Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 2562-2568
Received: 11-03-2019
Accepted: 15-04-2019

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Development and validation of RP-HPLC and HPTLC method for the estimation of curcumin in haridrakhand polyherbal tablet formulation

Seema Gosavi, Shweta Kasar, Pooja Warule and Sarita Pawar

Abstract
Haridrakhand is polyherbomineral Ayurvedic formulation employed in skin disorders, allergies, shitapitta, urticaria, and psoriasis. A simple, specific, precise RP-HPLC and HPTLC method for estimation of Curcumin in Haridrakhand tablet formulations were developed and validated as per ICH guidelines Q2(R1). The development was done using precoated TLC plate's lino mat IV sample spotter. Toluene: ethyl acetate: methanol (4: 5: 1 % v / v / v) were used as mobile phase. Measurements were performed at 421 nm in using Server vision cats-server. The developed HPLC method involved use of C18 ODS, (250× 4.6) mm, 5 µ column. Acetonitrile: 0.05% orthophosphoric acid (70:30) was used as mobile phase at 0.7 mL/min flow rate and 394 nm wavelength maxima for detection. The linearity range 50-1000 ng/spot was set for HPTLC and for HPLC 1-25 µg/ml. Rf value and retention time by was found to be 0.421±0.03 and 8.387 by HPTLC and HPLC respectively.

Keywords: Haridrakhand, curcumin, RP-HPLC, HPTLC

Introduction
Haridrakhanda is the important polyherbal formulation for the treatment of allergy. It also helps in improving immunity [1-3]. The main and active ingredient of Haridrakhand formulation is Haridra i.e. turmeric. It acts as a histamine mediator for internal and external allergic reactions [2].

The major ingredients of Haridrakhand are Haridra (*Curcuma longa*), Haritaki (*Terminalia chebula*), Nishottar (*Operculina turpethum*), Darve (*Berberis aristata*), Ajmoda (Carum coxburghinanum), Musta (*Cyperus rotundus*), Yavani (Trachyspermum annani), Chitrak (Plumbago zeylanica), Katuka (Picrorrhiza kur- roo), Jeerak (*Cuminum cyminum*), Pipali (Piper on- gum), Ela (Clettaria cardamomum), Twak (*Cinnamomum zeyhnicum*), Tejpatra (*Cinnamomum tamala*), Vidanga (*Embelia ribes*), Guduchi (Tinospora cardi- ofolia), Kostha (*Saussurea lappa*), Triphala (Terminalia chebula, Terminalia belerica, Emblica officinalis), Dha- nyak (*Coriandrum sativum*), Loha bhasma (Ash of iron), Sharkara (Sugar) etc [1, 3].

Curcumin is the main alkaloid present in haridra which inhibit non-specific and specific mast cell-dependent allergic effects. It is proposed as the best anti-inflammatory agent in many researches works [2].

Curcumin is 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6- heptadiene-2,5-dione (Fig. 1) having yellow colored phenolic pigment collected from the powdered rhizome of *Curcuma longa* Linn. (Family: Zinziberaceae) [4, 5]. It blocks the synthesis of certain prostaglandins, reduces pro-inflammatory cytokine synthesis [6] inhibits pro-inflammatory arachidonic acid as well as neutrophils aggregation when inflammatory conditions occur. Curcumin is unstable at basic pH and undergoes alkaline hydrolysis in alkali/higher pH solution. Decomposition of Curcumin in Hydrolytic decomposition is reported in in-vitro physiological condition (isotonic phosphate buffer, pH 7.2) [7-9]. It undergoes photodegradation while exposing to light in solution as well as in solid form. There are already various methods developed for the analysis of Curcumin in the literature like UV, HPLC [10], TLC [20, 21] and HPTLC but there are very few reports on analytical methods for the estimation of curcumin in haridrakhand polyherbal tablet formulations [11].

For analysis of drugs, the analytical method should be simple, sensitive, accurate and rapid for the evaluation of herbs, herbal drugs and their formulation as per the ICH guideline [12-14]. Therefore, the purpose of the present work was to develop and validate an accurate, simple, precise and robust method for the study of Haridrakhand containing curcumin as a major ingredient in form of turmeric powder by using RP-HPLC and HPTLC and the method will be acceptable for routine analysis or quality control testing of an ayurvedic formulation Haridrakhand [21].

