferin composition is very complicated, and it is not simple to synthesize through chemical reactions, and isolating mangiferin from natural materials is an excellent choice for its development. Mangiferin is obtained by different natural herbal drugs like *Mangifera indica*, *Swertia chirayita* and *Canscora decussate* etc (Acton, 2013; Aritomi and Kawasaki, 1969; Chauhan and Dutt, 2012).

There is a dearth of analytical methods of mangiferin as a chemical and biological marker for quality control of these important medicinal plants. Hence, it was considered worthwhile to develop precise, cost effective and stability indicating UPLC method for determination of mangiferin in *M. indica, S. chirayita,* and *C. decussate*. The UPLC analytical method has advantages over another analytical method for study of botanicals, herbal crude drugs and standard markers (Nangare and Mendhulkar, 2014; Xiejun et al. 2014). The aim of the present investigation was to develop simple, sensitive, rapid, accurate and economical UPLC methods for estimation of mangiferin in methanolic extract of *Mangifera indica, Swertia chirayita* and *Canscora decussate* as per international conference on harmonization (ICH) guideline Q2 (R1).

MATERIALS AND METHODS

Procurement and identification of plant material

The leaves of *M. indica,* and aerial parts of *S. chirayita* and *C. decussate* were procured from the local market in Dammam, Saudi Arabia and were authenticated by pharmacognosist and phytochemist Dr. Wasim Ahmad, Department of Pharmacognosy, Mohammad Al-Mana Pharmacy College for Health Sciences, Dammam, Saudi Arabia.

Chemicals and Reagents

All analytical grade chemicals were obtained from S.D. Fine chemicals Ltd., Dammam Saudi Arabia and HPLC grade chemicals were obtained from E. Merck, Darmstadt, Germany. Mangiferin (99%) was purchased from Sigma Aldrich, United State.

Extraction

The plant material of *M. indica* (Leaves), aerial parts of *S. chirayita*, and *C. decussate* were dried in sun light properly. The dried leaves and aerial parts were powdered using mixer grinder. These crude powders (50 g) were defatted separately with 300 mL of petroleum ether by the maceration method. The defatted powder was extracted with methanol (300 mL) for 6h by Soxhlet method and concentrated using rotary evaporator (Buchi, R-215; Switzerland). The concentrated extracts were stored in air tight glass container at 5-10°C for further study. The % extraction yield was calculated by applying the given formula below:

$$\%\ \text{Extraction yield} = \frac{\text{Weight of dried extract}}{\text{Weight of drug sample}} \times 100$$

Identification of mangiferin

Thin layer chromatography (TLC) technique

For identification and presence of mangiferin, standard and samples solutions of mangiferin were spotted on precoated silica gel aluminium TLC plate $60F_{254}$ (Merck, Darmstadt, Germany) and run with using of different solvent system. The TLC plates were being dried at 110 °C for 30 min in oven. The chromatographic method was chosen on the basis of the R_f (Retention factor) value of mangiferin. The R_f value was calculated by the using of given stat.

$$\text{Rf value} = \frac{\text{Distance travelled by solute (cm)}}{\text{Distance travelled by solvent (cm)}}$$

Infrared spectroscopy

NICOLET Is50 Fourier transform-infrared spectroscopy (FT-IR) spectrophotometer (Thermo Fischer Scientific, 5225 Verona Road, Madison, WI 53711, USA) was used to record the IR spectrum for mangiferin. In details, mangiferin (100 µg) and 100 mg KBr was mixed via pressed to make a pellet and further subjected to IR analysis.

NMR Spectroscopic Analysis

Mangiferin 5 mg were dissolve in 1 ml DMSO-d6 solvent and transferred into a 5 mm diameter NMR spectroscopy tube for analysis. ^{1}H NMR analysis was recorded using a Bruker Biospin AG 300 MHz (Bruker Biospin, Germany). The NMR system was operated using software TopSpin 3.2 pl7 operating at 300 MHz for ^{1}H at 295 K with 5 MM probe. Proton 90° pulse widths was 12.25 µs. Chemical shifts were expressed in ppm relative to tetra methyl silane (TMS) as an internal standard. About number of scans 16 was gathered with a recycle period of 2–3 seconds to get excellent spectra for ^{1}H NMR. A region from 0–15 ppm was scanned for the sample for ^{1}H NMR.

