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Development of an Efficient Extraction Method for Separating Solasodine, an Steroidal Alkaloid, From an Oily Matrix: A Comparison Between LLE and SPE

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MM and SKK designed the study and wrote the protocol. Authors SKK, MP and FS managed the analyses of the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors FS and AP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In many references of Traditional Iranian Medicine (TIM), there are methods to produce medicinal products from plant fruit based on extraction of active substances into oils. One of these recommendations is to import cucurbit or pumpkin seed oil into an entirely ripe fruit of Solanum melongena plant. In this study for the first time, the extraction of solasodine from the oily matrix has been investigated to find a precise method with suitable extraction recovery.

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Study Design: Original Research Article.
Place and Duration of Study: The study took place in herbal and traditional medicines research center of Kerman, Iran from February 2015 to November 2017.
Methodology: Solasodine, an active steroidal alkaloid metabolite of the plant, was used for standardization of the product. Two methods of (Liquid-liquid extraction) LLE and (Solid phase extraction) SPE were evaluated and their conditions were optimized by assessment of seven effective factors at five levels using HPLC as the method of choice for solasodine determination.
Results: Results showed that LLE and SPE in optimized conditions had recoveries of about 76.5 and 94.7%, respectively. For the extraction of low concentrations of the analyte, the SPE method had better accuracies (88 to 105%), but its precision was less than that of LLE method. In contrast, the LLE method had a higher precision in the whole range while its relevant accuracy was lower. The LOD and LOQ of SPE-HPLC method were 0.2 and 0.6 µg/ml and those of the LLE-HPLC method were 0.2 and 0.7 µg/ml, respectively.
Conclusion: In general, both methods of LLE-HPLC and SPE-HPLC showed acceptable validation parameters including linearity, precision, accuracy, LOD, and LOQ; and they can be used for routine extraction and determination of solasodine in traditional medicine products.

Keywords: Cucurbit; eggplant; extraction; LLE; pumpkin oil; Solanum melongena; solasodine; SPE.

1. INTRODUCTION

Traditional drugs and medicinal plants, due to the complexity of their compounds and processes of production in different conditions, sometimes may produce different therapeutic effects, which overshadow their therapeutic use and extend their resistance to use. Today, there has been a tremendous request for traditional medicines which is increasing every day. On the other hand, manufacturers of traditional drugs are confronted with several problems, such as lack of access to high-quality raw materials and the appropriate methodology for large scale production of the batches of these drugs. Reproducibility, impact and safety of these preparations depend on the quality of the production or, in other words, the standardization of these products.

Hemorrhoid is a common disease in world population. More than 50% of men and women aged 50 years and older will experience hemorrhoid or piles symptoms during their life [1]. Its treatment is more symptomatic rather than surgical one. Many traditional drugs are of particular interest for healing the injuries of the disease. "Dohn al-Badanjan" drug is recommended topically for the treatment of hemorrhoids in TIM literature such as "Tohfat al-Momenin" by "Mohammad Momen Hosseini Tonekaboni" [2] and “Makhzan-ol-Adviyah” by "Seyyed Mohammad Hossein Aghili Alawi Khorasani Shirazi" [3]. For preparation of this drug, pumpkin and cucurbit seed oils have been used and delivered to the eggplant fruit and extraction has been completed using heat in a special procedure.

Eggplant with the scientific name *Solanum melongena* is a flowering plant belonging to the Solanaceae family whose fruit is edible and has a nutritional value [4]. Various chemical compounds including flavonoids, tannins, steroids and alkaloids exist in different parts of the plant. The most important compounds of the plant fruit are glycosylated steroidal alkaloids [5]. The main glycoalkaloids of the plant are solasonine and solamargine which have several medicinal and therapeutic properties [6]. Solasodine (Fig. 1) is common aglycone of both glycoalkaloids of solasonine and solamargine and has a very high lipophilic property and some of its medicinal products are used for cancer treatment [7]. In this research solasodine has been used for standardization of "Dohn al-Badenjan" drug preparation [2,3].

Different analytical techniques such as high performance thin layer chromatography (HPTLC) [8], gas chromatography (GC) [9], high performance liquid chromatography (HPLC) [10] and potentiometry [11,12] have been reported for the determination of steroidal alkaloids in herbal drugs. Most of these compounds have a low volatility and are susceptible to elevated temperatures, so GC is not usually the method of choice for analysis of steroidal alkaloids [13], whereas, HPLC has been used for the determination of a variety of steroids and alkaloids [10].

