

HPLC/MS ANALYSIS OF SOLANINE IN *PHYSALIS ALKEKENGII* AND *SOLANUM DULCAMARA*

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Abstract

We developed a HPLC/MS method for the identification and determination of solanine in plants. This method was applied for samples of *Solanum dulcamara* (from Iași and Vânători Neamț areas in Romania) and *Physalis alkekengi* (from Bucium and Breazu, Iași, Romania) during August-September 2009. Isolation of solanine was performed by extraction with a mixture consisting of water/methanol/acetic acid 49/49/2 (v/v/v) in an ultrasonic bath. Solanine was determined in concentrations ranging between 0.7963 µg/g vegetal product (leaves, Zimbru, Romania) and 4.2166 µg/g (leaves, Iași area) in *Solanum dulcamara* and between 0.092 µg/g (fruits, Bucium) and 0.2818 µg/g (stems, Bucium) in *Physalis alkekengi*. For *Physalis alkekengi*, in leaves (sample from Bucium) roots and fruits (sample from Breazu) the concentration values of solanine were below the detection limit. The mean concentration of solanine was 21.67 times greater in samples of *Solanum dulcamara* than in *Physalis alkekengi*. For *Solanum dulcamara*, the highest concentration of solanine was recorded in stems.

Rezumat

Lucrarea prezintă o nouă metodă HPLC/MS pentru identificarea și dozarea solaninei din plante. Prin această metodă s-a analizat solanina din părțile plantelor *Solanum dulcamara* (recoltată din zona Iași și Vânători Neamț) și *Physalis alkekengi* (recoltată din Bucium și Breazu, Iași) recoltate în perioada august-septembrie 2009. Izolarea solaninei s-a realizat prin extracție cu un amestec de apă/metanol/acid acetic 49/49/2 (v/v/v) în baia cu ultrasunete. Solanina a fost determinată în concentrații de 0,7963 µg/g produs vegetal (frunze, Zimbru) și 4,2166 µg/g produs vegetal (frunze, zona Iași) în *Solanum dulcamara* și între 0,092 µg/g produs vegetal (fructe, Bucium) și 0,2818 µg/g produs vegetal (tulpini, Bucium) în *Physalis alkekengi*. Pentru *Physalis alkekengi* probele de frunze din Breazu și cele de rădăcini și fructe din Breazu au prezentat valori sub limita de detecție. Valoarea medie a concentrației solaninei în probele de *Solanum dulcamara* a fost de 21,67 ori mai mare decât cea din probele de *Physalis alkekengi*. Pentru *Solanum dulcamara*, cele mai mari concentrații s-au determinat în tulpinile plantelor.

Keywords: solanine, *Solanum dulcamara*, *Physalis alkekengi*, HPLC/MS

Introduction

Solanine is a glycoalkaloid toxine in species of the *Solanaceae* family. It can occur naturally in any part of the plant, including the leaves, fruits and tubers. Solanine has fungicidal and pesticidal properties and it is one of the plant's natural defenses. Solanine was first isolated in 1820 by Desfosses from the berries of the European black nightshade (*Solanum nigrum*), after which it was named. This toxin has neurological and gastrointestinal effects. Historically, solanine was used in the treatment of epilepsy and asthma, in controlled doses. This practice is no longer common. Solanine from unripe fruits of *Solanum dulcamara* has antimicrobial activity by inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus* [5,7].

Recent studies demonstrated the antiproliferative activities of solanine against human colon (HT29) and liver (HepG2) cancer cells. The effectiveness against the liver cells was greater than against the colon cells. Other studies confirmed the anticarcinogenic effects of solanine against human cervical, liver, lymphoma and stomach cancer cells [1, 4, 6]. The most analyzed solanine is that from potato. There are few studies on solanine from other plants of the *Solanaceae* family. This compound is a potential treatment for infections and cancer, this is why, we should explore other sources [2,3].

Within phytochemical research of *Physalis alkekengi* and *Solanum dulcamara*, we determined solanine qualitatively and quantitatively by high-performance liquid chromatography tandem mass spectrometry (HPLC/MS) [8].