UPLC Analysis

Preparation of Standard and sample Solution

Stock solution of the mangiferin was ready by dissolving 10 mg of accurately weighed mangiferin in 10 mL in UPLC grade methanol in volumetric flask to obtain the final concentration 1mg/mL. This solution was further diluted in the different dilution series of 100– 1200 ng/mL for plotting of calibration curve. The all dilutions were filtered by 0.45 µm syringe filters and then reserved for UPLC analysis.

Dried methanolic extract 10 mg each of *M. indica*, *S. chirayita* and *C. decussate* were extracted by Soxhlet method and were dissolved in 100 mL UPLC grade methanol to attain a final concentration. All sample solutions were then filtered by 0.45 µm syringe filters and reserved for UPLC study.

Instrument and UPLC Conditions

ThermoScientific™Vanquish™UPLC system (Thermo scientific, Germany), made up of a binary solvent release system along with photodiode array detector (Chromeleon (c) DionexVersion 7.2.8.10783, Germany), was used to perform UPLC. For chromatographic isolation, the apparatus used with specifications, i.e., Pinnacle DB Cyanom C18 column (1.9 µm; 30 mm × 2.1 mm), degassed mobile phase of HPLC-grade solvent, i.e., acetonitrile (ACN): water (50:50 *v/v*) with isocratic elution, flow rate of mobile phase (0.300 mL/min) with retention time (0.680 min.) as well as injection sample volume of 10 µL as injected at every run. The total run time was 2.0 min with software Chromeleon (c) DionexVersion 7.2.8.10783.

Method Validation

The method was developed and validated according to ICH guidelines (ICH, 2005).

Linearity

Linearity was estimated by injecting the standard solution of mangiferin in the dilution range of 100-1200 ng/mL. The standard plot was made for mangiferin by plotting the peak area versus the different dilution range. Regression study was carried out in order to estimate the linearity, in terms of r^2, of the calibration curve.

Precision

According to the ICH guidelines, precision should be analyzed at two phase, interday and intraday precision. Interday precision observe to applying the analytical method in different days over a specific period of time by the same analyst with unchanged instrument. Intraday was determined by the applying of analytical method within a day at different period of time by the same analyst with the same instrument.

Accuracy

To evaluate the consistency and appropriateness of the developed procedure, recovery studies were performed. These experiments were carried out by using standard addition method. Known quantity of standard mangiferin was added to pre-studied samples and their recovery was matched with the theoretical value.

Selectivity

Selectivity is the aptitude to evaluate standard compound in the existence of other phytochemicals. The selectivity of the method was determined through comparing the run time and UV spectrum of mangiferin found in the plant extracts through those of standard.

Robustness by Design Expert

A Box-Behnken statistical screening design was applied to optimize the compositional parameters and to estimate quadratic effects of the column temperature (℃), flow rate of mobile phase (mL/min) and wavelength (nm). Seventeen experiments with three center points were carried out by selection of three factors, column temperature (A), wavelength (B), flow rate of mobile phase (C) and peak area were chosen as the response. The nominal value of these three factors A, B and C was 27℃, 0.2 mL/min and 380 nm; respectively. The statistics generated were examined using Design-Expert (Version 11.0.3.0, Stat-Ease Inc., Minneapolis, MN, USA) statistical software. The significance of the factors was calculated using Fisher's statistical test for Analysis of Variance (ANOVA) model that were estimated. These components were then used to compute an F-ratio to evaluate the effectiveness of the model. If the F-ratio probability is low, the model is considered a better statistical fit for that data. All experiments were carried out in randomized order to reduce the bias effects of uncontrolled factors.

Limit of detection and quantification

The limit of detection (LOD) of the analytical procedure refers to that lowest concentration of the reference marker in the sample that can be successfully examined based on visual estimation. Limit of quantification (LOQ) is the minimum quantity of reference marker in the sample that can be quantitatively estimated via appropriate accuracy and precision. The LOD and LOQ were calculated by the standard deviation method using calibration curve.

LOD = 3.3 SD/a

LOQ = 10 SD/a

where SD is the standard deviation of intercept, and a represents the slope of the calibration plot.

Quantification of mangiferin content in Plant Samples

The amount of mangiferin in *M. indica, S. chirayita* and *C. decussate* were examined via developed and validated procedure by calibration curve. The plant extract solutions were injected in tripliin column of UPLC and area of each triplicate samples chromatogram were used for study of quantity of marker by linearity equation.The results of triplicate evaluation were uttered as mean quantity of mangiferin in % *w/w*.

