The Pharma Innovation Journal 2019; 8(12): 146-149

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(12): 146-149
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www.thepharmajournal.com
Received: 14-10-2019
Accepted: 17-11-2019

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Quantitative determination of Diosgenin from *Dioscorea bulbifera* Linn. Bulbils powder using reverse phase liquid chromatography

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Abstract
A simple rapid and precise reverse phase high performance liquid chromatographic method has been developed for the quantitative determination of Diosgenin from the extract of dried powder of *Dioscorea bulbifera* Linn. Tubers. Chromatographic analysis was carried out on Inertsil ODS-3V-C_{18} (25cm x 4.6 mm i.d., 5 um) with a mobile phase of mixture of water and methanol in the volume ratio of 95:5, at a flow rate of 1.0 mL min^{-1}. Quantitation was performed using a UV – visible detector at 211 nm. Good linearity was obtained over the range of 1.00 ug/cm^3 to 12.00 ug/cm^3 for Diosgenin.

Keywords: *Dioscorea bulbifera* Linn., Diosgenin, HPLC

Introduction

Dioscorea bulbifera Linn. is common throughout India, ascending upto 1800 m. It is found mainly in Himalayas, Chota Nagpur, Bihar, Orissa, Konkan and found wild on W. coast.

Dioscorea bulbifera Linn. is a perennial, slender, bulb bearing Twinner with a tuberous root. Leaves are broadly ovate, cordate, alternate and simple. It propagates by seeds, bulbils and tubers.

Some of the chemical constituents of bulbils of *Dioscorea bulbifera* Linn. are D-sorbitol, Furanoid, Norditerpenes, Diosgenin, Lucentis, Neoxant, Violaxanthin, Zeaxanthin, Auroxanthin, cryptoxanthin [1].

Diosgenin, 3 furanoid norditerpenes-diosbulbin A, diosbulbin D and F, Sinodiosgenin, β-sitosterol, D-sorbitol, diosbulbin B and D [2, 3, 4].

Uses and pharmacology of plant

The bulbils of *Dioscorea bulbifera* Linn. are Anorexiant, Diuretic, hunger suppressant [2]. It is anthelmintic, aphrodisiac, diuretic, antiseptic [5], it is also used in the treatment of thyroid and cancer [6], bitter tubers are used in treatment of leprosy and tumers [6, 7]. It has diuretic and anti-inflammatory activity [8], it is used for sore throat and struma[6]. Bulbs are used to treat piles, dysentery, syphilis, ulcer [9].

Experimental

Reagents, chemicals and standards

HPLC grade methanol (purity 99.9%), were obtained from Qualigens Fine Chemicals (Mumbai, India). Standard Diosgenin (98.5%), was procured from Sigma-Aldrich Chemie GmbH (Aldrich Division, Steinbeim, Germany)

Plant material

The bulbils of *Dioscorea bulbifera* Linn. Were collected from All India coordinated Research on Tuber Crop Plants, Vakoali, Taluka-Dapoli (No: BSKKV/FC/439/11). The bulbils were cut into small pieces and dried in the shade, and finely powered. The powder was passed through an 85 – mesh sieve and stored in an airtight container at room temperature (28 ± 2 ^{0}C).

Sample preparation

Accurately weighed (1000 mg) powder of the leaves of *Dioscorea bulbifera* Linn. Was vortex mixed with 10.0 cm^3 of methanol and 1 ml of Conc HCl for 15 minutes. The extract was filtered through a Whatman no. 1 qualitative filter paper, pore size 11 µm. The contents were then evaporated to dryness and final volume was adjusted to 10 cm^3 with methanol in a volumetric flask

Preparation of stock and working standard solution of Diosgenin

About 10.0 mg of Diosgenin was accurately weighed and transferred to a 100.0 cm^3 standard volumetric flask. The content of the flask were initially dissolved in 50.0 cm^3 of methanol, followed by sonication and then the content was diluted upto the mark with methanol. The obtained concentration of stock solution of diosgenin was 100.0 ug/cm^3 into a seris of 10.0 cm^3 standard flask, aliquots of 100.0 uL, 200.0 uL, 400.0uL, 800.0ul, 1000.0uL and 1200.0 uL were drawn from Diosgenin stock solution of concentration 100.0 ug/cm3 and the content of each flask were diluted up to the mark with methanol, to obtain the concentration range of 1.0 ug/cm^3 to 12.0 ug/cm^3 respectively.

