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Novel HPLC method for simultaneous estimation of gallic acid, protocatechuic acid and mangiferin in mango

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Abstract

Mango (*Mangifera indica*) is the main tropical fruit of Asia and has developed its prominence all over the world. Mango wastes such as peels and seeds generated from fruit are a good source of functional ingredients out of which important ones are phenolic compounds with potent antioxidant properties. The quantification of gallic acid (GA), protocatechuic acid (PCA) and mangiferin (MGF) in the seed and peel extracts of three different varieties of mango fruit was performed by a novel, simple, specific, linear, precise, accurate and economic RP-HPLC (reversed-phase high- performance liquid chromatography) method. The chromatographic separation was achieved on the C18 analytical column with a flow rate of 1.2 mL/min in an isocratic mode using 0.2% ortho-phosphoric acid and acetonitrile in the ratio 90:10 as the mobile phase. The column temperature was maintained at 40 °C and observations were recorded at 242 nm. The retention time of gallic acid, protocatechuic acid and mangiferin was observed at 3.41, 5.50, and 14.6 min respectively. The developed method when validated as per ICH guidelines was found to be specific, linear, precise, robust and accurate

Keywords: *Mangifera indica*, peel, seed, phenolic acids, HPLC.

INTRODUCTION

Mangifera indica, is commonly called as mango, amra, manga or mangot (Family –Anacardiaceae). Extracts of *M. indica* Linn has been reported to possess antiviral, antibacterial, analgesic, anti-inflammatory, immunomodulatory, antiamoebic, cardiotonic, and diuretic properties [1].

India produces more than 18.7 million tonnes of mangoes every year and the world production is nearly 42 million tons per year [2, 3]. After consumption or industrial processing of mangoes, approximately 40 to 60% of waste (including peels, seeds, and kernels) is generated during the processing of mangoes [2].

Peel and seeds of mango fruits are discarded as agro-industrial waste but they may have significant potential benefits due to the high content of polyphenolic compounds, including gallic acid, syringic acid, gentisic acid, protocatechuic acid, mangiferin, ellagic acid, and quercetin, ferulic acid, catechin, kaempferol, coumarin, caffeic acid, vanillin, cinnamic acid [3-7].

Mangiferin is reported to possess the anti-tumor activity, used for metabolic regulation, anaemia, it is also used in cosmetics due to its antioxidant and UV-protecting properties. Gallic acid is a well-known polyphenol with potential antioxidant activity, making it an effective, bioactive constituent in neuropsychological diseases, gastrointestinal diseases, and oral health issues. Protocatechuic acid is also a bioactive compound reported possessing anti-ageing, anti-asthma, antiulcer, antispasmodic property [8].

Literature survey reveals HPLC methods are reported for estimation of these markers in mango, but in combination with other compounds, however, there has not been any reported method for the simultaneous RP-HPLC of gallic acid, protocatechuic acid, and mangiferin.

Hence, it was thought worthwhile to develop a novel, simple, economic isocratic HPLC method for the simultaneous estimation of these polyphenol constituents. The developed novel RP-HPLC method was also applied for the first time for the quantification of selected polyphenolic in peels and seeds of different cultivars of mango.

MATERIALS AND METHODS

Plant material

The cultivars studied were Dasheri, Badami, and Totapuri which were brought from the local market (Mumbai). The fresh peels and seeds of selected cultivars of mango fruits were subjected to sun drying for 45 days. Dried peels were powdered using a mixer grinder, while the seeds were powdered using Hanningfield Uni-Mill M10B (Gansons Pvt. Ltd.) having screen size 039R and 040G respectively.

Chemicals

Gallic acid, protocatechuic acid and mangiferin (purity 95%) were procured from Yucca Enterprises, Mumbai, India. HPLC grade solvents such as methanol, acetonitrile, *ortho*-phosphoric acid were purchased from Thomas Baker Chemicals Pvt. Ltd. Mumbai, India**.** RP-HPLC Shimadzu (LC 2030) model with "Lab Solution" software was used in this method. The analytical column used for the separation of analytes was RP-shim-pack HPLC C18 (250 X 4.6 mm, 5 µm).