Assessment of % Scavenging activity by DPPH-UV method

The free radical scavenging power of the plant samples were observed using DPPH-UV method (Amir et al. 2011). DPPH (0.004%) was dissolved in methanol. The stock solution should be freshly prepared daily for experiment. The DPPH solution was used as control. The DPPH solution (1 mL) was added to 1 mL of plant extracts (10 mg/mL) or standard (quercetin 1 mg/mL) with 3 mL of methanol. The solution mixture was shaken strongly and kept to place at 20℃ in the dark light for 10 min. The decline in absorbance of the resulting solution was observed at 517 nm after 10 min. The variation in the decrease of absorbance between the control and the plant extract/standard was applied for calculating the %

radical scavenging activity. Each result was examined in triplicate**.**

$$\text{Radical Scavenging } (\%) = \frac{(A_C - A_S)}{A_C} \times 100$$

Where, A_C = Absorbance of control, A_S = Absorbance of sample

RESULTSAND DISCUSSION

Extraction yields

The volume of plant extract provided by an extraction process in a specific solvent is also an estimated measure of the quantity of specific compound found in the plant material. The extraction process was carried out in methanol by Soxhlet method. During the study, time of extraction, temperature of extraction and plant sample, and solvent ratio were kept constant. The % extract yield of *M. indica*, *S. chirayita* and *C. decussate* were found to be 12.83 ± 0.053, 9.61 ± 0.059 and 8.92 ± 0.061, respectively.

Identification of mangiferin

Thin layer chromatography is mainly applied by which the quality control and fingerprint of plant phytochemicals can be studied. TLC has exceptional resolution and, thus, allows instantaneous detection of an extensive series of materials in a single run. Various mobile phases were tried by hit and trial procedure for the identification and confirmation of mangiferin and also its presence in plant extracts (Figure 2). Chromatographic separation was achieved in solvent system consisting of ethyl acetate: methanol: formic acid (10:1:0.5; *v/v/v*). The mangiferin was detected at 380 nm and the results were found satisfactory. The R_f value of mangiferin was found to be 0.41 ± 0.083. The IR spectrum also showed the confirmation of mangiferin (Figure 3). The spectra of 1^1H NMR analysis of mangiferin showed the same as previous literature (Figure 4) (Taniya et al. 2015).

UPLC Analysis

UPLC method optimization

Best possible chromatographic conditions were found after tested various mobile phases with a reverse phase C_{18} column. Acetonitrile was chosen over methanol as solvent system since its use resulted enhanced isolation. Several isocratic systems of mobile phase were tried for the good separation of chromatograms. The mobile phase contains acetonitrile and water (50:50; *v/v*) was pumped in an isocratic mode using C_{18} column (27℃). The mobile phase was pumped at a flow rate of 0.3 mL/min, injection volume was 10µL, and wavelength for detection was set to 380 nm. The separation was observed with 2 min without any peak interference. A well resolved, balanced and sharp peak was found for mangiferin at 0.690 min retention time (Figure 5).

Method Validation

Linearity

The linearity of an analytical procedure is its facility to draw analysis results that are directly, or by means of well-defined mathematical alteration, proportional to the concentration of standard in samples within a defined range. The linear regression data found for the standard plot (n=6) showed an outstanding linear relationship over different concentration range 100-1200 ng/mL for mangiferin (Figure 6) with respect to peak area. The plot shows the y-intercept values were low and standard deviations of slope were also quite low. The r^2value was found to be 0.997 ± 0.097

Precision

The precision of an analytical procedure is the level of agreement among particular test results achieved when the process is useful to numerous sampling of a homogenous sample. It is an evaluation of the reproducibility of the complete analytical method under standard working conditions. The precision of sample application and observation of chromatogram area were represented in terms of %RSD and results are illustrated in table 1.

Accuracy

Accuracy articulated the closeness of understanding between the values which is established as an ordinary genuine value or an established reference value and the observed obtained. This test permits the evaluation of probable bias. The UPLC analysis was used for the selection of accurate mobile phase for estimation of mangiferin in herbal compounds after injecting via 50%, 100% and 150% of added references standard, which showed best recovery of 98.52-102.95%. The results of % of mangiferin recovered and % RSD are shown in table 2.

