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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CURCUMIN AND DIOSGENIN FROM POLYHERBAL FORMULATTION

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ABSTRACT

Curcumin with Diosgenin reduces inflammations, improves blood sugar levels and reduces stress, anxiety and fatigue. A simple, selective, rapid, precise and economical reverse phase HPLC method has been developed for simultaneous estimation of Curcumin and Diosgenin from polyherbal dosage forms. The method was carried out on a Phenomenex C18 column (250 mm *4.5 mm *5µm) with a mobile phase consisting of KH2PO4 buffer (PH adjusted to 3): Acetonitrile (60:40 %v/v) at flow rate of 1ml/min and PDA detection at 210 nm. The retention time of Curcumin and Diosgenin was found to be 2.6 and 4.5 min respectively. The drugs were found to be linear with correlation coefficient which was nearly 0.9992 and 0.9991 for Curcumin and Diosgenin respectively with linearity range of 500 – 1500 µg/ml. Percentage recoveries from recovery studies were found to be 99.33% and 99.61% for curcumin and Diosgenin individually. LOD and LOQ was found to be lower, hence the method is sensitive.

Key words: Curcumin, Diosgenin, RP HPLC, Method Validation

1. INTRODUCTION

Curcumin is used as anti-inflammatory agents that are non-steroidal in nature. [1] In addition to anti-inflammatory actions, they have analgesic, antipyretic, a platelet inhibitory action, antifertility action, in

respiratory disease and to lower the blood cholesterol level. [1-2] Externally applied in pains and bruise. [1-2] Curcumin with Diosgenin reduces stress, anxiety, fatigue and reduces inflammation. The mechanism of action by which curcumin shows anti-inflammatory effect is by attenuating inflammatory response of TNF-α stimulated human endothelial cells by interfering with NF-kB.[3] Furthermore, curcumin is also capable for preventing platelet derived growth factor (PDGF).[3] Diosgenin is a steroidal saponin processing estrogenic and anti-tumor properties. It has a role as an apoptosis inducer ,an antiviral agent, an antineoplastic agent .[3] Literature review of curcumin revealed several analytical methods reported on different technique namely HPLC, HPTLC, UV Spectrophotometric, LC-MS/MS capillary electrophoresis[9-30] and literature review of diosgenin showed there are few analytical methods reported on HPLC, HPTLC, TLC for its quantification.[31-35] From the literature review it is found that there is no HPLC method reported for simultaneous estimation of curcumin & diosgenin in their combined dosage form. So, the aim of the present work is to develop a simple, reproducible & sensitive RP HPLC method for quantification of selected curcumin & diosgenin and to validate the newly develop method to ensure their accuracy, precision, reproducibility & other analytical method validation parameter as per ICH Q2(R1) Guideline.[36]

2. MATERIAL & METHOD

2.1 Material:

Curcumin and diosgenin were obtained from AUM Research Labs.Pvt. Ltd, Ahmedabad, Gujarat, India, as a gift sample. ACN & KH2PO4 were purchased from Rankem, Ahmedabad & merk chemicals Ltd, Ahmedabad respectively and both were HPLC grade. OPA was of analytical grade & was purchased from finer chemical Ltd., Ahmedabad. Two formulation collected in which one was in house formulation and Another one was marketed formulation. In house formulation, label claim state that both of the drug is 50 mg of curcumin &50mg of diosgenin and marketed formulation label claim of both the drug is 250mg.

2.2 Method development:

A. HPLC Instrumentation & chromatographic condition:

Alliance 2693 HPLC system with Phenomenex C18 column (250mm*4.6mm*5 µm), UV detector & 10µl Hamilton syringe. waters Phenomenex C18 column was used at Ambient column temperature. The optimized mobile phase consisted a KH2PO4: ACN (60:40%V/V) adjusted with OPA to PH 3 at flow rate 1.0ml/min with run time 10 min and UV spectrometric wavelength at 210 nm.

B. Diluent Preparation:

Mobile Phase A:

An accurately weighed quantity of KH2PO4 (27.218gm) was taken in 100 ml beaker, dissolved with triple distilled water. adjust the PH with OPA to PH 3. buffer was taken filtered with Whatman filter paper.

