

Development and Validation of HPTLC Method for Quantification of Solanesol in Various Parts of *Nicotiana tabacum* Collected from Different Geographical Regions of India

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Abstract. Solanesol is the starting material for many high value biochemicals, including Co-enzyme Q10 and vitamin-K analogues. The aim of the current study was to develop and validate a reliable and fast analytical procedure for the determination of solanesol in *Nicotiana tabacum* using high-performance thin layer chromatography (HPTLC) method. The method was developed on TLC aluminium plates precoated with silica gel 60F-254 using solvent system hexane: ethyl acetate (5:1, v/v), which gives compact spot of solanesol (R_f value 0.41 ± 0.02). Densitometric analysis of solanesol was carried out in the absorbance mode at 210 nm. The linear regression analysis data for the calibration plot showed good linear relationship with r = 0.9978 with respect to peak area, in the concentration rang 100-5000 ng per spot of solanesol. The limit of detection and quantification were 13 and 30 ng per spot, respectively. The proposed method was applied for quantitative estimation of solanesol in different parts of *Nicotiana tabacum* from different geographical regions in India, which showed that maximum amount of solanesol was found to be present in leaf sample collected from Karnataka i.e. 3.52 mg/g. Statistical analysis proved that the method is repeatable, selective and accurate for the estimation of solanesol in *Nicotiana tabacum*.

Introduction

Solanesol is a trisesquiterpenoid alcohol (Fig. 1) which was first isolated from tobacco [1]. Tobacco belongs to family Solanaceae and the plant is considered to be a good source of a large number of bioactive substances and is the starting material for many high value bio-chemicals, including Co-enzyme Q10 and vitamin-K analogues. Solanesol is also a potentiating agent in many medicines. The research shows that after introducing "solanesol" radical into the structure of some medicines, the effect are increased distinctly. Solanesol can counteract the bacteria, diminish inflammation and hemostasis. It also has strong activity of counteracting the cancer. With solanesol as its primary material, Co-enzyme Q is useful in the treatment of heart diseases, cancers, ulcers, wound and so on. [2-10]. The content of solanesol in tobacco leaves is higher than that in any other organ [11]. A few methods for determination of solanesol in tobacco by HPLC [12-13], by GC [14], by HPLC-APCI-MS [15], and by LC-TMS [16], etc. have been reported. Till date, to best of our knowledge no HPTLC method for quantitative estimation of solanesol in different parts of *Nicotiana tabacum* from different geographical regions has been reported. Consequently a simple, precise and accurate HPTLC method for rapid quantification of solanesol was developed and the method was validated to achieve the satisfactory precision and recovery.

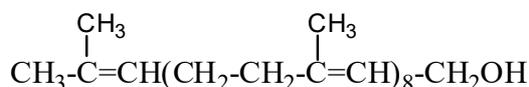


Fig. 1. Structure of solanesol (3,7,11,15,19,23,27,31,35-nonamethyl hexa triaconta-2,6,10,14,18,22,26,30,34-nonaen-1-ol)

Experimental

Drugs and chemicals

The *Nicotiana tabacum* plants were collected from the local farms of Jhajjar (Haryana, India), Shimoga (Karnataka, India), Ayodhya (U.P., India) and Satara (Maharashtra, India). The leaf, stem and root parts were separated and dried at normal room temperature. The drugs were authenticated by Taxonomist of Department of Botany, Hamdard University. Reference standard Solanesol was obtained from Sigma Aldrich, China and all chemicals and reagents used were of analytical grade and were purchased from Merck, India.

HPTLC instrumentation and conditions

The samples were spotted in the form of bands of width 4mm with a Camag microlitre syringe on percolated silica gel aluminium plate 60F-254 (20 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat V applicator (Switzerland). A constant application of 100 nL/s was employed and space between two bands was 8.3 mm. The slit dimension was kept at 4mm × 0.45mm, and 20mm/s scanning speed was employed. The mobile phase consisted of hexane: ethylacetate (5:1). Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase. The optimized chamber saturation time for mobile phase was 20 min at room temperature. The length of chromatogram run was 80 mm. Subsequent to the development TLC plates were dried in a current of air-dryer. Densitometric scanning was performed on Camag TLC scanner IV in the absorbance mode at 210 nm. The source of radiation utilized was deuterium and tungsten lamp.

Calibration curves of solanesol

The stock solution of solanesol (1000 µg/ml) was prepared in hexane. Different volumes of stock solution, 0.1, 0.2, 0.4, 0.5, 1, 2, 4, 5 µL were spotted in duplicate on TLC plate to obtain concentration of 100, 200, 400, 500, 1000, 2000, 4000, 5000 ng per spot of solanesol, respectively. The data of peak height/area versus drug concentration were treated by linear least-square regression.

Validation

The proposed analytical method was validated as per the latest ICH guidelines [17, 18] and the statistical analysis was done using Excel 2000 (MS Office).

Accuracy

The recovery studies of solanesol were carried out by standard addition method. The pre-analyzed samples were spiked with extra 50, 100 and 150 % of the standard solanesol and the mixtures were reanalysed by the proposed method. The experiment was conducted in triplicate.