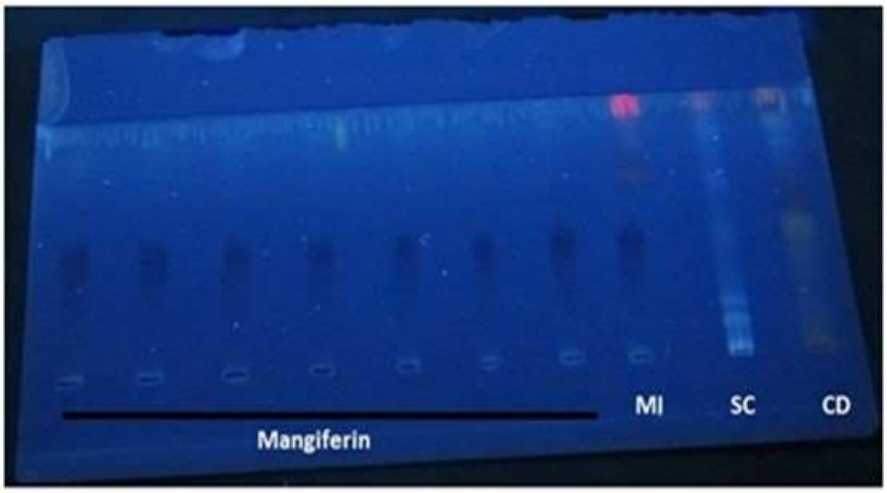

Figure 2: Identification of Mangiferin by TLC in *Mangifera indica* (MI), *Swertia chirayita* (SC) and *Canscora decussate* (CD)

