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# Rapid and quantitative determination of solanesol in *Nicotiana tabacum* by liquid chromatography–tandem mass spectrometry

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**Abstract**

A rapid and sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS) method with multiple reaction monitoring (MRM) was developed for the determination of solanesol in *Nicotiana tabacum*. Sample preparation was performed by ultrasonic extraction with methanol for 20 min and then supernatant was extracted with hexane. The method used atmospheric pressure chemical ionization (APCI) detection in positive-ion mode. The separation of solanesol was performed on a Symmetry Shield™ RP18 column with a mixture of acetonitrile and isopropanol (1:1, v/v) containing 2 mM ammonium acetate as mobile phase. Quantification of solanesol was performed by the standard addition method. The limit of quantification (LOQ) and limit of detection (LOD) of solanesol were, respectively, 5.0 ng/ml (S/N = 10) and 1.5 ng/ml (S/N = 3). The relative standard deviations of peak area were 0.89 and 1.12% for intra-day and inter-day, respectively. The recoveries of solanesol ranged from 97.72 to 99.67% and the corresponding R.S.D.s were less than 2.7%. Analysis took 5 min, making the method suitable for rapid determination of solanesol in *N. tabacum*. The proposed method has been successfully applied to the analysis of solanesol in various organs of *N. tabacum*.


*Keywords:* LC–MS/MS; MRM; APCI; *Nicotiana tabacum*; Solanesol

## 1. Introduction

*Nicotiana tabacum* belongs to the Solanaceae family and the plant is considered to be a good source of a large number of bioactive substances. Recently, the chemical compositions of *N. tabacum* have attracted considerable attentions in the world [1–4]. Solanesol, a 45-carbon, all-*trans*-nonaprenol (see Fig. 1), was first isolated from flue-cured tobacco [5]. Solanesol itself can be used as antiulcer and hypertension treating agent [6,7]. In addition, solanesol is a necessary medical intermediate in the industrial synthesis of coenzyme $Q_{10}$ [8–10], which is an excellent medicine in cardiovascular disease, cancer, atherosclerosis and so on [11–15].

Solanesol is in fact found in many plants from the Solanaceae family, one member of which is the *Nicotiana* genus. Other members of the family known to contain solanesol include tomato plants, potato plants, eggplants and pepper plants [16]. However, it was reported that the content of solanesol in *N. tabacum* was considerably higher than that in other plants and thus this plant represented the most convenient source for large-scale isolation of solanesol [17–19]. So it is very important to determine the content of solanesol in *N. tabacum*.

Many analytical methods including of column chromatography weight, thin-layer chromatography (TLC), coulomb's analysis, gas chromatography (GC) and high performance liquid chromatography (HPLC) have been documented for the determination of solanesol [20–28]. All of the methods above suffered from some limitations, such as, column chromatography weight method had low recovery, the precision obtained by TLC was poor, coulomb's analysis had significant error, the GC method was complicated by the interference of solanesenes, produced from the pyrolysis of solanesol at high temperatures in the GC oven and the breakdown of solanesol hindered the direct quantification of solanesol. The HPLC methods above had poor selective and sensitivity compared with the LC–MS/MS methods proposed in the study.

At present, liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been accepted by more and more people as a useful method for identification and determination of compounds [29,30]. Especially, it is very effective in the analysis of

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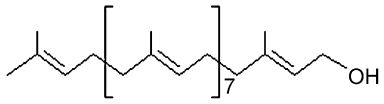


Fig. 1. Structure of solanesol.

compounds from complex samples because of its low detection limit, high sensitivity and the possibility for short run time [31–33]. Signal suppression or enhancement of the target extracts by matrix components is a common phenomenon in LC–MS/MS analysis and should be considered [34]. Moreover, interfering matrix components can affect accuracy of the proposed method and maybe lead to some compromising or erroneous results [35]. In order to avoid the problems related with matrix effect, some authors preferred to select the optimization of sample preparation, the optimization of the chromatographic system and MS/MS detection [36–38]. In addition, some authors refered to the necessity of implementation of the standard addition method as a form of eliminating matrix effects [39,40].

