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Original Article

Development and Validation of an HPLC Method for Diosgenin, A Bioactive Compound in the Rhizomes of *Chamaecostus cuspidatus*

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ABSTRACT

Chamaecostus cuspidatus is an important medicinal herb, with rhizomes which are rich in diosgenin content and are mainly used as an antidiabetic and anticancer. Diosgenin is a steroidal sapogenin (5α-Spirosten-3β-ol) and belongs to the spirostanol group. A validated High-Performance Liquid Chromatography (HPLC) method has been developed to estimate diosgenin. *C. cuspidatus* is native to India and Sri Lanka. In this study, diosgenin was separated by HPLC using a welchrom 5 µm-C_{18} column, at a flow rate of 0.5 mL /min, by keeping the injection volume 10 µL. The column temperature was maintained at 25ºC and detection was carried out at wavelength 232 nm. The retention time for diosgenin was found to be 7.086 min. Data analysis was performed using lab solution software. The Limit of Detection (LOD) was found to be 0.753 µg/mL for diosgenin. The Limit of Quantification (LOQ) was found to be 2.341 µg/mL for diosgenin. Recovery of diosgenin was found to be 91.90%. The proposed HPLC method was validated as per ICH guidelines and was found to be selective, sensitive, and accurate for the estimation of diosgenin from the rhizome of *C. cuspidatus*. To the best of our knowledge, there are limited studies on the HPLC quantification of diosgenin from *C. cuspidatus* this is the novel study on HPLC of diosgenin from the rhizomes of *C. cuspidatus.*

Keywords: *Chamaecostus cuspidatus* rhizomes, Diosgenin, Validated HPLC method.

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INTRODUCTION

Chamaecostus cuspidatus (Nees and Mart.) is distributed to India, Sri Lanka,[1] and South America.[2] It is a member of the Costaceae family, the largest group of angiosperms.[3] And has eight genera.[4] The tuberous rhizome is penetrated by the rhizomatous shrub *C. cuspidatus* (Nees and Mart).[5] Asthma, bronchitis, intestinal worms, fever, rash, and conditions affecting the eyes, stomach, neck, jaws, tongue, and mouth have all been treated using rhizomes. *C. cuspidatus* Nak (also known as *Costus pictus* Don, *C. mexicanus* Liebm, or *C. congenital* Rowle) is sometimes referred to as Fiery Costus, Step ladder, Spiral flag, or Insulin plant.[6] Diosgenin is a steroidal sapogenin that is bioactive (5α-Spirosten-3β-ol). It is frequently used in medicines and is a member of the spirostanol group.[7] Diosgenin (Figure 1) is a unique multi-target substance that has been utilized as a dietary component in alternative, traditional, and contemporary medicine to treat a variety of illnesses.[8] As a main and suggestive precursor in the production of synthetic steroids, it acts as a primary substrate for the synthesis of corticosteroids, sex hormones, oral contraceptives, and other related steroidal medicines.[9,10] Clinical studies are now being conducted to examine diosgenin's possible uses as an antioxidant agent,[11] anti-inflammatory agent,[12] and anti-metastatic therapy.[12] Furthermore, it is being evaluated for its antifungal properties[13] and its anti-thrombin effect as a megakaryocytic differentiation inducer in HEL cells.[14] Research on animals has demonstrated that diosgenin is effective in treating disorders of the neurological system, including Parkinson's and Alzheimer's illnesses.[15-17] Diosgenin has also been shown to alter cyclooxygenase activity in osteosarcoma cells[18] cause cell cycle arrest, and also affect TxS (Thromboxane Synthase). Past research shows various methods of analyzing diosgenin such as microscopy, spectrophotometrics, GLC,[19] HPLC,[20] and HPTLC.[21] This research aims to develop and validate an HPLC method for the estimation of diosgenin present in *C. cuspidatus*.

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MATERIALS AND METHODS

Plant material

The fresh rhizomes of *C. cuspidatus* (Nees and Mart.) were collected from Nadiad, Gujarat. The rhizomes were collected, shade-dried, and then pulverized into a mortar pestle with HCl.

Chemicals and reagents

Acetonitrile (HPLC grade) was purchased from Merck Life Science Private Limited (supelco). Water (HPLC grade) was obtained from Finar Limited. Methanol (HPLC grade) was purchased from S D Fine Chem Limited. Chloroform was obtained from Central Drug House Limited.

Selection of wavelength

Absorption spectra of 1 mg/mL of diosgenin were recorded and found highest absorbance on 232 nm of wavelength using a single beam UV-vis spectrophotometer.