Selection of wavelength

Appropriate wavelength was determined by separate scanning of the standard solutions in the range from 200-400 nm using a UV spectrophotometer. It was then overlapped to determine the common UV maxima.

Chromatographic conditions

The optimized chromatographic conditions include a RP- shim-pack HPLC C18 column (250 X 4.6 mm, 5 µm), 0.2% *ortho*-phosphoric acid and acetonitrile in the ratio 90:10 as a mobile phase, at a flow rate of 1.2 mL/min, column temperature maintained at 40 °C and a detection wavelength of 242 nm using a UV-visible detector. The total run time was 16 min.

Preparation of standard solution

100 mg of GA, PCA, and MGF standards were accurately weighed and transferred into 100 mL volumetric flask respectively. About 70 mL methanol was added in which the standards are soluble then sonicated to dissolve and diluted up to the mark using methanol (1000 ppm). The stock solution was suitably diluted for further HPLC injections.

Preparation of sample

Accurately about 4 g of mango peel and seeds of each variety were extracted separately by refluxing with hydro-alcohol in a round bottom flask for 20 min. The sample solution was filtered, made up to 100 mL, and a clear solution was obtained. Each stock solution was injected into HPLC after suitable dilutions.

Validation of developed method

The developed optimized HPLC method was validated by means of specificity, linearity, precision, robustness, and accuracy as per ICH guidelines [9].

RESULTS AND DISCUSSION

Method Development

The standard solutions were scanned using a UV spectrophotometer and the detector wavelength was then set at 242 nm as the best compromise to reach maximum absorbance for all compounds of interest.

A series of trials were conducted using a varying proportion of acetonitrile/methanol along with acetic acid/water/phosphate buffer for getting proper resolution of the peaks. The selected polyphenols in each sample were identified by comparing chromatographic peaks with the retention time (RT) of individual standards. When methanol: acetic acid (20:80) was tried as a mobile phase, all the standards were resolved properly but the RT of mangiferin was more than 19 min as shown in Fig. 1. With methanol: 0.2% *ortho*-phosphoric acid (30:70) as a mobile phase, the resolution of peaks was poor in the sample. After several other trials, the optimum separation was achieved using acetonitrile: 0.2% *ortho*-phosphoric acid (10:90) as a mobile phase with a flow rate of 1.2 mL/min in an isocratic mode. The optimized chromatographic conditions are tabulated in Table 1 resulting in the elution of GA at 3.41 min, PCA at 5.50 min and MGF at 14.6 min. The typical chromatogram of mixed standards of GA, PCA, and MGF is shown in Fig. 2.

Table 1: Optimized chromatographic conditions

Parameters	Optimized conditions
Column	Prontosil C18 (250 X 4.6 mm, 5 µm)
Mobile phase	Acetonitrile: 0.2% orthophosphoric acid (10:90)
Detector	UV detector
Detection wavelength	242 nm
Column temperature	40 °C
Injection volume	10 µl
Flow rate	1.2 mL/min
Run time	16 min
Retention time	3.41, 5.50 and 14.6 min

Quantification of GA, PCA, and MGF in different cultivars of Mango

The optimized method was applied for the quantitative determination of selected markers in peels and seeds of different cultivars of mango. The results are tabulated in Table 2. Fig. 3, 4, and 5 represent chromatograms of Badami, Totapuri, and Dasheri seed samples respectively, whereas Fig. 6, 7 and 8 represent chromatogram of peel samples of Badami, Totapuri, and Dasheri respectively. The total run time was 16 min.

Table 2: Percent content of GA, PCA, and MGF in different mango cultivars.

