Talanta 81 (2010) 565–571

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Sequential injection analysis with lab-at-valve (SI-LAV) for the determination of solasodine in Solanum species

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ARTICLE INFO

Article history:
Received 8 October 2009
Received in revised form
20 December 2009
Accepted 21 December 2009
Available online 4 January 2010

Keywords:
Sequential injection analysis
Lab-at-valve (LAV)
Semi-automated liquid–liquid extraction
Spectrophotometric determination
Solasodine
Solanum species fruits

ABSTRACT

The development of sequential injection analysis with lab-at-valve (LAV) semi-automated system on-line liquid–liquid extraction is demonstrated for spectrophotometric determination of solasodine in various Solanum species fruits. The main proposed is semi-automated extractive determination of solasodine using methyl orange as colorimetric reagent. After optimization of the system, sample, reagent and organic solvent were sequentially aspirated into an extraction coil connected to the center of a selection valve, where extraction took place by flow reversal. The aqueous and organic phases were separated in a lab-at-valve unit attracted to one of the ports of the selection valve. The absorption of ion-pair solasodine–methyl orange complex in the organic phase was measured spectrophotometrically at 420 nm. The method performances, including reproducibility, linearity, sensitivity and accuracy, were also evaluated. The proposed method is simple, reproducible and accurate. It was successfully applied to the determination of solasodine in *Solanum aculeatissimum* Jacq., *Solanum violaceum* Ortega., *Solanum melongena* Linn. and *Solanum indicum* Linn. fruits in Solanaceae family. Results obtained were in good agreement with those obtained by batch wise spectrophotometric method. It is also suitable and useful for determination of solasodine in other medicinal plants.

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1. Introduction

Solasodine, a spiroketal alkaloid sapogenin with a hetorocyclic nitrogen atom (Fig. 1), is used for the production of steroid drug in medical industry. It is also used in the preparation of contraceptive drug. Solasodine is present in a number of Solanum species (Solanaceae) such as *S. khasianum*, *S. xanthocarpum*, *S. nigrum*, *S. gracile*, *S. laciniatum*, etc. [1]. A number of traditional herbs containing solasodine have been used in the Indian System of Medicine [2–5]. Solasodine have been reported to provide anticancer [5], insecticidal [6], antiaccelerator cardiac activities [7], and antioxidant activities of the chloroform extract [8]. A number of analytical methods such as high performance thin layer chromatography [9–12], high performance liquid chromatography [13–21], capillary electrophoresis [22–24], gas chromatography [25–28] and colorimetric method [29–36] are available for determination of solasodine from its plant. Solasodine does not have a conjugated double bond in its structure. The nitrogen is protonated and forms complexes that are extractable into organic solvent like chloroform.

Liquid–liquid extraction is one of the most versatile techniques for sample matrix separation. It has been applied to various analytical fields. However, manual extractions present a series of drawbacks such as high consumption of sample and toxic organic solvents, low sampling frequency and loss of analyte through manipulation. Among them, the successful techniques are probably the on-line liquid–liquid extraction using flow systems [37–39]. A variety of flow-based systems have been reported for the on-line liquid–liquid extraction. Flow injection liquid–liquid extraction was proposed by Karlberg and Thelander [40] and Bergamin [41]. Consumption of reagent and organic solvent are lower than those in manual procedures, consequently, reducing the waste generated.

Sequential injection analysis (SIA) has also been employed for on-line liquid–liquid extraction by sequential aspiration of a small volume of organic solvent and aqueous sample into contact before determination resolve them using phase separator [42,43].

In recent years, special attention was given for the development of automated analytical procedures. A lot of new instrumental methods were described via sequential injection analysis [44] and bead injection analysis [2]. SIA has been introduced in 2000 by Ruzicka [44] with lab-on-valve (LOV) module. A number of SIA pro-

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doi:10.1016/j.talanta.2009.12.044

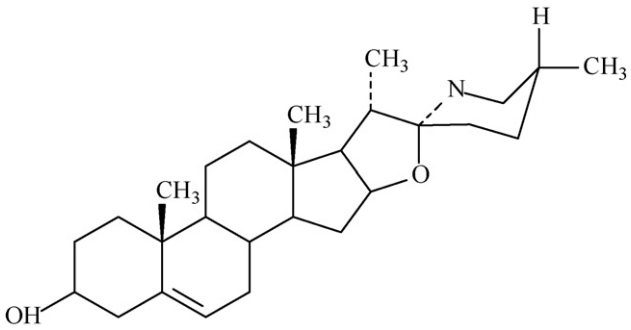

Fig. 1. Structure of solasodine.

cedures have been described for determination variety of samples with various matrices. For example, Makchit et al. [46] descried the SIA method for determining cefadroxil in pharmaceuticals. Leelasattarathkul et al. [47] reported the LOV procedure for determination of small amounts of copper (II) in wastewater.