Solanesol is a nonaprenol containing a linear hydrocarbon chain, which is made up of nine isoprenoids. $CoQ_{10}$ is an ubiquinone, whose side chain is made up of 10 isoprenoids. Structurally, solanesol and $CoQ_{10}$ have some similarities. LC–MS/MS method with MRM have been reported for the determination of $CoQ_{10}$ in *N. tabacum* [41]. As far as we are aware, no report on determination of solanesol in *N. tabacum* by LC–MS/MS method up to now. Considering it, the aim of this study is to propose a validated LC–MS/MS method with multiple reaction monitoring (MRM) for separation and determination of solanesol in *N. tabacum*. Regarding that matrix components in *N. tabacum* are complex, in order to avoid the problems related with matrix effect, each step mentioned above (sample preparation, chromatographic system and MS/MS detection) was carefully optimized in the study. At the same time, the standard addition method was used as a quantification method to further minimize the matrix effect in the study. We have found this technique to be suitable for the rapid and sensitive quantification of solanesol in *N. tabacum*. Based on this work, the contents of solanesol in various organs of *N. tabacum* are determined and compared in this paper.

## 2. Experimental

### 2.1. Equipments

LC–MS/MS analysis was performed on an API3000 (Applied Biosystems, Canada) triple-stage quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) interface and an Agilent 1100 series HPLC from Agilent technologies (Agilent, CA). A Model '11' single syringe pump (Harvard Apparatus Inc., Holliston, USA) was also used.

The Agilent HPLC system consisted of a G1312A HPLC binary pump, a 7725i manual injector and a G1379A degasser. A reverse phase Symmetry Shield$^{TM}$ RP18 column (5 μm, 93 Å, 66% porosity, 3.9 mm Ø × 150 mm, Waters Company, USA) was used.

### 2.2. Reagents and materials

Acetonitrile and isopropanol were of HPLC grade (Krackeler Scientific, Inc., Albany, NY). Ammonium acetate was HPLC grade (Dima Techmology Inc., USA). Methanol, ethanol, acetone and hexane were of analytical grade (Beijing Chemical Reagents Company, China). Solanesol standard (>90%) was purchased from Sigma Company (USA).

*N. tabacum* were collected from the arboretum in Northeast Forestry University, China. Roots, stalks, leaves, flowers, seeds and fruits materials were separated from *N. tabacum* for the determination of solanesol by LC–MS/MS.

### 2.3. LC–MS/MS conditions

Chromatographic analysis was carried out by a Symmetry Shield$^{TM}$ RP18 column. Column temperature was maintained at 25 °C. The mobile phases was a mixture of acetonitrile and isopropanol (1:1, v/v) containing 2 mM ammonium acetate. Elution was performed at a flow rate of 1.0 ml/min. The injection volume was 5 μl. For operation in MS/MS mode, a mass spectrometer fitted with an APCI source interface was used for analysis. Ionization of solanesol was achieved in the positive ionization mode. The infusion experiment was performed using a single syringe pump '11'. MRM was performed with 150 ms dwell time. Nitrogen was used for the nebulizing, curtain and collision gas. The mass spectrometer was programmed to monitor the precursor ion $[(M-H_2O)+H]^+$at *m/z* 613.7 via the first quadrupole filter (Q1), the product ion at *m/z* 69.2 was monitored via Q3. Finally, all MS parameters were manually fine-tuned to obtain the highest MRM signals. Under the above conditions, the ion source was thermally stabilized for 30 min before injection. Peak areas obtained from the MRM were utilized for the quantification of solanesol. Data were processed by the analyst software Version 1.4 and the optimized MS parameters for the detection of solanesol were listed in Table 1.