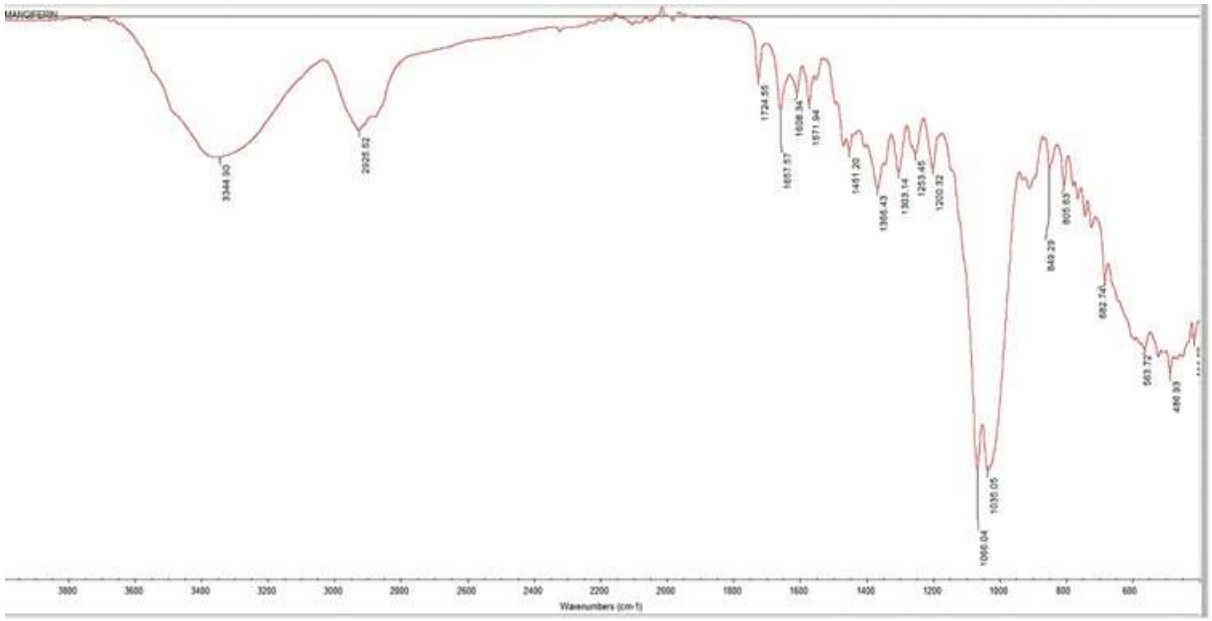

Figure 3: IR spectra of Mangiferin

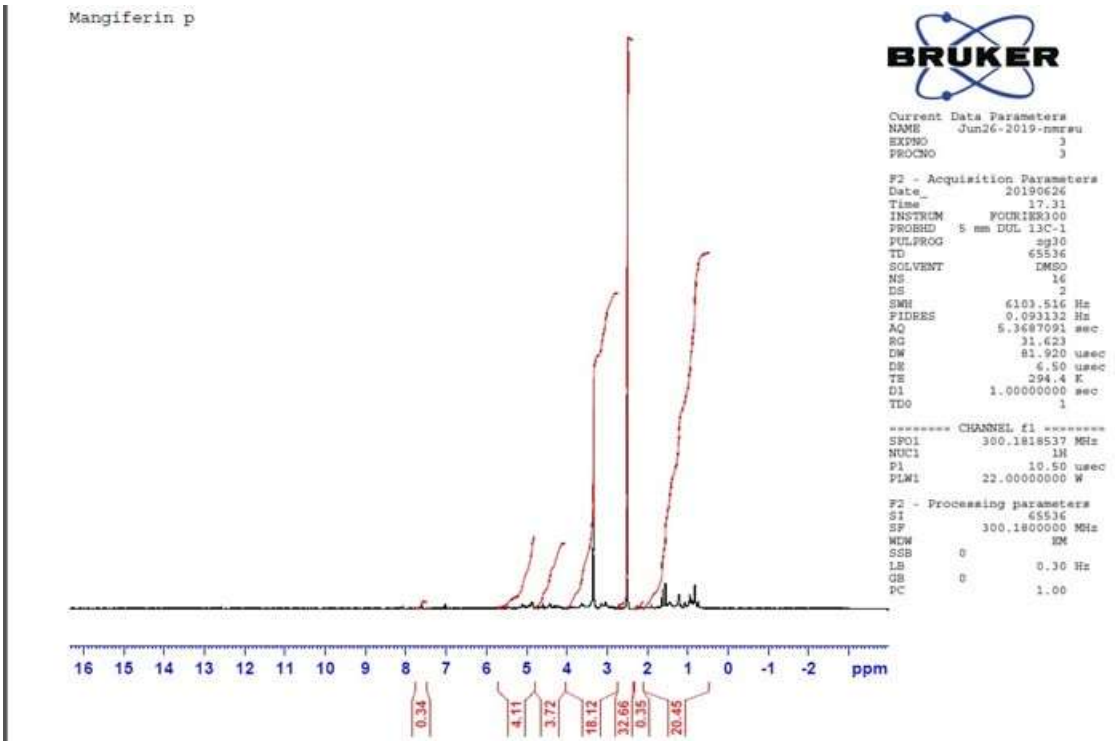

Figure 4: NMR spectra of Mangiferin

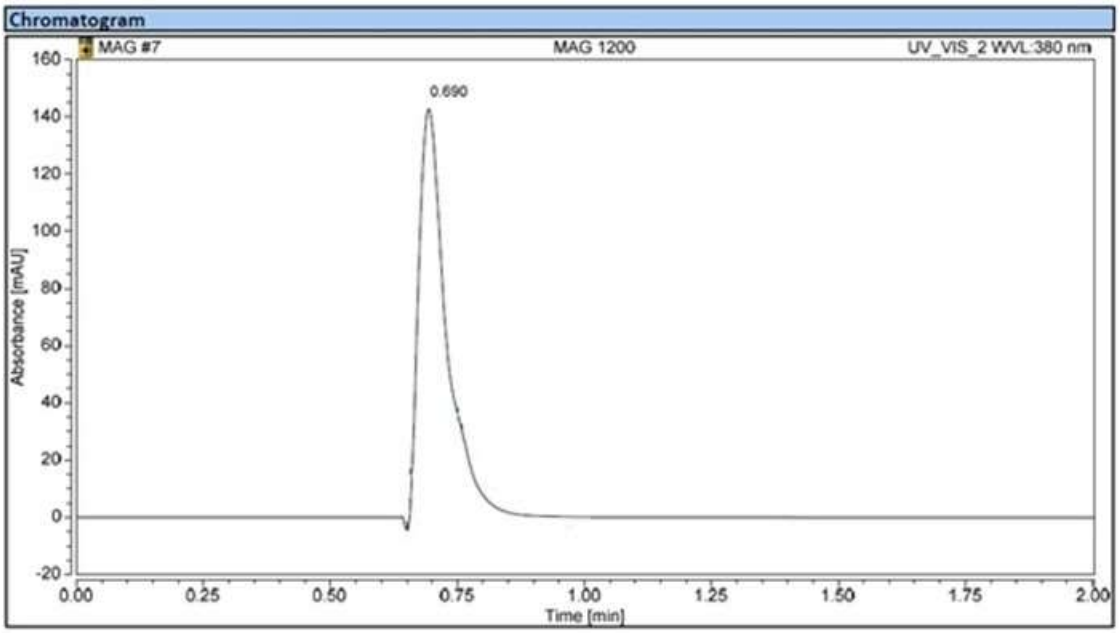

Figure 5: A typical UPLC chromatogram of Mangiferin (Rt =0.690)