Cultivars	**% Content (GA)**	**% Content (PCA)**	**% Content (MGF)**
Badami seed	0.422	0.0013	0.058
Totapuri seed	0.408	-	0.037
Dasheri seed	0.466	-	0.056
Badami peel	0.055	0.0443	0.280
Totapuri peel	0.097	-	0.119
Dasheri peel	0.284	-	0.010

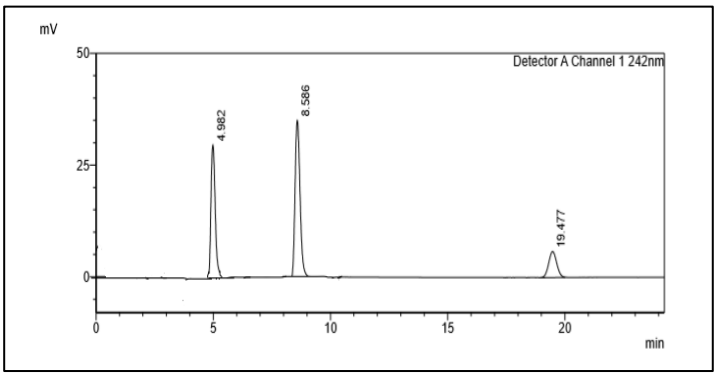

Figure 1: HPLC chromatogram of standard mixture of GA, PCA, and MGF obtained using methanol: acetic acid (20:80) as mobile phase

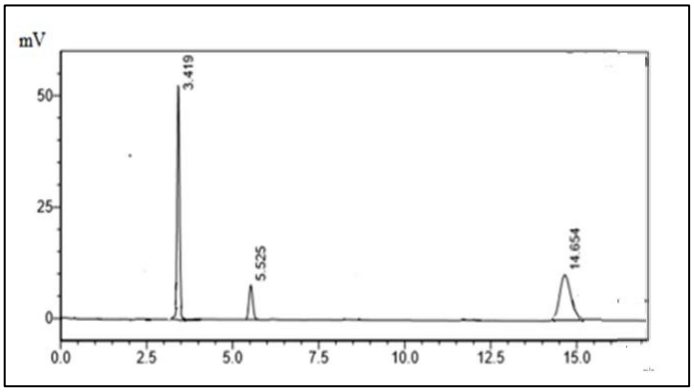

Figure 2: HPLC chromatogram of standard mixture of GA, PCA, and MGF obtained using optimized conditions

