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Article

Liquid Chromatography-Orbitrap Mass Spectrometry Method for the Determination of Toxic Glycoalkaloids and their Aglycons in Potato Upper Soil

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O destino de toxinas naturais no meio ambiente tem estado em foco nos últimos anos, assim como compostos produzidos a partir de plantas que podem ser introduzidos no meio ambiente por processos como lixiviação, decomposição de resíduos de plantas ou exsudação. Dois glicoalcaloides (α-solanina e α-chaconina) e suas agliconas (demissidina e solasodina) produzidos pela batata (*Solanum Tuberosum* L.) foram encontrados na camada superior de solo coletada cada semana, durante dois meses, em diferentes níveis. Um novo método analítico que usa cromatografia líquida Orbitrap com espectrometria de massas foi desenvolvido para a análise de dois glicoalcaloides e duas agliconas em camadas superiores de solos com batata e a validação do método incluiu sensibilidade, recuperação, linearidade, exatidão e precisão e especificidade. A aplicação desse método para a triagem de rotina desses compostos foi alcançada e este estudo representa um método simples, rápido e confiável para a quantificação desses compostos em camadas superiores de solos com batata.

The fate of natural toxins in the environment has been in focus for the past years. Also, plant produced compounds that can be introduced to the environment by processes such as leaching, decomposition of plant residues or root exudation. The two toxic glycoalkaloids (α-solanine and α-chaconine) and theirs aglycons (demissidine and solasodine) produced by the potato plant (*Solanum Tuberosum* L.) have been found in upper soil collected each week in two months in varying levels. A new analytical method that uses liquid chromatography Orbitrap mass spectrometry has been developed for the analysis of two glycoalkaloids and two aglycons in potato upper soils, and method validation was performed including sensitivity, recovery, linearity, accuracy and precision and specificity. The application of this method for routine screening of these compounds has been achieved and this study represents a simple, fast and reliable method for the quantification of these compounds in potato upper soils.

Keywords: glycoalkaloids, aglycons, soil, Orbitrap MS, high resolution MS

Introduction

Many plants and microorganisms produce toxins and there has been an increased interest in the fate of natural toxins in the terrestrial environment within the last decade. Several natural toxins have been detected in the soil or in surface, drainage, or soil water. The presence of natural toxins in the terrestrial environment may have unintended effects on various organisms or because they may contaminate valuable drinking water resources. One of the most important crops in the world, the potato plant, produces biologically active secondary metabolites like glycoalkaloids and their aglycons, which may have both adverse and beneficial biological effects in the diet.[1] These compounds are present in all parts of the potato plant, and previous studies indicate that they may be relatively lasting in the terrestrial environment. Hence, the potato glycoalkaloids could possibly be a risk in the terrestrial environment. A vast number of toxins are produced by plants and microorganisms and alone in the human diet 5,000-10,000 natural toxins are estimated to be present.[2] The group of natural toxins are diverse in terms of structure, toxicity, and properties.[3]

The potato plant is one of the most important crops in the world. More than 321 million tons were produced

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worldwide in 2007 using 19 million hectare. Potatoes are often grown on sandy soils with low water to holding capacity. These soils are in general vulnerable to leaching, because they contain little sorption material. The potato fields are heavily irrigated, which will result in large percolation and lead to an increased risk of leaching. The potato plant produces high levels of glycoalkaloids, such as α-chaconine and α-solanine, which are known to be toxic to human as well as to many other organisms including fungi, snails, and insects[4-8] and produces also other aglycons like demissidine and solasodine.