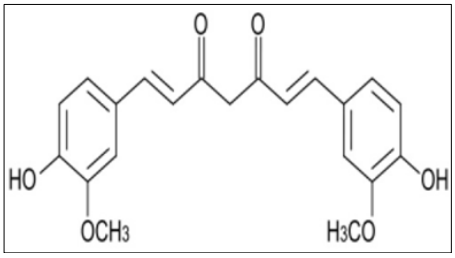


Fig 1: Structure of curcumin

Material and Methods

Material

Haridrakhand (Satyam Healthcare Pvt. Ltd., Vadodara, Gujarat, India) was purchased from local market at Baroda, Gujarat.

Chemicals and instruments

The reagents used for present study are as follows HPLC grade Water and Methanol, Acetonitrile, Toluene, Ethyl acetate, ortho-phosphoric acid. Double beam UV/visible Spectrophotometric (SHIMADZU 1800) with 10mm quartz cuvettes were used for spectral measurements. Isocratic HPLC (SHIMADZU), HPTLC linomat IV sample spotter.

Preparation of extract from haridrakhand formulation

Maceration: Take a 1 gram of dried powder of Haridrakhand with 30 ml of methanol (curcumin is soluble in methanol) in a shaker with 210 rpm at room temperature for 2 days. The extract was filtered through whatman filter paper. Other portions of the solvent were added to the solids and the extraction was repeated until the reactant was colorless. The extracts were combined and filtered [15].

Chromatographic conditions

Chromatographic separation were achieved using Shimadzu HPLC with spinchrom, ODS, (250× 4.6) mm, 5 µ column at ambient temperature. The mobile phase consisted of Acetonitrile: 0.05% OPA (70:30). The isocratic elution was carried out with the flow rate of 0.7ml/min and a wavelength of 394 nm was used for detection. The retention time of curcumin was found to be 8.387 for HPLC.

In HPTLC the mobile phase consisting of the mixture of Toluene: ethyl acetate: methanol (4:5:1) resolved Curcumin spot with better peak shape with ($R_f = 0.421 \pm 0.03$)

Standard stock solution preparation

Standard curcumin 10 mg was accurately weighed and transferred to a 100 ml volumetric flask and the volume was made with acetonitrile for HPLC and for HPTLC 100 ng/µl concentration was prepared.

Preparation of sample solution

Ten tablets were weighed and finely powdered. The powder equivalent to 10 mg of the curcumin was weighed, mixed with 25 ml of methanol and sonicated for 15 min. The solution of tablet was filtered through Whatman filter paper and the residue was thoroughly washed with methanol. The filtrate and washings were combined in a 100 ml volumetric flask and diluted to the mark with methanol to get the final concentration of 100 µg / ml of curcumin. 0.1 ml was taken from this stock solution and the volume made up to 100 ml to get a concentration of about 100 ng / µl.

Method validation [14, 15]

Specificity

The specificity of the method was confirmed by analyzing the interference of principle peak with the excipients peak in the formulation by HPLC and HPTLC identified from Retention time and RF values of Curcumin respectively.

Linearity

For HPLC linearity range for Curcumin was found to be 1-10 µg/ml. Solutions of 2, 4, 6, 8, 10µg/ml were made by transferring the aliquot from the stock solution which dilute with acetonitrile and for HPTLC, linearity range was set as 80- 400 ng/band. The different volumes of stock solution 6, 8, 10, 12 and 16 µl were spotted on the HPTLC plate.

Precision

To measure the degree of repeatability of an analytical method, inter-day and intra-day precision calculated by taking 6 replicates of the freshly prepared standard solution of same concentration on the same day and on 2 different days respectively.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection and limit of quantification is the lowest concentration of analyte in a sample which can be detected and quantified with acceptable accuracy and precision. LOD and LOQ of the developed method were calculated from the standard deviation of the response and slope of the calibration curve.

Accuracy

% Recovery: Accuracy of the method was evaluated by calculating recovery studies of addition of standard drug curcumin solution to the prepared sample solution at different concentration levels 80%, 100% and 120% (n = 3) within the range of linearity of the drug.

Assay: 10mg of sample in 10ml methanol solution was examined. The amount of curcumin present in the sample solution was determined by the area values of peaks corresponding to curcumin into the equation of the line representing the calibration curve of curcumin.

Results and Discussion

Estimation of curcumin in the formulation by HPLC analysis: [16]

specificity

The specificity of the method was confirmed by analyzing the interference of principle peak with the excipients peak in the formulation. Retention time was found to be 8.387 by RP-HPLC.

