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Quantitative analysis of diosgenin in *costus speciosus* extracts by HPTLC with ultraviolet absorption densitometry

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ABSTRACT

Diosgenin is one of the phytoconstituent present in *Costus speciosus* (Koen) J.E. Sm. This plant and its extracts are used for treatment of fever, snake bites, jaundice, and as a purgative, astringent and antibacterial. In the present study an attempt has been made to develop a HPTLC method for quantitative estimation of diosgenin in fractions of methanol extract. This HPTLC method was found to be reproducible, accurate and precise and could detect and quantitate nanogram level concentrations of diosgenin. The developed HPTLC method would be an important tool in the evaluation of diosgenin content in polyherbal formulations.

KEYWORDS

HPTLC;
Diosgenin;
Fractionation;
Densitometer;
Costus speciosus.

INTRODUCTION

Costus speciosus (Koen) J.E. Sm. Family: Zingiberaceae is an erect herbaceous plant up to 2 m high with long lanceolate leaves and white fragrant flowers in terminal clusters. The plant flowers during the months of July and August, the aerial parts withering away during the winter season. It has wide distribution in India, occurring throughout the sub-Himalayan tract from Himachal Pradesh to Assam, Vindhya and Satpura hills in central India and the western ghats of Maharashtra, Karnataka, Kerala[1].

Costus speciosus has recently gained importance as a source of diosgenin, a precursor for the synthesis of steroidal hormones[2]. The rhizomes are well known for their diosgenin content and also for several saponins[3]. Literature survey revealed that there is no HPTLC method for analysis of diosgenin in Costus speciosus. The purpose of this research is to establish such a method and, after validation in accordance with International Conference on Harmonization (ICH) guidelines and the directives for good laboratory practice, to use the method for analysis of the diosgenin content in other plants.

EXPERIMENTAL

Chemicals and reagents

All chemicals used for this study were of analytical grade and purchased from E. Merck Ltd., Mumbai. Standard diosgenin was obtained as a gift sample from Sahyadri Chemicals, Mumbai.

Preparation of standard solutions

A stock solution containing 1mg mL^{-1} of diosgenin was prepared in methanol. Calibration solutions containing 300, 600, 900, 1200, 1500, 1800 ng μL^{-1} were prepared by dilution of stock solution. These solutions were used to apply 300 to 1800 ng diosgenin to the plates.

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Preparation of sample solutions

C.speciosus was collected from the forest of Bahuligaon Tal- Igatpuri Dist-Nashik Maharashtra, India. The entire plant was sent to Botanical Survey of India, Pune for authentication and obtained certificate of authentication. A voucher specimen was deposited in herbarium of our laboratory. The shed dried rhizomes were pulverized and dry powder (25g) of rhizome was extracted with 100 ml methanol by Soxhlet apparatus. The residue was obtained after exhaustive cycles of siphoning. The dry extract (2.5g) was obtained by distilling off the solvent. The dry extract was hydrolyzed with 2N hydrochloric acid for 15 hrs with methanol and water (60:40:20), filtered, washed with methanol exhaustively to produce a yield of 2g residue.

The residue was spotted on TLC plate and the TLC plates was developed using n-hexane, ethyl acetate (4:1) solvent system giving four spots one corresponding to authentic diosgenin R_f 0.34. The residue was subjected to column chromatography over silica gel (about 60-120 mesh) using n-hexane, ethyl acetate (4:1) solvent system. The fraction having R_f 0.34 was collected and identified by GC-MS. This fraction of extract (FE) was used for the HPTLC analysis and spotted with std. diosgenin.

Thin layer chromatography

HPTLC was performed on 10 cm × 10 cm aluminium-backed HPTLC plates coated with 0.2 mm layers of silica gel 60 F_{254} (E. Merck, Darmstadt, Germany). Before use, the plates were washed with methanol and stored in a desiccator. Samples were applied to the plates as 6-mm bands, 11.6 mm apart and 10 mm from the lower edge, by means of a Camag (Muttenz Switzerland) Linomat V applicator equipped with a Hamilton (Reno, Nevada, USA) 100-µL micro syringe. The rate of application was 15 s μL^{-1}. Ascending development of the plates, with n-hexane- ethyl acetate, 4:1 (v/v), as mobile phase was performed at 25 ± 2^0C in a Camag twin-trough TLC chamber previously saturated with the mobile phase for 15 min. The development distance was 70 mm. After development the plates were dried for 5 min in an oven at 50^0C. The plates were sprayed with concentrated sulphuric acid: methanol (1:2 v/v) and dried in hot air oven at 105^0C for 5 minutes. Densitometric scanning was then performed at 428 nm (λ_{max} for the compounds) with a Camag TLC scanner 3 equipped with Wincats Software Version 1.3.0, using the deuterium light source. The slit dimensions were 6.00 mm × 0.45 mm.