Pre-analysis treatment of samples and extraction of the analytes of interest from the complicated matrixes are of great importance in HPLC technique. Various methods such as liquid-liquid extraction (LLE) and solid phase extraction (SPE) have been proposed to extract active metabolites from oily bases. Some investigations have been carried out for separation of toxic alkaloids which have been extracted from the seeds during the oil extraction process [13-18]. However, the extraction of steroidal glycoalkaloids from the oil bases is not a common practice; and to the best of the authors' knowledge no investigation has been conducted on the extraction of steroidal alkaloids from oily matrixes using SPE and LLE. Hence, the purpose of this study was to optimize, validate and compare LLE and SPE for extraction of solasodine from oily medium used in certain TIM.

2. METHODOLOGY

2.1 Materials, Chemicals and Solvents

All the reagents and solvents used in this study were of analytical or HPLC grade. Standard solasodine was purchased from Santa Cruz Biotechnology Inc. (USA). Manila TM pumpkin seed oil was prepared from Kimiagran Pesteh Co (Kerman, Iran). HPLC grade methanol and acetonitrile were purchased from Duksan Pure Chemicals Company Ltd. (South Korea). Deionized water was prepared using a MilliporeTM Direct-Q deionizer (USA). Buffers and mobile phases were filtered using 0.45 µm-47 mm PTFE filters (Sartorius, Germany) by a MilliporeTM vacuum pump filtration set (USA). The pH of aqueous buffers was adjusted with a Metrohm digital pH meter (Switzerland). INOPAK® silica gel cartridges (100 mg, 1 ml)(Korea) were used for solasodine SPE extraction; and the extraction steps were carried out using a SPE manifold equipped with a membrane vacuum pump (Macherey-Nagel GmbH & Co., Germany).

2.2 Methods

2.2.1 Preparation of solasodine oily base

To prepare a standardized oily specimen containing solasodine, 5 mg of solasodine was dissolved in 100 mL methanol, and then one ml of the stock solution was vortex-mixed with equal volume of pumpkin seed oil. The organic solvent of the spiked oily sample was then evaporated under gentle stream of nitrogen gas for 15 minutes.

2.2.2 HPLC instrumentation and method

The analysis of standard solutions, the LLE and SPE extracts of spiked oily samples were performed using a Smartline® HPLC system (Knauer®, Germany) consisted of pump 1000, PDA-UV detector 2600, manger 5000, autosamplers 3950, membrane vacuum degasser and column temperature compartment. Separations were carried out on a Knauer® C18 column (250×4.6 mm, 5 µm) (Germany) kept at 25°C, using KH2PO4 buffer (pH 2.5):MeOH (25:75%v/v) as mobile phase in an isocratic elution mode. A volume of 50 µL of the standard and samples were automatically injected into the system and analyzed at flow rate of 1 ml/min in a 12 min run time while the UV detector was set at 205 nm. The software ChromGate® version 3.1.7 (Knauer®, Germany) was utilized for data acquisition and processing.

2.2.3 Liquid-liquid extraction

Sample preparation and LLE method of solasodine extraction was composed of four main stages including: I) acid hydrolysis of the steroidal glycoalkaloid, II) partitioning of ionized form of solasodine (aglycone) into aqueous phase, III) back-extraction of non-ionized form of the analyte into organic phase, IV) evaporation of the organic phase and reconstitution the residue for HPLC analysis. Briefly, the oily phase containing the analyte was treated with acid modifier and extracted with water. After basifying the aqueous phase with a suitable alkali, the analyte was extracted into chloroform using sonication or dispersion procedures. The chloroform phase was then washed with distilled water and evaporated under gentle stream of N_2 to dryness. The residue was finally reconstituted with mobile phase and analyzed using HPLC method.

To optimize the LLE extraction conditions, the following parameters (variables) were assessed: type and volume of the hydrolyzing acid (designated with codes A and B); type and volume of the alkali added to aqueous phase (encoded with C and D); volume of the extractant (indicated E), time of shaking oily and aqueous phases (encoded F), and the number of consecutive extraction on a sample (designated G). In our experimental approach, different levels for each of the above-mentioned variables were defined; and then the effect of the variables on the recovery of LLE method was evaluated. Table 1 shows the variables and their tested

levels and Table 2 shows the corresponding results of the recovery assessment. The recovery efficiency was calculated and expressed as the percent ratio of extracted spiked sample to non-extracted standard sample with the same concentration.