Mobile Phase B: Acetonitrile

Diluent proportion was kept 60:40 %v/v

C. Preparation of standard stock solution:

I. Curcumin standard stock solution (10,000 µg/ml)

An accurately weighed quantity of curcumin (100 mg) was taken in 10 ml of volumetric flask volume was made up to the mark with diluent to make 10,000µg/ml.

II. Diosgenin standard stock solution (10,000 µg/ml)

An accurately weighed quantity of diosgenin (100mg) was taken in 10ml volumetric flask & volume made up to the mark with diluent to make 10,000 µg/ml.

D. Preparation of test/sample solution:

Take 20 tablets, each containing 50 mg of curcumin & 50mg diosgenin were accurately weighed &finely powdered. A quantity of tablet powder equivalent to 50 mg of curcumin & 50mg diosgenin was weighed transferred into 50 ml volumetric flask. 20ml of mobile phase was added & warm for 15 min in water bath & solution was made up to 50ml with mobile phase & filter the solution using Whatman filter paper.

2.3 Method Validation:

The method was validated according to ICH Q2(R1) guideline for linearity, precision, accuracy, limit of detection and limit of quantification, Specificity, Robustness & System suitability studies.[36] The linearity response was determined by analysing 5 independent levels of calibration curve range of 500-1500 µg/ml for Curcumin and Diosgenin. The accuracy of the proposed method was determined by a recovery study, while was carried out by spiking standard (500µg/ml) to sample at three different level (80%, 100%, 120%) and % recovery was calculated for Curcumin and Diosgenin. The precision of the assay was investigated by measurement of both repeatability and Intermediate precision. Precision of analytical method is expressed as the standard deviation and relative standard deviation. The system precision of the test method was performed by injecting 6 replicates of standard preparation (1000 µg/ml.). Determination of limits of detection and quantitation was based on the standard deviation of the y-intercepts of regression lines and the slope of the calibration plots. The system suitability study conducted as per ICH Q2(R1). The parameters like tailings factor, retention time, number of theoretical plates and resolution were calculated by injecting six replicates of standard concentration 1000 µg/ml of Curcumin and Diosgenin. Specificity of proposed method was determined by injecting blank solution, placebo and combined standard solution 1000 µg/ml of

Curcumin and Diosgenin and test samples into the chromatographic system and interfaces was checked for each. Robustness of the method was carried out by deliberately made small changes in the PH, mobile phase ratio and lamda maximum by analysing combined standard solution (1000 µg/ml) of Curcumin and Diosgenin and % RSD was calculated.

4. Result and Discussion

4.1 Optimization of Chromatographic Conditions

Initially various mobile phases, flow rate and detection wavelength were tried for HPLC analysis to achieved best chromatograph by using Phenomenex C18 column (250mm*4.5mm*5µm) with KH2PO4 buffer (PH adjusted to 3.0): Acetonitrile(60:40%v/v) as mobile phase in Isocratic mode at a flow rate 1ml/min and PDA detection at 210 nm. The chromatograph of Curcumin and Diosgenin standard and sample solution were shown in Figure 1,2 and 3 respectively. The retention time was 2.6 and 4.5 min for Curcumin and Diosgenin respectively. Result of standard chromatograph was shown in Table 1.