Validation of the method

The method was validated in accordance with ICH guidelines and chromatographic methods[4-8].

Linearity

Amounts of standard solutions equivalent to 300, 600, 900, 1200, 1500, 1800 ng per spot diosgenin were applied to the plates and the plates were developed, dried, and scanned as described above. Calibration graph was constructed by plotting peak areas against the corresponding amounts (ng spot-1) of analyte. Each amount was chromatographed five times and mean was employed for the calibration graph which was linear.

Sensitivity

The sensitivity of measurement of diosgenin was estimated as the limits of quantification (LOQ) and detection (LOD), which were calculated by the use of the equations LOD = 3 × N/B and LOQ = 10 × N/B, where N is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of the noise, and B is the slope of the corresponding calibration plot.

Precision

The precision of the method was assessed by replicate (n = 5) analysis of authentic diosgenin. Intra-day precision (% R.S.D.) was determined by analysis of the solutions three times on the same day. Inter-day precision (% R.S.D.) was assessed by analysis of the solutions on three different days over a period of one week.

Accuracy

The accuracy of the method was determined by the method of standard additions at three different levels, i.e. by multiple level recovery studies. Sample stock solution containing 400 ng mL^{-1} of diosgenin prepared from and spiked with amounts of the drugs equivalent to 0, 50, 100, 150 % of the amounts present in the original solution. These solutions were then analyzed as described above.

Specificity

Peak purity for diosgenin was tested by comparing

TABLE 1: Results of analysis of standard diosgenin

Conc./spot(ng)	Peak Areas					Avg.	SD	%R.S.D
	Set 1	Set 2	Set 3	Set 4	Set 5			
300	1663.18	1689.32	1687.99	1710.3	1665.11	1683.18	19.5118	1.1592
600	3216.36	3202.98	3208.43	3210.91	3193.13	3206.36	08.8238	0.2752
900	4780.04	4832.17	4841.80	4835.18	4823.54	4822.54	24.6511	0.5111
1200	6572.72	6588.43	6642.42	6627.59	6632.46	6612.72	30.3433	0.4588
1500	8435.90	8358.21	8401.78	8344.99	8288.92	8365.9	56.1463	0.6711
1800	9729.08	9879.17	9840.39	9909.799	9786.96	9829.08	72.3301	0.7358

spectra acquired of at the start (S), apex (A), and end (E) of the peaks.

Repeatability

The six spots of sample solutions having concentration 600 ng were spotted and the peak areas for all were measured.

Ruggedness

The six spots of sample solution having concentrations 900 ng were spotted by different analysts. And the peak areas were found.

Robustness

The sample 300 ng spotted on different three plates and the distance traveled by solvent front were changed as 7, 7.5, 8 cm and peak areas were analyzed.

Determination of diosgenin content in fraction of the extract[9,10]

Quantification of diosgenin was done by applying fraction of extract on TLC plate with standard diosgenin.

RESULTS AND DISCUSSIONS

Validation of method

Linearity

Response to diosgenin was linear in the concentration range 300- 1800 ng spot^{-1}. The regression equation for diosgenin was y = 5.5236x - 46.533 where y is response and x the amount chromatographed. The correlation coefficient, r, was 0.9991, over these concentration ranges.

Sensitivity

The limits of quantification (LOQ) and detection (LOD) for diosgenin was 63.91 and 21.08 ng, respectively.

