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Short communication

Rapid determination of solanesol in tobacco by high-performance liquid chromatography with evaporative light scattering detection following microwave-assisted extraction

Hua-Ying Zhou [a,b], Chun-Zhao Liu [a,b,*]

[a] State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100080, PR China
[b] Graduate school of the Chinese Academy of Sciences, Beijing 100049, PR China

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Abstract

Solanesol is the starting material for many high-value biochemicals, including coenzyme Q_{10} and Vitamin-K analogues. The aim of the current study was to develop a reliable and fast analytical procedure for the determination of solanesol in tobacco using high-performance liquid chromatography (HPLC) with evaporative light scattering detection (ELSD) coupled with microwave-assisted extraction (MAE) as an efficient sample preparation technique. The HPLC conditions were Agilent C18 column using acetonitrile–isopropanol (60:40, v/v) as mobile phase at a flow rate of 1 ml/min. ELSD conditions were optimized at nebulizer-gas flow rate of 1.5 l/min and drift tube temperature of 65 °C. The method was validated to achieve the satisfactory precision and recovery, and the calibration range was 0.1–1.5 mg/ml. The developed analytical procedure was successfully applied to determine solanesol content in tobacco samples from different growing regions in China.

Keywords: Evaporative light scattering detection; High performance liquid chromatography; Microwave-assisted extraction; Solanesol; Tobacco

1. Introduction

Solanesol, long-chain terpenoid alcohol whose structure was shown in Fig. 1, is the starting material for many high-value biochemicals, including coenzyme Q_{10} and Vitamin-K analogues. Solanesol itself can be used as cardiac stimulant, lipid antioxidant and antibiotic [1]. Clinical trials are also going on for the usage of solanesol as anti cancer drug. Solanesol mainly exists in plants of *Solanaceae* family, especially in tobacco leaves [2]. Because of its special molecule structure, the wavelength of solanesol for maximal UV absorption is around 200 nm that results in low sensitivity for detection with UV detectors. Analysis results with UV detection will be seriously interfered by solvent, because many frequently used solvents have maximal UV absorption around 200 nm [3]. HPLC with diode array detection (DAD) was proposed to analysis solanesol in tobacco [4], but DAD is sensitive to the variation of mobile phase and the temperature of the column, so its sensitivity and reproducibility remain a problem. GC was also used to determine solanesol in tobacco [5], but solanesol is easy to be destroyed under high column temperature. Evaporative light scattering detection (ELSD) is increasingly being used coupled with HPLC during the last decade. The principle of its operation involves nebulizations of the column effluent to form an aerosol, followed by solvent evaporation in a heated drift tube and then the detection of the remaining nonvolatile solute particles in the light scattering cell [6]. ELSD is a kind of mass and non-selective detector, it can analyse all samples which have low volatility than mobile phase, especially for non-UV-absorbing phytochemicals [7].

The conventional extraction methods used for HPLC analysis of solanesol are room temperature extraction, heat-reflux extraction and Soxhlet extraction. Microwave-assisted extraction (MAE) has been used for extraction of interested components from a wide variety of sample matrices and has been used as an alternative sample preparation technique for a number of applications [8–16]. Compared with conventional methods, MAE can considerably reduce both extraction time and solvent consumption [17,18]. The objective of the current research is to develop a valuable method to determine solanesol content in tobacco

* Corresponding author. Tel.: +86 10 82622280; fax: +86 10 82622280.
E-mail address: czliu@home.ipe.ac.cn (C.-Z. Liu).

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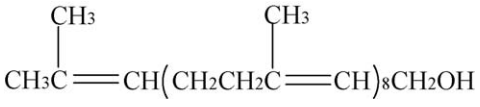

Fig. 1. The chemical structure of solanesol.

leaves by HPLC-ELSD in couple of rapid sample preparation process with automatic microwave-assisted extraction.