Chromatographic conditions

HPLC separation was performed with a Jasco system, consisting of PU-980 isocratic pump, AS-1555-10 auto sampler, UV-970 detector and 20ul loop. The instrument was equipped with PU-970UV visible detector. A zorbax (5ul) reverse phase C-18 column (150x4.6mm) was used the mobile phase comprising of a mixture of methanol and distilled water in the ratio of 95:5 was delivered at a flow rate of 1.0 mL min $^{-}$ [1]. The data were collected at 211 nm. The Diosgenin peak was identified by comparison with the retention time of standard Diosgenin.

Results

Method Validation

Method Validation Validation of the developed HPLC method was carried out as per the International Conference of Harmonization (ICH) guidelines for specificity, sensitivity, linearity, accuracy, precision, repeatability, and robustness.

Linearity

The concentration range of diosgenin solution selected for linearity was Into a series of 10.0 cm^3 standard volumetric flask, aliquots of (100.0 μL, 200.0 μL, 400.0 μL, 600.0 μL, 800.0 μL, 1000.0 μL and 1200 μL) were drawn from Diosgenin stock solution of concentration (100.0 μg/cm^3) and the contents of each flask were diluted up to the mark with the mobile phase used, to obtain a concentration range of 1.00 μg/cm^3 to 12.00 μg/cm^3 respectively.

Twenty Microlitres, of each of these solutions, were injected into the chromatographic system under the optimized chromatographic conditions (Table 1), in triplicate. The chromatograms were recorded and the peak areas of Diosgenin were noted for each concentration of the working standard solutions of Diosgenin, applied in triplicate. The values of mean peak areas, standard deviation and the percent relative standard deviation of a Diosgenin for each injected concentration were calculated. The results are tabulated in Table 1.

Table 1: High performance liquid chromatographic determination of diosgenin results of the linear working range of diosgenin

Obs. No	Concentration of diosgenin (μg/cm^3)	Peak area of diosgenin			Meanpeak area	S.D	%R.S.D
1	1.0	49850	49652	49923	49808.3	140.2	0.28
2	2.0	96786	96574	96445	96601.6	172.1	0.17
3	4.0	195120	194832	196252	195401.3	750.6	0.38
4	6.0	299457	299206	299560	299407.6	182.0	0.06
5	8.0	390596	390289	390496	390460.3	156.5	0.04
6	10.0	487557	487239	487953	487583.0	357.7	0.07
7	12.0	585473	585460	585840	585591.0	215.7	0.03

A graph of mean peak area values of diosgenin (Y-axis) against the corresponding concentration of diosgenin (X-axis) was plotted, which showed a linear response in concentration range of 1.00 μg/cm^3 to 12.00 μg/cm^3. (Figure 1)

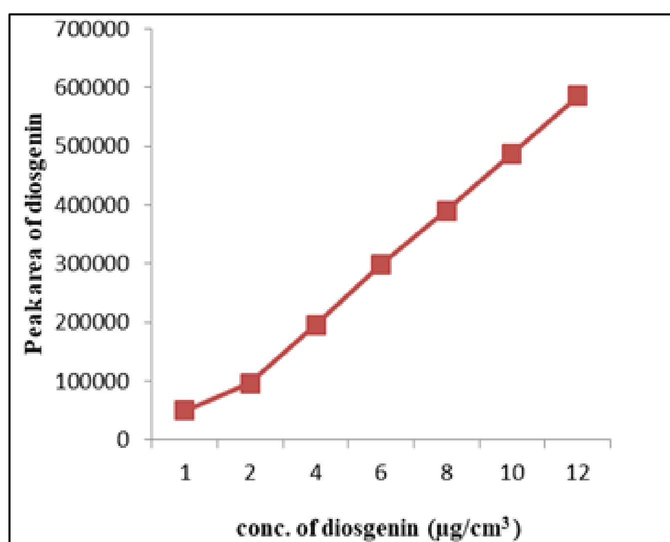

Fig 1: High Performance Liquid Chromatographic determination of diosgenin Calibration curve of standard diosgenin

From the above graph, it is observed that a linear response exists in the concentration range of 1.00 μg/cm^3 to 12.00 μg/cm^3 of diosgenin.