Sequential injection analysis with a simple approach called "lab-at-valve" (LAV), introduced by Rodjana et al. [45,48]. The technique is simple and economic which become an alternative cost effect systems for on-line automated extraction. Less consumption of the sample, reagent and organic solvent was achieved and compared to the conventional batch and FIA method. No published articles have been described for determination of solasodine in Solanum species fruits based on SIA and related methods (LOV and LAV).

In the present work, the SIA–LAV system was developed for simple semi-automated on-line liquid–liquid extraction. A desired component; a separation chamber was attached at one port of a multi-position selection valve. The sample solution, reagent and organic solvent were sequentially aspirated into an extraction coil. After that, the aqueous and organic phases were separated in a conical separating chamber. The organic phase containing sample product complex was measured by spectrophotometer. The proposed method has been successfully applied to the determination of solasodine in various Solanum species fruits.

2. Experimental

2.1. Chemicals and reagents

All of reagents used were analytical reagent grade. Deionized water of milli-Q water purification system, Millipore Co., USA was used throughout the experiments. Solasodine reference standard of 99% purity was purchased from Sigma (No. 204-774-2, Sigma Chemical Co., USA).

Stock standard solution of solasodine (1000 μg mL^{-1}) was prepared by accurately weighed 50 mg of solasodine reference standard into a 50 mL volumetric flask and dissolving in 20% acetic acid which was diluted to the mark with 20% acetic acid and stored in plastic vial cap in the freezer. Working standard solutions with lower concentrations were freshly prepared by diluting the stock solution with 20% acetic acid to obtain appropriate serial concentrations.

Stock solution of methyl orange (0.04%, w/v) was freshly prepared daily by dissolving 0.04 g of methyl orange in 100 mL of deionized water and kept in a suitable reagent bottle.

2.2. Instrumentations

The developed SIA manifold (Fig. 2) was arranged using the following equipment: a FIAlab® 3000 system (FIAlab® Instruments, USA) consisting of a syringe pump (syringe reservoir 2.5 mL) and a six-port selection valve (Valco Instrument Co., USA), which is connected to a four-port switching box. The four ports undergo the following functions:

Port A is connected to a syringe control (CAVRO XL 3000 stepper motor-driven syringe pump).
Port B is available for other instruments.
Port C is connected to a valve control unit.
Port D is connected to Jenway 6300 Spectrophotometer.

A Jenway 6300 Spectrophotometer (Jenway, Dunmow, Essex, UK) equipped with a model QS1.000 Hellma flow cell (10-mm path length, 120 μL inner volume) over the wavelength range 320–1000 nm. The syringe pump was connected to the center of the selection valve via the extraction coil (0.635 mm i.d. × 150 cm PTFE tubing around the small test tube). A separating chamber type conical shape [45] (8 mm i.d. of the wider end × 7 cm long) modified from a 1.0 ml pipette tip (Eppendoft, Germany) was placed at port-1 of the selection valve. The absorbance signal of the colored complex was passed through a flow-through cell in a spectrophotometer. An absorbance signal can be retrieved directly from a Jenway spectrophotometer via the RS232 interface. The absorbance signal was measured at 420 nm through a 10 mm path length flow cell. All electrical devices of the manifold were connected to a personal computer controlled by means of a homemade program written