### 2.4. Preparation of standard solutions

Solanesol standard was purchased from Sigma Company and the standard was additionally purified by silica gel column chromatography. Then, stock solution of solanesol was prepared by accurately weighing 7.50 mg of powder and dissolving this in 10 ml of the mobile phase. The stock solution was stored at 4 °C. From the stock solution, working standards were prepared by dilution with mobile phase. All standard solutions were filtered through 0.45 μm membrane filter (Millipore).

### 2.5. Preparation of sample solution

Sample solution was prepared by the method of Ref. [42]. For the plant sample, plant material (fresh weight) was grinded to a fine powder in liquid nitrogen. Subsequently, 2.0 g of the fine powder was placed in a closed stainless steel vessel and 20 ml of methanol were added. The optimized extraction was carried out in an ultrasonic washer for 20 min at 25 °C. The extract was centrifuged at 12,000 rpm for 8 min. After filtering,

Table 1
Parameters of mass spectrometric conditions

| Parameters | Value |
|---|---|
| NEB | 14 |
| CUR | 12 |
| CAD | 5 |
| IS (V) | 4000 |
| Ion source TEM (°C) | 350 |
| AUX (psi) | 70 |
| Q1 mass (precursor ion [(M−$H_2O$)+H]$^+$) | 613.7 |
| Q3 mass (product ion) | 69.2 |
| DP (V) | 60 |
| EP (V) | 10 |
| FP (V) | 400 |
| CEM (V) | 2000 |
| CE (V) | 35 |
| CXP (V) | 8 |
| MRM (amu) | 613.7/69.2 |

NEB: nebulizing gas; CUR: curtain gas; CAD: collision-activated dissociation; IS: ion spray voltage; TEM: temperature; AUX: auxiliary gas; DP: declustering potential; EP: entrance potential; FP: focusing potential; CEM: channel electron multiplier; CE: collision energy; CXP: collision cell exit potential; MRM: multiple reaction monitoring.

the supernatant was extracted with hexane. The above procedure was repeated for three times and the hexane phases were combined. Combined hexane phase was concentrated to dryness and the dried extract was dissolved in the chromatographic mobile phase. After filtering, the supernatant was injected directly.

### 2.6. Recovery studies

The recovery experiment of solanesol was performed by adding solanesol standards to the extract solutions of *N. tabacum* leaves, stalks, roots, flowers, seeds and fruits, respectively. Several portions of the same volume of extract solutions were, respectively, spiked with the same volume of solanesol standard solution at different mass concentrations. All samples were vortex-mixed and filtered through a 0.45 μm Millipore filter. Samples were determined three times by LC–MS/MS. The recovery was calculated as follows:

$$\text{Recovery}\,(\%) = \frac{(A - B)}{C} \times 100\%$$

where $A$ is the amount detected, $B$ the amount of extract without added standard, $C$ is the added amount of the standard.

## 3. Results and discussion

### 3.1. Optimization of sample preparation

Fresh *N. tabacum* leaves were used as extraction material. Anhydrous ethanol, methanol and 95% ethanol were chosen as extraction solvent. At the same time, different extraction methods (ultrasonic, Soxhlet and reflux) were compared. Solanesol in fresh *N. tabacum* leaves were extracted according to the procedure described in Section 2.5. The extracts obtained by the above three extraction methods were injected, respectively. The extraction yields of solanesol in *N. tabacum* leaves were determined under the LC–MS/MS conditions of Section 2.3. The results are shown in Fig. 2.