2. Experimental

2.1. Materials and reagents

Tobacco leaves from different areas of China were kindly provided by local companies. Solanesol standard was purchased from Sigma (USA). Acetonitrile and isopropanol (HPLC grade) were purchased from Cakedib Laboratories Ltd. (Georgetown, Ont., Canada). Ultra-pure water was produced by Milli-Q system (Millipore, Bedford, MA, USA). Other reagents used were analytical grade purchased from local chemical company.

Solanesol stock solutions were prepared by dissolving 10 mg solanesol in 10 ml hexane and stored at −20 °C. The standard working solutions at the concentration of the calibration range were prepared by serial dilutions of stock solutions with hexane.

2.2. HPLC conditions

The Agilent 1100 HPLC system was equipped with a quaternary pump, an on-line solvent vacuum degasser and an auto sampler with 20 μl injection loop. The detector was ELSD 2000ES (Alltech, USA). The data were required and processed by means of HP chemstation for LC software.

An Agilent C18 column (150 mm × 4.6 mm I.D., 5 μm) fitted with a Alltech C18 guard cartridge (8 mm × 4.6 mm I.D., 5 μm) was used at a column temperature of 25 °C. The mobile phase was acetonitrile–isopropanol (60:40, v/v) at a flow rate of 1 ml/min. ELSD conditions were optimized in order to achieve maximum sensitivity: nebulizer-gas flow rate 1.5 l/min and drift tube temperature 65 °C.

2.3. Sample preparation for HPLC analysis

Tobacco leaves were dried and milled to 60 mesh powder as extraction sample. The extraction solvent was a mixture of hexane and ethanol (1:3, v/v). For room temperature extraction, 10 g tobacco sample was extracted in a sealed flask (250 ml) containing 100 ml extraction solvent under room temperature for a given time. Heat reflux extraction using a water-bath was performed with 10 g tobacco sample and 100 ml extraction solvent in a flask (250 ml) with mechanical stirrer and the temperature was kept at 60 °C. For Soxhlet extraction, 10 g tobacco sample was put into 200 ml Soxhlet thimble which was fitted with 250 ml round bottom flask containing 100 ml extraction solvent. A household microwave oven was modified in our laboratory with the addition of a magnetic stirrer, water condensor, temperature measurement and time controlling for automatic microwave-assisted extraction [9]. Ten grams of tobacco sample was mixed with 100 ml extraction solvent, and the suspensions were irradiated automatically with microwaves in a pre-setting procedure for 30 min (for microwave power was switched on to reach the desired temperature of about 60 °C and then heated for 2 s and the power was switched off for cooling for 2 s). The microwave irradiation power was set at 700 W.

After extraction using various methods, all supernatants were centrifuged at 4250 × *g* for 5 min, and filtered through 0.45 μm membrane before HPLC analysis. In the present work, the percentage extraction of solanesol was defined as follows: percentage extraction of solanesol (w/w) = mass of solanesol extracted/mass of material (tobacco sample) × 100%.

3. Results and discussion

3.1. Optimization of the chromatographic system

To effectively separate solanesol from other compounds in the tobacco sample, various HPLC mobile phase systems were tested. The increase of solvent volatility reduces the droplets condensation of the wall of nebulization chamber, which enhances the ELSD response [19]. Acetonitrile–methanol, acetonitrile–isopropanol, hexane–isopropanol were examined and acetonitrile–isopropanol was found to give the best resolution and sensitivity. The optimal mobile phase was acetonitrile–isopropanol (60:40, v/v) which was employed in the following experiments.

ELSD conditions were optimized in order to achieve maximum sensitivity. The nebulizer-gas flow rate and drift temperature are the main instrumental parameters affecting signal response. Large droplets are formed at slow gas flow rate that results in spiked and noisy signals, however, increase of gas flow rate results in a decrease of signal response [20]. An optimal gas flow was obtained at 1.5 l/min of nitrogen gas. Solvent evaporation is not complete at low temperature, however, the detector response is decreased at high temperature [21]. The best result was obtained with a drift tube temperature at 65 °C. The total time between injections was 12 min (Fig. 2). Identification of solanesol was based on retention time, co-injection with standard.