2.2.4 Solid phase extraction

INOPAK® silica gel cartridge (100 mg, 1 ml) as a polar SPE sorbent was used to extract solasodine form the pumpkin oil preparation. Sample preparation and SPE procedure was consisted of the following stages: I) acid hydrolysis of the steroidal glycoalkaloid, II) conditioning of SPE cartridge, III) loading and retention of ionized form of solasodine on the solid phase, IV) washing out the interferences, V) elution of the analyte form the SPE sorbent, VI) evaporation of the organic phase and reconstitution the residue for HPLC analysis.

In order to have adequate interaction between the solvents/analyte and the solid phase, the low-pressure condition applied on the manifold during SPE stages was adjusted to a flow rate of 25 drops/min. In conditioning stage of SPE, 1 ml of pure MeOH passed through the columns to activate the silica beds and then the cartridges were treated with acidic mixture of MeOH:H2O (20:80% v/v) to equilibrate the columns before loading of the samples. Following loading of samples, 3 ml acidic mixture of MeOH:H2O (20:80% v/v) was applied on the cartridges to wash out the interferences. In the elution stage of SPE, alkaline MeOH was passed through the cartridges to desorb the non-ionized solasodine form the sorbent bed. Then, a centrifugation step (10 min at 4000 rpm) was carried out on the elution effluent and the alkaline methanolic phase was evaporated to dryness under gentle stream of N2. The residue was reconstituted in 1 ml MeOH, vortex-mixed, and finally analyzed by HPLC method.

In order to optimize the extraction conditions of SPE method, the following variables were assessed: type (encoded A), concentration (designated B) and volume (indicated as C) of the acid modifier added to MeOH in SPE conditioning stage; type (encoded D), concentration (designated E) and volume (indicated F) of the alkali added to MeOH in SPE elution stage; the number of consecutive extraction on a sample (encoded G). Similar to LLE study, the same approach was utilized to evaluate the effect of the variables on the recovery of SPE method (Tables 4 and 5).

2.2.5 Validation of optimized extraction methods

To validate the extraction methods, several parameters were considered including linear range, precision, accuracy, LOD, and LOQ. Relative standard deviation (RSD%) of triplicate analyses was the criterion for assessment of the precision at intra- and inter-day (intermediate) levels. Accuracy was also evaluated by calculation the ratio percent of the measured value to the actual one at three solasodine concentrations of 1, 6 and 25 µg/ml. To calculate LOD and LOQ, several analytical blanks were analyzed and the signal from noise observed at or close to the retention time (t_R) of the analyte was measured and considered as a criterion. Formulas I and II were used to calculate these two parameters.

(I) LOD = 3.3SDblank/Slope
(II) LOQ = 10 SDblank/Slope

SD blank : Standard deviation of analytical blanks
Slope : The slope of the standard calibration curve

3. RESULTS AND DISCUSSION

Solasodine has a steroidal non-polar structure with a logP of about 5 and a weak secondary amine part with a pKa of 7.7 [19, 20] (Fig. 1). Due to its lipophilic nature, solasodine extraction from the oily matrixes such as cucurbit and pumpkin oil is quite a big challenge. Controlling of ionized and non-ionized forms of the analyte(s) is of high importance in different stages of extraction techniques. Having chosen a certain volume of a proper acid and/or alkali with an appropriate concentration, one can manage to maximize the extraction recovery and efficiency.

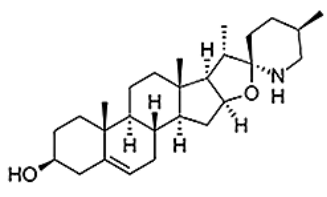

Fig. 1. Chemical structure of solasodine

3.1 Optimization of LLE Extraction

In LLE method, the glycoalkaloid has been firstly hydrolyzed and converted into water-soluble salt by acid addition; and solasodine enters the aqueous phase. By addition of an alkali, the alkaloid salt turns into its free base alkaloid and can be extracted by low polarity solvents such as chloroform. The results of optimization of LLE method are presented in Table 2. Among 0.1 M formic, hydrochloric, acetic, and sulfuric acids used for hydrolysis of the glycoalkaloid, the best result was obtained using formic acid leading to a recovery of 25.3%. Different volumes of the acids (0.4, 0.8, 1.2, 1.6, 2, 2.4 and 2.8 ml) were tested and the optimum recovery was achieved with 2 ml 0.1 M formic acid. The results of LLE optimization (Tables 1 and 2) showed that the use of organic acids exhibited much better recoveries than that of inorganic ones during hydrolysis of glycoalkaloid and ionization of solasodine. Volume and concentration of the applied acid were also evaluated as an effective factor. If the amount of acid is sufficient it can ionize all free solasodine in the oil; meanwhile choosing proper amount of acid reduces the need for alkali in the next steps. The optimum acidic condition was provided by addition of 2 ml 0.1 M formic acid into the oily base.

