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Development and validation of HPLC method for simultaneous estimation of ellagic acid and curcumin in herbal formulation

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Abstract

A rapid, simple, selective and precise UV- Visible Spectrophotometric method has been developed for the determination of ellagic acid and curcumin in bulk forms and solid dosage formulations. The spectrophotometric detection was carried out at an absorption maximum of 366 nm using methanol as solvent. The method was validated for specificity, linearity, accuracy, precision, robustness and ruggedness and the results were noted down.

Key-words: HPLC, Curcumin, Ellagic acid

Introduction

In the post genomic era, World Health Organization (WHO) estimated about 80% of the world population uses herbs and other traditional medicines for their primary health care needs.[1] Tremendous raise in the use of herbal medicine is leading to a fast-growing market of polyherbal formulations worldwide.[2] Whereas, according to WHO guidelines, standardization of herbal products is essential in order to assess the quality, clinical safety and efficacy before releasing into the market.[3]

In complementary and alternative medicine therapies, curcumin and ellagic acid ahas significant role either alone or in combination with other herbs. A variety of polyherbal formulations of turmeric and ellagic acid extracts have been marketed commercially in which the percentage is vital to ensure physiological benefits. Conversely, several without marketed polyherbal formulations are available labeled claim on relative percentage composition of individual phytoconstituents.[4-5]

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Several methods were reported for the estimation of curcumin and ellagic acid in different pharmaceutical and herbal formulations by using UV, HPLC, UPLC, HPTLC, FT-IR and others hyphenated methods.[6-12]However, these techniques are not suitable for analyzing compounds in combinations of polyherbal formulations like Ayurvedic or Chinese medicinal products, since they contain more than one herb. While UV-spectrophotometric methods are more suitable for this objective, studies on dedicated UV-spectrophotometric methods to quantify the curcumin and ellagic acid in polyherbal formulations are very limited. Therefore, in the present study, HPLC method was developed and validated for the quantitative estimation of curcumin and ellagic acid in polyherbal formulations.

Material and Methods

Selection of wavelength

Suitable wavelength for the HPLC analysis was determined by recording UV spectrums in the range of 200-400 nm for individual drug solutions of ellagic acid (ELA) and curcumin (CUR) then overlapped. UV overlain spectra of these two markers showed that the drugs absorb appreciably at 366 nm and hence 366nm was taken as a detection wavelength for HPLC analysis (Fig. 1)

Chromatographic conditions

The method was developed using reverse phase, shim-pack HPLC C18 column (250 X 4.6 mm, 5 µm). The run time was of 10 min. The mobile phase

used was 0.02 M potassium dihydrogen orthophosphate buffer (pH adjusted to 3.5 with orthophosphoric acid) and acetonitrile in the ratio 60:40 at a flow rate of 1.2 ml/min, column temperature maintained at 35 °C and a detection wavelength of 255 nm using a UV-visible detector.

Preparation of 0.02 M phosphate buffer (pH 3.5)

About 2.72 g of potassium dihydrogen orthophosphate was accurately weighed and dissolved in 950 ml of water. The pH was adjusted to 3.5 with orthophosphoric acid and the volume was made up to 1000 ml in volumetric flask. The solution was then filtered using 0.45 µ membrane filter.

Preparation of standard solution

100 mg of ellagic acid (ELA) and curcumin (CUR) standard were accurately weighed and transferred into 100 ml volumetric flask respectively. About 70 ml solvent (Methanol) was added, sonicated to dissolve and diluted up to the mark using solvent (1000 ppm). Final concentration of ellagic acid (ELA) and curcumin (CUR) were made to 10 ppm and 7 ppm respectively by suitable dilutions.

Sample preparation

Accurately about 100 mg of tablet powder was extracted with 100 ml methanol. The sample solution was filtered to obtain a clear solution. The stock solution after suitable dilutions was used for further analysis.