3.2. Method validation

3.2.1. Calibration

In ELSD, second-order polynomial calibrations (peak area against amount) were observed in the range of 0.1–1.5 mg/ml. After log-transformation, the data provided a linear calibration following the equation: $Y = A + BX$, where Y is the log value of the peak area, X the log value of sample amount, A the intercept, and B is the slope [22]. The mean equation of the calibration curve ($n = 6$) obtained from six points was as follows: $Y = 6.27 + 1.53X$. The regression coefficient is 0.9998.

3.2.2. Precision

The precision injection was evaluated by repeated injection of the sample solution six times. The R.S.D. of peak area and retention time is less than 2.0%.

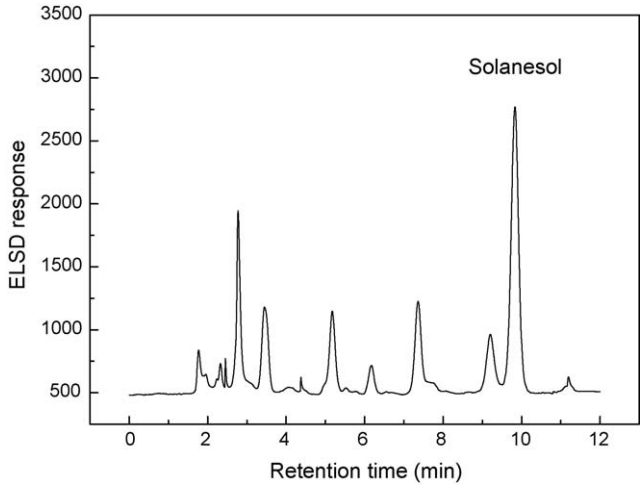

Fig. 2. Representative chromatogram of solanesol from tobacco leaves. Column: reversed-phase Agilent C18 (150 mm × 4.6 mm I.D., 5 μm) fitted with a Alltech C18 guard cartridge (8 mm × 4.6 mm I.D., 5 μm); mobile phase: acetonitrile–isopropanol (60:40, v/v); flow rate: 1 ml/min; ELSD conditions: nebulizer-gas flow rate 1.5 l/min, drift tube temperature 65 °C.

The acceptable intra- and inter-day precisions (relative standard deviation, R.S.D.) and accuracy (relative error, RD) were set as <10% and between −5% and 5%, respectively [23]. Intra- and inter-day variabilities were determined by analysis of average amount of solanesol in quality control samples prepared by standard solutions at low, medium and high concentrations on three different days. The quality control samples were prepared as a single batch on the same day at each concentration, and then divided into aliquots that were stored at −20 °C until analysis. The calculated R.S.D. and RD values from repeated measurements are summarized in Table 1. The assay precision was ranged from 0.68% to 5.16%, and the accuracy was greater than 98.20%.

3.2.3. Limit of quantification (LOQ) and limit of detection (LOD)

LOQ was defined as the lowest concentration of solanesol at which the signal was larger than 10 times of the baseline noise (S/N = 10), and LOD was defined as S/N = 3. The measured LOQ and LOD values of the described method were observed for 80 μg/ml and 20 μg/ml, respectively, which

Table 1
Intra- and inter-day precision and accuracy

Actual concentration (μg/ml)	Detected concentration (mean ± S.D., $n = 3$)	R.S.D. (%)	Accuracy (RD) (%)
Intra-day			
200	207.67 ± 2.52	1.21	103.83
500	495.00 ± 16.09	3.25	98.20
1000	989.67 ± 22.30	2.25	98.97
Inter-day			
200	209.00 ± 1.41	0.68	104.50
500	491.06 ± 20.92	4.26	98.21
1000	1019.83 ± 46.94	4.60	101.98

S.D., standard deviation; R.S.D., relative standard deviation; RD, relative error.