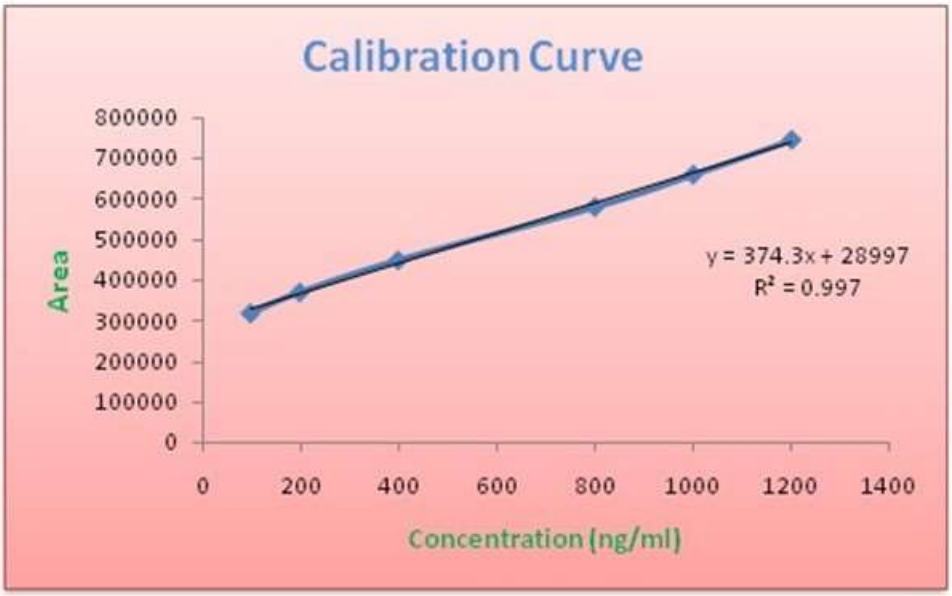

Figure 6: Calibration curve with respect to area of chromatogram at various dilution range of Mangiferin

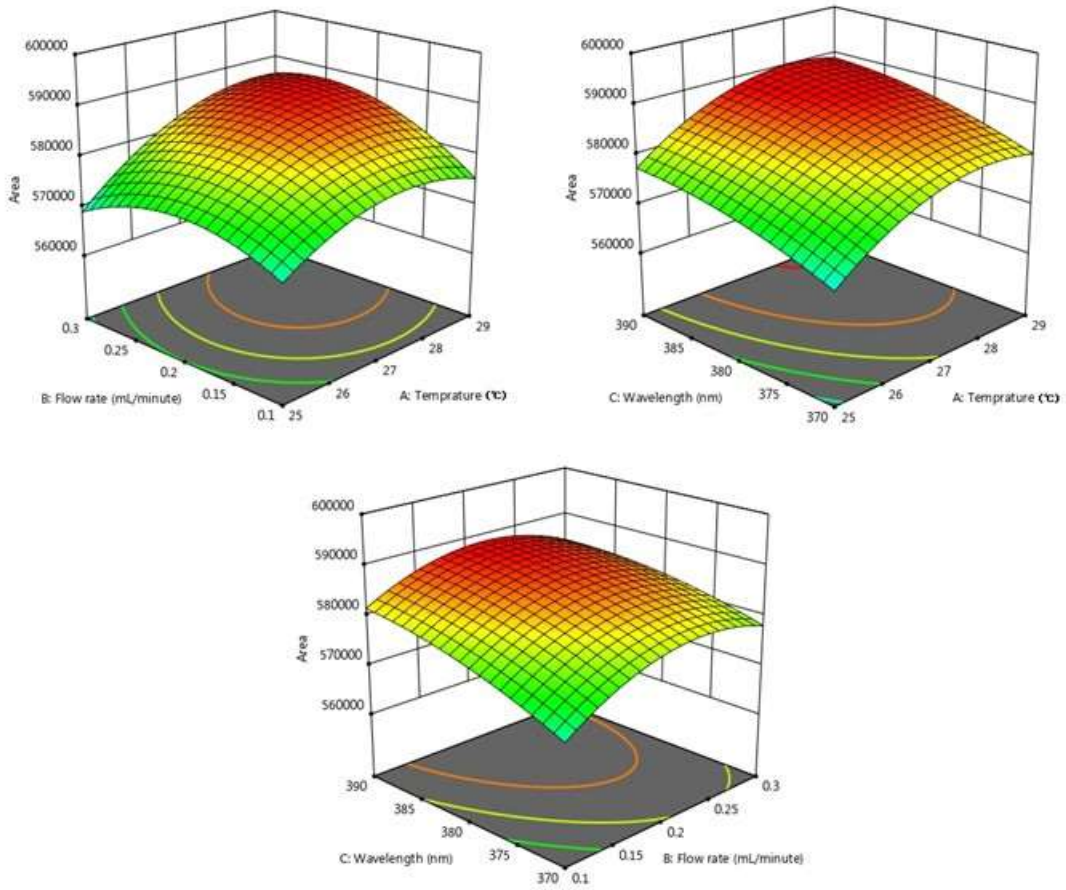

Figure 7:Three-dimensional graphs of robustness for Mangiferin by using Box-Behnken experimental design

