International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Production of High Purity Solanesol from Impure Low Assay Tobacco Extract by Single Step Normal Phase Chromatography Method

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Abstract: ***Solanesol is an essential intermediate for the synthesis of valuable vitamins and anticancer drugs. In impure form, solanesol is available as low purity extracts of tobacco. This extract is a complex composition of waxes and various polar and non-polar constituents with solanesol. The work presented one step chromatography method for the generation of high purity (95 wt.%) solanesol from the crude 18 wt.% assay extract of tobacco. The mobile phase for the purification was selected on the basis of miscibility characteristics of impure extract with various solvents. Suitable adsorbent for purification was selected on the basis of elution selectivity and desorption ratio. Alumina was selected as an adsorbent for purification due to high binding capacity of 35.2 mg/(mL of adsorbent), high elution selectivity and desorption ratio. Optimized process produces solanesol of 95 wt.% purity. The process provides single step scalable purification of solanesol with high purity and recovery.***

Keywords: Solanesol, Normal phase chromatography, Alumina, Miscibility, Purification

1. Introduction

Solanesol is a polyisoprenoid alcohols or polyprenols. It is found in many botanical species including tomato, potato, eggplants and pepper plants [1-2]. It is used for the synthesis of valuable chemicals like Vitamin E, Vitamin K and Coenzyme Q10 [3]. It is also an important intermediate of anti-cancer drug preparations [4-5]. The widely available crop "Tobacco" is the richest source of solanesol. Zhao *et al.* (2007) and Severson *et al.* (1977) have conducted significant work to extract solanesol from *N. tabacum* (tobacco) and presented detail investigation of the distribution of solanesol in various parts it [6-7].Therefore, various methods have evolved with time for the extraction of solanesol from this cheap tobacco plant. Rowland *et al.* (1956) first isolated solanesol by extracting tobacco with mixture of methanol and ether composition [8]. Another method suggests the use of petroleum ether for extraction; the extract was further concentrated under vacuum [9]. According to reported patents, n-hexane was used as extracting solvent and a pasty solanesol was obtained after two extractions followed by concentrations [10]. In another process, solanesol was extracted from moldy or broken tobacco by extraction with solvents such as n-hexane, ultrafiltration and concentration by membrane evaporation [11].

All these reported methods produce solanesol extracts of different assay purity. Among these extracts 18-20 wt.% and 50 wt.% assay solanesol extracts are more common and available in the market due to their commercial value. But solanesol is mostly utilized in the pharmaceutical industry for the preparation of drug intermediates, therefore much higher purity (> 90%) is desirable. Isolation of solanesol from this 18 wt.% extract is challenging and complex due to the presence of wide range of polar and non-polar impurities in it depending on the temperature and solvent conditions used for extraction. Conventional methods reported for high purity solanesol or for the enrichment of solanesol from such low assay extracts involve supercritical extraction followed by crystallization [12]. Another method involves adsorptive separation using multiple steps silica columns [13]. Du et al. (2006) reported a high-speed countercurrent chromatography method for 90 wt.% purity solanesol production from such extracts [14]. All these reported method involved specialized operating conditions, multiple operating steps and unit operations or involve large consumption of solvents for purifying a small amount of solanesol [14-15]. Therefore, there is a need of modified purification method for the production of high purity (> 90%) from these commercially available solanesol extracts.

To address this present work presented a single step adsorptive chromatographic method for the production of 95 wt.% purity solanesol from 18 wt.% commercial solanesol extract. The purification method was applied by studying the miscibility of crude extract in different solvent compositions. Normal phase chromatography method was selected because of the miscibility of 18 wt.% extract with non-polar solvents. Alumina was used for the developed of purification process due its high equilibrium capacity and desorption selectivity towards solanesol. Column experiments were used for high purity of solanesol. Different modulators and their concentration in non-polar hexane were applied for the optimization of elution conditions. This involves through discussion of effect of modulators concentration on elution characteristics, purity and recovery of purified solanesol. The method provides 95 wt.% purity solanesol in single chromatographic column with recovery as high as 79% of loaded solanesol at ambient temperature and pressure conditions

2. Experimental Section

2.1 Tobacco extracts

The crude tobacco extract containing 18 wt.% assay of solanesol was obtained as a gift sample form Sami Labs Ltd.,

India. Pure 99 wt.% solanesol for calibration curve was purchased from Sigma Ltd., India.