Symptoms of glycoalkaloids poisoning include colic pain in the abdomen and stomach, gastroenteritis, diarrhea, vomiting, fever, rapid pulse, low blood pressure, and neurological disorders.[9] Total glycoalkaloids content of different potato varieties varied from few (about 10) to many (about 580) mg kg^{-1}.[10] Duke[11] and Friedman *et al.*[12] refer the contents of the major glycoalkaloids potato constituents are α-solanine, α-chaconine and solanosolone. The compounds are present in all parts of the potato plant, where the highest concentrations are found in the above ground plant material.[13-16]

Several analytical methods have been used for quantification of the glycoalkaloids,[17] the preferred method is high performance liquid chromatography (HPLC)-UV.[18-20] Though, due to the poor UV absorption of the compounds, a more sensitive and selective detector would be preferable, which leads to the choice of using mass spectrometry (MS) for detection. A few methods using liquid chromatography-mass spectrometry (LC-MS) for potato glycoalkaloids detection have been published recently[21-23] but not in soil. Overall, this indicates that the glycoalkaloids could be relatively persistent in the environment. The high biomass of the potato plant and the high amount of glycoalkaloids in the plant result in a high potential glycoalkaloids load to the soil environment from a potato field. The circumstances, under which the potato plants are grown, constitute a general high risk of leaching. The possible persistence of the compounds in the environment may increase the risk of leaching, because of the prolonged lifetime in the soil. Overall, in addition to the worldwide importance of this crop, this is the motivation for the investigation of the fate of the glycoalkaloids in the environment. The aim of the work is to develop and validate for the first time an Orbitrap method for the separation and determination of two glycoalkaloids (α-solanine and α-chaconine) and two aglycons (demissidine and solasodine) in potato upper soil using up-to-date chemical instrumentation; an HPLC system connected to a LTQ Orbitrap XL (Thermo Scientific, San Jose, CA, USA), through a heated electrospray interface (HESI; Thermo Fisher Scientific, San Jose, CA, USA), that can be suitable as an accurate technique for regulatory monitoring purposes in analysis.

Material and Methods

Materials and standards

Analytical standards of α-solanine (99.0%) and demissidine (99.0%) were purchased from Sigma Aldrich (Dublin, Ireland), analytical standards of solasodine (99.0%) and α-chaconine (98.0%) were purchased from ABCR (Karlsruhe, Germany). Stock solutions at concentration of 1000 μg mL^{-1} were prepared from the pure compound standards using methanol/formic acid 0.2%. Standard working solution, at various concentrations, were daily prepared by appropriate dilution with methanol of aliquot of the stock solution.

Formic acid analytical grade was purchased from Prolabo (Manchester, UK). All solvents including HPLC grade methanol and HPLC grade water were purchased from Fisher (Dublin, Ireland). Prior to HPLC injection, the samples were filtered through a 0.45-μm PTFE filter from Supelco (Bellefonte, PA, USA). Cartridges solid phase extraction (SPE) Strata C18-E (200 mg/3 mL) were purchased from Phenomenex (Torrance, CA, USA).

Sample collection

Soil samples were collected in potato crops of the Cork city area at seven different week during the months of July and August 2013. They were dried in stove at about 80 °C for at least 24 hours prior analysis.

Extraction procedure for potato upper soil

5 g of soil sample are weighed in 50 mL centrifuge tube and homogenized with Ultraturrax with 15 mL methanol. Then the sample is centrifuged for 5 min at 3000 rpm. Supernatant organic solution is transferred into another centrifuge tube. The residue is treated twice more with 15 mL of methanol and then centrifuged for 5 min at 3000 rpm. Supernatants are combined, they are then filtered using a 0.45 μm nylon membrane filter, evaporated, and made up with 20 mL of water before SPE.

The solid phase extraction tube used for the clean up glycoalkaloids was the SPE Strata C18-E extraction tube purchased from Phenomenex (CA, USA). Each SPE cartridge was conditioned with HPLC methanol (3 mL) followed by HPLC water (6 mL). Samples were loaded onto each cartridge using a vacuum pump (KNF Laboport,

Carl Stuart, Dublin, Ireland) set at 10 psi. The cartridge was washed with water (4 mL) to remove the undesirable components of the matrix and eluted using HPLC methanol. The eluted solution was evaporated to dryness under nitrogen using a Turbo Vap LV evaporator (Zymark, Caliper Technologies, Russelsheim, Germany) and reconstituted in HPLC methanol (0.5 mL). The solution was transferred to a Teruno Syringe (5 mL) and filtered using a 0.45 μm nylon membrane filter, and transferred into an amber HPLC vial (10 mL, Thermo Scientific, Hemel Hempstead, Hertfordshire, UK).