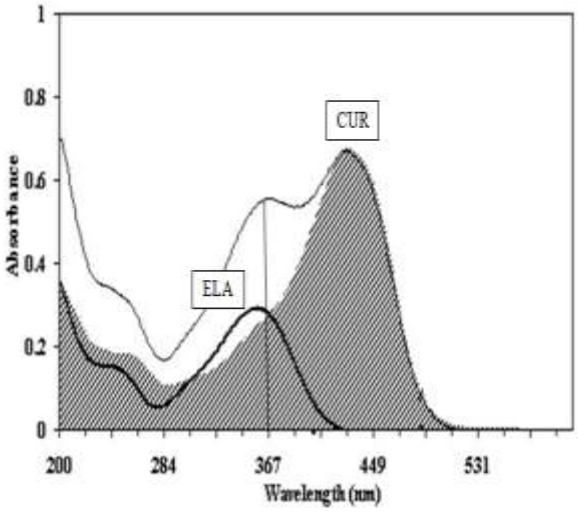
Composition
Antidiabetic herbal formulation (Tablet 100 mg)

Common name (Hindi name)	Botanical name	Part used	Family	Composition (%)
Black berry(Jamun)	*Syzygium cumini*	Seed	Myrtaceae	10
Bitter gourd (Karela)	*Momordica charantia*	Fruit	Cucurbitaceae	10
Indian gooseberry (Amla)	*Embelica officinalis*	Fruit	Euphorbiaceae	20
Ram's horn (Mesha Shringi)	*Gymnema sylvestre*	Leaves	Asclepiadaceae	10
Nagajivha (Chota chirayata)	*Enicostemma littorale*	Entire plant	Gentianaceae	10
Neem	*Azadirachta indica*	Leaves	Meliaceae	10
Gulancha tinospora (Guduchi)	*Tinospora cordifolia*	Root	Menispermaceae	10
Turmeric (Haridra/Haldi)	*Curcuma longa*	Rhizome	Zingiberaceae	18

Results and Discussion

Suitable wavelength for the HPLC analysis was determined by recording UV spectrums in the range of 200-400 nm for individual drug solutions of ellagic acid (ELA) and curcumin (CUR) then overlapped. UV overlain spectra of these two markers showed that the drugs absorb appreciably at 366 nm and hence 366nm was taken as a detection wavelength for HPLC analysis (Fig. 1)

A reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. resolution factor between peaks, tailing factor, number of theoretical plates, runtime and the cost effectiveness. The developed optimized method resulted in the elution of ellagic acid at 1.65 min and curcumin at 2.94 min. Fig. 2 and 3 represent chromatograms of ellagic acid and curcumin standard solution respectively. The total run time was 10 min.