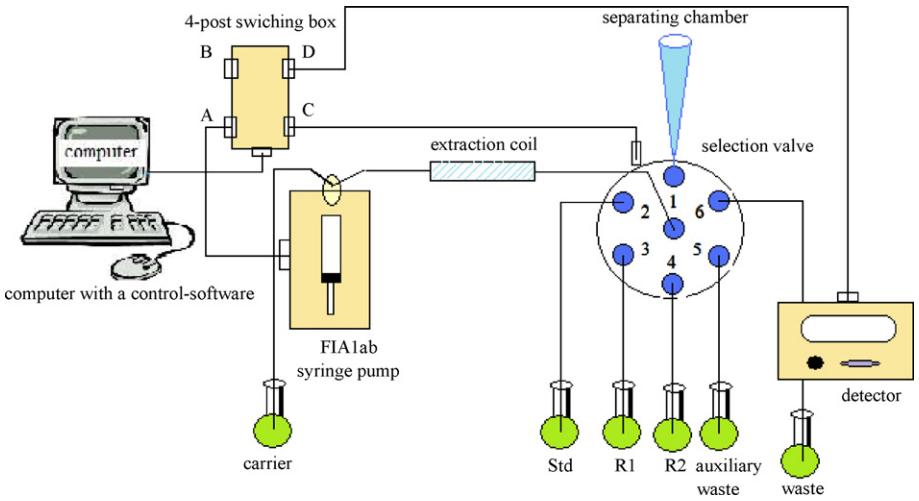

Fig. 2. Schematic diagram of SIA system with lab-at-valve (LAV).

in Microsoft Visual Basic 6.0 developed by Alpha Flow Research Group [46,47].

2.3. Sequential injection method

A four-port RS232 switching box receives an activation command from the PC through master port. When the system is initialized, it activates port A (Fig. 2) move the piston of the syringe to zero position. It also activates port C to actuate with the valve at position 6. Then, it activates port A to drive the syringe to aspirate the carrier with the desired volume. After that, it activates port C to actuate the valve at position 6 and sending empty syringe. Then, it again activates port C to actuate the chloroform (valve at position 3) was firstly aspirated into the extraction coil. Next, the solasodine standard solution (valve at position 2) was introduced. Then, methyl orange (valve at position 4) was aspirated and the extraction was done by programming the syringe control to aspiration and dispensed modes. After extraction in the extraction coil, the solution was propelled to the separating chamber, where the separation between aqueous and chloroform phases occurred. The chloroform phase containing ion-association compound was re-aspirated into the extraction coil for transportation to a detector. While the PC is sending the empty syringe command through port A, it activates port D and receives absorbance signals from the spectrophotometer and drives the plot module to plot the SIA grams on the screen. The maximum peak heights were also detected at 420 nm and displayed in this process. The time required to analyze one sample is approximately 5 min. Table 1 lists the steps of the procedure entered to the FIAlab® for windows software.

Table 1
Experimental protocol as shown in the FIAlab® for Windows software.

Fill syringe
Loop Start # 1
SyringePump Flow rate (microliter/sec) 100
SyringePump Valve In
SyringePump Delay Until Done
SyringePump Aspirate (μL) 2500
SyringePump Valve Out
SyringePump Delay Until Done
Standard/sample and reagents to extraction coil
Loop Start # 8
SyringePump Flow rate (microliter/sec) 100
Valve port 3
SyringePump Aspirate (μL) 75
SyringePump Delay Until Done
Valve port 4
SyringePump Aspirate (μL) 12.5
SyringePump Delay Until Done
Valve port 2
SyringePump Aspirate (μL) 37.5
SyringePump Delay Until Done
Loop End
SyringePump Valve In
SyringePump Fill
SyringePump Delay Until Done
Extraction
Loop Start # 3
SyringePump Valve Out
Valve port 1
SyringePump Flow rate (microliter/sec) 100
SyringePump Dispense (μL) 1000
SyringePump Delay Until Done
SyringePump Valve Out
Valve port 1
SyringePump Aspirate (μL) 1000
SyringePump Delay Until Done
SyringePump Valve Out
Valve port 1
SyringePump Flow rate (microliter/sec) 100
SyringePump Aspirate (μL) 1000
SyringePump Delay Until Done
SyringePump Valve Out
Valve port 1
SyringePump Dispense (μL) 1000
SyringePump Delay Until Done
Delay (sec) 30
Send standard/sample to detector
Valve port 1
SyringePump Flow rate (microliter/sec) 100
SyringePump Aspirate (μL) 250
SyringePump Delay Until Done
SyringePump Valve In
SyringePump Fill
SyringePump Delay Until Done
SyringePump Valve Out
Valve port 6
SyringePump Flow rate (microliter/sec) 50
SyringePump Empty
SyringePump Delay Until Done
Clean extraction coil
Valve port 1
SyringePump Flow rate (microliter/sec) 100
SyringePump Aspirate (μL) 1000
SyringePump Delay Until Done
SyringePump Valve In
Valve port 5
SyringePump Empty
SyringePump Delay Until Done
SyringePump Valve In
Valve port 1
SyringePump Dispense (μL) 1500
SyringePump Delay Until Done
SyringePump Aspirate (μL) 1500