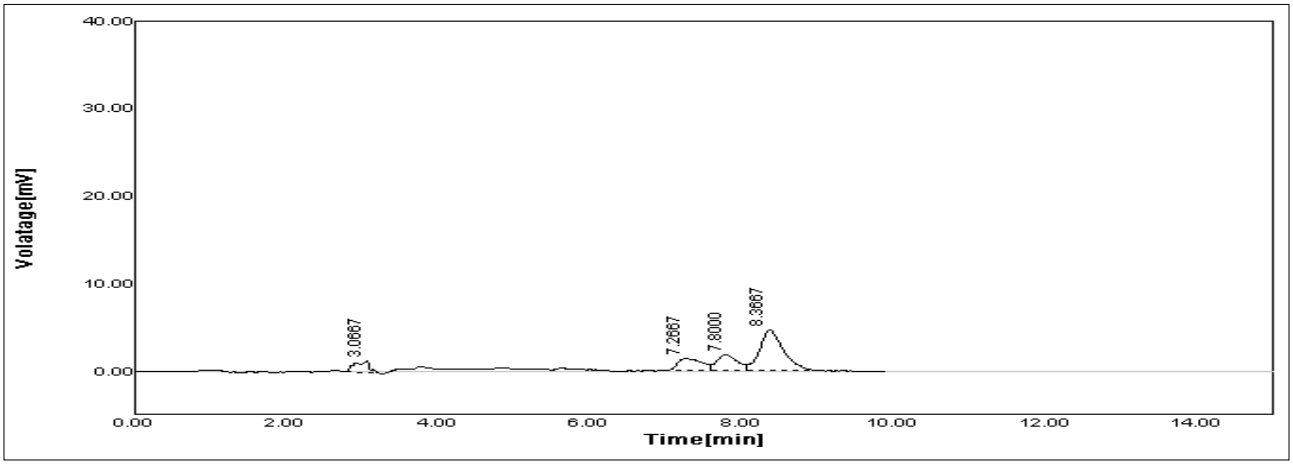


Fig 2: Chromatogram of Specificity

Linearity

The linear regression data for the calibration curves (N= 5) as shown in Table 1. Results showed a good linear relationship over the concentration range 2-10µg/ml with respect to peak height and peak area, slope 0.999, Y= 401.09x – 2.941 (Figure 3).

Table 1: Linearity Study by HPLC

S. No.	Conc	Area-i	Area-ii	Mean	SD	% RSD
1	2	179.8	184.36	182.08	3.22	1.77
2	4	331.64	334.99	333.32	2.37	0.71
3	6	505.78	511.8	508.79	4.26	0.84
4	8	690.3	697.02	693.66	4.75	0.69
5	10	882.16	872.29	877.23	6.98	0.80