Selectivity

The selectivity was determined by evaluating the standard mangiferin and plant extracts. The method was determined to compare the retention time of mangiferin obtained in the plant extracts with those of the standard mangiferin.The retention time of the mangiferin in the tested herbal extract was found to be same as in standard mangiferin (RT=0.690 min).The ultraviolet spectrum of the target constituents was analyzed in same manner of the reference standard. The peak purity of mangiferin was studied by comparing the spectrum at three various stages, viz. peak begin (S), peak top (M) and peak finish (E) positions (Figure 5).

Robustness by design expert

A three-factorial, Box-Behnken statistical experimental design was performed using 17 experiments. The independent variables and the responses for all 17 optimized runs are given in Table 5. It was observed that the best-fitted model was the quadratic model and the comparative values of SD and % CV for the different proposed models. Only statistically significant ($p< 0.05$) Coefficients were included in the equation. A positive value represents an effect that favors the optimization, while a negative value indicates an inverse relationship between the factor and the response (Myers and Montgomery, 2002). It is clear from the equation that the factor temperature (A) and flow rate (B) has a positive effect and wavelength (C) has a negative effect on the response (Y) (Figure 7). It also shows that the relationship between responses and factors is not always linear. Used at different levels in an analysis or when more than one factor is changed simultaneously, a factor can produce different degrees of response. For an experimental design with three variable factors, the suitable model fitting to the data was the quadratic model. The polynomial equations for the response factors are given below:

Peak Area (Y) $+5.861E+05+5825.29*A+2673.67*B+4399.39*C+2969.75*A*B-0.7500*A*C1226.25*B*C-5416.78*A^2-5608.76*B^2-1857.03*C^2$

Where A is the temperature (ºC), B is the flow rate (mL/min) and C is the wavelength (nm)

Limit of Detection and Limit of Quantification

The limit of detection (LOD) is explained as the lowest amount of a reference standard in a sample that can be detected, but not essentially quantified. It is a limit test that indicates whether a reference standard is high or lowers a specific value. The limit of quantification (LOQ) is explained as the lowest amount of a reference standard in a sample that can be quantified with suitable precision and accuracy under the stated operational circumstances of the process. In order to determine the limit of detection (LOD) and limit of quantification (LOQ), blank methanol was spotted six times. The signal-to-noise level was examined. LOD was measured as 3:1 and LOQ as 10:1. The LOD with S/N ratio of 3:1 was obtained 24.68 ng/mL and LOQ with S/N ratio of 10:1 was obtained 74.75 ng/mL.

Quantification of mangiferin in extract of Mangifera indica, Swertia chirayita and Canscora decussate

The amount of mangiferin in the methanolic extract of *M. indica, S. chirayita* and *C. decussate* were determined via developed and validated chromatographic method by calibration curve. A well-defined and resolved sharp peak of mangiferin was observed at 0.690 min retention time in the chromatograms of the samples. The mangiferin content in the *M. indica, S. chirayita* and *C. decussate* were found to be 8.6 ± 0.21 % *w/w,* 4.94 ± 0.16 % *w/w* and 2.09 ± 0.19 % *w/w*, respectively, with low % RSD value indicated the suitability of this process for regular study of mangiferin during the formulation development. The results of the analysis are given in table 4.

Table 1: Inter-day and intra-day precision of the UPLC method (n=6) for mangiferin

Amount (ng/mL)	Inter-day precision		Intra-day precision	
	Mean peak area ± SD	%RSD	Mean peak area ± SD	%RSD
Mangiferin				
400	446196.33 ± 3970.84	0.889931	438248.00 ± 10012.14	2.284584
800	585691.66 ± 8327.81	1.421876	583512.33 ± 6273.98	1.0752104
1000	668621.00 ± 7957.165	1.1900860	664072.66 ± 4947.16	0.7449736