2.4. Sample preparation

The steroids based of the solasodine group occur naturally as the glycoside usually containing three sugars. The hydrolysis of the glycoside yield the steroid alkaloid in the aglycone forms for example, the glycoside solanine, solasodine and glucose, galactose and rhamnose. Alkaloid content of this material is usually determined by extraction of the dried material using continuous extraction apparatus, removal of the solvent and precipitation of the bases, follow by dissolving in acid.

About 1 g of each dried fruit (previously dried at 50 °C for 4 h) was accurately weighed and transferred into a clean mortar. The sample was ground and 20 mL of 95% ethanol was added and mixed thoroughly, then transferred into a—250 mL beaker. The solution was heated in a water bath at 70 °C for 30 min, and then filtered through Whatman No. 1 filter paper into a—50 mL volumetric flask, followed by washing with several portions of 95% ethanol. Each solution was adjusted to 50 mL with 95% ethanol.

Aliquot of 5.0 mL of ethanolic extract from each sample was transferred into a—20 mL test tube and completely removed ethanol by holding tubes at 70 °C while gently blowing an air current into each tube. To each tube, 3.0 mL 1.0 mol L^{-1} hydrochloric acid was added, and the temperature of water bath was increased to 100 °C. The tube containing each acidified sample solution was then held at 100 °C for 2 h. The acidic sample solution was neutralized by adding 3.0 mL 1.0 mol L^{-1} sodium hydroxide followed by addition of 2.0 mL glacial acetic acid into each tube. Each solution was transferred into a 25 mL volumetric flask and then diluted to the mark with distilled water.

3. Results and discussion

Solasodine contains heterocyclic nitrogen but without conjugated double bonds in its structure. Therefore it does not give absorption spectrum in UV region. After acid hydrolysis, it yields aglycone which can be complexed with methyl orange forming

Table 1 (*Continued*)

SyringePump Delay Until Done
SyringePump Valve In
Valve port 5
SyringePump Empty
SyringePump Delay Until Done
Loop End

an ion-pair complex (solasodine–methyl orange complex). After the colored complex is extracted into chloroform it exhibited an absorption spectrum with maximum absorption at 420 nm [29].

3.1. Spectral characteristics

A preliminary experiment was carried out to investigate the spectral characteristics of the above complex. The hydrolysis product of solasodine (aglycone) was complexed with methyl orange solution at pH 3–4 which was then extracted into chloroform yielding a yellow colored complex. The absorption spectrum of such a complex was studied by batch wise spectrophotometrically, the absorption spectrum of ion-pairing solasodine–methyl orange complex in chloroform was obtained by scanning the wavelength over the range of 240–600 nm. It was found that the yellow color of solasodine–methyl orange complex formed at pH 3–4 which showed maximum absorption at 420 nm.

3.2. Investigation of the experimental parameters

The conditions for the determination of solasodine were investigated by studying the influences of various parameters such as operational sequence, the number of flow reversals, reagent/carrier flow rates and reagent concentrations of the respective measurements. The optimal value for each parameter was judging from maximum response of the detector, minimum noise of the baseline and relative standard deviation.

3.2.1. Aspiration order of reagents and sample

In reactions involving multiple zone penetrations, it is essential to examine the aspiration order of reagents and sample [49]. The sequence order of operation is one of the important factors that determine the time and efficiency that the aqueous and organic phases are in contact for improving the extraction efficiency. Two sequential orders as shown in Fig. 3 were examined. It can be seen