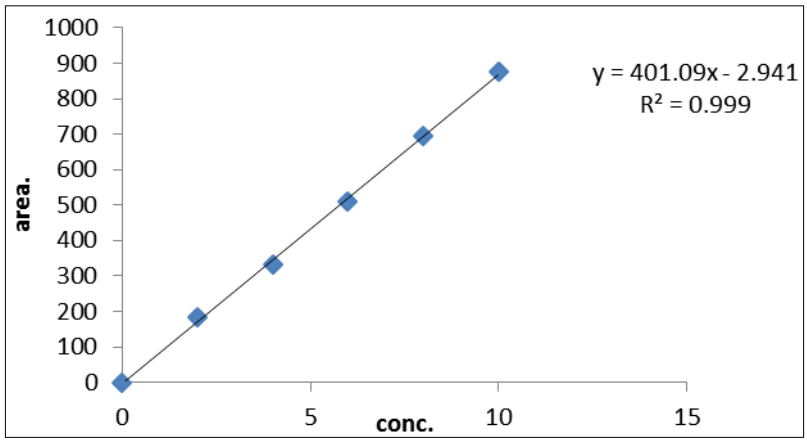


Fig 3: Calibration curve of curcumin by HPLC

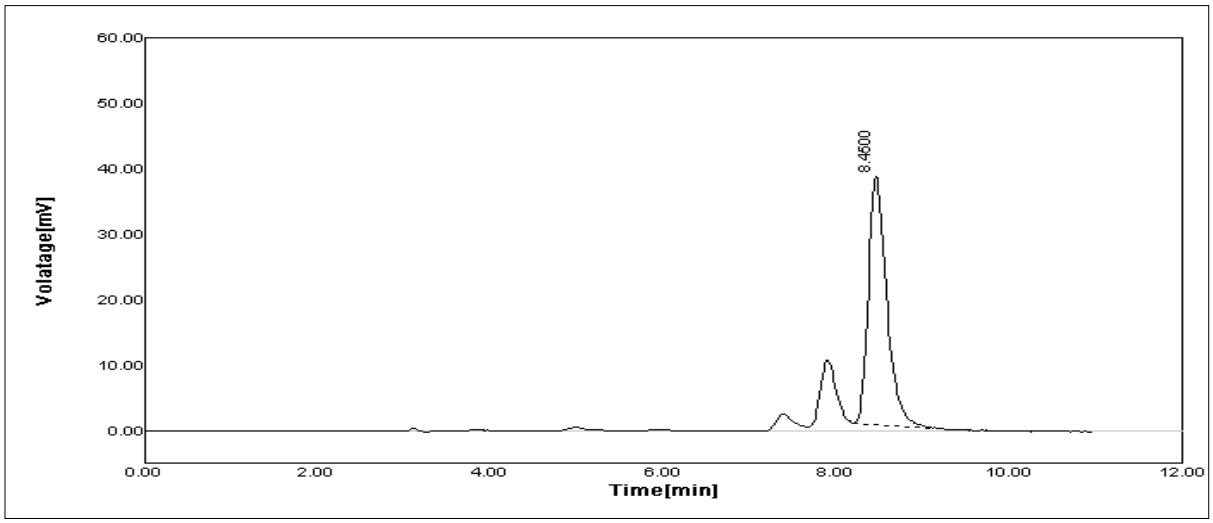


Fig 4: Chromatogram of standard curcumin

Precision

Intraday and inter-day precision

The inter and intra- day precision (N= 9) is shown in Table 2 results expressed in terms of %RSD, which describes intra- and inter-day variation of curcumin at 3 different concentration with 3 replicates levels (N= 9).

Table 2: Intra- day and Inter-day precision of HPLC method

S. No.	Conc.	Intraday precision	SD	%RSD	Inter-day precision	SD	% RSD
1	4	336.37	2.18	0.65%	337.65	0.329	0.097%
2		336.56			338.23		
3		332.68			337.67		
4	6	509.54	2.93	0.57%	507.90	6.68	1.30%
5		506.76			510.56		
6		512.67			520.56		
7	8	689.45	4.82	0.69%	691.75	3.91	0.56%
8		698.70			699.56		
9		696.65			695.35		

Limit of detection and limit of quantification

Detection limit and quantification limit were calculated using the formula LOD = 3.3 x σ/S, LOQ = 10 x σ/S Where, σ = standard deviation of the response, S = slope of the calibration curve LOD and LOQ were found to be 0.16 µg/ml and 0.49 µg/ml respectively, which shows the sufficient sensitivity of the method.

Accuracy

Accuracy was determined by means of recovery experiments by the determination of % mean recovery of sample at three different levels (80%-120%). At each level three determinations were performed. Percent mean recoveries were within a limit which is found to be 100.83% (Table 3). All observed data were within the required range which indicates good recovery values and hence the accuracy of the method developed can be confirmed

Table 3: % Recovery of Haridrakhand tablet

S. No.	Level	Amt. of sample	Amt. of drug	conc. of drug	% Recovery
1	80%	1	1.6	1.54	96.25%
2	100%	1	2	2.0	102.5%
3	120%	1	2.4	2.49	103.75%
				Mean % Recovery	100.83%

Assay

Assay of Haridrakhand tablet was carried out by using the proposed developed method. Sample solutions were prepared and injected into RP-HPLC system. The sample solution was scanned at 394 nm. The % drug estimated was found to be 83.43%.

Estimation of curcumin in the formulation by HPTLC analysis [17-20]