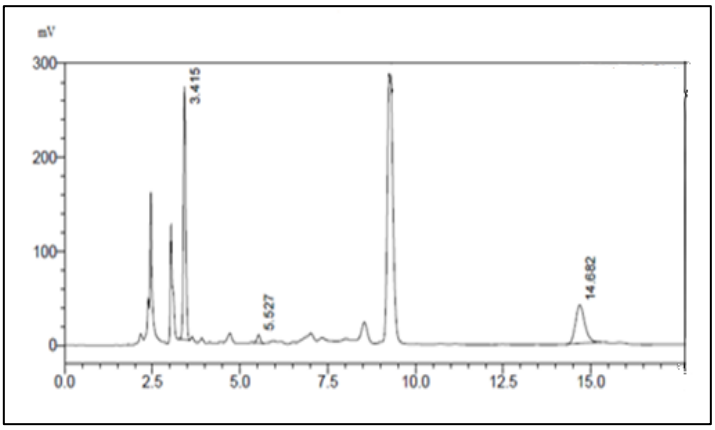

Figure 3: HPLC chromatogram of Badami seed sample using optimized conditions

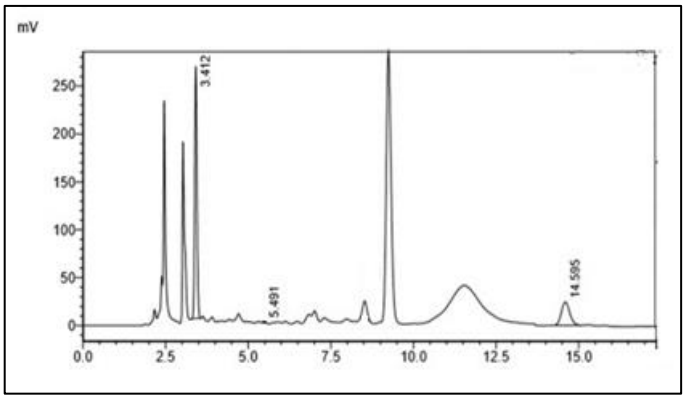

Figure 4: HPLC chromatogram of Totapuri seed sample using optimized conditions

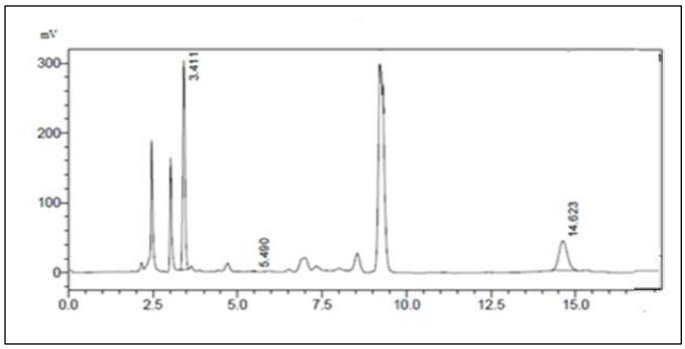

Figure 5: HPLC chromatogram of Dasheri seed sample using optimized conditions

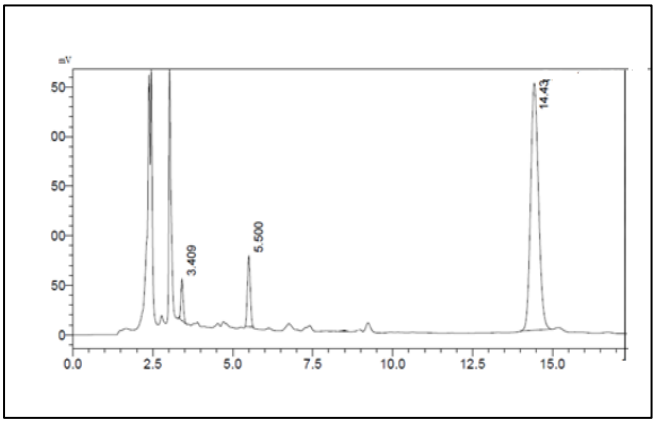

Figure 6: HPLC chromatogram of Badami peel sample using optimized conditions

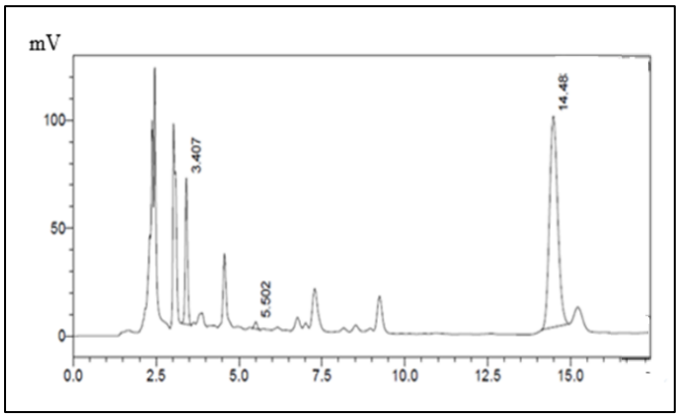

Figure 7: HPLC chromatogram of Totapuri peel sample using optimized conditions

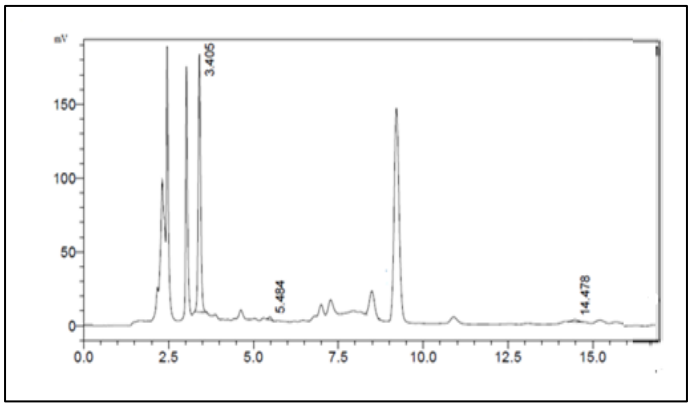

Figure 8: HPLC chromatogram of Dasheri peel sample using optimized conditions

Method Validation

Specificity

The developed method was found to be specific as there was no interference of any other constituents at the RT of all the three markers GA, PCA and MGF as shown in Fig. 2.