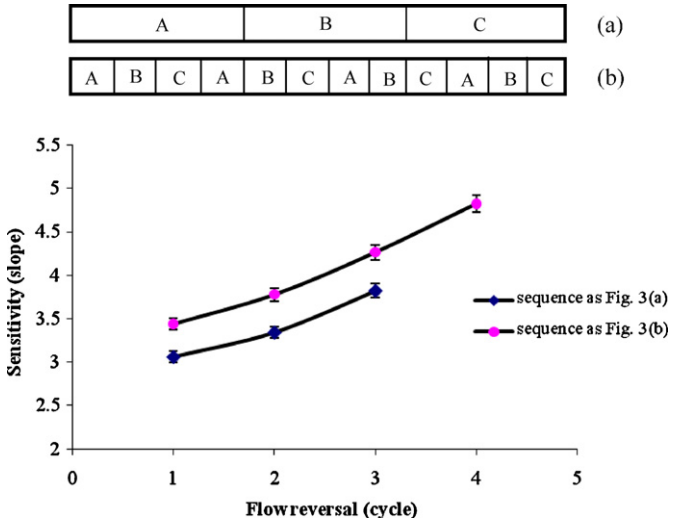

Fig. 3. Sequence order of the SIA–LAV system and flow reversal for the micro-extraction of solasodine: A: chloroform; B: methyl orange; C: sample and/or standard.

that the operational sequence in which the sample, reagent and organic solvent were sequentially aspirated as small segments (see Fig. 3(b)) provided the higher slope of calibration graph (a plot of absorbance vs. solasodine concentration) than that obtained by another operational sequence (Fig. 3(a)). This might be due to the fact that the higher degree of contact between the two phases the higher sensitivity was obtained. In addition, the sensitivity increased with increasing number of the flow reversals. It was found that the higher slope of calibration graph could be reached by using a repeatedly segmented sequence, although 4 cycles of the flow reversal was performed. Therefore, the chloroform was firstly aspirated into the extraction coil, then the standard/sample solution was introduced, and methyl orange was then aspirated. The extraction step was performed in the extraction coil by programming the syringe control unit to aspiration and dispensed modes. The extraction process took place in the extraction coil. After that, the aqueous and organic phases were propelled into the separating unit where separation of the two phases occurred. The ion-association complex was left for 30 s and the absorption was measured spectrophotometrically at 420 nm. The separating LAV unit was then cleaned before starting the next determination.

3.2.2. Sample, reagent and organic solvent aspiration volumes

The aim for investigation of sample, reagent and organic solvent aspiration volumes is to minimize the consumption of reagents and sample while maintaining the best sensitivity, accuracy and reproducibility of the procedure for the analyte of interest.

3.2.2.1. Organic solvent aspiration volume. The influence of the organic solvent volume was investigated over the range of 300–700 μL. It was found that the maximum peak height was obtained at a volume of 600 μL for chloroform volume and it gave the best sensitivity.

3.2.2.2. Sample and reagent volumes. The influences of volume of the sample and methyl orange solutions aspirated were studied between 50 and 350 μL. The volume of chloroform was kept constant at 600 μL. It was found that the maximum response was obtained at a volume of 300 μL for sample volume and it gave the best sensitivity. For methyl orange volume, it was found that as the aspiration of methyl orange volume increased the sensitivity increased up to 100 μL. A methyl orange volume of 100 μL was chosen as an optimum reagent volume for subsequent measurements.

3.2.2.3. Volume of sending sample to detector. The volumes of sending sample to detector were investigated from 50 to 400 $\mu L\,s^{-1}$ at every 50 $\mu L\,s^{-1}$ interval. It was found that the maximum response was obtained at a volume of 250 μL for sending sample to detector because it gave the best sensitivity.

3.2.3. Flow rate

In any flow-based analysis procedure the response is dependent on the reagent and sample flow rates and thus it is necessary to optimize them to achieve the greatest sensitivity, sample throughput, etc.

3.2.3.1. Sample and reagents flow rates. It was obvious that the flow rate of aspiration of sample and reagents were significant with the peak height. The sample and reagents flow rates were investigated from 50 to 100 $\mu L\,s^{-1}$ at every 10 $\mu L\,s^{-1}$ interval while the flow rate of sending sample to detector kept constant at 100 $\mu L\,s^{-1}$. It was found that the flow rate increases with the peak height increases up to 100 $\mu L\,s^{-1}$.