Overlapped wavelength 366 nm

Fig. 1: UV Spectra of ELA and CUR

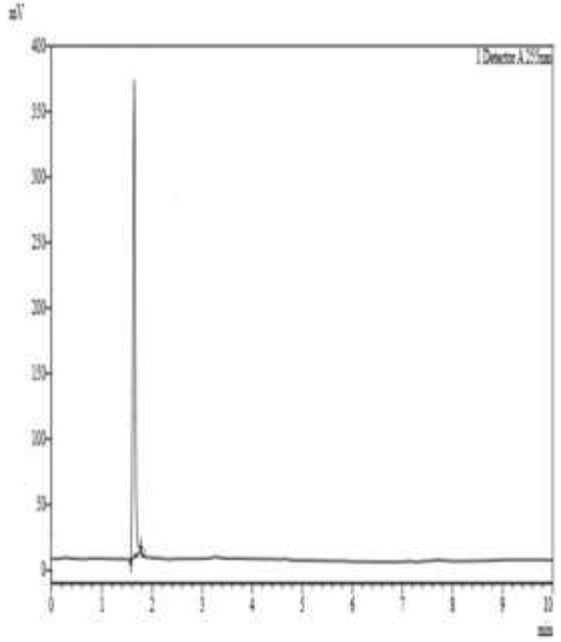


Fig. 2: Chromatograms of ellagic acid standard solution

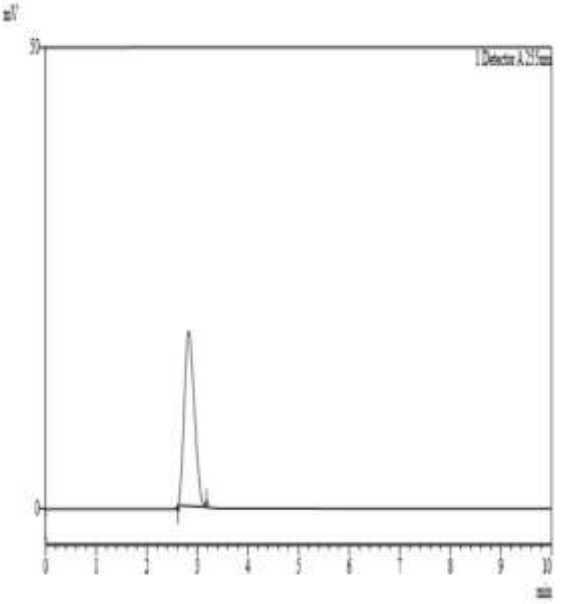


Fig. 3: Chromatograms of curcumin standard solution

System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time, number of theoretical plates, peak resolution and peak tailing factor were evaluated replicate injections of the standard working concentration.

The results given in table 1 were within the acceptable limits.

Table 1: System suitability studies results

S/No.	Parameters	Acceptance Limit	Ellagic acid (ELA	Curcumin (CUR)
1.	Retention time	-	1.68	2.98
2.	Resolution factor	NLT 2	-	8.14
3.	No. of theoretical plate	NLT 2000	3618	3889
4.	Tailing factor	NMT 2	1.05	1.31

In order to test the applicability of the developed method to an herbal formulation, tablet powder extracts were chromatographed and it is shown in fig. 4.

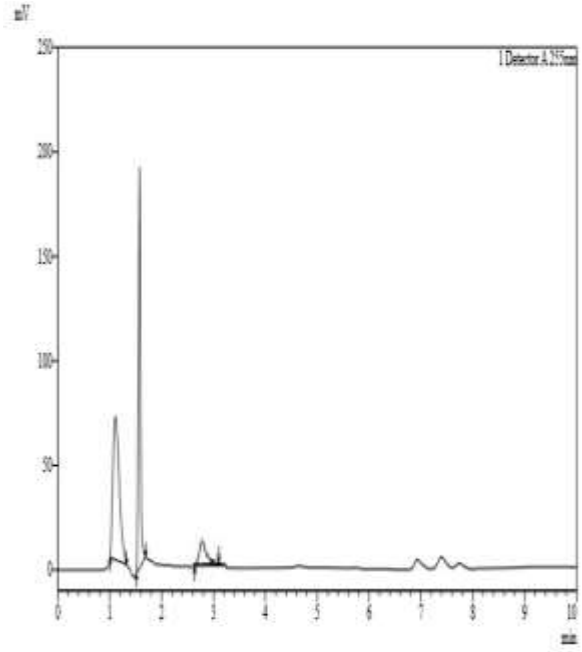


Fig. 4: Chromatograms of herbal formulation

The sample peaks were identified by comparing the relative retention times with standard markers (fig. 2,3). System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of separated peak area was done and each marker concentration was determined by using the peak area concentration relationship obtained in the standardization step. For the analysis of sample, extract of 100 ppm of tablet powder was

injected in triplicate and quantified for two active markers using linear regression equation.

The results of tablet powder extract analysis are reported in table 2.

Table 2: Analysis of herbal tablet powder extract

Formulation	Marker	Amount present (ppm); n=3	Content (%)
Herbal Formulation (Tablet powder extract) 100 ppm	Ellagic acid (ELA)	3.92	0.81
	Curcumin (CUR)	2.98	0.63

Method validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. The developed HPLC method was validated according to ICH guidelines for validation of analytical procedures. The method was validated for the parameters like linearity, accuracy, system precision, method precision, robustness, limit of detection and limit of quantitation.

Specificity

Fig. 2-4 for standard drug solutions and sample chromatograms reveals that the peaks obtained in the standard solutions and sample solution at working concentrations are only because of the drugs as blank has no peak at the retention time of ellagic acid and curcumin accordingly, it can be concluded that the method developed is said to be specific.

Precision

System precision

Six replicate injections of the standard solutions at working concentration showed percent relative standard deviation (% RSD) less than 2 concerning peak area for each marker, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in table 3.

Method precision

Method precision was determined by performing the analysis of the sample under the test of repeatability at working concentration. Three injections of the sample from the same homogeneous mixture at working concentration showed % RSD less than 2 concerning content of two markers indicate that the method developed is precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (table 4).