TABLE 2 : Results from precision interday and intraday studies

A. Interday studies

Conc./ Spot (ng)	Peak Area	Amount found (ng)	% Amount found	S.D.	% R.S.D.
600	3220.19	591.41	98.56		
600	3240.36	595.06	99.17	0.3073	0.3110
600	3228.15	592.85	98.80		
	Average		98.84		
600	3212.5	590.01	98.33		
600	3201.4	587.46	97.91	0.312143	0.3176
600	3218.76	591.15	98.52		
	Average		98.25333		
600	3210.40	589.63	98.27		
600	3204.45	588.56	98.09	0.1374	0.1400
600	3201.36	588.00	98.00		
	Average		98.12		

B. Intraday studies

Conc./Spot (ng)	Peak Area	Amount found	% Amount found	S.D.	% R.S.D.
600	3225.40	592.38	98.72		
600	3218.12	591.03	98.50	0.3143	0.3913
600	3204.86	588.63	98.10		
	Average		98.44		
600	3223.84	592.07	98.67		
600	3208.8	589.34	98.22	0.341516	0.3474
600	3201.64	588.05	98		
	Average		98.29		
600	3240.41	595.07	99.17		
600	3215.51	590.56	98.42	0.4895	0.4963
600	3209.84	589.53	98.25		
	Average		98.61		

Precision and accuracy

Results and statistical data from replicate (n = 5) analysis of diosgenin is reported in TABLE 1 and figure 1. Results from determination of intra-day and inter-day precision, by analysis of standard solutions covering the entire calibration range, are listed in TABLE 2(A,B). Results from determination of recovery of diosgenin and sample solutions are reported in TABLE 3.

Specificity

The mobile phase used resolved the drugs very ef-

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TABLE 3 : Results from accuracy, recoveries studies

Amt Taken (Std) ng/spot	Amt of fraction of extract added ng/spot	Peak Area	Amt Recovered ng	% Recovery	S.D.	% R.S.D.
			0%			
600	0	3216.07	590.66	98.44		
600	0	3203.45	588.38	98.06	0.1942	0.1976
600	0	3212.05	589.92	98.32		
	Average			98.27333		
			50%			
600	300	4828.44	882.57	98.06		
600	300	4815.75	880.27	97.8	0.0130	0.1328
600	300	4822.35	881.46	97.94		
	Average			97.93333		
			100%			
600	600	6614.45	1205.91	100.49		
600	600	6620.4	1206.99	100.58	0.0754	0.0750
600	600	6610.75	1205.24	100.43		
	Average			100.5		
			150%			
600	900	8360.75	1522.06	101.47		
600	900	8370.57	1523.84	101.58	0.0776	0.0764
600	900	8358.8	1521.56	101.43		
	Average			101.49		

TABLE 4 : Results from Repeatability studies

Conc./spot (ng)	Area	Amt found	% Amt found	Mean	S.D.	%R.S.D.
600	3215.89	590.63	98.43			
600	3202.75	588.25	98.04			
600	3217.36	590.89	98.48	98.31	0.2372	0.2412
600	3222.87	591.89	98.64			
600	3204.54	588.57	98.09			
600	3208.75	589.34	98.22			

TABLE 5 : Results from ruggedness studies

Analysts	Conc. /Spot (ng)	Area	Amt. found	% Amt. found	S.D.	% R.S.D.
	900	4818.64	880.79	97.86		
	900	4812.74	879.59	97.73		
Analyst I	900	4826.29	882.12	98.01	0.3613	0.3694
	900	4825.6	882.05	98.00		
	900	4830.01	882.85	98.09		
	900	4820.87	881.20	97.11		
	Average			97.80		
	900	4819.47	880.94	97.88		
	900	4834.97	883.75	98.19		
Analyst II	900	4812.68	879.71	97.74	0.1501	0.1532
	900	4825.9	882.11	98.01		
	900	4820.57	881.14	97.9		
	900	4823.69	881.71	97.96		
	Average			97.94		