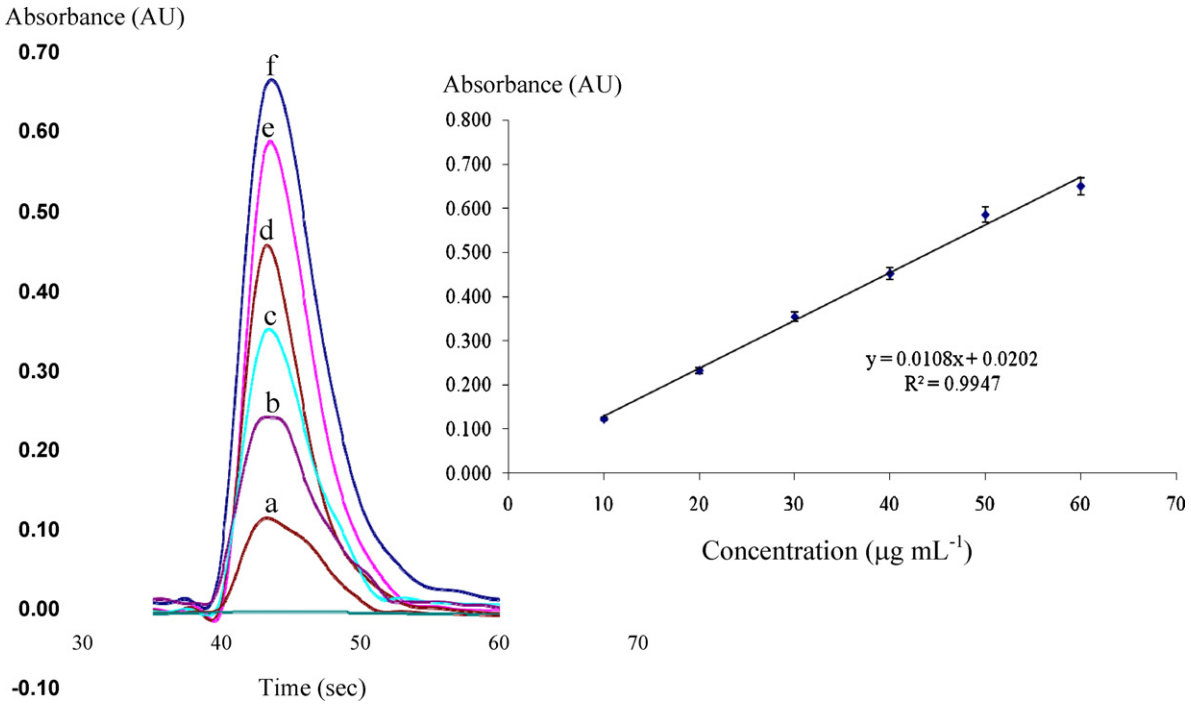

Fig. 4. Typical SI-grams and calibration graph of solasodine: $a = 10\ \mu g\ mL^{-1}$, $b = 20\ \mu g\ mL^{-1}$, $c = 30\ \mu g\ mL^{-1}$, $d = 40\ \mu g\ mL^{-1}$, $e = 50\ \mu g\ mL^{-1}$, $f = 60\ \mu g\ mL^{-1}$, respectively.

3.2.3.2. Flow rate of sending sample to detector. The flow rates of sending sample to detector were investigated from 10 to 50 $\mu L\ s^{-1}$ at every 10 $\mu L\ s^{-1}$ interval while the flow rate of aspiration of sample and reagent were kept constant at 100 $\mu L\ s^{-1}$. It was observed that the peak height increased with increasing in the flow rate up to 50 $\mu L\ s^{-1}$. Thus, a flow rate of 50 $\mu L\ s^{-1}$ was chosen and used for subsequent measurements.

3.2.4. The effect of methyl orange concentration

The effect of methyl orange concentrations was studied over the range 0.01–0.06%. The slopes of the calibration equation indicated that the sensitivity increased with increasing the methyl orange concentration. The equations of the calibration curves were $y = 1.3472x + 0.0114$, $y = 2.8797x - 0.0143$, $y = 4.2181x - 0.0337$, $y = 4.5184x - 0.0381$, $y = 3.9994x - 0.0168$ and $y = 3.9238x - 0.0542$ where y is absorbance in AU and x is percentage of methyl orange concentration (%), for methyl orange concentrations of 0.01%, 0.02%, 0.03%, 0.04%, 0.05% and 0.06%, respectively. The concentration of methyl orange at 0.04% gave the best sensitivity hence this concentration was chosen for further works.