Limit of Detection (LOD) and Quantitation (LOQ)

The limit of detection (LOD) and limit quantitation (LOQ) were determined at signal to noise ratios of 3:1 and 10:1, respectively. The values LOD obtained for Diosgenin were 0.01μg/cm^3 and the LOQ was found to be 0.30 μg/cm^3.

Precision

Inter-day, Intraday and instrumental precision were studied for Diosgenin. Intraday precision was evaluated by having a single operator analysis, 3 quality – control replicate samples within a day. Inter day and instrumental precision was assessed by replicating the analysis of quality samples for 3 days. The instrumental precision was studied by repetitive injections (n=6) of standard solution of diosgenin (6μg/cm^3). The values of% RSD of Peak area of diosgenin for Inter day, Intraday and instrumental precision were determined. The value of % RSD for Inter-day, Intraday and instrumental precision was found to be 0.18, 0.09 and 0.11 respectively.

Solution Stability

Solution stability was determined by injecting freshly prepared, working standard solution of diosgenin of concentration 6.0 μg/cm^3. The samples were drawn at the end of 0, 12, 24 and 48 hours for analysis and injected into the chromatographic system under the optimized chromatographic conditions. The chromatograms were recorded and the peak area values were noted for each injected concentration of diosgenin.

The result show that for all the solutions, the peak area almost remained unchanged (RSD was less than 2%) and no significant degradation is observed within the given period, indicating that the solution of diosgenin was stable for minimum 48 hours.

System suitability

System Suitability of diosgenin was carried by injecting standard solution of diosgenin (6.0μg/cm^3) 5 times in the chromatographic system under optimized chromatographic conditions. The values and retention times of diosgenin were noted for each injection concentration of standard. As the % RSD was less than 2%, the system was found to be suitable.

Accuracy

Accuracy of the experiment were carried out for bulbils powder of *Dioscorea bulbiferra* Linn. using standard addition method. The recovery of the added standard was studied by ad+ding pure standard Diosgenin at three different levels to the powder of *Dioscorea bulbifrra* Linn. Bulbils. The accuracy was expressed as the percentage of Diosgenin recovered by the assay. Mean percentage recoveries for Diosgnin from the bulbils powder of *Dioscorea bulbiferra* are shown in table 2. The result indicates good accuracy of the method for the quantitative determination of the Diosgenin form Dioscorea bulbils powder.

Table 2: High performance Liquid Chromatographic determination of diosgenin Results of recovery experiment

Level	Wt. of sample (mg)*	Wt. of std. added (mg)	Amount of diosgenin found (mg)							Mean * (mg)	S.D	% R.S.D
			1	2	3	4	5	6	7			
0	100.5	0	0.062	0.061	0.063	0.062	0.063	0.061	0.060	0.061	0.001	1.802
1	100.7	0.06	0.120	0.121	0.122	0.119	0.117	0.118	0.124	0.120	0.002	2.006
2	100.4	0.08	0.142	0.140	0.138	0.141	0.137	0.139	0.138	0.139	0.002	1.291
3	100.6	0.09	0.152	0.151	0.149	0.148	0.150	0.147	0.149	0.149	0.001	1.149

* Sample: Dried bulbils of *Dioscorea bulbiferra* Linn.
**Mean amount of Diosgenin found.

Quantitation of Diosgenin

The quantitation of diosgenin was done using above validated HPLC method. The optimized chromatographic conditions were set on the HPLC system and the system was monitored to attain a stable base line. Twenty microliters of solution was injected into the chromatographic system under the optimized chromatographic conditions.

The identity of peak of diosgenin in the sample solution was confirmed by comparing the chromatogram of the sample (Figure 2) with that of the diosgenin standard solution having retention time as 8.51minutes (Figure 3). Amount of diosgenin present in the sample solution was determined from the calibration curve by using the peak area of diosgenin in the sample solution.

To ascertain the repeatability of the method, the assay experiment was repeated seven times. The values of amount of diosgenin present in *Dioscorea bulbifera* Linn., standard deviation and the percent relative standard deviation were calculated. The results of assay are given in Table 3.