ficiently, as shown in the figure 2. The R_F value of diosgenin was 0.34

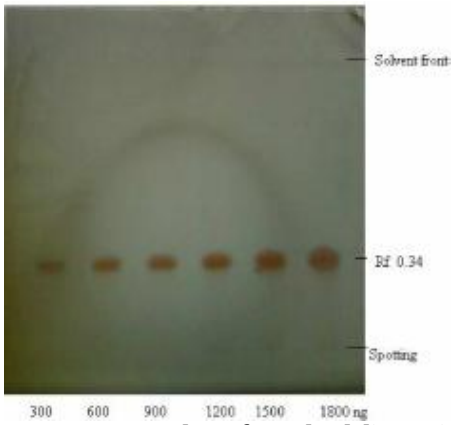

Figure 1 : HPTLC plate of standard diosgenin

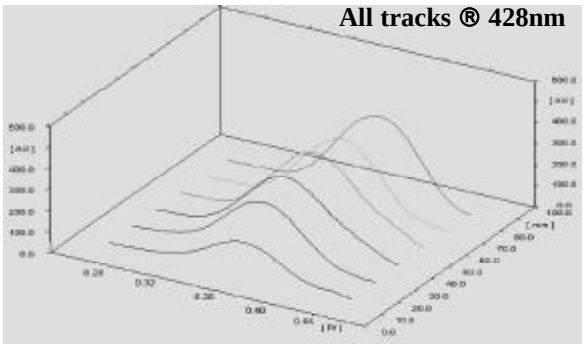

Figure 2: TLC plate analyzed by densitometer showing the tracks of diosgenin, The spot concentrations were 300,600,900,1200,1500,1800 ng/spot

Repeatability

Measurement of the repeatability of diosgenin application showed RSD for peak height and peak area were 0.23% and 0.24% respectively as in TABLE 4.

When the same spot was scanned seven times without changing the position of the plate RSD for measurement of peak height and peak area were 0.26% and 0.09% for diosgenin.

Ruggedness

Measurement of R.S.D. of % amount found of diosgenin from sample by two analysts were 0.36% and 0.15%. The results obtained were in TABLE 5.

Robustness

No substantial variation in % amount found observed from the robustness studies TABLE 6.

Determination of diosgenin content in fraction of the extract

Quantification of diosgenin was done by applying

TABLE 6 : Results from robustness studies

Solvent front (cm}	Conc./Spot ng	Area	Amt. found	% Amt. found	S.D.	% R.S.D.
	300	1675.08	311.68	103.89		
7	300	1683.18	313.14	104.38	0.8082	0.7769
	300	1665.09	309.87	103.29		
	Average			104.02		
	300	1680.36	312.63	104.21		
7.5	300	1675.05	311.67	103.89	0.6325	0.6099
	300	1660.11	308.97	102.99		
				103.69		
	300	1670.75	310.89	103.63		
8	300	1684.19	313.33	104.44	0.7265	0.7007
	300	1660.18	308.98	102.99		
	Average			103.68		

TABLE 7 : Quantification of diosgenin in fractions of methanol extracts of *Costus speciosus*

ng/spot	Max. R_f	Peak Area	Assigned substance	Amount found ng	% Amount
1200	0.34	6640.57	Diosgenin	1210.77	100.89
1500	0.34	8372.64	Diosgenin	1524.38	101.62
1200	0.34	267.49	Extract fraction	56.85	4.73
1200	0.34	270.58	Extract fraction	57.41	4.78
1200	0.34	247.39	Extract fraction	53.21	4.43
1200	0.34	261.88	Extract fraction	55.84	4.65

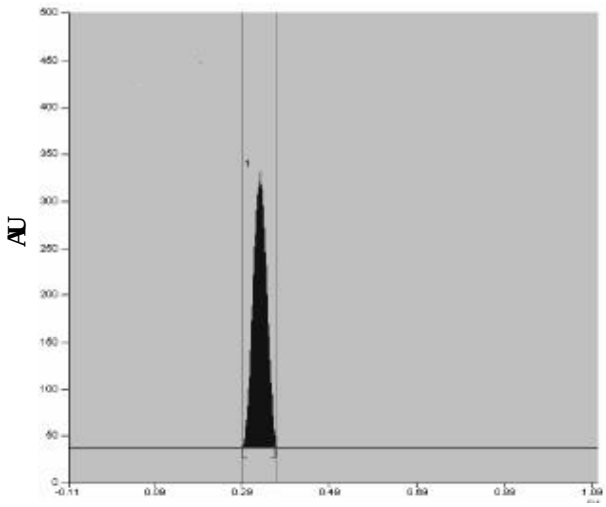

Figure 3: Peak of FE* of concentration 1200 ng(* Fraction of extract)

the 1200 ng /spot on TLC plate and the peak area was evaluated as in figure 3 and TABLE 7.

CONCLUSION

High Performance Thin Layer Densitometry after detection with derivatization of diosgenin is attractive alternative for determination of diosgenin in Costus speciosus rhizomes with regard to reproducibility, accuracy and sensitivity. This powerful method could be widely applied to analysis of various extracts. Additional advantages over known methods are related to simplicity of extraction, the low detection quantification limits. This method is suitable for quality assurance of diosgenin and related extracts.

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