Experimental work

Instrumental Conditions For HPLC

HPLC analysis was carried out using an instrument from Shimadzu Manufacturing Inc., 1900 SE 4th Ave., Canby, Oregon, 97013, USA, consisting of a quaternary pump (LC 20 AD), autosampler (SIL-30 AC), column oven (CTO-10 AS VP), and a detector (SPD-M20A). The outlet of the detector was linked to a splitter for splitting of the flow. The welchrom 5 µm C18 (4.6×250 mm) column was used, and the column temperature was maintained at 25°C. The mobile phase used was acetonitrile-water in the ratio of 92:08 v/v. The analysis was done by keeping the injection volume at 10 µL and the flow rate at 0.5 mL/min.[22]

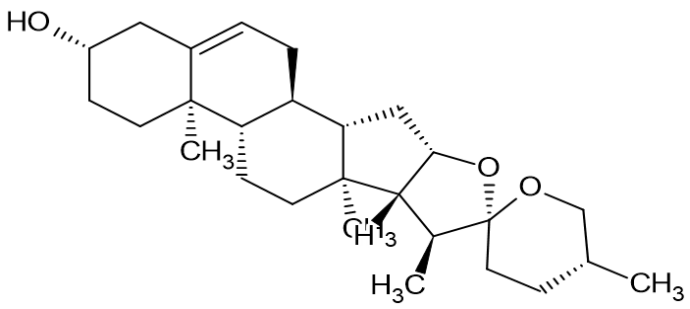

Figure 1: Structure of diosgenin.

Preparation of sample solution

Accurately weighed (1.67 g) powder of rhizomes of *C. cuspidatus* (Nees and Mart.) was extracted with 50 mL of 2 N HCl for 60 min. The extract was filtered and concentrated to a sticky mass. 0.02g of the extract was dissolved in 2 mL of methanol (HPLC grade).

Preparation of standard solution

The stock solution for standard diosgenin was prepared by weighing 10 mg in 10 mL solvent of methanol. Then, 1 mL from the above solution was taken and diluted up to 10 mL.

Linearity

The linearity of an analytical procedure represents its capacity to generate test results that are approximately equal to the analyte concentration in the sample. Standard methanolic solutions of different concentrations of diosgenin were prepared to determine the method linearity at multiple concentrations of 2, 4, 6, 8 and 10 ppm as per Table 1.

Accuracy

The accuracy was determined as the percent recovery of the added amount of known analyte in the sample concerning the actual value, together with the Relative Standard Deviation (%RSD).

Table 1: Linearity for diosgenin.

Concentration (ppm)	Area	Mean	Standard Deviation	%RSD
2	155998	157867	1869	1.18
	157871			
	159732			
4	299783	303873	4094	1.347
	303865			
	307971			
6	438681	445780	7096	1.591
	445786			
	452873			
8	584911	595887	10963.52	1.839
	595912			
	606838			
10	732805	741894	9086.5	1.224
	741899			
	750978			

Precision

The developed method's precision was verified by repeated injections of the *C. cuspidatus* (Nees and Mart.) rhizome extract at multiple concentrations of 15, 20, 25, 30, and 35 ppm. The intra-day and inter-day variations were evaluated by Relative Standard Deviation (RSD) values of active compounds in the *C. cuspidatus* (Nees and Mart.) rhizome extract.

Intra-day precision (repeatability)

A variation in the result within the same day is called intra-day precision. It was determined by repeating the calibration curve 3 times on the same day with 5 different concentrations.

Inter-day precision (repeatability)

A variation in results amongst days is called inter-day precision. It was determined by repeating the calibration curve 3 times on different days with 5 different concentrations.

LOD and LOQ

The LOD is the lowest concentration of an analyte in a sample that can be detected but not quantified under analytical conditions. LOQ is the lowest concentration of an analyte that can be quantified under the analytical conditions.

Recovery

The analytical recovery was performed by analyzing the analytes by spiking with the four reference standards into rhizomes extracts.

Robustness

Several deliberate changes to the analytical procedure were introduced to evaluate the robustness of the method.

System suitability studies

System suitability was established by injecting six replicate injections of mixture standard solution, and the % Relative Standard Deviation (% RSD) of peak areas, resolution factor, tailing factor, and theoretical plates were determined.

RESULTS AND DISCUSSION

Detection of diosgenin

Diosgenin is identified by comparing the retention times R_t of the standard is 7.079 min and the sample is 7.086 min, as shown in Figures 2 and 3. Diosgenin content was found to be 0.05 %w/w in *C. cuspidatus* rhizomes extract.