To alkalize the aqueous phase for converting solasodine into its free base form in back-extraction step of LLE method, 25% w/v solutions of NaOH and NH_4OH were tested. The best results obtained using NH_4OH with a recovery of 25.6%. Different volumes (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ml) of the alkaline solution were examined and the optimum recovery was achieved using 200 µl of NH4OH. The use of organic alkali (NH4OH) brought about much better results in comparison with inorganic one (NaOH). It was revealed that a volume of 200 µl of concentrated ammonia solution is enough to fully suppress the ionization of solasodine during its back-extraction into organic phase.

In the step of back-extraction of solasodine with water-immiscible organic solvent, volumes of 1, 1.5, 2, 2.5 and 3 ml chloroform were examined from which 2 ml extractant resulted in the highest recovery about 25%. For entire mixing the hydrolyzed oily sample and aqueous layer, two phases had to be vortex-mixed vigorously. Hence, periods of 1, 2, 3, 4, and 5 min were tested in this regard where 2 min shaking of phases showed the highest result with a recovery of 25.3%. Another examined variable was the number of repeat extractions carried on a sample; the results showed that the best recovery was observed after 6 sequential extractions on one oily sample with a recovery efficiency of 52.4%; less repeats diminished the total efficiency of the LLE method.

Table 1. The type and volume of acid and alkali, volume of extractant, shaking time and number of extraction, codes assigned to each variables and their examined levels in the LLE method

Variable	Code	Examined levels of variable						
		Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7
Hydrolyzing acid (0.1 M)	A	HCOOH	CH3COOH	HCl	H2SO4	-	-	-
Volume (ML) of acid	B	0.4	0.8	1.2	1.6	2	2.4	2.8
The base used for alkalization	C	NH4OH (25%w/v)	NaOH (25% w/v)	-	-	-	-	-
Volume (ML) of alkalizing base	D	0.1	0.2	0.3	0.4	0.5	0.6	-
Volume (ML) of extractant	E	1	1.5	2	2.5	3	-	-
Time (MIN) of shaking oily & aqueous phases	F	1	2	3	4	5	-	-
No. of sequential extractions on a sample	G	1	2	3	4	5	6	7

Table 2. Comparison of the effect of different variables at various levels to optimize the recovery of the LLE method

Extraction stage	Fixed conditions in each stage	Extraction recovery (%) at different levels of variable						
		Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7
Hydrolyzing acid	B5.C1.D2.E3.F2.G1	25.3	22.2	16.6	13.7	-	-	-
Volume of the acid	A1.C1.D2.E3.F2.G1	6.4	8.3	12.9	19.1	24.9	24.8	25
Alkalyzing base	A1.B5.D2.E3.F2.G1	25.6	8.9	-	-	-	-	-
Volume of the base	A1.B5.C1.E3.F2.G1	14.5	25.4	24.9	25.3	24.8	25.5	-
Volume of extractant	A1.B5.C1.D2.F2.G1	18.9	22.2	25	24.9	25.1	-	-
Time of shaking	A1.B5.C1.D2.E3.G1	20.9	25.3	25.1	25.2	25.1	-	-
No. of sequential extractions	A1.B5.C1.D2.E3.F2	25.4	38.3	41.4	45.6	48.1	52.4	52.5

Table 3. Comparison between sonication and dispersion procedures and the effect of repeat partitioning on solasodine recovery percentage from an oily matrix

No. of repeats in A sequential LLE partitioning	LLE average recovery % (N=3)		
	Without sonication	With sonication	With dispersion
1	25.4	31.3	36.1
2	38.3	40.7	47.0
3	41.4	43.6	58.0
4	45.6	47.1	68.0
5	48.1	48.4	74.0
6	52.4	51.9	76.5
7	52.5	52.3	76.6