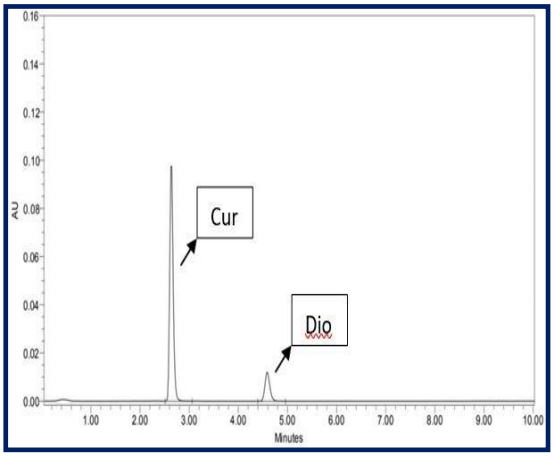

Figure 1

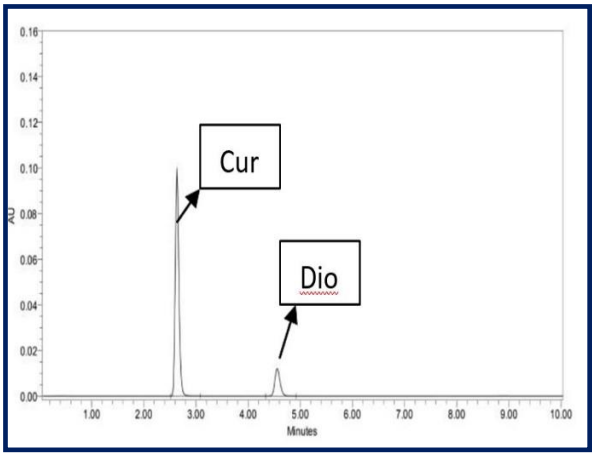

Figure 2

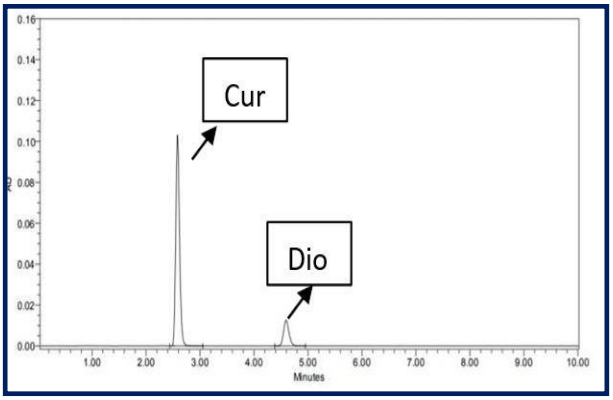

Figure 3

Figure 1: HPLC Chromatogram of Standard, Figure 2: HPLC Chromatogram of Formulation 1, Figure 3: HPLC Chromatogram of Formulation 2.

Table 1: Result of Standard Chromatogram

Sr.No.	Peak Name	RT	Area	%Area	USP plate count	USP Tailing	USP Resolution
1	Curcumin	2.64	4776313	85.19	7139	1.15	-
2	Diosgenin	4.59	830658	14.81	10059	1.15	7.45

4.2 Validation and Assay results of RP-HPLC method:

The linearity data of standard Curcumin and Diosgenin are shown in Table 2 & 3. The calibration curve for Curcumin and Diosgenin was obtained by plotting the peak area of both the drug versus concentration over the range of 500-1500 µg/ml and it were found to linear with r^2 of Curcumin = 0.9992 and r^2 of Diosgenin = 0.9991.(Fig. 4 & 5) The LOD of Curcumin and Diosgenin was found to be 9.49 µg/ml and 2.46 µg/ml respectively.(Table 4) The LOQ of Curcumin and Diosgenin was found to be 28.7 µg/ml and 7.46 µg/ml respectively.(Table 4) The % RSD for interday precision of Curcumin and Diosgenin was found to be 0.01 and 0.10.(Table 5) The % RSD for intraday precision of Curcumin Diosgenin was found to be 0.03 and 0.22 .(Table 6) The result of accuracy of formulation 1 and 2 were show in Table 7 & 8 .The % RSD for different parameters of robustness was found to be less than 2 %. (Table 9 ,10&11).

Table 2: Linearity data of Curcumin

Sr. no.	Concentration(µg/ml)	Area Mean ± SD	% RSD
1	500	2300978±339.2	0.01
2	800	3848837±8691.0	0.23
3	1000	4708411±10690.7	0.23
4	1200	5661562± 14916.6	0.26
5	1500	7198608±21315.3	0.30

Table 3: Linearity data of Diosgenin

Sr. no.	Concentration(µg/ml)	Area Mean ± SD	% RSD
1	500	399872±100.1	0.03

2	800	673874±576.2	0.09
3	1000	831612±4642.0	0.56
4	1200	1000818 ± 365.3	0.04
5	1500	1284211± 9131.8	0.71