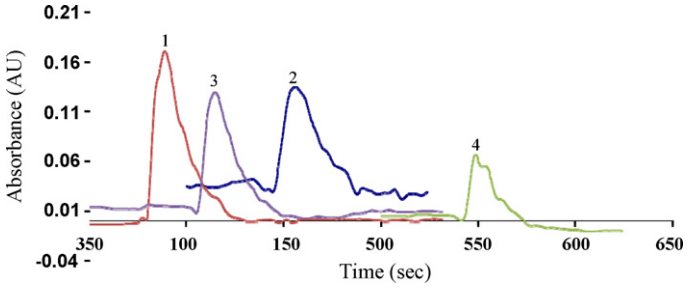

Fig. 5. Overlay SI-grams of four Solanum species: 1 = *Solanum aculeatissimum* Jacq., 2 = *Solanum violaceum* Ortega., 3 = *Solanum melongena* Linn., and 4 = *Solanum indicum* Linn.

3.2.5. Analytical characteristics

Fig. 4 shows the calibration graph and typical SI-grams of solasodine. Using the selected conditions, the analytical characteristics of the proposed SIA–LAV system were evaluated by examining the linear range, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ) and sampling frequency.

3.2.5.1. Linearity of calibration graph. The linearity of calibration graph was determined using the optimal experimental parameters in Table 1. Six standard solutions ranging from 10 to 60 $\mu g\ mL^{-1}$ in concentration, in three replicates each, were injected into the SI system. The calibration graph was obtained by plotting the absorbance of the solutions against the standard concentrations. Linear calibration graph over the concentration range 10–60 $\mu g\ mL^{-1}$ of solasodine was obtained with the regression equation $y = 0.0108x + 0.0202$ where y is absorbance in AU and x is solasodine concentration in $\mu g\ mL^{-1}$ and a correlation coefficient of 0.9947 (Fig. 4).

Solasodine contents in the sample solutions were determined by reference to this calibration curve.

3.2.5.2. Detection limit and quantification limit. Limit of detection (LOD) of solasodine was estimated from the calibration curve using the expression 3.3SD/S where SD is standard deviation of the blank (or the intercept of the calibration curve) and S is the slope of calibration curve and limit of quantitation (LOQ) = 10SD/S [50].

The limit of detection (LOD) was found to be 1.41 $\mu g\ mL^{-1}$. The limit of quantitation (LOQ) value was found to be 4.28 $\mu g\ mL^{-1}$.

3.2.5.3. Precision and accuracy. Precision: The precision of the method was determined by measuring the repeatability (intra-day precision) and the intermediate precision (interday precision), both expressed as relative standard deviation (RSD). The precision was evaluated by assaying six replicate injections of 10, 20 and 30 $\mu g\ mL^{-1}$ of solasodine. The repeatability was evaluated each sample on the same day under the same experimental conditions, 3.35%, 1.12% and 5.86%, respectively. The intermediate precision

Table 2
Percentage recovery of solasodine found using the proposed SIA–LAV method.

Samples	Solasodine concentration ($\mu g\,mL^{-1}$)		%Recovery[a]
	Added	Found	
Solanum aculeatissimum Jacq.	10	9.61	95.39 ± 0.012
	20	19.27	
	30	28.11	
Solanum violaceum Ortega.	10	8.73	97.03 ± 0.014
	20	19.92	
	30	31.24	
Solanum melongena Linn.	10	8.25	97.65 ± 0.024
	20	21.30	
	30	31.18	
Solanum indicum Linn.	10	8.99	96.58 ± 0.007
	20	19.67	
	30	30.44	

[a] Mean ± SD of three determinations.

Table 3
Comparative determination of solasodine contents in various Solanum species using the SIA–LAV method and the spectrophotometric method.

Samples	Amount of solasodine found ($mg\,g^{-1}$)		*t*-Values[c]
	SIA–LAV proposed method[a]	Spectrophotometric method [29][b]	
Solanum aculeatissimum Jacq.	4.70 ± 0.40	4.42 ± 0.08	1.93
Solanum violaceum Ortega.	0.84 ± 0.20	0.71 ± 0.09	1.67
Solanum melongena Linn.	1.40 ± 0.42	1.36 ± 0.05	0.48
Solanum indicum Linn.	0.67 ± 0.15	0.63 ± 0.13	0.71

[a] Mean ± SD of five determinations. The calibration equation; $y = 0.0109x + 0.0163$.
[b] Mean ± SD of five determinations. The calibration equation; $y = 0.0076x + 0.0181$.
[c] Tabulated *t*-value for $P = 0.05$ and four degrees of freedom is 2.7764.