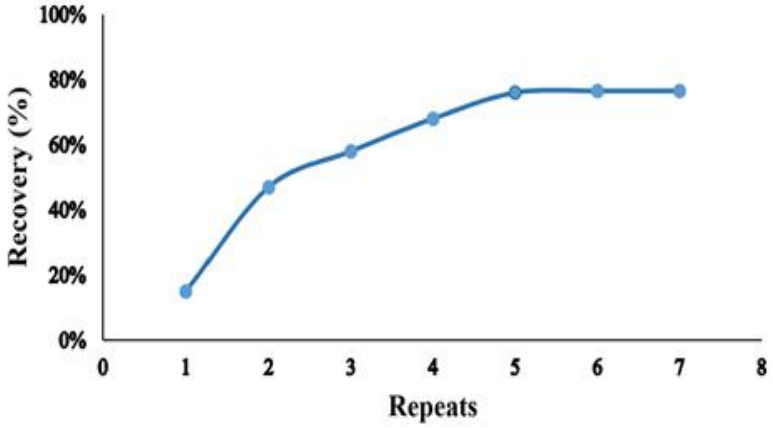

Fig. 2. The effect of repeats of LLE partitioning on the recovery% of solasodine from one sample using dispersion procedure

3.2 Comparison between Sonication and Dispersion Techniques on LLE Efficiency

In LLE methods, it is very essential to have a high level of contact between two phases for optimum partitioning of the analyte into the extractant. In the current study, sonication and dispersion techniques were used in this regard. [10] As shown in (Table 3), sonication exhibited slightly improved recovery average on the first four repeats of a sequential extraction without definite effect on recovery on the 5th, 6th, and 7th repeats. Dispersing method was carried out

Table 4. Type and volume of acid and alkali, volume of extractant, the numbers of extraction, codes assigned to each variable and their examined levels in the SPE method

Variables	Code	Examined levels of variable				
		Level 1	Level 2	Level 3	Level 4	Level 5
Type of acid used in conditioning stage	A	HCOOH	CH3COOH	HCl	-	-
Concentration of acid (% v/v)	B	0.05	0.1	0.25	0.5	1
Volume of acidic solution (ml)	C	1	2	3	4	5
Type of alkali used in elution stage	D	NH4OH	NaOH	-	-	-
Concentration of alkali (% v/v)	E	1	1.5	2	2.5	3
Volume of alkaline solution (ml)	F	2	4	6	8	10
Consecutive extraction on a sample	G	1	2	3	4	5

Table 5. Comparison of the effect of different variables at five levels to optimize the recovery of the SPE method

Extraction stage	Fixed conditions in each stage	Extraction recovery (%) at different levels of variable (n=3)				
		Level 1	Level 2	Level 3	Level 4	Level 5
Acid used in conditioning stage	B2.C3.D1.E4.F3.G2	91	78	56	-	-
Concentration of acid	A1.C3.D1.E4.F3.G2	65	88	85	83	76
Volume of conditioning	A1.B2.D1.E4.F3.G2	59	71	90	88	89
Alkali used in Elution stage	A1.B2.C3.E4.F3.G2	81	32	51	-	-
Concentration of alkali	A1.B2.C3.D1.F3.G2	47	69	83	89	88
Volume of elution	A1.B2.C3.D1.E4.G2	39	75	92	86	87
Consecutive extraction on a sample	A1.B2.C3.D1.E4.F3	49	89	90	90	89

using a long-needle syringe which resulted in enhancement of the recovery of solasodine from 25.4% to 36.1% during just in one step of extraction. With more repeat extractions, the recovery increased gradually so that six consecutive partitioning of chloroform with a sample using the dispersing method led to a considerable increase in recovery up to 76.5% (Table 3 and Fig. 2). Formation of small droplets of chloroform within the aqueous phase in dispersing method causes a substantial increase in the interface of two phases leading to a faster and efficient transfer of the analyte into the extractant.

3.3 Optimization of the SPE Method

Silica gel cartridges as normal phase SPE sorbents have been widely used for the easy separation of polar and non-polar compounds from edible oils. [21] Similar to LLE, the physic-chemical properties of the analyte, solvents, pH-modifiers, ion-pairing reagents, and in the case of SPE surface chemistry of the sorbent have critical roles for efficient and selective extraction of the analytes and elimination of the interferences. In this regard, adjusting the pHs of conditioning solvent of SPE cartridges, sample solvent, washing out solvent, and elution solvent are important parameters. The type, concentration, and volume of the acid and/or the alkali used for pH control and the organic solvents used in different stages of SPE were optimized in the current study.