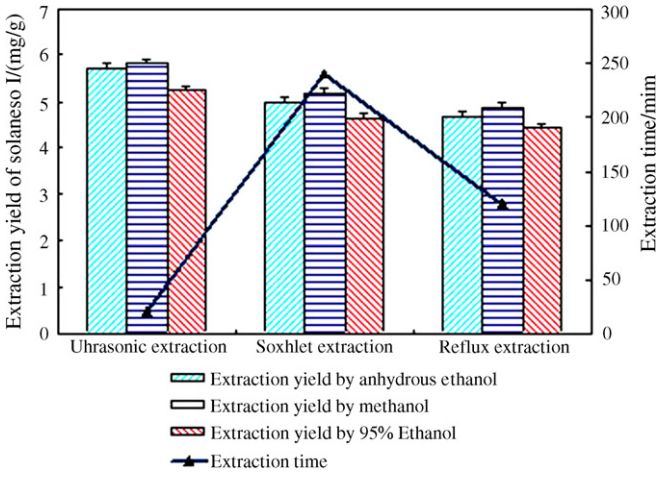


Fig. 2. Effect of different sample preparation methods on extraction yield of solanesol.

As seen in Fig. 2, the extraction yield of solanesol obtained with 95% ethanol is obviously low, that obtained with anhydrous ethanol is higher and that obtained with methanol is the highest. Therefore, in this work, methanol was considered a safe and more effective solvent for extraction of solanesol from *N. tabacum* leaves. It can also be seen from Fig. 2 that the extraction yield of solanesol obtained by ultrasonication is highest, that obtained by Soxhlet is lower and that obtained by reflux is obviously lowest. In addition, the extraction completeness by Soxhlet depends to a large extent on the extraction time, the extraction completeness of ultrasonication is almost independent on time. Ultrasonic extraction gave better result within 20 min than Soxhlet extraction did within 240 min. Ultrasonic extraction needs a shorter time compared to reflux and Soxhlet extraction. With regard to extraction yields and time, methanol was regarded as extraction solvent, ultrasonic extraction was considered to provide effective conditions for the extraction of solanesol from *N. tabacum* and was used in the following tests.

### 3.2. Optimization of chromatographic conditions

It is vital to select an appropriate mobile phase to ensure that the peak of solanesol could be separated and well resolved within a reasonable analysis time. In order to assure optimal chromatographic conditions for solanesol, the mobile phase systems were optimized. The organic solvent, methanol or acetonitrile is commonly used as one component of the mobile phase. Since solanesol readily dissolves in isopropanol, various proportions of methanol–isopropanol and acetonitrile–isopropanol were initially tested as mobile phases. The results showed that the solanesol responses increased with increasing percentage of methanol or acetonitrile. However, because of acetonitrile's lower viscosity as compared to methanol, from the standpoint of pressure, acetonitrile was chosen as one component of the mobile phase. In succession, mixtures of acetonitrile and isopropanol in different ratios were tested. Eventually, it was found that acetonitrile–isopropanol (1:1, v/v) gave the best separation

of solanesol offset only by slightly off-maximum sensitivity for solanesol.

It was reported that addition the ionizing agents to mobile phase had the greatest effect on the ionization of compound and the improvement of sensitivity [43–46]. To enhance the sensitivity and ionization efficiency, different percentages of ammonium acetate were used in the mobile phase. The results showed that solanesol was completely separated and the peak shape of solanesol was better when the concentration of ammonium acetate was in the range of 2–10 mM. However, when the concentration of ammonium acetate was below 2 mM, the ionization was poor. Considering both ionization and sensitivity, the optimized ammonium acetate concentration was 2 mM. As a result, a mixture of acetonitrile and isopropanol (1:1, v/v) containing 2 mM ammonium acetate was confirmed as the optimum mobile phase. Under these conditions, the retention time of solanesol was $2.2 \pm 0.1$ min.