Instrumentation

The separation of the analytes were carried out using an HPLC system (Thermo Scientific Accela; Thermo Scientific, San Jose, CA, USA) equipped with a Luna PFP analytical column of 150 mm × 2.0 mm and 3 μm particle size (Phenomenex, Torrance, CA, USA). The injected sample volume was 10 μL.

Mobile phase A and B were water and acetonitrile, respectively, both containing 0.01% formic acid. The gradient program for the separation was: 0 min 80% A, 0-9 min 20% A. Finally, phase A was increased at 80% from 9 to 9.10 min and held at 80% until end of the run at 13 min. The flow rate during analysis was 200 μL min^{-1}.

The HPLC system was connected to an LTQ Orbitrap XL (Thermo Scientific, San Jose, CA, USA), through a heated electrospray interface (HESI; Thermo Fisher Scientific, San Jose, CA, USA), operating in positive ionization mode using the following parameters: capillary temperature 240 °C, vaporization temperature 250 °C, sheath gas flow 35, aux gas flow 30, source voltage 4 kV, source current 100 μA, capillary voltage 52 V, tube lens 120 V.

The scan type settings used for the analysis are: scan 1, analyzer: Fourier transform mass spectrometer (FTMS) operating in full scan; resolution: 30,000 FWHM (full width at half maximum); polarity: positive. Scan 2-5, were operated in: ion trap mass spectrometer (ITMS) mode using the $[M + H]^+$ ions and their optimized collision energies (CE) 852.51 at 42% CE, 868.5 at 39% CE, 400.35 at 38% CE and 414.35 at 26% CE for confirmation.

Result and Discussion

Method validation

Percentage recovery experiments were conducted by spiking known concentrations of α-chaconine, α-solanine, demissidine and solasodine in soil samples at the beginning of the extraction procedure. The percentage recoveries of the monitored analytes are illustrated in Table 1.

It is clear from these results that the percentage recoveries are acceptable. α-chaconine and α-solanine show the best percentage of recovery with ≥ 89.9% recovery. The percentage of recovery of demissidine and solasodine are also quite good, with ≥ 69.5%. The repeatability of the method, evaluated 5 times on each kind of soil, was expressed by % CV, that proved to be lower than 7.9 %.

In Figure 1, a chromatogram of a spiked soil samples at a concentration of 0.5 mg kg^{-1} is shown.

The specificity of the method was attained using the retention time stability. The stability in retention of each analyte was analyzed over 5 days, with a total sample number equal to 50. The retention time of this method is stable over a five-day period, with a relative standard deviation of ≤ 1.69 % (Table 1).

The linear range of this method was investigated using different ranges for each compound and was calculated in HPLC grade methanol. The linear range for demissidine and solasodine was from 0.0025-0.1 mg kg^{-1} with a sample number equal to five (n = 5) and the linear range for

Table 1. Calibration data including linear range, correlation coefficient (R^2), limits of detection (LODs; mg kg^{-1}), limits of quantitation (LOQs; mg kg^{-1}), retention time stability (RSD% in parenthesis), and percentage recovery of glycoalkaloids and their aglycons in soil samples (n = 5)

Analyte	Linear range / (mg kg^{-1})	R^2	LOD[a] / (mg kg^{-1})	LOQ[b] / (mg kg^{-1})	Ret. time stability / (RSD %)	Spiked concentration / (mg kg^{-1})	Recovery / %	RSD / %
α-solanine	0.025-1	0.9975	0.01	0.025	4.65 (1.07)	0.05	90.3	6.3
						0.5	99.0	3.7
α-chaconine	0.025-1	0.9967	0.01	0.025	4.68 (1.00)	0.05	89.9	3.4
						0.5	108.9	7.9
solasodine	0.0025-1	0.9958	0.0001	0.00025	6.25 (1.69)	0.005	83.0	6.8
						0.05	84.7	1.8
demissidine	0.0025-1	0.9933	0.000075	0.0001	6.32 (1.47)	0.005	69.5	3.4
						0.05	72.1	3.3

[a]LOD: limit of detection; [b]LOQ: limit of quantitation.