2.2. Loading Sample

The sample for batch and column studies was prepared by mixing crude extract with hexane to get the final loading sample of solanesol concentration 7.8 mg/mL.

2.3 Materials

The matrices like SEPABEADS EB-HG and Tulsion A2X-MP were obtained as a gift sample form Resindion SRL., Italy and Thermax India Ltd., India. Alumina used for the purification process was procured from the local market. The solvents like n-Hexane, Iso-Octane, Isopropyl alcohol, ethyl acetate, methyl alcohol, acetone and acetonitrile (ACN) were purchased in bulk from Chem Udyog Pvt. Ltd., Mumbai, India.

2.4 Columns and Pump

Two glass columns of 1.0 cm × 30 cm and 1.0 cm × 50 cm, (Diameter×length) dimensions were purchased from Omega glass works, Mumbai. Organic solvent compatible SS316 adaptors of 25 cm length were designed in the lab and got fabricated from the local vendor. The packed bed of specific height in these glass columns was prepared by using these adaptors. An eight roller Masterflex (Cole-Parmer) peristaltic pumps was used for the external loading of feed sample on to the column. Teflon tube used for pumping the crude samples and solvent by external pump was purchased from local market.

2.5 HPLC Analysis Method

The HPLC analysis for Solanesol was done by using normal phase 4 mm× 250 mm silica Licrosphere column of Merck packed with silica gel of 5 µm diameter. IPA:Hexane in 4:96 (v/v) ratio at a flow rate of 0.4 ml/min was used as mobile phase. The sample injection volume was 20 µL. The wavelength of 215 nm was used for the detection of Solaensol and associated impurities. The retention time of solanesol was 9.0-9.5 min depending on the solvent composition. The calibration curve using the sigma standard was prepared for the estimation of concentration of solanesol in unknown samples. The calibration curve showed linearity over the range of 0.5-20 mg/mL. The regression line for solanesol was y = 69107 x + 15211(R^2 = 0.999), where y is peak area and x is the concentration of solanesol in mg/mL.

2.6 Wt.% assay Method

The assay method involved measurement of specific quantity of sample containing solanesol. This volume was then subjected to evaporation at temperature of 60 ^{0}C under vacuum of 100 mbar for the removal of other solvents. After complete removal of solvent from the sample the remaining residue was weighed. Hexane of known volume (10 ml) was then added in to this residue to get a dissolved solution. 20 µl of this solution was then injected to HPLC and area corresponding to solanesol peak was recorded. The same volume of standard solanesol sample of known concentration (10 mg/ml) was also analyzed by HPLC and corresponding area was determined. Then the assay purity of the sample was estimated by the Equation 1.

$$wt.\%\ Assay = \frac{Wt.\ Std.}{Wt.\ Sample} \times \frac{Sample\ Area}{Std.\ Area} \times \frac{Std.\ inj.\ vol}{Sample\ inj.\ vol} \times Std.\ purity \quad (1)$$

2.7 Miscibility study of 18 wt.% assay solanesol extract

The study involves mixing of 18 wt.% assay crude with solvent composition in 1:2 proportion. Therefore, 10 mL of solvent was mixed with 5 mL of crude extract under mild stirring condition of 200 rpm for 1 hour at temperature of 35 °C and 70 °C. After which the mixture was allowed to stand for 30 min for phase separation. If the phase separation observed with the added solvent then the volume of sample phase and solvent phase were measured. Using the volume of phases obtained after phase separation, miscibility with respect to added solvent system for solanesol crude extract was measured in terms of miscibility as defined by Equation (2).

$$Miscibility = \frac{V0 - V_e}{V0} \quad (2)$$

Where, V_0 is the initial volume of crude extract, V_e is the volume of raffinate (extract sample) after phase separation. Equation (1) indicates that if V_e = 0 then the crude extract is completely (miscibility = 100%) miscible with the added solvent or solvent composition.