### 3.3. Optimization of MS/MS detection conditions

Electrospray ionization (ESI) and atmospheric pressure chemical ionization techniques were, respectively, tested in positive and negative ion mode. The results showed that APCI in positive mode was superior to APCI in negative mode and to ESI in positive or negative mode. Solanesol was determined with much better sensitivity on using the APCI source in positive mode. Thus, the APCI source in positive mode was chosen for the detection of solanesol. Infusion experiments were carried out to examine ionization and fragmentation patterns of the solanesol standards using a syringe pump. First, a standard solution of solanesol was chosen to obtain a constant signal in the Q1 scan mode. A full scan spectrum of the solanesol standard was acquired with a scan range of 500–700 amu, a dwell time of 1.5 s and a step size of 0.1 amu. The declustering potential (DP) was optimized using the quantitative optimization function of Analyst 1.4 to achieve the highest signal response. The main ions were observed at *m/z* 613.7 $[(M-H_2O)+H]^+$ with smaller signals visible at *m/z* 648.7 $[M+NH_4]^+$ and 631.9 $[M+H]^+$ when the data were acquired in the Q1 scan mode (see Fig. 3). Compared with $NH_4^+$ adducts at *m/z* 648.7 and the protonated $[M+H]^+$ ion at *m/z* 631.9, the $[(M-H_2O)+H]^+$ ion at *m/z* 613.7 showed the highest intensity signals, the $[(M-H_2O)+H]^+$ ion at *m/z* 613.7 was chosen as precursor ion of solanesol. Secondly, we used product ion scans to look for the most abundant product ion. The collision energy (CE) was optimized to achieve highest sensitivity. The product ion spectrum obtained, which is shown in Fig. 4, consisted of an intense ion at *m/z* 69.2 and a second intense ion at *m/z* 81.1 when CE was 35 V. The two base ions at 69.2 and 81.1 were characteristic ions of polyprenol [47,48]. Among the product ions, that at *m/z* 69.2 was the most abundant and was therefore chosen for the quantitative determination of solanesol. Finally, the precursor/product ion pair of *m/z* 613.7/69.2 was chosen for the MRM scan. MRM was performed with 150 ms dwell time. Peak areas obtained from the MRM of solanesol standards were utilized for the quantitative determination of solanesol. Sample solutions of *N. tabacum* leaves extract (prepared according to the procedure described in Section 2.5) were injected directly,

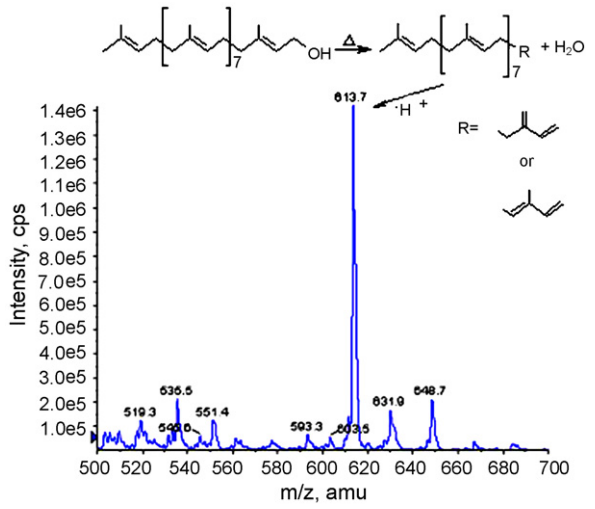


Fig. 3. Positive-ion Q1 mass spectrum of solanesol standard.

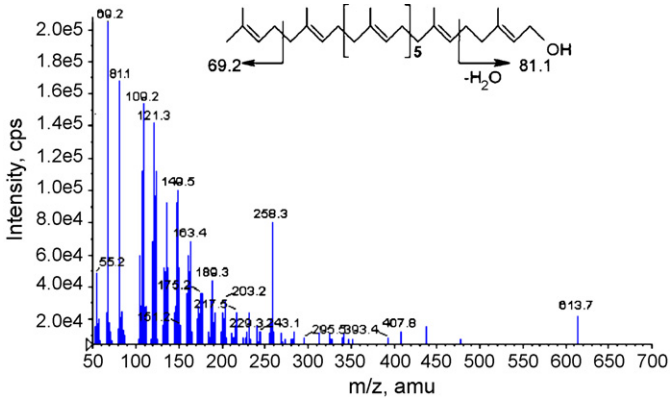


Fig. 4. Production ion spectrum and fragmentation pattern of solanesol standard.