Table 4
Comparison of the proposed method with selected earlier reported methods.

Techniques	Samples	Concentration range ($\mu g\,mL^{-1}$)	%Recovery	Limit of detection; LOD ($\mu g\,mL^{-1}$)	Limit of quantitation; LOQ ($\mu g\,mL^{-1}$)	References
Spectrophotometric method	*S. laciniatum*	100–4000	98.87	–	–	Birner [29]
	S. avicular		99.75			
Ion-paring RP-HPLC	*S. ptycanthum*	–	100.85	–	–	Ghazi and Matthees [15]
RP-HPLC	*S. linnaeanum*	4–100	–	1.60	–	Eanes et al. [21]
	S. melongena					
Non-aqueous capillary electrophoresis	*S. elaeagnifolium*	50–500	101.27	3.0	9.0	Cherkaouia et al. [24]
SIA with lab-at-valve	*S. aculeatissimum*	10–60	95.39	1.41	4.28	Proposed method
	S. violaceum Ortega.		97.03			
	S. melongena		97.65			
	S. indicum		96.58			

was evaluated by assaying each sample on three different days, 4.15%, 3.58% and 4.17%, respectively.

Accuracy: The recoveries were determined by using standard addition method. Solasodine standards (10, 20 and 30 $\mu g\,mL^{-1}$) were added and mixed with known aliquots of sample solutions, the sample was extracted and analyzed using the proposed method. The mean recovery ($n = 3$) of solasodine in *Solanum aculeatissimum* Jacq., *Solanum violaceum* Ortega., *Solanum melongena* Linn. and *Solanum indicum* Linn. were found to be 95.39%, 97.03%, 98.96% and 95.29%, respectively (Table 2).

3.2.6. Application to samples

The proposed SI method was applied to the determination of solasodine in *S. aculeatissimum* Jacq., *S. violaceum* Ortega., *S. melongena* Linn. and *S. indicum* Linn. in Solanaceae family. Each sample was prepared according to Section 2.4 (sample preparation) and the content of solasodine in each sample solution was determined using the optimum conditions as presented in Table 1. The samples gave well-defined peaks. There is no interference peak present in each sample as shown in Fig. 5. Comparative determination of solasodine in the same sample solutions was also carried out by the previously reported method [29]. The contents of solasodine in various Solanum species are shown in Table 3. Results obtained by both methods compared favorably verified by student *t*-test at 95% confident level. Statistical analysis of the results by using *t*-test indicated that there were no significant differences between both methods at 95% confidence level. Comparison of the proposed method with the selected earlier reported methods which used for determining solasodine indicated that the proposed method is more sensitive than other methods with equal accuracy (Table 4).

4. Conclusion

Sequential injection analysis with lab-at-valve approach for alternative simple on-line liquid–liquid semi-automated extrac-

tion was exploited. A simple fabricated lab-at-valve unit, a separating chamber, attached at one of the ports of a conventional multi-position selection valve offers an on-line automated extraction in a micro-scale. Therefore, consumption of sample, reagent and organic solvent also waste generation are tremendously reduced. The developed SIA–LAV system is precise, selective, accurate and robust for determination of solasodine in Solanum species. Thus this method involves a simple extraction–hydrolysis step, selective extraction of the solasodine and quantification of the yellow colored complex at its λ_{max} 420 nm without any interference. The method developed for determination of solasodine is also applicable to similar nitrogen-containing alkaloids. The color complex with methyl orange is form only by an aglycone after hydrolysis. The glycosides do not form such complexes. This study incorporates systematic instrumental analysis of solasodine from *S. aculeatissimum* Jacq., *S. violaceum* Ortega., *S. melongena* Linn. and *S. indicum* Linn. without any interferences.

Acknowledgements

The authors gratefully acknowledge the Thailand Research Fund (TRF) that supported a Royal Golden Jubilee Ph.D. research assistantship to Wisanu Thongchai (Project no. PHD/0223/2548 Code 5.G.CM/48/A.1). They also would like to express their sincere thanks to the Graduate School and Faculty of Pharmacy, Chiang Mai University for partial support. Additionally, Saisunee Liawruangrath would like to express her special thank to Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC); Chemistry Department, Faculty of Science, Chiang Mai University; TRF and Commission on Higher Education Ministry of Education for their very kind partial support.

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