Various solvents like iso-octane, heptanes, hexane, ethyl acetate, acetone, isopropyl alcohol, ethanol and methanol were used for miscibility study. Miscibility of crude 18 wt.% extract with the range of polar and non-polar solvents is an indication of presence of close characteristics polar and non-polar impurities in it.

2.8. Batch adsorption studies for thermodynamic selection of matrix for the separation

Thermodynamic selection of the adsorbent for the said separation was done by batch adsorption studies [16]. For the study, 2 mL of adsorbent under investigated was equilibrated with hexane in a glass bottle of 40 mL size. After 1 hr equilibration, the supernatant hexane was decanted from the bottle. In this equilibrated adsorbent 15 ml of loading sample (solanesol = 7.8 mg/mL) was added. The adsorbent was contacted with loading sample for sufficient time (5 hr) in stirred condition. After establishment of equilibrium conditions, supernatant solution was removed and analyzed for the solanesol content. The resin obtained was washed with hexane for the removal of unadsorbed components. The washing solution was also removed and analyzed for solanesol content. The bound Solanesol was then desorbed in 10 ml of 4% ethyl acetate in hexane as a desorbing solvent. The desorbent was analyzed by the HPLC method for the checking xylitol content. The suitable adsorbent for purification was selected on the basis of high equilibrium capacity and desorption selectivity of solanesol in elution fractions.

2.9. Column experiments for the purification of solanesol from crude 18 wt.% tobacco extract

Column experiments for purification were carried out in 1.0 cm × 25 cm glass column packed with alumina. Depending on the miscibility characteristics of crude extract and polarity of adsorbent, non-polar hexane was used as equilibration solvent. Therefore the type of chromatography involved in the said purification is normal phase chromatography. This 20 mL of packed alumina was equilibrated by two bed volume (BV) of equilibration solvent. After that loading sample of amount 1.5 BV was loaded on the equilibrated alumina bed. Further, the washing volume and composition of the unadsorbed components was optimized. After that eluent composition and quantity was optimized for the selective elution and desired purity of bound solanesol. This was achieved by changing the separation factor by preparing different composition of polar and non-polar solvents. For that elution strength of hexane was modified by adding various polar solvents like isopropyl alcohol, acetonitrile, ethyl acetate or acetone.

Results and Discussion

3.1 Miscibility Characteristics and selection of mobile phase for purification

Miscibility characteristics of crude extract with polar and non-polar solvents is shown in Table 1. Complete miscibility of the crude extract was obtained in non-polar isooctane, n-heptane, n-hexane, acetone, ethyl acetate solvents. The extract also showed complete miscibility with polar isopropyl alcohol but limited miscibility of 0.91 and 0.62 for high polar ethanol and methanol respectively. The crude extract was immiscible with water.

Table 1: Miscibility characteristics of 18 wt.% crude solanesol extract with various solvents

Solvent type	*Solvent*	*Miscibility Factor*
High Non-polar	Iso-octane	1.0
	Heptane	1.0
	Hexane	1.0
Weak non-polar	Ethyl acetate	1.0
	Acetone	1.0
	Acetonitrile	1.0
Polar	IPA	1.0
	Ethanol	0.92
	Methanol	0.62
	Water	0.00

Depending on miscibility characteristics, hexane was selected as mobile phase solvent for chromatographic purification of solanesol.

3.2. *Selection of adsorbent for purification*

Table 2 shows the comparison of adsorption and elution selectivity of various adsorbents under investigation. Alumina gives higher binding capacity of 35.1 mg/mL compared to 28.6 and 15.8 mg/mL of SEPABEADS EB-HG and Tulsion A2X-MP. This is due to the high surface area and pore structure of alumina compared to other matrices. But this does not imply that alumina could provide selective separation of Solanesol over the impurities associated with it. Therefore the 4% (v/v) EA eluted fraction obtained for these three matrices were analyzed on HPLC. The batch elution obtained from Alumina showed selective elution of solanesol compared to associated impurities.