Materials and methods

Plant samples were harvested around Iași, Romania as follows:

- *Solanum dulcamara*: August-September 2009 – Iași and Vânători Neamț area.
- *Physalis alkekengi*: August-September 2009 – Bucium and Breazu, Iași.

At 100 mg powder of plant product 5 mL water/methanol/acetic acid 49/49/2 (v/v/v) was added and was let at rest for 24 hours in the dark at room temperature. After that the samples were placed in an ultrasonic bath for 30 minutes. 1 mL of solution was introduced into an Eppendorf tube and was centrifuged for 5 minutes at 12,000 rpm. 200 μ L were further used for the HPLC/MS analysis.

A standard of solanine (Fluka) was used for the quantitative determination (figure 1).

The HPLC tandem mass spectrometer equipment was composed of HP 1100 Series binary pump, autosampler and thermostat, mass spectrophotometer Agilent Ion Trap 1100 SL. The HPLC column was Zorbax SB-C18 100 mm x 3.0 mm i.d., 3.5 μ m. The mobile phase was a mixture of 0.1% formic acid and methanol 75/25 (v/v), isocratic elution, flow rate: 1 mL/min, temperature: 40°C. The MS detection was performed by isolation and ion fragmentation with m/z 869, corresponding to protonated solanine, then ions with m/z 706.4 and 722.4 were monitored by MS/MS spectrum of the analyte.

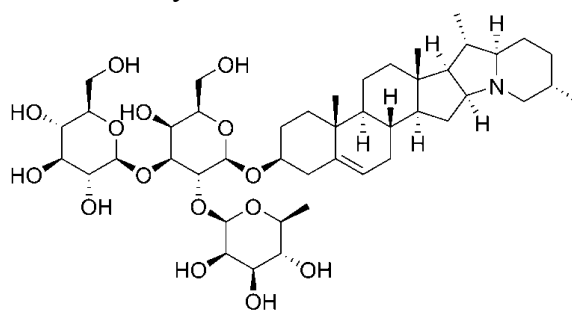


Figure 1

Chemical structure of solanine

Results and discussion

HPLC/MS analysis of solanine standard

Full-scan spectrum of a solution with solanine is presented in figure 2. According to the molecular mass of solanine ($M=868.0$) and in accordance with the positive ionization mode, the expected ion would be the ion with m/z 869.0, adequate for the protonated molecule.

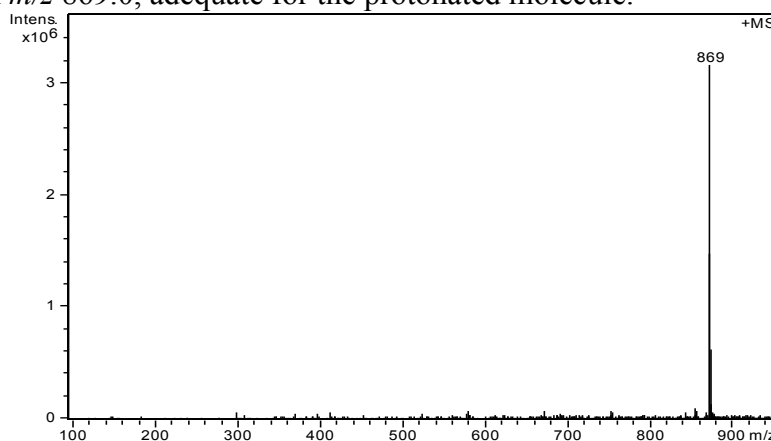


Figure 2

Full-scan spectrum of solanine in mobile phase

In order to increase the selectivity of the HPLC/MS method, characteristic ion of solanine fragmentation was achieved and MS spectrum was recorded (figure 3).