Methanol as the first choice solvent was used for activation of solid phase bed due to its good wetting and penetration properties in the silica sorbent. Meanwhile, the solubility of solasodine in this solvent is very good; therefore, it is strong

enough for efficient elution of the analyte in the final stage of SPE, i.e. elution stage. In an acidic environment, solasodine is mostly in its ionized form due to the protonation of its secondary amine; therefore, solasodine distribution into aqueous phase is facilitated at lower pHs than its pKa. The mixture of acidic MeOH:H2O (20:80% v/v) was used as conditioning solvent for SPE cartridges. As shown in (Tables 4 and 5), HCOOH as acid modifier at concentration of 0.1% v/v could give the highest recovery among the examined acids, i.e. formic, acetic, and hydrochloric acids. In addition, it was found that a volume of 3 ml acidic MeOH:H2O (20:80% v/v) is sufficient to equilibrate the SPE cartridges before loading of the oily sample. The greater volumes of the solvent did not result in higher recoveries.

Table 6. Average recovery of multiple SPE extraction of spiked (50 µg/ml) oily samples using the optimized method

Sample	Recovery %
1	91.1
2	98.3
3	90.9
4	94.2
5	95.6
6	97.9
Mean±SD	94.7±3.2

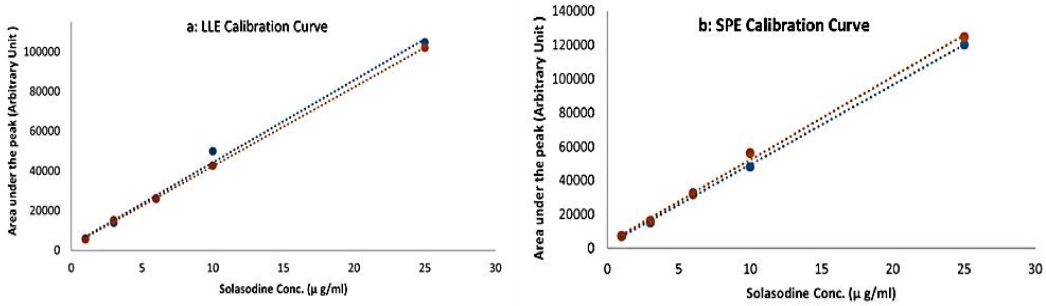

Fig. 3. Calibration curve for LLE (a) and SPE (b) extraction of solasodine from oily matrix
Range: 1-25 µg/ml (Mean±Sd, n=3); blue and red lines are relevant to intra- and inter-day precision levels, respectively

Table 7. Linearity parameters of LLE & SPE methods for solasodine extraction in the range of 1-25 µg/ml

Linearity parameters	LLE		SPE	
	Intra-day	Inter-day	Intra-day	Inter-day
R^2	0.99975	0.99364	0.99764	0.99916
Slope	3987.8	4145.6	4918.3	4720.6
Intercept	2519.1	2862.3	3116.2	2204.4

Table 8. Precision of LLE & SPE methods for solasodine extraction from oily matrix at intra- & inter-day levels

Conc. (µg/ml)	LLE precision (RSD %)		SPE precision (RSD %)	
	Intra-day	Inter-day	Intra-day	Inter-day
1	5.3	5.4	9.5	8.1
6	3.1	3.7	11.9	13.7
25	4.4	2.8	10.5	15.7

Table 9. Accuracy of LLE & SPE method for solasodine extraction from oily matrix at different concentrations

Expected conc. (µg.ml-1)	Measured conc. (µg.ml-1)		Accuracy %	
	LLE	SPE	LLE	SPE
1	0.8	0.9	76.1	88.1
6	5.6	5.9	93.2	98.1
25	24.6	24.7	98.4	99.0

Ammonia and NaOH were tested to alkalize MeOH as the elution solvent to desorb solasodine from the SPE cartridges in the final stage. The best results were obtained with 6 ml ammoniacal MeOH at a concentration of 2.5% v/v. It was found that 3×2 ml aliquots of this elution solvent with a 30-second soaking time between the portions resulted in a recovery of 92%. It was revealed that formic and acetic acids gave rise to a better recovery than inorganic acid (HCl). Also, adjustment of the pH of MeOH with organic alkali (NH4OH) in elution stage of SPE yielded better extraction efficiencies than that of inorganic alkali, i.e. NaOH.