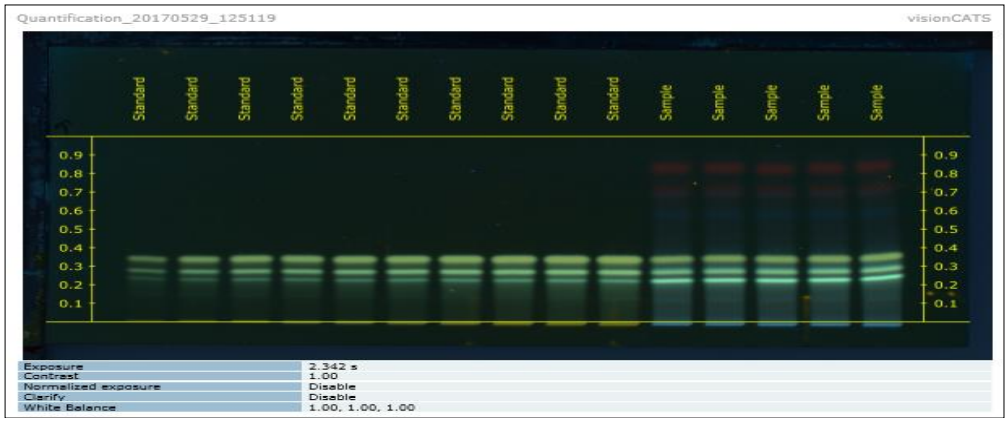
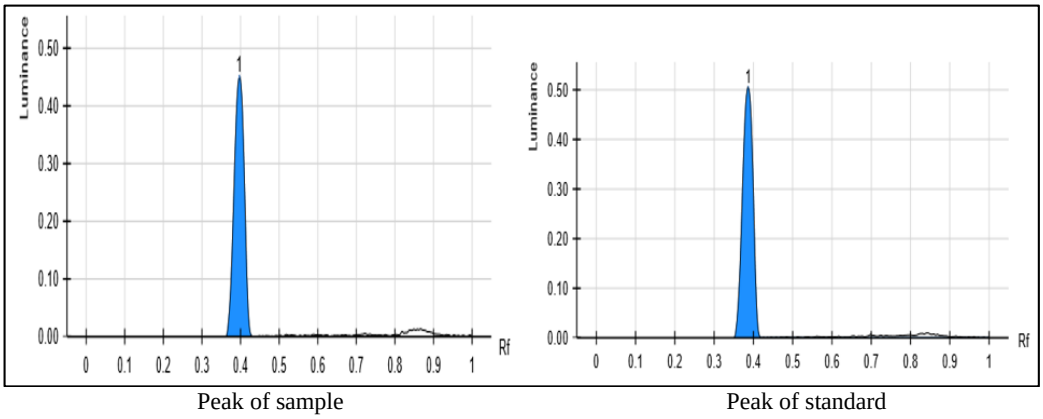


Fig 5: Chromatogram showing the separated spots of curcumin in sample and in standard

Specificity

The chromatogram of the polyherbal formulation obtained using the developed method showed only one peak at *Rf* of 0.419 for sample, and was found to be at the near *Rf* (0.431) for standard drug. There was no any interference of Mobile phase and diluent at the RF values of Curcumin, indicating the specificity of method in the presence of various excipients (Figure 6).



Peak of sample Peak of standard

Fig 6: Peak purity spectra of curcumin in formulation (Sample) with the corresponding standard

Linearity

A representative calibration curve of curcumin was obtained by plotting the mean peak area of curcumin against the concentration over the range of 6– 14 µl / spot. The regression coefficient was found to be 0.991, y = 0.001x + 0.015. (Figure 7).

Table 4: Linearity study by HPTLC

S. No	Concentration (µl/spot)	Average Peak Area	Correlation Coefficient	LOD (ng/spot)	LOQ (ng/spot)
1	6	0.0215	0.991	9	30
2	8	0.0227			
3	10	0.0253			
4	12	0.0271			
5	14	0.0289			