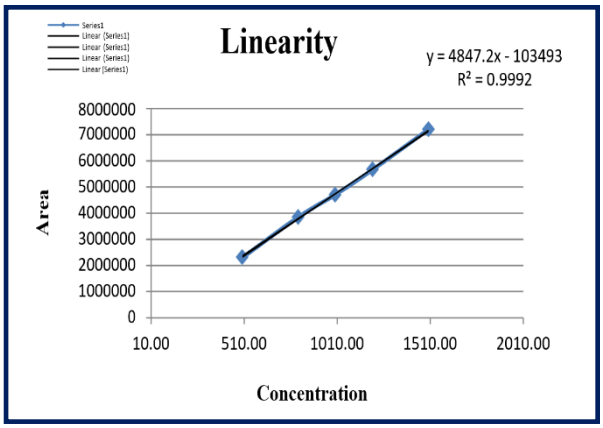

Figure 4: Calibration Curve of Curcumin

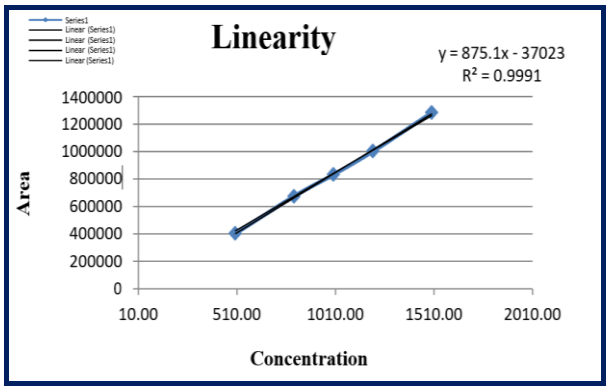

Figure 5: Calibration Curve of Diosgenin

Table 4: LOD and LOQ

Drug	LOD	LOQ
Curcumin	9.49µg/mL	28.7µg/mL
Diosgenin	2.46µg/mL	7.46µg/mL
Drug	**LOD**	**LOQ**
Curcumin	9.49µg/mL	28.7µg/mL
Diosgenin	2.46µg/mL	7.46µg/mL

Table 5: Results for Interday Precision

Time	Area	
	Curcumin	**Diosgenin**
10:00 am	4787797	837430
2:00 pm	4788601	835756
6:00 pm	4788268	836425
MEAN	4788222	836537
SD	403.969	842.601
% RSD	0.01	0.10

Table 6: Results for intraday Precision

Day	Area	
	Curcumin	**Diosgenin**
DAY 1	4788402	838583
Day 2	4789446	837542
Day 3	4786172	841133
MEAN	4788006	839086
SD	1672.419	1847.587
% RSD	0.03	0.22

Table 7: Recovery data for formulation 1

Parameter	Curcumin			Diosgenin		
	80%	100%	120%	80%	100%	120%
Sample conc. (µg/ml)	500	500	500	500	500	500
Std.conc. (µg/ml)	400	500	600	400	500	600
Total conc. (µg/ml)	900	1000	1100	900	1000	1100
%Recovery	99.14%	100.07%	98.78%	99.08%	98.89%	99.86%

Table 8: Recovery data for formulation 2

Parameter	Curcumin			Diosgenin		
	80%	100%	120%	80%	100%	120%
Sample conc. (µg/ml)	500	500	500	500	500	500
Std.conc. (µg/ml)	400	500	600	400	500	600
Total conc. (µg/ml)	900	1000	1100	900	1000	1100
%Recovery	99.55%	99.96%	99.61%	99.70%	99.89%	99.68%

Table 9: Variations in parameters of robustness

Parameter	Variations	
pH (3.0)	2.8	3.2
Mobile phase Ratio (60:40 % v/v)	55:45	65:35
λmax (210 nm)	208 nm	212 nm

Table 10: Results of pH

pH	Drug	Conc. (µg/ml)	Mean ± %RSD
2.8	Curcumin	1000	5947937±0.41
	Diosgenin		1026554±0.63
3.2	Curcumin	1000	3924402±0.49
	Diosgenin		691493±0.57

Table 11: Results of λmax

λmax	Drug	Conc. (µg/ml)	Mean±%RSD
208nm	Curcumin	1000	4788977±0.12
	Diosgenin		833239±0.29
212nm	Curcumin	1000	4771150±0.41
	Diosgenin		833133±0.30

Table 12: Results of Mobile Phase Ratio

Mobile phase ratio	Drug	Conc. (µg/ml)	Mean ± %RSD
55:45 % v/v	Curcumin	1000	5304359±0.78
	Diosgenin		920516±0.36
65:35 % v/v	Curcumin	1000	4325121±0.46
	Diosgenin		763315±0.34

5.CONCLUSION

The developed RP-HPLC method for simultaneous estimation of Curcumin and Diosgenin in tablet dosage forms was simple, precise, specific, highly accurate and less time consumption for analysis.

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