Linearity

The selected markers, GA, PCA and MGF showed a linear response in the tested concentration range of 1-200 µg/mL, 0.5-20 µg/mL and 1-200 µg/mL respectively. The linearity was validated by the high value of correlation coefficients (R^2) 0.9932, 0.9979 and 0.9956 for GA, PCA and MGF, respectively, which meets the acceptance criteria for method

validation. The results are tabulated in Table 2 and plots obtained are given in Figures 9, 10 and 11 respectively.

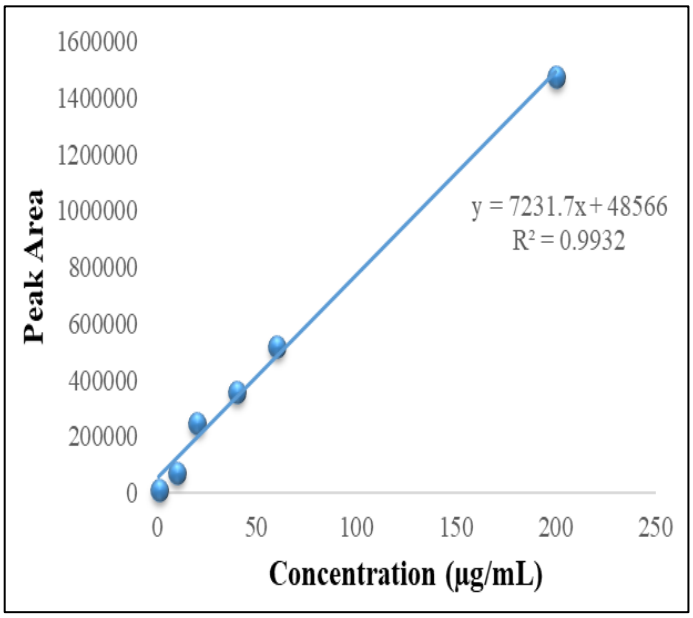

Figure 9: Calibration curve of gallic acid

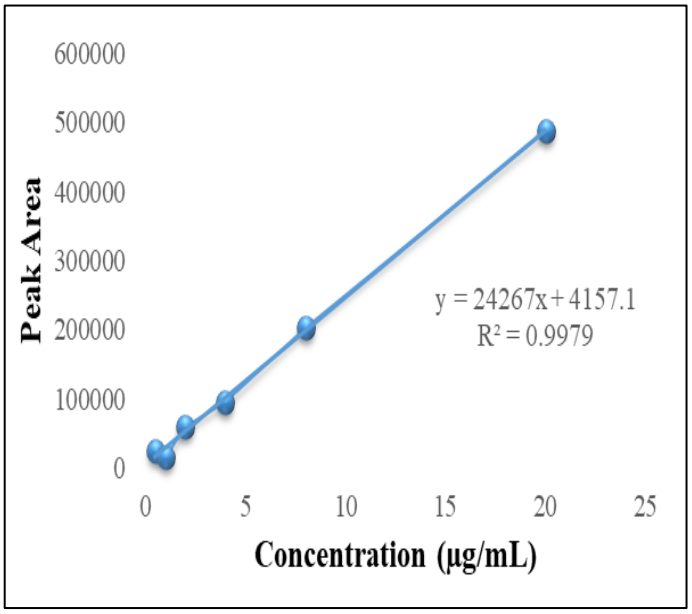

Figure 10: Calibration curve of protocatechuic acid

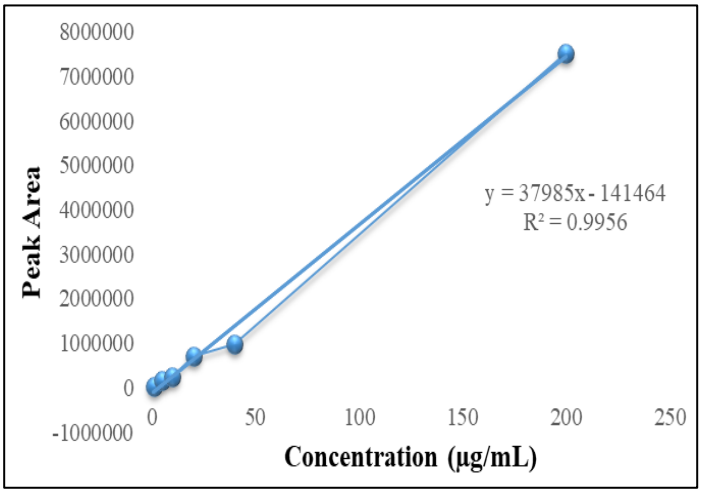

Figure 11: Calibration curve of mangiferin

Table 3: Linear regression data obtained from the calibration curves of GA, PCA, and MGF