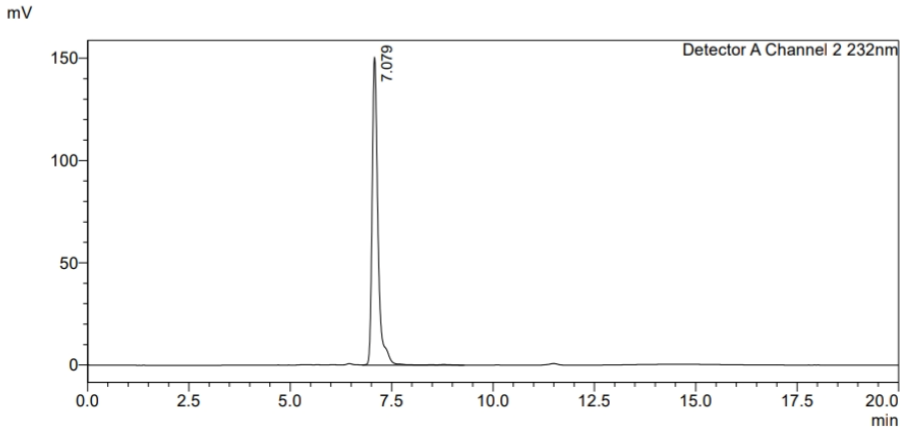

Figure 2: HPLC chromatogram of standard diosgenin.

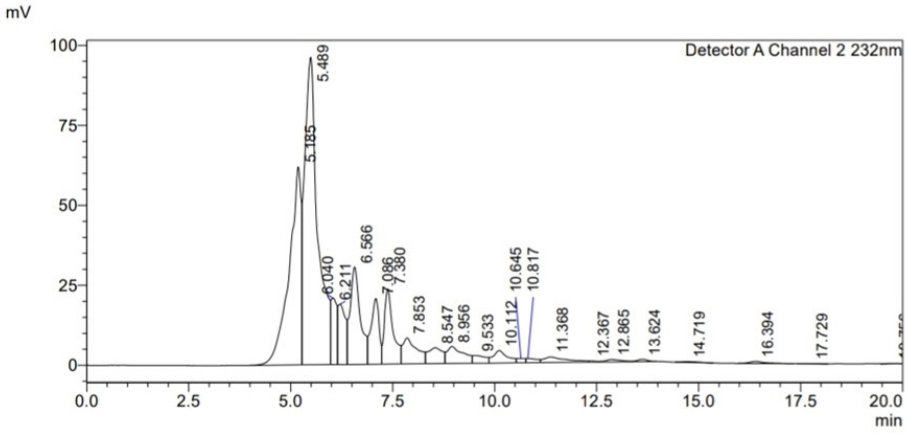

Figure 3: HPLC chromatogram of extract of diosgenin.

HPLC method optimization

C. cuspidatus (Nees and Mart.) rhizome extract was prepared using a reflux extraction process. To optimize peak responses, a relatively high-sensitivity experiment was performed at 232 nm wavelength. Detection of diosgenin was achieved after repeated trials, with different combinations of acetonitrile and water as the mobile phase.

HPLC method validation

The developed HPLC method was validated as per ICH guidelines. The validation included examination of specificity, linearity, limit of detection, limit of quantification, precision, accuracy, recovery, and robustness.

The calibration curve for diosgenin was prepared using 2, 4, 6, 8, and 10 ppm concentrations following regression equation. The regression equation was found to be y=73003x+11040 for diosgenin is shown in Figure 4 for diosgenin.

Intra-day precision and accuracy were assessed by analyzing six sets of samples, each prepared at concentrations of low, medium, and high levels of the calibration curve over intervals of 2 hr in a single day. Inter-day precision and accuracy were examined over six days by the analysis of samples prepared each day as shown in Table 2.

The result of the Precision of the developed method also shows reproducibility as shown in Table 3.

Recovery of diosgenin was found to be 91.90% proving that the developed method is quite sensitive as shown in Table 3.