Table 3: High performance Liquid Chromatographic determination of diosgenin Results of assay Experiment

Obs. No.	weight of bulbils powder	Peak area of diosgenin	Amount of diosgenin present mg/g
1	100.5	303789	0.620
2	100.8	304198	0.621
3	100.3	303952	0.620
4	100.7	304315	0.621
5	100.6	304489	0.622
6	100.5	304096	0.621
7	100.4	303948	0.620
Mean	100.5	304112.4	0.621
S,D	0.171	240.76	0.004
% RSD	0.170	0.079	0.079

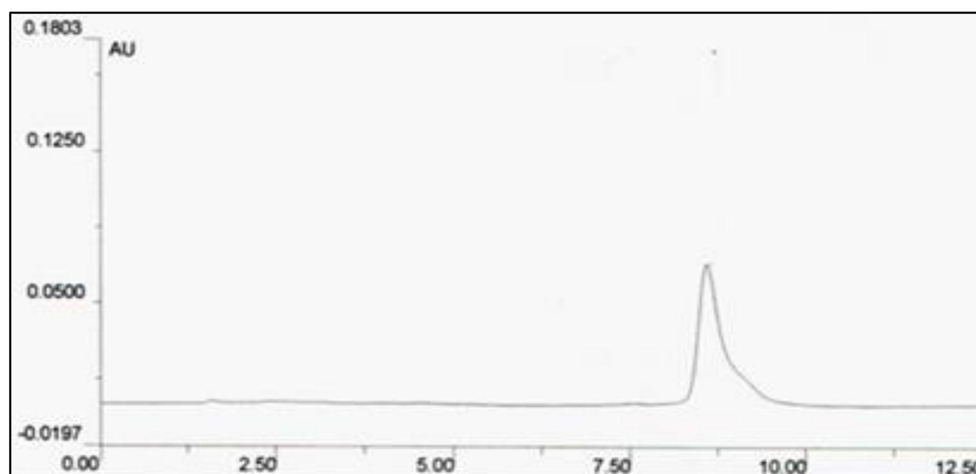

Fig 2: High Performance Liquid Chromatographic determination of diosgenin A typical HPLC chromatogram of standard diosgenin.

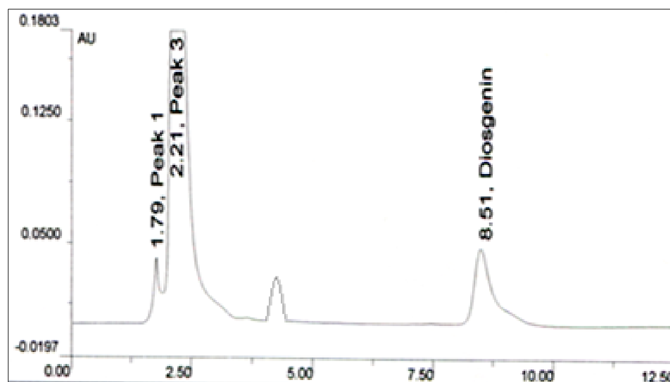

Fig 3: High Performance Liquid Chromatographic determination of diosgenin. A typical chromatogram of methanolic extract of dried bulbils powder of *Dioscorea bulbifera* Linn.

Discussion

The mobile phase used in the present research work for Quantitation of diosgenin from methanolic, dried bulbils powder extract of *Dioscorea bulbiferra* Linn. is methanol and water in the volume ratio of 95:5 (v/v) which is relatively simpler as compared to the mobile phase used in the reported methods [10, 11, 12].

The retention time for diosgenin was found to be 8.51 minutes which is relatively less than the retention time reported in the literature (18.06 min., 11.08 min., and 15.05 min. respectively) [10, 11, 12].

The method used in the present research work was also found to be sensitive to measure the concentration as low as 0.01 μg/cm^3, whereas in the reported method [1-3], the detection limit was 0.037 μg/cm^3, 0.04 μg/cm^3 and 10.0 μg/cm^3 respectively.

The column used in the present research work, comprised of Octadecyl bonded to silica phase. Due to the length of the column, (250.0 mm) and small particle size of silica (5.0 μm), a good resolution of diosgenin from different components of bulbils powder of *Dioscorea bulbiferra* Linn. was obtained.

Hence the HPLC method used in the present research work was found to be simpler, sensitive and accurate than other reported methods.

Conclusion

An HPTLC method developed for the quantitation of diosgenin from the bulbils powder of *Dioscorea bulbifera* Linn. is simple, precise and accurate and can be used for routine quality control analysis of bulbils powder of *Dioscorea bulbiferra* Linn.

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