

PHCOG MAG.: Research Article

A high performance thin layer chromatography method for quantitative estimation of Diosgenin in *Solanum nigrum* Linn.

Suthar A. C.* and Mulani R. M.

Botany Research Laboratory, Seth L. U. J. & Sir M. V. Collage, Andheri (East) Mumbai 400 069. India.

Author for Correspondence : acsuthar@gmail.com

ABSTRACT

The present paper deals with development and standardization of HPTLC method used for quantification of Diosgenin in *Solanum nigrum*. *Solanum nigrum* Linn. (Makoi) Solanaceae is known in Ayurveda for its effective treatment in cough, cold and asthma from ancient times. It contains many phyto-constituents such as Diosgenin, Solasodine & other steroidal alkaloids. As there is no analytical method for quantification of Diosgenin, which can be used for standardization of extract of *Solanum nigrum*, an attempt has been made to quantify Diosgenin in *Solanum nigrum* fresh fruit, dried crude fruit and extracts by HPTLC method. The lowest detectable limit of Diosgenin was found upto 200 nanograms and provides good resolution and separation of Diosgenin from other constituents of *Solanum nigrum* Linn. Further, recovery values of Diosgenin were found to be about 98%, which shows the reliability and suitability of the method. This HPTLC method was found to be reproducible, accurate and precise. The structure of isolated Diosgenin was characterized and confirmed by various advanced spectroscopic methods.

KEY WORDS - HPTLC, *Solanum nigrum* Linn., Makoi, Diosgenin

INTRODUCTION

Solanum nigrum Linn. Solanaceae is well known medicinal plant in Ayurveda, which possess a range of medicinal properties like anti-microbial, anti-oxidant, cytotoxic properties, antiulcerogenic, and hepatoprotective activity (1-3). There are various medicinal plants reported to have anti-cancer as well as anti-inflammatory activity in the Ayurvedic system of medicine, *Solanum nigrum* Linn. (Indian name - Makoi) is one of them with proven anti-cancer as well as anti-inflammatory activity (4-7). *Solanum nigrum* Linn. is a potential herbal alternative as anti-inflammatory and anti-cancer agent and one of the active principles reported to be responsible for this action is Diosgenin (8-10). It is therefore important to standardize the extract of *Solanum nigrum*.

There are no titrimetric, colorimetric, spectrophotometric or quantitative TLC methods available for estimation of Diosgenin. Therefore an attempt has been made to develop a HPTLC method, which is fast, precise, sensitive and reproducible with good recoveries for standardization of crude or/and extract of *Solanum nigrum* Linn.

MATERIALS AND METHODS

The fruits of *Solanum nigrum* Linn. Solanaceae were collected from two different location near Mumbai,

Maharashtra, India and used as samples for the quantification of Diosgenin.

Preparation of the extract

100 gms of *Solanum nigrum* powder was extracted with 500 ml solvent (chloroform, methanol and water) by stirring at 50°C for 1 hr. The filtered extract was concentrated under reduced pressure to remove the solvent. The extraction carried out for two times with the above mentioned protocol. The extract was obtained by drying the concentrated pooled extract under vacuum. These extracts were used for estimation and comparison of Diosgenin content.

Pure Diosgenin was isolated from the dried fruits of *Solanum nigrum* Linn. Purity and structure of isolated compound (Diosgenin) was confirmed by HPTLC, melting point and spectral analysis like NMR & MS.¹¹ This isolated Diosgenin was used as working standard for quantification of content in fresh fruits, dried crude and extracts.

Sample preparation

Accurately weighed 100 mg of aqueous extract and chloroform extract of dried Makoi fruit powder, 1 gm of crude dried powder or 10 gms of fresh purple fruit pulp of *Solanum nigrum* Linn. were separately extracted with methanol (10 ml x 3) by vortexing and allowed to stand for 5 min. at room temperature. The methanol extract was then filtered through Whatmann

no.42 filter paper; extracts were pooled and concentrated to dryness under vacuum. Final volume was made to 10 ml with methanol in volumetric flask. Diosgenin content was then analyzed after subjecting to HPTLC.