Process steps	Matrices		
	Alumina	SEPABEADS EB-HG	Tulsion A2X-MP
Loading solanesol conc (mg/mL)	7.8	7.8	7.8
Supernatant conc Solanesol (mg/mL)	3.12	3.98	5.69
Equilibrium capacity (mg/mL adsorbent)	35.1	28.65	15.82
Washing	Hexane	Hexane	Hexane
Elution	4:96, EA:Hexane	4:96, EA:Hexane	4:96, EA:Hexane
Elution purity	71.10%	64.20%	63.80%
Elution selectivity	√	×	×

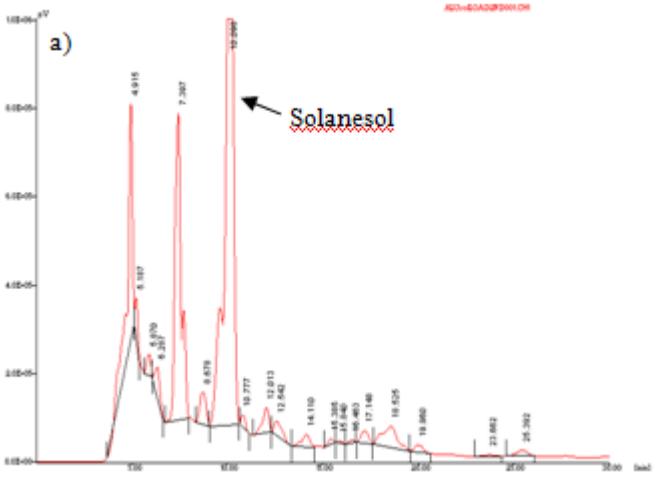
EA = Ethyl acetate; × = Poor conditions; √ = Desirable conditions

This can be confirmed by the comparison of the HPLC chromatogram of 18 wt.% crude extract and eluted fraction from alumina as shown in Fig.1a and 1b. It can be observed from Figure. 1 that purity of loaded impure solanesol is improved to 71% in alumina elution fraction compared to that of 64% and 63% obtained in the elution fractions from SEPABEADS EB-HG and Tulsion A2X-MP.

Therefore alumina was selected as adsorbent with hexane or iso-octane as mobile phase for the development of single step purification process for Solanesol.

3.3 Development and optimization of purification process

Development and optimization of purification protocol is governed by the purity and yield constraints. Optimization involves the selection of efficient binding and elution conditions. Therefore depending on batch adsorption behaviour and to obtain maximum binding strength in column operation hexane was used as equilibration solvent for the purification of solanesol. While the elution conditions were optimized in such a way that selective elution of solanesol from the associated impurities takes place.



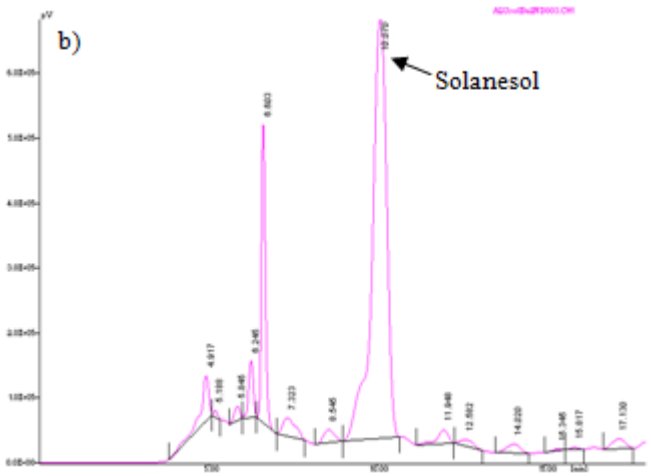


Figure 1: (**a**) HPLC chromatogram of solanesol, dilution factor = 100 (a) 18 wt.% tobacco extract (b) Batch elution fraction from Alumina [elution by 4% EA in hexane; % Area purity of solanesol = 71 %]