Precision

The intra and inter day variation and inter analyst precision of six replicates for the determination of as carried out at three different concentration levels of 500, 1000, 5000 ng per spot. The % RSD of peak area was calculated.

Robustness of the method

By introducing small changes in the mobile phase composition, the effects on the result were examined. Mobile phases having different composition of hexane and ethyl acetate were tried at 1000 ng concentration per spot and % RSD of peak area was calculated.

Limit of detection and limit of quantification

In order to estimate the limit of detection (LOD) and limit of quantification (LOQ), blank hexane was spotted six times. The signal to noise level was determined. LOD was considered as 3:1 and LOQ as 10:1 [19].

Determination of solanesol in different samples of *Nicotiana tabacum*

All dried powdered samples of *Nicotiana tabacum* (1gm each of leaf and stem sample and 2 gm of root samples) were sonicated with petroleum ether (25ml) for thirty minutes. The filtered petroleum ether extract obtained were concentrated under reduced pressure to dryness which were re-dissolved separately, in 1ml of chromatographic grade petroleum ether. Further, 1 μ L each of leaf, 5 μ L each of stem and 10 μ L each of root samples were applied in duplicate on pre-coated silica gel 60F₂₅₄ aluminium sheets for quantification using proposed method.

Result and Discussion

Selection of mobile phase

Several easily available solvents in different combination and concentrations were tried to obtain good resolution, compact spot and better separation of spot. Lastly, the mobile phase consisting of hexane: ethyl acetate (5:1v/v) was selected which gives a sharp and well defined peak of solanesol at R_f value of 0.41. It was found that the solvent system has very good resolution for the separation of solanesol from other components of the extracts.

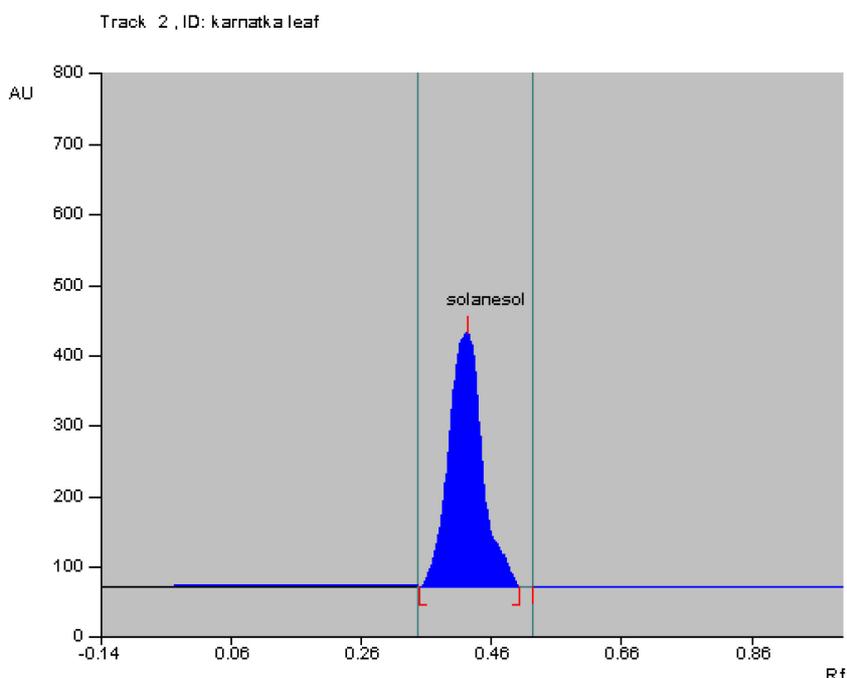


Fig. 2. A typical HPTLC chromatogram of solanesol ($R_f = 0.41$)

Calibration Curves

Linearity of the method

The calibration curve ($n=3$) was plotted by using peak area against concentration and was found linear in the range of 0.1 μ g/ml to 5 μ g/ml with a good correlation coefficient of 0.9978. Calibration data, with their standard deviation of slope and intercept were given in Table 1. Values obtained from the table indicated the method to be precise and reproducible. The linear regression data for the calibration plot were indicative of a good linear relationship between peak area and concentration over a wide range.

Validation of Proposed Method

The method was validated with respect to parameters including accuracy, precision, robustness, LOD and LOQ.

Recovery studies

The proposed method when used for extraction and consequent estimation of solanesol after spiking with 0, 50, 100 and 150 % of the standard solanesol produced mean recovery of 100.475 %. The %RSD values after spiking with 0, 50, 100 and 150 % of additional drug were found in the range of 0.86-2.7. The result for the recovery has been incorporated in Table 2. The values of recovery study revealed the method is accurate for a wide range of concentration.

Precision

The proposed analytical method for repeatability and intermediate precisions were calculated and reported in terms of %RSD in Table 3 and 4. Intermediate precisions included data of intra day, inter day and inter analyst precision. The low values of RSD indicated the reproducibility of method. The Intraday, Inter day and Inter analyst precision of solanesol were determined at three different concentration of 500, 1000, 5000 ng per spot. The % RSD was found in the range of 1.99-1.5%, 1.9-2.06 % and 3.01-1.73 % for repeatability and reproducibility.