HPTLC method

Silica gel 60 F₂₅₄ precoated plates (10 x 10 cm) were used with Petroleum ether : Acetone (80:20) as solvent system. 10 µl of sample was spotted on pre-coated TLC plates. Ascending mode was used for development of thin layer chromatography. TLC plates were developed upto 8 cms. The TLC plates were scanned at 210 nm and then were sprayed with 10% Sulphuric acid methanolic reagent (10 ml concentrated sulphuric acid added in 90 ml of methanol with cooling and the reagent must be prepared freshly), heated at 110°C for 2-3 min and brought to room temperature. Brownish spot with R_f ≈ 0.52 - 0.55 was visible and scanned under visible light wavelength (535 nm).

Procedure: -1 (Calibration curve of standard Diosgenin)

One milligram of working standard Diosgenin was dissolved in 10 ml of methanol to yield stock solution of 100 µg/ml concentration. Calibration curve from 1 µg to 8 µg was prepared and checked for reproducibility, linearity and validating the proposed method. The correlation coefficient, coefficient of variance and the linearity of results were calculated.

Procedure: -2 (Calibration curve using extract spiked with Diosgenin)

The content of Diosgenin in fresh, dried crude and extracts was determined by comparing with the calibration curve of the working standard of Diosgenin. The extract, which showed lowest content of Diosgenin was then used as blank. This blank was then used to spike with the working standard of Diosgenin. Different samples with varying amount of standard Diosgenin in range of 0.15 mg to 0.55 mg were spiked separately in 100 mg of blank extract in which the content of Diosgenin had already been estimated. Procedure for sample preparation was followed as mentioned above. In each sample preparation, 10 µl of spiked solution were then subjected to HPTLC with 10 µl of blank solution for comparison. The percent recovery of Diosgenin standard was calculated. Reproducibility, precision and validation of the method was achieved by analyzing six replicate of spike sample solutions. Correlation coefficient, coefficient of variance was calculated.

RESULTS AND DISCUSSION

Standard Diosgenin showed single peak in HPTLC chromatogram (Figure I). The UV spectrum of Diosgenin before and after derivatization help to select the UV maxima for quantification of Diosgenin in various samples of *Solanum nigrum* Linn. (Figure II & III). The calibration curve of Diosgenin was obtained by spotting standard Diosgenin on HPTLC plate after scanning at 210 nm. Fresh fruits, dried fruits and various extracts of *Solanum nigrum* were analysed by the proposed method and the data are recorded in table I.

Chromatographic precision and recoveries from spike sample solution

Specificity

It was observed that the other phytoconstituents present in the extract or herb did not interfere with the peak of Diosgenin. Therefore the method was specific and help in separation of Diosgenin from other constituents of herb and hence, help to get the exact content of Diosgenin.

Limit of Detection

By applying the proposed method, the minimum detectable limit was found to be 600 ng / spot at 210 nm.

Linearity

The linearity of the method was checked with standard Diosgenin with the calibration curve in the concentration range of 1 - 8 µg based on a 10 µl sample volume. The regression equations ($Y = 264.082 + 415.070 * X$) and correlation coefficient were obtained with 6-8 replicate analysis for each concentration. Correlation coefficients were obtained in the range of 0.9918-0.9952 indicated excellent linearity of the procedure for standard Diosgenin analyzed. Calibration curve of standard Diosgenin is shown in figure IV.

Precision

Six replicate HPTLC analysis of spiked Diosgenin samples performed as per the procedures were carried out to check chromatographic precision. The results are recorded in table II.

Accuracy and precision

The method was applied to determine concentration of spiked Diosgenin samples in the range of 2.5 - 6.5 µg for assessing the accuracy & precision of the procedure. Table III represents the mean values and Coefficient Variance (C.V.) results indicates the levels in the above range can be estimated with accuracy and precision.