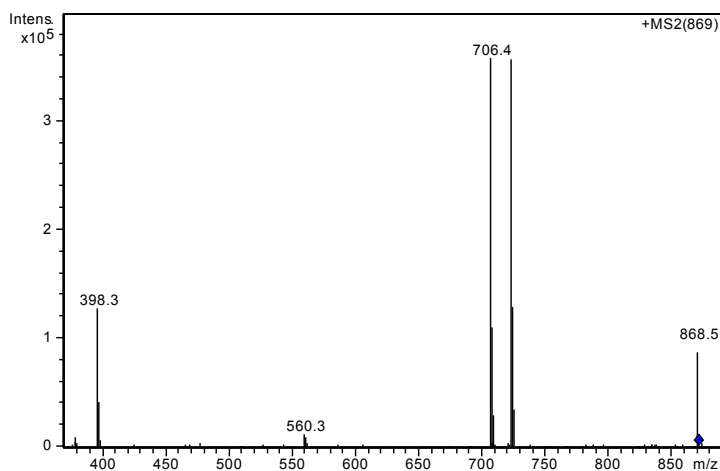


Figure 3

MS/MS spectrum of solanine in mobile phase

Note that by fragmentation, solanine is split into four main fragments with m/z 398, 560, 706 and 722. The most intense ions with m/z 706 and 722 were chosen for quantification. Figure 4 shows a chromatogram of solanine after MS/MS detection.

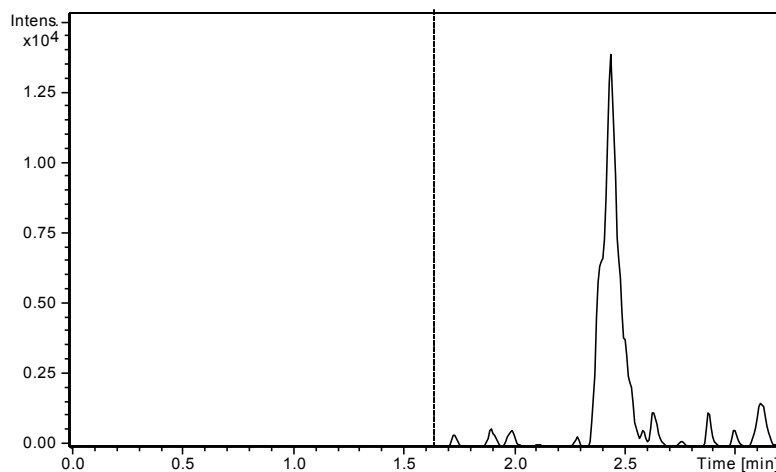


Figure 4

The solanine chromatogram by MS/MS detection,
at limit of the method quantification

The calibration curve of solanine was performed in the concentration range 6 - 180 ng/mL.

HPLC/MS analysis of solanine from *Physalis alkekengi* and *Solanum dulcamara*

The results are expressed in μg solanine/g vegetal product and are presented in tables I (for *Solanum dulcamara*) and II (for *Physalis alkekengi*).

Table I
Solanine concentration in *Solanum dulcamara*

Part of plant	Location	Solanine ($\mu\text{g/g}$ vegetal product)
leaves	Iași	4.2166
leaves	Zimbru	0.7963
stems	UMF	3.8303
stems	Zimbru	3.2025
roots	UMF	1.6007
roots	Zimbru	1.5116
fruits	UMF	1.3639
fruits	Zimbru	1.5501
	Maximum	4.2166
	Mean	2.2590
	Minimum	0.7963

For *Solanum dulcamara* (figure 5), the highest concentration value of solanine was recorded in the stems (mean $3.51 \mu\text{g/g}$ vegetal product); quantities are similar in fruits (mean $1.45 \mu\text{g/g}$ vegetal product) and roots (mean $1.55 \mu\text{g/g}$ vegetal product).