Parameter	Gallic acid	Protocatechuic acid	Mangiferin
Equation of regression line	y=7231.7x + 48566	y=24267x + 4157.1	y=37985x – 141464
Coefficient of correlation (R^2)	0.9932	0.9979	0.9956
Slope	7231.7	24267	37985
Intercept	48566	4157.1	-141464

Precision

The % RSD of the peak areas obtained was <2; hence the developed method was found to be precise. The data of intra-day and inter-day precision are tabulated in Table 3.

Table 4: Intra-day and Inter-day Precision of markers (GA, PCA and MGF).

Markers	Intra-day Precision (% RSD)	Inter-day Precision (% RSD)
Gallic acid	1.57	1.85
Protocatechuic acid	1.72	1.80
Mangiferin	0.88	1.27

Robustness

The % RSD of the peak areas obtained was <2; this ensured that the method is robust. The results of robustness are shown in Table 5.

Table 5: Robustness of markers (GA, PCA, and MGF)

Parameters	Deviation	Gallic acid		Protocatechuic acid		Mangiferin	
		Area	RT (min)	Area	RT (min)	Area	RT (min)
Flow rate (mL/min)	1.1 mL	270022	3.72	62476	6.00	242312	15.77
	1.3 mL	236441	3.14	54393	5.06	220043	13.40
Column temperature	39 °C	251027	3.41	58378	5.52	221880	14.88
	41 °C	265924	3.38	61256	5.43	258953	14.24
Wavelength	241 nm	244637	3.40	55701	5.47	248994	14.36
	243 nm	261083	3.99	61343	5.46	232816	14.36

Accuracy

The acceptance limit for percent recovery ranges from 98% to 102%. The mean % recovery of GA, PCA, and MGF in Badami cultivar (both in seed and peel) was found to be within the range, whereas in seeds and peels of Totapuri and Dasheri cultivar, GA and MGF was found to be within the range except PCA which was not found to be quantifiable. From the % recovery results, it was found that the method was accurate.

The accuracy results are tabulated in the Table 6.

Table 6: Average % recovery of the seeds and peels of mango cultivars

Cultivars	**Average of 3 samples % recovery**		
	Gallic acid	**Protocatechuic acid**	**Mangiferin**
Badami seed	99.4	99.6	100.5
Totapuri seed	100.2	-	100.4
Dasheri seed	99.1	-	99.6
Badami peel	98.8	99.2	100.3
Totapuri peel	98.6	-	99.3
Dasheri peel	100.2	-	99.6

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CONCLUSION

Being a really standard plant, particularly among the tropics and due to its individuation of components (peel and seed) being used domestically and industrially, the mango so may be a low cost and pronto on the market provider of polyphenols. As tonnes of mango wastes such as seeds and peels are generated annually, it's necessary to search out the acceptable application of those waste merchandise. The developed method has been applied to analyze GA, PCA, and MGF from three different cultivars (Badami, Totapuri, and Dasheri) of mango. A good separation of peaks was achieved and the above method developed is novel, simple, sensitive. Validation procedures were conducted and therefore the methodology was established to be specific, linear, precise, robust and accurate. Other advantages are shorter run time, shorter sample preparation time and economic. The presence of a significant amount of respective bioactive components ensures its unequivocal recommendation for the employment within the pharmaceutical and nutraceutical sector.

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Abbreviations used

HPLC, high-performance liquid chromatography; UV−vis, ultraviolet−visible; %RSD: Percentage relative standard deviation; SD, standard deviation; GA, gallic acid; PCA, protocatechuic acid; MGF, mangiferin

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