Table I: Percentage of Diosgenin in different samples of *Solanum nigrum* fruits and extract by measuring area in HPTLC method

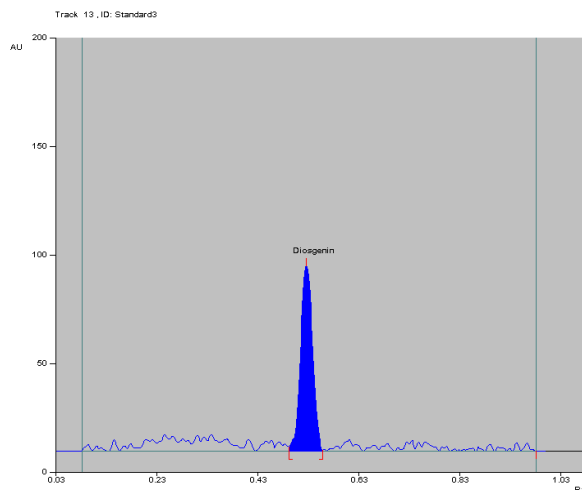
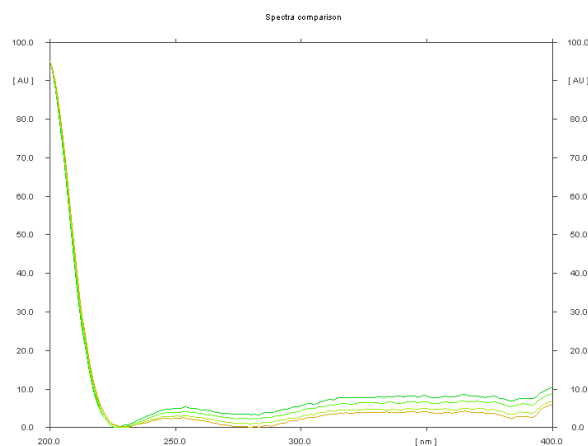
Sr.	Sample name	Region of collection	Solvent used for extraction	Diosgenin content (in mg)
1	Fresh fruits	Kelwe Road	-	6
2	Dried fruits	Kelwe Road	-	34
3	Fresh fruits	Borivali National Park, Mumbai	-	8
4	Dried fruits	Borivali National Park, Mumbai	-	40
5	Dried fruit extract - 1	Borivali National Park, Mumbai	Chloroform	750
6	Dried fruit extract - 3	Borivali National Park, Mumbai	Methanol	590
7	Dried fruit extract - 3	Borivali National Park, Mumbai	Water	120

Table II: Chromatographic precision and recoveries of spiked Diosgenin sample

Standard Diosgenin spiked concentration (ng/ml)	Precision (C.V.)	Recovery (%)
1.5	4.15	95.35
2.5	3.78	95.18
3.5	4.74	94.84
4.5	4.09	94.41
5.5	4.32	94.09

Table III: Precision & accuracy of the method applied to spiked Diosgenin samples

Amount added (µg / ml)	Amount found (µg /ml) (Mean ± S.D., n=6)	Precision / Reproducibility (C.V.)	Mean Recovery (%)
2.5	2.37 ± 0.11	4.64	95.02
4.5	4.23 ± 0.22	5.20	94.17
6.5	6.08 ± 0.31	5.09	93.63

**Figure I: TLC Chromatogram of standard Diosgenin****Figure II: UV spectrum of Diosgenin before derivatization with spraying reagent**

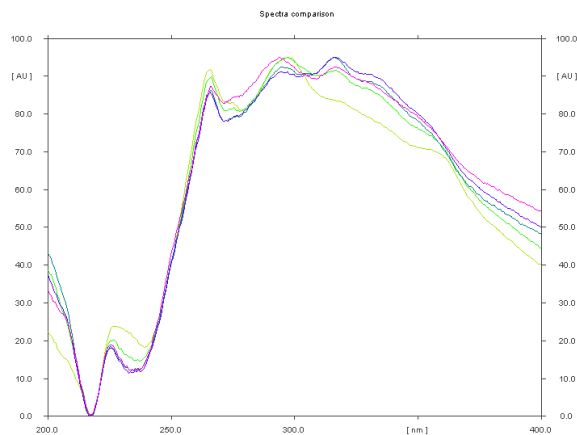


Figure III: UV spectrum of Diosgenin after derivatization with spraying reagent

CONCLUSION

The structure of Diosgenin was confirmed by NMR, Mass and IR spectroscopy. The lowest detectable limit of Diosgenin was found upto 600 ng. and provides good resolution and separation of Diosgenin from other constituents of *Solanum nigrum* Linn. Further, recovery values of Diosgenin were found to be about 95%, which shows the reliability and suitability of the method. The proposed HPTLC method is rapid, simple and accurate for quantitative monitoring of Diosgenin in *Solanum nigrum* Linn fresh fruits, dried fruits and extracts.