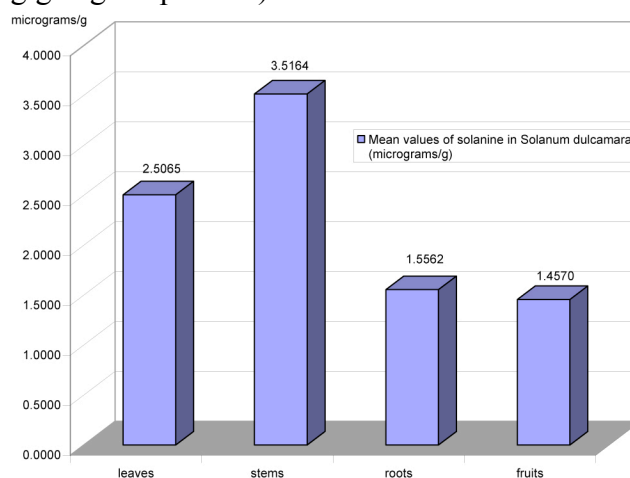


Figure 5

The variation of mean values of solanine concentration in *Solanum dulcamara* ($\mu\text{g/g}$ vegetal product)

Table II
Solanine concentration in *Physalis alkekengi*

Part of plant	Location	Solanine ($\mu\text{g/g}$ vegetal product)
leaves	Bucium	0.0000
leaves	Breazu	0.1442
stems	Bucium	0.2818
stems	Breazu	0.0920
roots	Bucium	0.2233
roots	Breazu	0.0000
fruits	Bucium	0.0926
fruits	Breazu	0.0000
	Maximum	0.2818
	Mean	0.1042
	Minimum	0.0920

For *Physalis alkekengi*, in leaves (sample from Bucium, Romania) roots and fruits (sample from Breazu, Romania) concentration of solanine was below the limit of detection. For *Physalis alkekengi*, the highest concentration of solanine was recorded in stems (mean $0.18 \mu\text{g/g}$ vegetal product), same as *Solanum dulcamara*, but the quantities are much lower than those obtained for *Solanum dulcamara*.

The mean concentration of solanine is 21.67 times greater in samples of *Solanum dulcamara* than in *Physalis alkekengi* and the minimum detectable quantity was 8.65 times lower in samples of *Physalis alkekengi* than the minimum value detected in *Solanum dulcamara*.

The concentration variations in all samples of *Solanum dulcamara* are shown comparatively to those of *Physalis alkekengi* in figure 6.

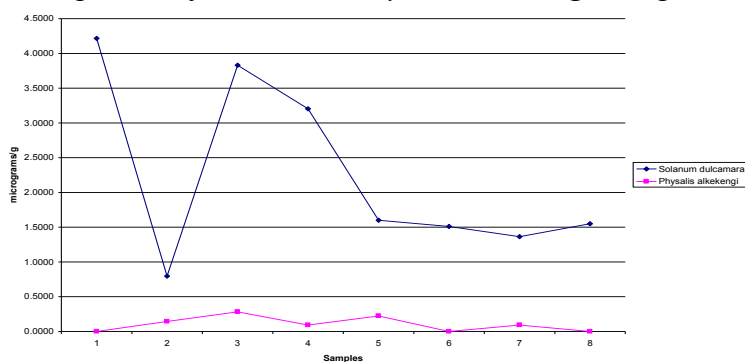


Figure 6

Solanine concentrations in *Solanum dulcamara* compared to *Physalis alkekengi*
($\mu\text{g/g}$ vegetal product)

Conclusions

We developed a HPLC/MS method for the identification and determination of solanine in plants. This method was applied for two plants: *Physalis alkekengi* and *Solanum dulcamara*. Solanine was determined in concentrations ranging between 0.7963 and 4.2166 µg/g vegetal product in *Solanum dulcamara* and between 0.092 and 0.2818 µg/g vegetal product in *Physalis alkekengi*. For *Physalis alkekengi*, in leaves (sample from Bucium, Romania), roots and fruits (sample from Breazu, Romania) the concentration of solanine was below the limit of detection. The mean concentration of solanine was 21.67 times greater in samples of *Solanum dulcamara* than in *Physalis alkekengi*. For *Solanum dulcamara*, the highest concentration of solanine was recorded in stems.

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