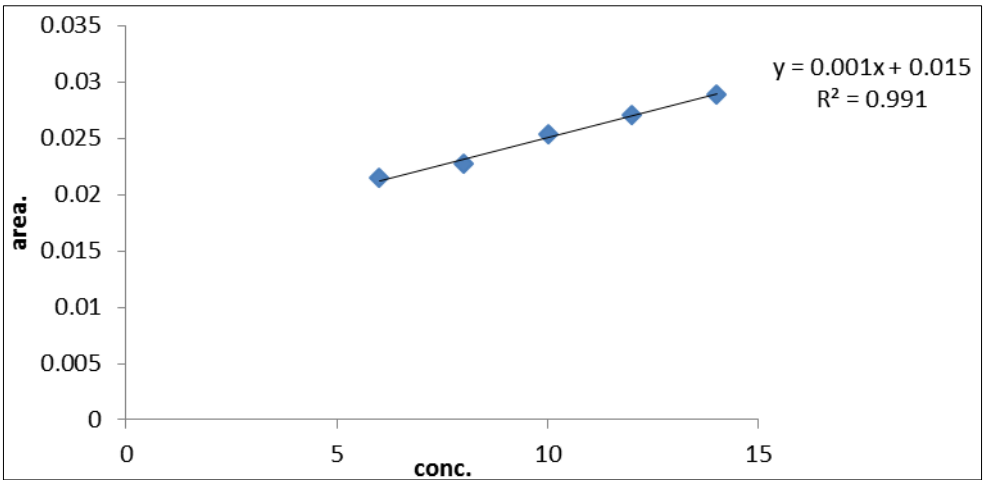


Fig 7: Calibration of Curcumin by HPTLC

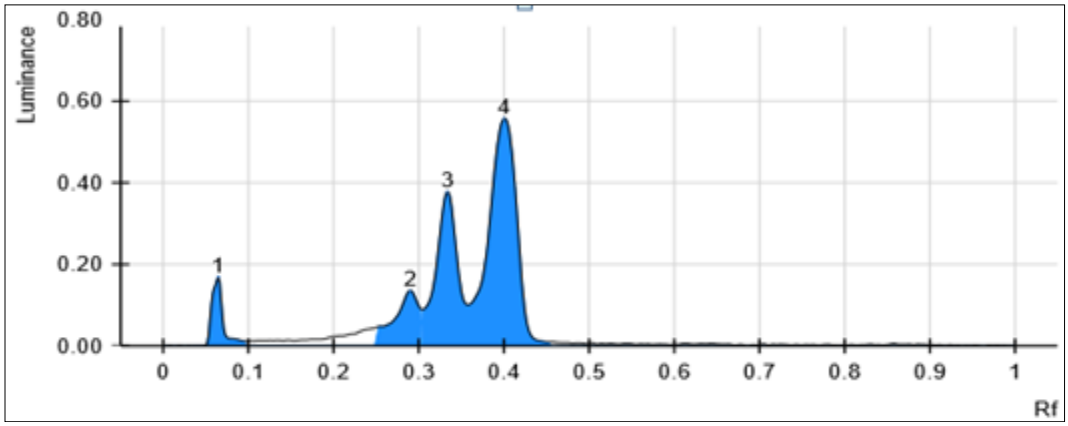


Fig 8: Chromatogram of standard curcumin

Precision

Intra-day variation, as RSD, was 1.76% and inter-day variation, as RSD, was 1.45% summarised in (Table 5). These low levels of the RSD show the repeatability and precision of the method was good.

Table 5: Intraday & Inter day Precision Study for Curcumin by HPTLC Method

S. No.	Concentration	Inter day Precision Peak area		Intraday Precision Peak Area	
		At first day	At Second day	At 0 hr.	After 5 hrs.
1	6	0.0215	0.0213	0.0211	0.0216
2	6	0.0212	0.0210	0.0215	0.0213
3	8	0.0226	0.0225	0.0229	0.0222
4	8	0.0221	0.0223	0.0230	0.0224
5	10	0.0253	0.0249	0.0252	0.0248
6	10	0.0251	0.0250	0.0251	0.0249
Mean S.D. % RSD		0.0234	0.0228	0.0231	0.0237
		0.00034		0.00041	
		1.45%		1.76%	

Accuracy

Recovery study results ranged from 98.33 to 102.5%. Results of recovery studies are reported in (Table 6).

Table 6: % Recovery Study

S. No.	Level	Amt. of sample	Amt of drug	Conc. of drug found	% Recovery
1	80%	5	8	8.2	102.5%
2	100%	5	10	10.1	101%
3	120%	5	12	11.8	98.33%
Mean % Recovery= 100.61%					

Assay

Assay of haridrakhand tablet was carried out by using the proposed developed method. The assay [%] was 80% in tablet formulation.

Table 7: Method performance parameters for validation of HPLC and HPTLC protocol

Analytical parameter	HPLC	HPTLC
Linearity-Correlation coefficient	0.999	0.991
Precision – S.D	3.21	0.00064
RSD	0.63	
%RSD		2.03%
Intraday Precision %RSD	0.63%	1.45%
Interday precision %RSD	0.67%	1.76%
Accuracy- %Recovery	100.83%	100.61%
LOD	0.16 µg/ml	9 ng/spot
LOQ	0.49 µg/ml	30 ng/spot
Assay %Practical Yield	83.43	80%

Conclusion

The present study of Estimation of curcumin in haridrakhand polyherbal tablet formulation using HPLC and HPTLC method confirms that the developed methods were simple, precise, specific, and accurate. HPLC is more sensitive and reliable method as compare to HPTLC. This work illustrates a simple, sensitive and robust HPLC method for estimation of Curcumin. The proposed method met the ICH validation criteria such as linearity, ranges, precision, accuracy and specificity.

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