Robustness of the method

The %RSD of the peak areas was calculated for change in solvent system composition at concentrations 1000 ng per spot in triplicate. The low values of %RSD (<4) obtained after introducing small deliberate changes in the developed HPTLC method indicated the robustness of method. The values are given in Table 5.

LOD and LOQ

The LOD and LOQ were determined by signal to noise ratio method and found to be 13 ng/ml and 30 ng/ml, respectively.

Table 1. Linear regression data for the calibration plot (n=3)

Linearity range (ng/ml)	100-5000
Regression equation	1308.45 + 3.902x
Correlation coefficient	0.9978
Slope±S.D	1341.0 ± 29.4
Intercept± S.D	3.809 ± 0.12

Table 2. Recovery /Accuracy of the method (n=3)

% of standard spiked to the sample	Amount of standard spiked (µg)	Amount of drug recovered (µg) ±S.D	% of drug recovered	% RSD
0	3520	3512.9 ±36.6	99.7	1.03
50	5280	5375.0 ± 60.3	101.8	1.13
100	7040	7307.5 ± 205.0	103.8	2.7
150	8800	7728.0 ± 66.4	96.6	0.86

Table 3. Repeatability of the method (n=3)

Concentration (ng/ml)	Mean area± S.D	Mean height ± S.D	R _f ± S.D	% RSD of area	% RSD of height	% RSD of R _f
500	3405.7±65.1	157.7±3.3	0.41±0.01	1.9	2.05	2.7
1000	5826.3±200.3	246±6.2	0.43±0.01	3.5	2.48	3.5
5000	20574±525.9	551.7±20.1	0.46±0.01	2.6	3.77	3.4

Table 4. Intermediate precision of the method (n=3)

Concentration (ng/ml)	Interday precision		Intraday precision		Inter analyst precision	
	Mean area±S.D (n=3)	%RSD	Mean area±S.D (n=3)	%RSD	Mean area±S.D (n=3)	%RSD
500	3266±67.09	2.06	3312±50.5	1.5	3308±56.22	1.73
1000	5785±145.6	2.6	5646±96.0	1.7	5714±137.3	2.45
5000	20692±400.4	1.9	20646±407.3	1.99	21402±659.5	3.01

Table 5. Robustness of the method (concentration 1000ng/ml)

Solvent system (Hexane : Ethyl Acetate)		Mean area ±S.D (n=3)	Mean R _f ±S.D (n=3)	% RSD of area	% RSD of R _f
Original	Used				
	4.9:1.1	5721±153.8	0.42±0.015	2.7	3.6
5:1	5:1.1	5737±78.3	0.44±0.015	1.4	3.5
	5:0.9	5715±68.1	0.44±0.017	1.2	3.7

Determination of solanesol in different samples of *Nicotiana tabacum*

The concentration of solanesol in different parts (leaf, stem and root) of *Nicotiana tabacum* from different geographical regions in India were determined by HPTLC and the result showed that the content of solanesol were significantly different from the different parts of *Nicotiana tabacum*. The maximum concentration of solanesol was observed in leaves, followed by stems and roots, respectively. The amount of solanesol was found to be 3.5204, 2.2010, 1.1590, 2.5020, 0.7339, 0.6944, 0.3468, 0.37190, 0.0823, 0.0078, 0.0817 mg/g sample collected from Karnataka (leaf), Maharashtra(leaf), U.P(leaf), Haryana(leaf), Karnataka(stem), Maharashtra(stem), U.P(stem), Haryana(stem), Karnataka(root), Maharashtra(root) and Haryana(root) respectively in drug. The maximum amount of solanesol was found to be present in leaf sample collected from Karnataka i.e. 3.52 mg/g. The root sample collected from Karnataka and Haryana were having almost same amount of solanesol. Root sample collected from Maharashtra contain minimum amount of solanesol i.e.0.0078mg/g. Root sample collected from U.P contain very less amount of solanesol which cannot be quantified. A very large variation in solanesol content of *Nicotiana tabacum* samples collected from different regions was found which may be attributed to several factors, such as local climate, growing conditions, harvest season, and so on. The low % RSD values indicated the suitability of this method for routine analysis of solanesol in crude drugs.

Conclusions

An efficient procedure using HPTLC has been developed for rapid analysis and quantification of solanesol in different parts of *N. tabacum* from different geographical regions in India and successfully validated. The proposed analytical procedure provided an alternative simple, precise, fast, accurate and cost effective method for rapid determination of solanesol in *N. tabacum*, and was successfully applied on samples from different growing regions in India. The statistical analysis of data obtained proves that the method is reproducible and selective and can be used for routine analysis of solanesol in crude drugs. Finally, the study confirmed that the content of solanesol in leaves was higher than that in stems and roots of *N. tabacum*.

Conflict of Interest

The authors declare that there is no conflict of interest.

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