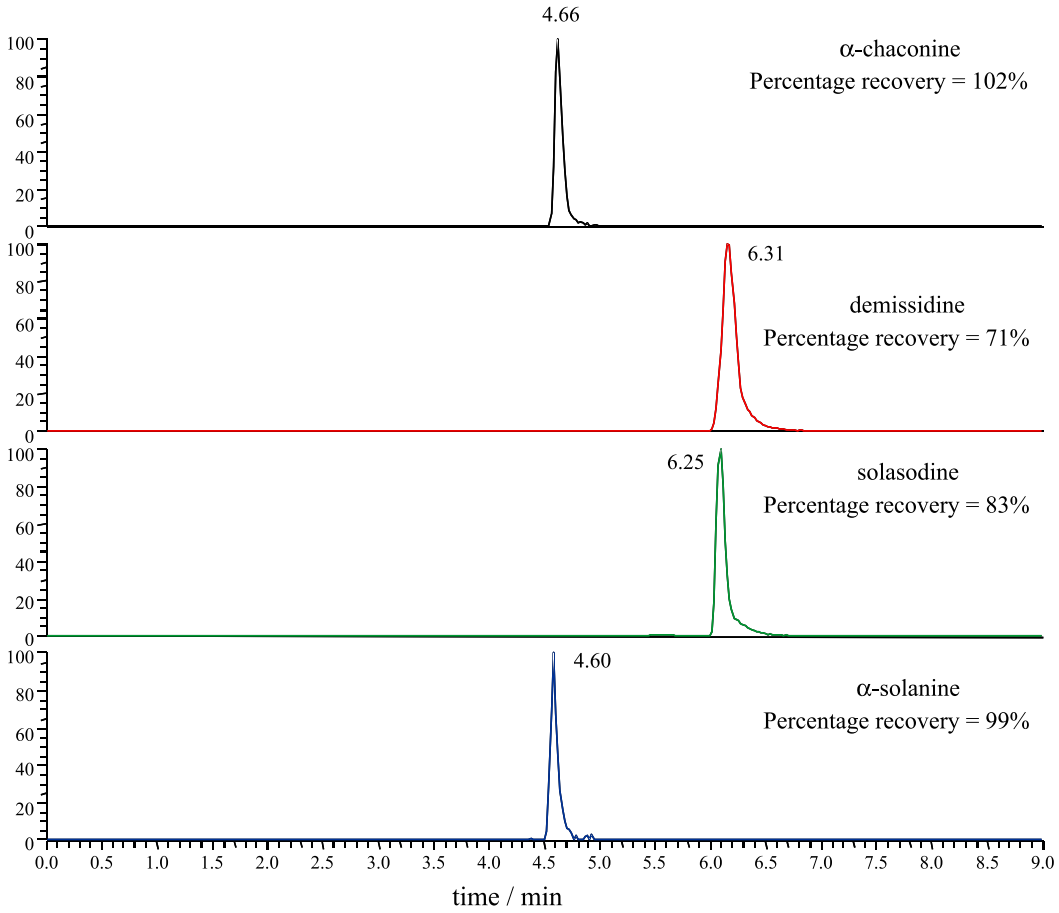

Figure 1. Spiked soil sample at a concentration of 0.5 mg kg^{-1} with the standards of the four compounds.

α-chaconine and α-solanine was from 0.025-1 mg kg^{-1}. This method showed good linearity for all the analytes with correlation coefficients (R^2) ≥ 0.9933 (Table 1).

Sensitivity was expressed by the instrumental limit of detection (LOD) and limit of quantitation (LOQ) for each target compound. Three times the S/N was used to determine the LOD and ten times the S/N was used to determine the LOQ (both calculated in HPLC grade methanol). The limits obtained are very low, especially for demissidine and solasodine. LOQs for demissidine and solasodine were 0.0001 and 0.00025 mg kg^{-1}, respectively. LOQs for α-chaconine and α-solanine were 0.025 mg kg^{-1} for both compounds. These results highlight the consistent and the appreciable sensitivity that can be obtained also using a full scan method like in the SPE-LC/LTQ orbitrap method developed (Table 1).