REFERENCES

1. Al-Fatimi, M., Wurster, M., Schroder, G. and Lindequist, U. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *J Ethnopharmacol.* **111** (3): 657-66 (2007).
2. Jainu, M. and Devi, C. S. Antiulcerogenic and ulcer healing effects of *Solanum nigrum* (L.) on experimental ulcer models: possible mechanism for the inhibition of acid formation. *J. Ethnopharmacol.* **104** (1-2): 156-63 (2006).
3. Raju, K., Anbuganapathi, G., Gokulakrishnan, V., Rajkaoppr, B., Jayakar, B. and Manian, S. Effect of dried fruits of *Solanum nigrum* Linn against CCl₄-induced hepatic damage in rats. *Biol Pharm Bull.* **26** (11): 1618-9 (2003).
4. An, L. Review about mechanisms of anti-cancer of *Solanum nigrum*. *Zhongguo Zhong Yao Za Zhi.* (15): 1225-6, 1260 (2006).
5. Lim, K. T. Glycoprotein isolated from *Solanum nigrum* L. kills HT-29 cells through apoptosis. *J Med Food.* **8** (2) : 215-26 (2005).

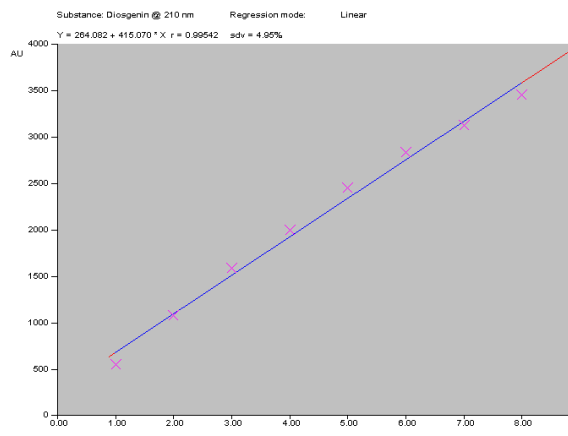


Figure IV: Calibration curve of standard Diosgenin with respect to the area under curve at various concentration

6. Lee, S. J. Glycine- and proline-rich glycoprotein isolated from *Solanum nigrum* Linne activates caspase-3 through cytochrome c in HT-29 cells. *Oncol Rep.* **14** (3): 789-96 (2005).
7. Zakaria, Z. A., Gopalan, H. K., Zainal, H., Mohd. Pojan, N. H., Morsid, N. A., Aris, A. and Sulaiman, M. R. Antinociceptive, anti-inflammatory and antipyretic effects of *Solanum nigrum* chloroform extract in animal models. *Yakugaku Zasshi.* **126** (11): 1171-8 (2006).
8. Yamada, T., Hoshino, M., Hayakawa, T., Ohhara, H., Yamada, H., Nakazawa, T., Inagaki, T., Iida, M., Ogasawara, T., Uchida, A., Hasegawa, C., Murasaki, G., Miyaji, M., Hirata, A. and Takeuchi, T. Dietary diosgenin attenuates subacute intestinal inflammation associated with indomethacin in rats. *Am J Physiol.* **273**: G355-64 (1997).
9. Shishodia, S. and Aggarwal, B. B. Diosgenin inhibits osteoclastogenesis, invasion, and proliferation through the downregulation of Akt, I kappa B kinase activation and NF-kappa B-regulated gene expression. *Oncogene.* **25**(10): 1463-73 (2006).
10. Raju, J. and Bird, R. P., Diosgenin, a naturally occurring furostanol saponin suppresses 3-hydroxy-3-methylglutaryl CoA reductase expression and induces apoptosis in HCT-116 human colon carcinoma cells. *Cancer Lett.* [Epub ahead of print] (2007)
11. Wawer, I., Nartowska, A. and Cichowlas, A. A. ¹³C cross-polarization MAS NMR study of some steroidal sapogenins. *Solid State Nucl Magn Reson.* **20**(1-2): 35-45 (2001).

Phcog Mag. Vol 4, Issue 14, Apr-Jun, 2008

Submitted on : 19th August, 2007

Revised on : 16th December, 2007

Accepted on: 10th January, 2008