separated, and detected under the optimum condition mentioned earlier (Section 2.3). The MRM chromatogram of solanesol in *N. tabacum* leaves is shown in Fig. 5. It can be seen from Fig. 5 that the retention time of solanesol was 2.24 min. The analysis

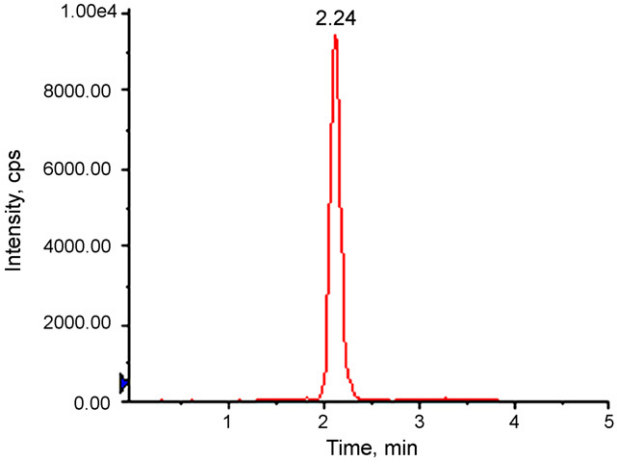


Fig. 5. LC–MS/MS chromatogram of solanesol.

procedure can be finished in a shorter analysis time. The results show the proposed method to be fast.

### 3.4. Method of standard addition

Quantification was based on the standard addition method with the *N. tabacum* extract solution spiked with solanesol standards of three different concentrations. The detector response can then be plotted against the added concentration of solanesol. This is referred to as a standard addition curve. The unknown solanesol concentration can be found by extrapolating the best fit line to the *x*-axis intercept. That intercept will be the unknown solanesol concentration. If the equation of the best fit line is written in the form $y = mx + q$ ($y$, peak area, counts; $x$, added solanesol concentration, μg/ml) then the *x*-axis intercept is equal to the *y*-axis intercept ($q$) over the slope ($m$).

### 3.5. Method validation

#### 3.5.1. Linearity

Six standard solutions of solanesol with concentrations between 0.075 and 750 μg/ml were determined by LC–MS–MS with MRM. Each individual standard with a certain concentration was consecutive injected for three times. The regression equation for solanesol (peak area, $A$, counts, versus concentration, $C$, μg/ml) was as follows:

$$A = 15.54\,C + 1.52 \quad (R^2 = 0.9997)$$

The regression equation was found to be linear in the ranges of 0.075–750 μg/ml with the good correlation coefficient ($R^2 = 0.9997$).

#### 3.5.2. LOQ and LOD

For the establishment of LOQ and LOD, 750 μg/ml of standard solutions of solanesol were gradually diluted with mobile phase. Each individual standard with a certain concentration was consecutive injected for three times. LOQ of 5.0 ng/ml was obtained for solanesol (S/N = 10). LOD of solanesol was 1.5 ng/ml (S/N = 3). Compared with other analytical methods, LOD obtained by the proposed method was lower than some results by column chromatography weight, thin-layer chromatography, coulomb's analysis, gas chromatography and high performance liquid chromatography methods [20–28]. The result showed the proposed method was highly sensitive.

#### 3.5.3. Precision and recovery

The intra-day and inter-day precisions (expressed as the relative standard deviation, R.S.D.) for peak area were determined by repeated analysis ($n = 5$). The result showed that intra-day and inter-day R.S.D.s for peak area were, respectively, 0.89 and 1.12%. Compared with other analytical methods [25–28], the precision obtained by the proposed method was better.