Table 3: Results of system precision

S/No.	Peak Area	
	ELA	CUR
1	1356600	405178
2	1361151	405276
3	1366110	404024
4	1366288	405671
5	1367917	402517
6	1370101	403210
Mean±SD	1363694.5±5200.94	403927.7±1268.50
%RSD	0.38	0.31

Note: Results are expressed as Mean±SD; n=6; SD: Standard deviation; RSD: Percent Relative Standard deviation

Table 4: Results of method precision results

Marker	Intra-day		Inter-day	
	Content (ppm); n=3	%RSD	Content (ppm); n=3	%RSD
ELA	3.90	0.77	3.92	0.81
CUR	2.92	0.59	2.98	0.63

Abbr.: n: number of injections (n=3), %RSD: Percent relative standard deviation

Linearity

Standard solutions of ellagic acid and curcumin at different concentrations level were prepared in triplicates. Calibration curves were constructed by plotting the concentration level versus corresponding peak areas for each marker. The results show an excellent correlation between peak areas and concentrations level within the tested concentration range of 6-14 ppm for ellagic acid and as that of 2-10 ppm for curcumin (table 5). The correlation coefficients were greater than 0.99 for each marker, which meet the method validation acceptance criteria and hence the method is said to be linear.

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of each compounds in the formulation at three different levels (80%, 100% and120%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in table 6. The accepted limits of mean recovery are 98%-102% and all observed data were within the required range,

which indicates good recovery values, affirming the accuracy of the method developed.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered, and the system suitability parameter tailing factor and peak area were evaluated. The solution was prepared as per the test method described earlier and injected at different variable conditions like column temperature (33 °C and 37 °C), flow rate (1.1 ml/min and 1.3 ml/min) and detection at specific wavelength. Robustness data clearly shows that the proposed method is robust at small but deliberate change. Robustness data are given in table 7.

Sensitivity

The sensitivity of measurement of ellagic acid and curcumin by use of the proposed method were estimated in terms of the limit of quantitation (LOQ) and limit of detection (LOD). LOQ and LOD were calculated by the use of the equations LOD = 3.3σ/S and LOQ = 10σ/S where σ is the standard deviation of intercepts of calibration plots and S is the average of the slopes of the corresponding calibration plot (table 8).

Table 5: Data from linearity studies

Marker	Conc. range (ppm)	R^2
ELA	6-14	0.997
CUR	2-10	0.991

Table 6: Recovery studies for two markers in herbal formulations

Compounds	Sample content (ppm)	Standard added (ppm)	Actual amount (ppm)	Total area found (n=3)	Amount recovered (ppm)	% Recovery
ELA	1.87	1.52	3.45	801381	3.39	98.34
		1.91	3.91	826651	3.82	99.50
		2.29	4.22	845421	4.18	99.01
CUR	1.58	1.12	2.54	384992	2.52	99.37
		1.40	2.82	386440	2.83	100.35
		1.68	3.10	388623	3.01	99.10

Table 7: Robustness data for two markers in herbal formulations

Parameters	ELA (10 ppm)		CUR (10 ppm)	
	Peak area	Tailing factor	Peak area	Tailing factor
Minus temp (33^0C)	1356600	1.1	405178	1.28
Plus temp (37^0C)	1361151	1.02	405276	1.34
Minus flow rate (1.1 ml/min)	1366110	1.01	404024	1.35
Plus flow rate (1.3 ml/min)	1366288	0.99	405671	1.32
Minus wavelength (365 nm)	1367917	1.1	402517	1.24
Plus wavelength (367 nm)	1370101	1.1	403210	1.28

Table 8: LOD and LOQ for two markers in herbal formulations

Marker	LOD (ppm)	LOQ (ppm)
ELA	0.63	1.89
CUR	0.15	0.46

Conclusion

In conclusion, we have developed and validated a method that was found to be eco-friendly, simple, cost-effective and was successfully applied for the estimation of curcumin and ellagic acid in polyherbal antidiabetic formulation Dihar tablets without involvement of any interference from other phytochemicals forming part of the formulation ingredients. Based on the results and statistical parameters demonstrate that this method could be the specific and remarkable for the analysis of curcumin and ellagic acid in polyherbal formulations.

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