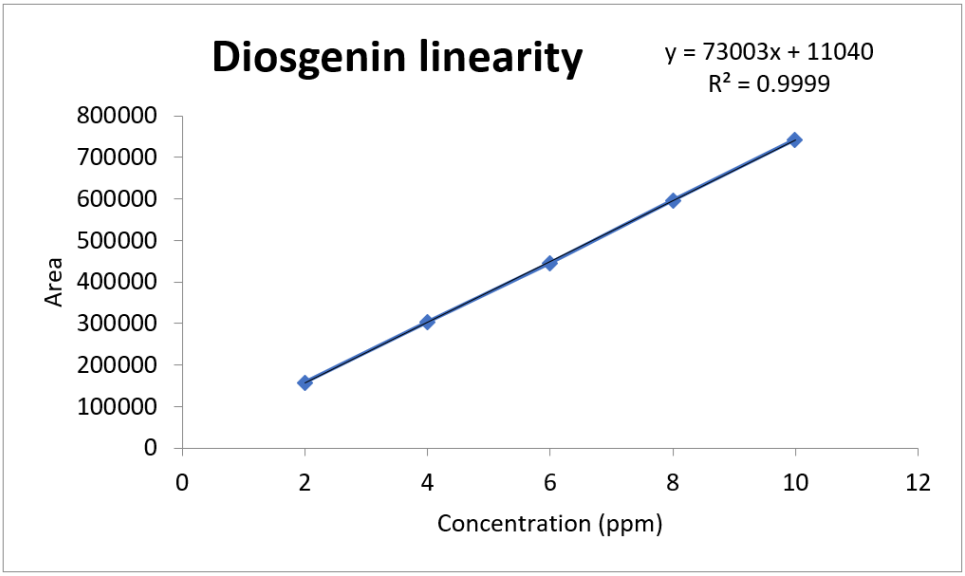

Figure 4: Linearity of diosgenin.

Table 2: (%RSD) Intra-day and Inter-day precision.

Compound	Concentration (ppm)	Intra-day precision (%RSD)	Inter-day precision (%RSD)
Diosgenin	15	0.735	0.897
	20	0.789	0.924
	25	0.812	0.956
	30	0.847	0.978
	35	0.889	0.981

Table 3: Precision (% RSD) (*n*=6) and Recovery (%) (*n*=6) in the developed HPLC method for Diosgenin.

Sl. No.	Parameters	Result
1	Recovery (%)	91.90
2	Repeatability	1.756
3	Reproducibility	3.163
4	Intermediate precision	2.056

Table 4: Retention time, linear regression, LOD, and LOQ in the developed HPLC method for Diosgenin.

Sl. No.	Standard	R_t (minutes)	Linear Regression equation	R^2	LOD (µg/mL)	LOQ (µg/mL)
1	Diosgenin	7.086	y=73003x+11040	0.999	0.753	2.341

LOD was found to be 0.753 µg/mL for diosgenin and the LOQ was 1.341 µg/mL respectively as shown in Table 4.

This study confirms that the HPLC method is robust and hence can give a high degree of accuracy and precision.

CONCLUSION

A validated HPLC analytical method has been developed for the estimation of diosgenin from the *C. cuspidatus* (Nees and Mart.) rhizomes extract. The proposed method is accurate, precise, and specific, and can estimate diosgenin. The developed method meets system suitability criteria, peak integrity, and resolution for the estimation of diosgenin. The limit of detection and the limit of quantification also describes that the method is quite sensitive. Diosgenin is commercially important and popularly used in traditional and modern formulations. The developed method would have utility in grading and quality evaluation of studied plant rhizomes collected from different habitats and seasons.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HPLC: High Performance Liquid Chromatography; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **RSD:** Relative Standard Deviation; **R_t:** Retention Time; **ppm:** Parts Per Million; **ICH:** International Conference of Harmonisation; **mL:** Millilitre; **mg:** Milligram; **µg:** Microgram; **µL:** Microliter; **GLC:** Gas Liquid Chromatography; **HPTLC:** High Performance Thin Layer Chromatography; **HEL:** Human Erythroleukemia; **HCl:** Hydrochloric acid; **nm:** Nanometer.

AUTHOR CONTRIBUTION DECLARATION

Mamta Shah: Identifying the research field, conceptualization, and shaping the design of experimental study for estimation of diosgenin using HPLC method and pivotal role in making the authors learn the skill of manuscript writing.

Hit Saliya: Sourcing, Collection, and Authentication of Plant Material.

Jagdish Madam and Harsh Patel: Optimized extraction method. Checked and confirmed the presence of diosgenin only in rhizomes with the help of a reference standard using the TLC method

Mihir Lakhani, Lakhandeep Shah, and Vivek Patel: Literature review for optimization of HPLC and method development. Performed Validation of developed HPLC method

Vidhi Vyas: The acquisition of data, data analysis and interpretation of data, and drafting of the article.

SUMMARY

Rhizomes of Chamaecostus cuspidatus are rich in diosgenin and are used as a remedy for the treatment of diabetes and cancer. Diosgenin is a steroidal sapogenin (5α-Spirosten-3β-ol) is an important precursor for corticosteroids, sex hormones, oral contraceptives, and other related steroidal compounds. A validated High-Performance Liquid Chromatography (HPLC) method has been developed to estimate diosgenin. The proposed HPLC method was validated as per ICH guidelines and was found to be selective, sensitive, and accurate for the estimation of diosgenin from the rhizome of *C. cuspidatus*.

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