To validate the propose method, the recoveries were obtained by the procedure described in Section 2.6 and the results were listed in Table 2. It was found that the recoveries of solanesol were closer to 100% of and corresponding R.S.D.s were all no more than 2.7%.

Table 2
Recovery of solanesol ($n = 3$)

| Samples | Solanesol added (μg) | Found (μg) | Recovery (%) | R.S.D. (%) |
|---|---|---|---|---|
| Leaves | 0.0 | 127.6 | – | 2.1 |
| | 30.0 | 157.5 | 99.67 | 2.2 |
| | 150.0 | 275.3 | 98.47 | 1.8 |
| | 750.0 | 867.2 | 98.61 | 1.9 |
| Stalks | 0.0 | 18.8 | – | 2.1 |
| | 7.5 | 26.2 | 98.67 | 2.0 |
| | 15.0 | 33.7 | 99.33 | 1.9 |
| | 30.0 | 48.4 | 98.67 | 2.0 |
| Roots | 0.0 | 1.9 | – | 1.7 |
| | 1.5 | 3.4 | 98.53 | 2.5 |
| | 3.0 | 4.8 | 97.72 | 2.0 |
| | 7.5 | 9.3 | 98.67 | 2.7 |
| Flowers | 0.0 | 7.0 | – | 2.0 |
| | 3.0 | 10.0 | 98.67 | 2.4 |
| | 7.5 | 14.4 | 99.23 | 2.5 |
| | 15.0 | 21.9 | 99.41 | 2.0 |
| Seeds | 0.0 | 4.6 | – | 2.6 |
| | 1.5 | 6.1 | 98.37 | 2.3 |
| | 3.0 | 7.6 | 98.99 | 2.0 |
| | 7.5 | 12.1 | 99.35 | 2.3 |
| Fruits | 0.0 | 2.8 | – | 2.7 |
| | 1.5 | 4.3 | 98.85 | 2.2 |
| | 3.0 | 5.8 | 99.23 | 1.9 |
| | 7.5 | 10.3 | 99.45 | 2.5 |

### 3.6. The determination of solanesol in N. tabacum

The contents of solanesol in various organs of *N. tabacum* were prepared and analyzed by the procedure described in Sections 2.5 and 2.3. The calculated contents of solanesol by standard addition method described in Section 3.4 were summarized in Table 3.

The results from Table 3 show that the contents of solanesol are different from various organs of *N. tabacum* and the content of solanesol in leaves of *N. tabacum* is the highest. The results also indicate that the contents of solanesol in *N. tabacum* are strikingly related to the organs of *N. tabacum*.

Table 3
Determination of solanesol in *N. tabacum* $n = 3$

| Organs | Standard addition curve[a] | Regression coefficient ($R^2$) | Content of solanesol (mg/g) | R.S.D. (%) |
|---|---|---|---|---|
| Roots | $y = 15.57x + 81.14$ | 0.9998 | 0.089 | 1.2 |
| Stalks | $y = 15.52x + 792.91$ | 0.9997 | 0.869 | 1.4 |
| Leaves | $y = 15.50x + 5292.59$ | 0.9995 | 5.908 | 1.6 |
| Flowers | $y = 15.60x + 301.04$ | 0.9996 | 0.325 | 1.8 |
| Seeds | $y = 15.72x + 191.53$ | 0.9997 | 0.215 | 1.6 |
| Fruits | $y = 15.72x + 117.03$ | 0.9995 | 0.129 | 2.1 |

[a] $y$, peak area (counts); $x$, added solanesol concentration (μg/ml).

## 4. Conclusions

In the present work, a LC–MS/MS method for the determination of solanesol in *N. tabacum* has been presented. The method makes analysis procedure be finished in a shorter analysis time with good recovery, precision and sensitivity. The contents of solanesol in various organs of *N. tabacum* were analyzed and compared using the method. At the same time, this method provides a reference for the analysis of solanesol in other samples.

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