Elution strength represents the adsorption energy of an eluent molecule per unit area of adsorbent. Various mobile phase modifiers are added in solvent system to achieve the desired elution strength. Batch elution studies indicate that addition of 4% (v/v) EA as a modifier in hexane eluted all of the Solanesol bound on the alumina. This was mainly due to polarity and elution strength of 0.48 of ethyl acetate for alumina. Another choice for mobile phase modifier was acetonitrile, because of its elution strength of 0.5, comparable with that of ethyl acetate. Initial trials with 4% (v/v) ethyl acetate or acetonitrile in hexane as elution phase did not yield desired purity solanesol. Therefore trials were carried out by varying the concentration of ethyl acetate and acetonitrile in the range of 1-4 % (v/v) in hexane. The effect of concentration of these modifiers in hexane phase on the purity, recovery and solvent consumption is shown in Figure 2(a) and 2(b).

The purification trials showed that the elution conditions with acetonitirle yielded lower purity than EA in the applied range of both the modifiers. The maximum purity achieved with the acetonitrile as modifier was 87.5 wt.% with only 75% recovery of it. This lower purity with acetonitrile was mainly due to its extra interaction ability with solute molecules bounded to resin by polarization forces. This high polarization and interaction ability of acetonitrile causes elution of impurities with solanesol even though its elution strength is similar to that of ethyl acetate.

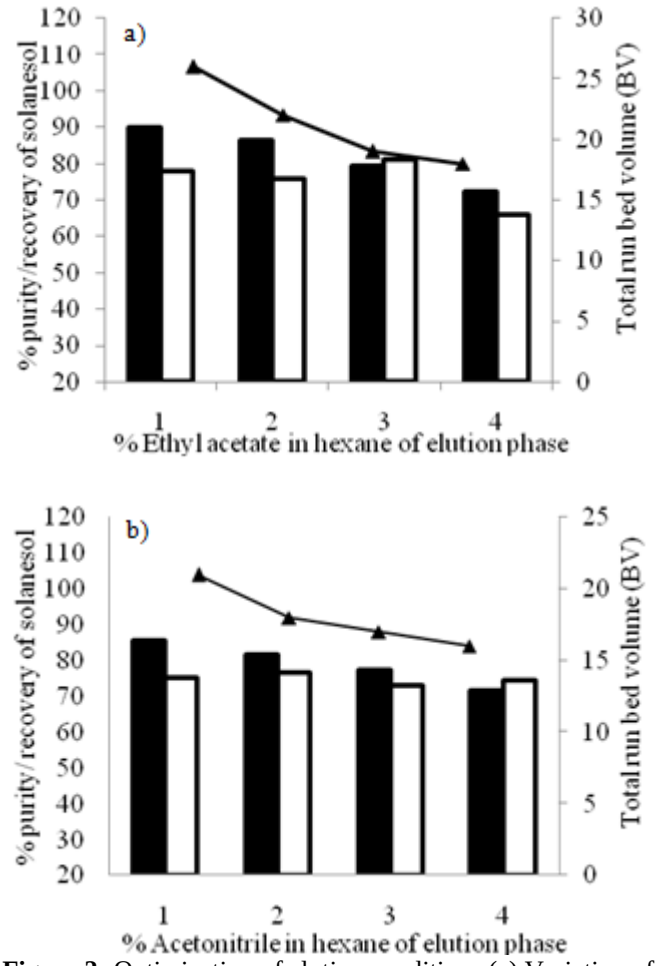


Figure 2: Optimization of elution conditions (a) Variation of volume percentage of ethyl acetate in hexane (b) Variation of volume percentage of acetonitrile in hexane [■ % purity of solanesol; □ % Recovery of purified solanesol; ▲total Bed volume]

In contrast to this, both purity and recovery of solanesol increases with decrease of ethyl acetate concentration in the hexane. Maximum purity of 96 % was obtained at 1% (v/v) concentration of ethyl acetate in mobile phase with recovery of 79%. This low elution strength was responsible for 26 BV of solvent requirement for the trial. This total solvent consumption was reduced by further optimization of purification protocol. In which the earlier quantities of equilibration was reduced to 1.5 BV from 2.0 BV and hexane washing was reduced to 1.5 BV from 4 BV. Isocratic 1% (v/v) ethyl acetate elution was split into: a) 3 BV, 0.5% EA in hexane b) 10 BV, 1% ethyl acetate in hexane. The optimized purification protocol for the purification of solanesol using alumina as adsorbent is shown in Figure 3.