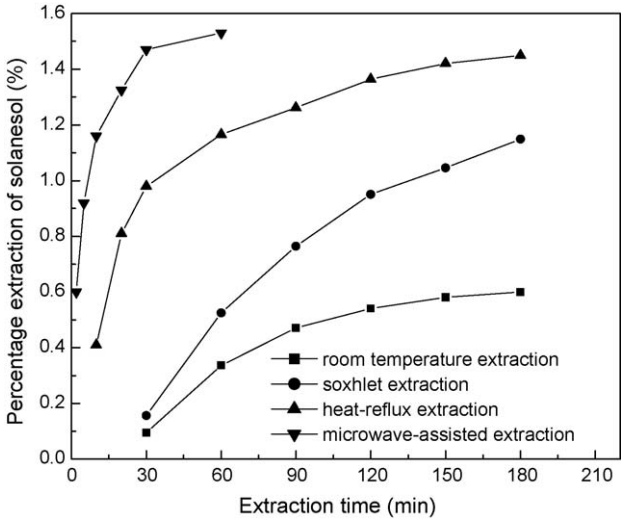

Fig. 3. Comparison of different extraction methods on solanesol recovery from tobacco leaf sample for HPLC-ELSD analysis.

will meet the requirements of validation for a new assay protocol.

3.3. Sample preparation and accuracy of MAE procedure

In order to develop an efficient extraction method of solanesol from tobacco leaf sample for HPLC analysis, various extraction methods including room temperature extraction, heat-reflux extraction, Soxhlet extraction and microwave-assisted extraction (MAE) were compared. As shown in Fig. 3, the highest percentage extraction of solanesol of 1.47% was achieved in the automatic microwave-assisted extraction with a shortest extraction time of 30 min among all extraction methods used.

In order to determine accuracy of the MAE procedure, the known amount (low, medium and high level) of solanesol was added into the tobacco leaf samples, and the resultant solutions were analyzed by the valid HPLC method. The results were shown in Table 2. The recovery of solanesol was between 97.80% and 101.92%, and R.S.D. was between 4.43% and 5.91%. The developed MAE sample preparation procedure not only showed high recovery and accuracy, but also was found to be suitable for rapid analysis of solanesol in tobacco.

3.4. Applications

The contents of solanesol in 16 tobacco leaf samples from different geographic areas of China were determined using the

Table 2
Accuracy of the MAE procedure

Amount of standard added (mg)	Recovered amounts (mg)			Mean recovery (%)	R.S.D. (%)
10	9.60	9.68	9.83	97.03	1.20
30	31.12	32.05	30.56	104.14	2.40
50	51.56	52.48	48.21	101.50	4.43

R.S.D., relative standard deviation.

Table 3
Contents of solanesol in the tobacco leaves from different growing regions

Sample sources	Content of solanesol (%)
Shandong	0.84 ± 0.015
Guizhou	0.39 ± 0.014
Henan	0.47 ± 0.012
Hubei	1.03 ± 0.024
Hainan	1.09 ± 0.031
Jilin	0.44 ± 0.017
Sichuan	0.53 ± 0.016
Hunan	1.69 ± 0.014
Guangxi	0.67 ± 0.025

above developed HPLC-ELSD method following the fast sample preparation method with automatic microwave-assisted extraction (Table 3). A very large variation was found among the samples collected from different regions, and the tobacco leaves collected from the areas of Hunan, Hubei, Hainan contained higher levels of solanesol than those from other regions. The variation of the solanesol content in tobacco is attributed to several factors, such as tobacco species, local climate, growing conditions, harvest season and so on.

4. Conclusions

An efficient procedure involving MAE, followed by analysis using HPLC-ELSD was developed for the rapid analysis and quantification of solanesol in tobacco. The benefits of MAE as a technique for sample preparation in comparison with the conventional extraction methods were shortened extraction time, reduced solvent consumption and high recovery of solanesol. The analytical procedure provided an alternative fast and accurate method for rapid determination of solanesol content in tobacco, and was successfully applied on samples from different growing regions in China. It seems possible to extend this methodological approach towards other non-UV absorption phytochemicals in many plant materials.

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