Consecutive extractions carried out on a sample could help to increase the recovery of SPE. Two-time passages of the acidified oily sample through SPE column could improve the recovery of solasodine extraction up to 89%; however, more than two times successive loading did not lead to much more increase in the recovery.

The overall optimum conditions of SPE were applied on 6 spiked oily samples containing 50 µg/ml solasodine in order to assess the repeatability of the method (Table 6). The average recovery was 94.7% with a SD of 3.2%.

3.4 Validation Parameters of the Methods

The calibration curve was plotted in the range 1-25 µg/ml for each of the optimized extraction methods. Regression line equations were assessed at intra- and inter-day levels (Fig. 3-a & 3-b). The linearity parameters, precision, accuracy, LOD, and LOQ of LLE-HPLC and SPE-HPLC methods are shown in (Tables 7, 8, 9, and 10) respectively.

3.5 Comparison between the LLE & SPE Methods

Reducing the amount of organic solvents, easier collection of the analyte, and the capability of simultaneous and multiple extractions were the main advantages of solasodine SPE method compared with the LLE one. Meanwhile, in low concentration of the analyte, average accuracy of the SPE method was much better than that of LLE. However, SPE method had less precision at both intra- and inter-day levels (RSD %) than LLE. Lower repeatability of the SPE method could be due to slight differences between SPE cartridges from one to another and also due to the influence of oily matrix on the polar sorbent bed of SPE cartridges. Both LLE and SPE methods exhibited repeatable regressions at intra- and inter-day levels in the examined concentration range; R^2 of the equation lines were in the range of 0.99364 to 0.99975.

Table 10. LOD & LOQ of LLE-HPLC and SPE-HPLC methods for solasodine determination in oily matrix

Method	LOD (µg/ml)	LOQ (µg/ml)
LLE-HPLC	0.2	0.7
SPE-HPLC	0.2	0.6

There are several studies which have been conducted to extract solasodine from non-oily media. Hock et al. [13] has been extracted solasodine from a non-oily base using SPE process with a C18 column. Harry H. Janker et al. [15] used CN SPE columns to clean up the potato samples and measure its steroid glycoalkaloids. Similar researches have been also done to extract and to remove the toxic alkaloids from oil seeds using LLE method. Ortiz & Mukherjee [16] reported the extraction of quinalozidine alkaloids from bitter lupin seeds. They extracted the oil by hexane and isolated oily fraction containing free base quinolizidine alkaloids using hydrochloric acid. In another study by Wretensjo & Karkberg [18], the effect of different processing steps on the purification of unsaturated pyrrolizidine alkaloids such as thesinine, crotaline and restoresine in Borage oil was investigated, and finally, the LLE method was used to extract the alkaloids. In the proposed method of Huizing et al.[14], sulfuric acid as acid modifier and chloroform as extractant were used to extract pyrrolizidine alkaloids. The organic phase was discarded and the remaining aqueous phase contained alkaline alkaloids was extracted with chloroform. In a research by Rahman et al. [17] for separation of the alkaloids, the oil of the Karanja seeds (*Pongamia pinnata* Linn) was extracted 3 times with 5% hydrochloric acid, then it was washed with water and the aqueous phase was used for alkaloid determination.

4. CONCLUSION

In this study for the first time, we tried to find a precise, efficient and low cost method for extraction of solasodine from oily matrixes. Two methods of LLE and SPE were investigated in this regard and their validation parameters including linearity, precision, accuracy, LOD, and LOQ were assessed. Both methods showed acceptable accuracies and good linearity parameters in the examined concentration range. The LLE method showed higher repeatability

levels than that of SPE while its recovery percentage was lower. The LLE method had several time-consuming stages with higher solvent consumption; in contrast, the SPE method had fewer steps than that of LLE whereas its main cost comes from supplying the cartridges.

In general, both methods can be used for routine extraction of solasodine from oily traditional medicine products. However, for studies in which the oily sample volume is minimized, the budget is enough, and there is limitation of the expert technicians, then SPE method could be the method of choice. In contrary, if the sample volume is greater, the funding is insufficient, and there is no limitation of expertise, then one can choose LLE method for solasodine extraction.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by any producing company rather it was funded by personal efforts of the authors and supported by Deputy of Research of Kerman University of Medical Sciences.

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