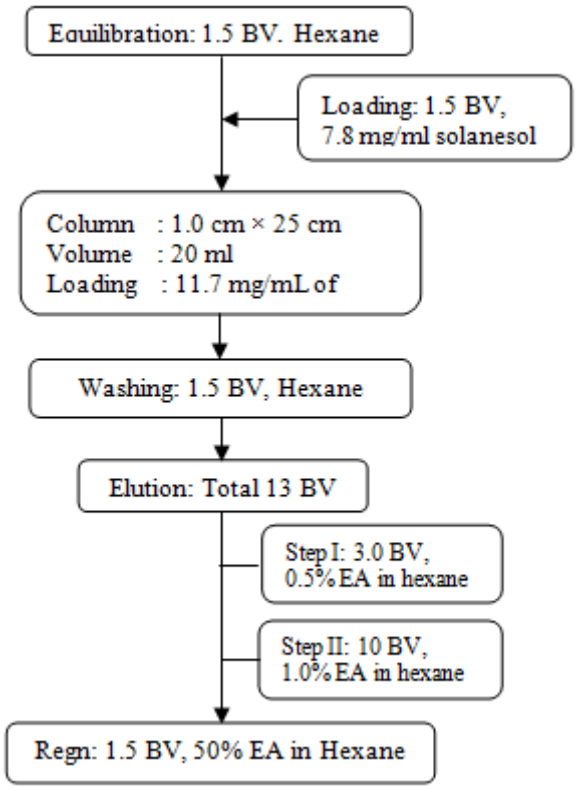


Figure 3:Process for chromatographic purification of Solanesol using Alumina

Using these modified elution conditions the overall solvent consumption was reduced down to 19 BV without affecting the 95% pooled purity of solanesol as shown in Figure 4**.**

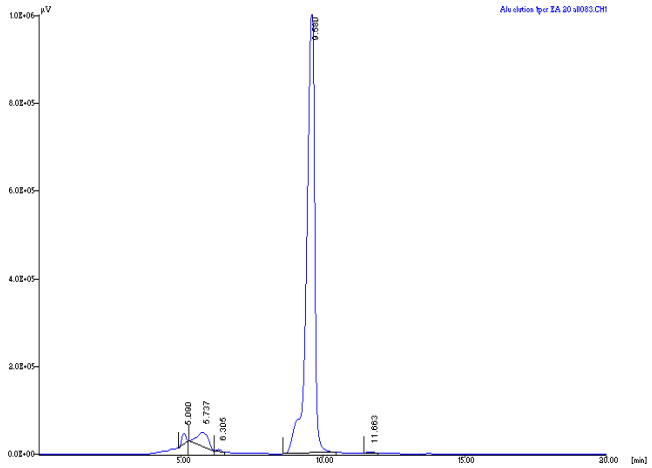


Figure 4: HPLC chromatogram of the purified Solanesol obtained during elution by 1% EA in Hexane

Conclusions

The gram scale preparative process for the purification of solanesol using Alumina as an adsorbent is successfully demonstrated in present study. The work shows miscibility characteristics of crude extract and batch desorption selectivity is useful for the designing of mobile phase and selection of adsorbent for solanesol purification. Loading conditions were designed by studying the batch equilibrium capacity. Elution conditions of 0.5% (v/v) followed by 1% (v/v) EA in hexane showed elution selectivity and maximum yield of purified solanesol. Depending on the above parameters, the developed separation process generates solanesol of 95 wt.% with recovery of 79%. Thus, developed method provides solution for the isolation of high purity solanesol from low assay crude tobacco extracts of solanesol. The loading and productivity per unit mass of solanesol can be significantly improved by applying the presented method for the high assay 50 wt.% or 75 wt.% tobacco extracts of solanesol.

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