RSD: Regressed Standard Deviation

Table 2: Accuracy of the UPLC method (n=6) for mangiferin

% of standard spiked to the sample	Theoretical content (ng)	Amount of drug recovered ng ± SD	% of drug Recovered	% RSD
0	800	823.66 ± 15.30	102.95	1.858512
50	1200	1182.33 ± 18.33	98.52	1.551118
100	1600	1640.33 ± 33.12	102.52	2.019468
150	2000	2030.33 ± 15.63	101.51	0.769881

RSD: Regressed Standard Deviation

Table 3: Estimation of mangiferin in selected herbal drugs by UPLC method

Samples	Mangiferin (% w/w)
Mangifera indica	8.6 ± 0.2 %
Swertia chirayita	4.94 ± 0.16 %
Canscora decussate	2.09 ± 0.19 %

Table 4: % Radical Scavenging activity of standard/samples

Samples	Conc. (µg/ml)	Absorbance at 517 nm	Radical Scavenging activity (%)	Mean ± SD
Quercetin	1	0.074, 0.082, 0.089	93.85, 93.18, 92.60	93.21 ± 0.625
Mangifera indica	10	0.221, 0.209, 0.201	81.64, 82.64, 83.30	82.52 ± 0.835
Swertia chirayita	10	0.358, 0.341, 0.349	70.26, 71.67, 71.01	70.98 ± 0.705
Canscora decussate	10	0.391, 0.385, 0.414	67.52, 68.02, 65.61	67.05 ± 1.272

Absorbance of Control (DPPH): 1.204

Table 5: Chromatographic Factors for Central Composite Response Surface Methodology

Std	Run	Factor 1 A:Temprature ℃	Factor 2 B:Flow rate mL/minute	Factor 3 C:Wavelength nm
15	1	27	0.2	380
3	2	25	0.3	370
8	3	29	0.3	390
7	4	25	0.3	390
5	5	25	0.1	390
13	6	27	0.2	363.182
6	7	29	0.1	390
1	8	25	0.1	370
17	9	27	0.2	380
14	10	27	0.2	396.818
16	11	27	0.2	380
10	12	30.3636	0.2	380
4	13	29	0.3	370
9	14	23.6364	0.2	380
11	15	27	0.0318207	380
12	16	27	0.368179	380
2	17	29	0.1	370

Assessment of % Scavenging activity by DPPH-UV method

It is renowned that the DPPH-UV method was used for a quick evaluation of pure active antioxidant constituent in complex mixtures, mainly herbal extracts. The additional quick absorbance reduces; the more potent antioxidant

efficacy of the constituent will be in terms of hydrogen-donating ability (Shirwaikar et al. 2006).This study proved that the plant samples have the proton giving capability and could provide as free radical inhibitors or scavengers, acting probably as main antioxidants. It was examined that the extract of *M. indica, S. chirayita* and *C. decussate*at dilution of (10 mg/mL) had Radical scavenging activity of 82.52 ± 0.835 %, 70.98 ± 0.705 and 67.05 ± 1.272 %, respectively which was comparable to that of the standard quercetin (1 mg/mL) (93.21 ± 0.625%). This result confirms that the method can be applied for a rapid screening of antioxidant compounds or more accurately radical scavenging activity of phytoconstituents (Table 5).

CONCLUSION

A new validated UPLC analytical method has been developed for the estimation of mangiferin in *M. indica, S. chirayita* and *C. decussate*. The developed method was found to be simple, specific, accurate, precise, less time consuming, low cost value and has the ability to isolate the marker compound from its other constituents with good linearity range and limits of determination. Statistical analysis confirmed that the method is appropriate for the regular analysis of mangiferin. The projected method is better as compared to other chromatographic densitometric methods, because it has been appropriately optimized, very less run time and well validated. The statistical evaluation of data obtained confirms that the method is reproducible and selective and can be used for regular qualitative and quantitative analysis of mangiferin in *M. indica, S. chirayita* and *C. decussate* and its multicomponent herbal formulations..

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

We are thankful to Imam Abdulrahman bin Faisal University, Dammam, KSA.

AUTHOR CONTRIBUTIONS

Conceptualization by Mohd Amir. Methodology by Mohd Amir, Niyaz Ahmad, Faisal Saad Mohammed Alqarni. Software by Niyaz Ahmad, Md Sarafroz, Formal analysis by Mohd Amir, Niyaz Ahmad, Faisal Saad Mohammed Alqarni,
Resources by Mohd Amir, Faisal Saad Mohammed Alqarni, Writing-original draft preparation by Mohd Amir, Md Sarafroz
Review and Editing by Mohd Amir, Md Sarafroz . All authors read and approved the final version.

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