The accuracy of these methods were determined using percentage relative errors at two different spiked concentrations (0.05 and 0.5 mg kg^{-1} for α-chaconine and α-solanine, 0.05 and 0.005 mg kg^{-1} for demissidine and solasodine) over five days (n = 5). The % relative error are ≤ 9.89 %. The lowest % relative error is 1.09 % obtained for α-chaconine. The precision of this method was determined by monitoring the % RSD over a five day period. All the % RSD obtained were ≤ 7.62% for the standards. The lowest % RSD was ≤ 1.45% for the standard of solasodine (data not shown).

Application of the LC/MS method to soil samples

The analytical data obtained in HPLC/MS LTQ Orbitrap of 7 soil samples collected in potato crops of the Cork city area at seven different week during the months of July and August 2013 are reported in Table 2, where sample date and concentrations of the glycoalkaloids and their aglycons, expressed in mg kg^{-1}, are shown.

The data display that the concentration of the glycoalkaloid α-chaconine range from 0.0108 in the sample of August 7th to 0.0668 mg kg^{-1} relative of a sample collected on August 14th. The levels of α-solanine range from 0.0099 mg kg^{-1} to 0.0782 mg kg^{-1} without important difference between samples. The trend of the content of α-chaconine and α-solanine in the seven monitored sample seems to be quite similar, with a maximum at august 14th and 21st. The amounts of the two aglycons, solasodine and demissidine, are lower than that one of the two glycoalkaloids. Also, in this case,

the trend of the concentration of the two aglycons in the monitored soil samples seems to be similar with a maximum also in this case on August 14th.

A few methods using LC-MS for potato glycoalkaloid detection have been published recently. However, these methods are all focusing on other applications, primarily analysis of samples from potato matrices.[23] Instead, it is well known that natural compounds may be released by different mechanisms from the plants; volatilization, leaching from plant parts, decomposition of plant residues, or root exudation[24] and the amount released from the plant is uncertain. The plants are also present in the field for a long period of time. This may overall lead to a more continuous application process.

In one study, an analytical method for the quantification of some glycoalkaloids (different from ours) has been performed and validated in environmental matrices.[25]

Jensen *et al*. found a maximum glycoalkaloid concentration of 2.8 mg kg^{-1} dry weight soil, determining that the leaching potential of the glycoalkaloids is to be considered small.[26] This value is higher with respect to our data. In fact, the highest total glycoalkaloids level, among the seven soil samples collected, has been found in the sample collected on August 14th in which the sum of the four analytes is equal to 1.383 mg kg^{-1}.

Our data (Table 2) indicate that the degradation proceeded relatively slow for glycoalkaloids in soil matrices as previously reported by Jensen *et al*..[27]

In Figure 2, a chromatogram of a soil sample collected on August the 7th, contained the four analytes at different concentration is reported. Additionally, mass error in ppm is shown for each one.

Accurate mass studies for standard solution and soil samples

Mass measurement experiments were carried out by using the LTQ Orbitrap MS and 1, 0.8, 0.75, 0.5, 0.3, 0.25, 0.1, 0.03 μg mL^{-1} standard solutions of α-chaconine, α-solanine, demissidine, solasodine. The accurate mass assigned to the $[M + H]^+$ ions of the analytes in each acquired mass spectrum was measured.

Thirty mass measurements for $[M + H]^+$ ions for the standards of α-chaconine, α-solanine, demissidine and solasodine were selected by using Orbitrap MS at a mass resolving power of 30,000 FWHM and scan cycle time 0.25 s and the mass error measured in ppm ranged from –2,09213 to –0,01842, showing the great accuracy of these data.

In addition, mass measurement experiments were carried out by using the LTQ Orbitrap MS and soil samples.

Table 2. Concentration of glycoalkaloids and aglycons in soil samples collected each week during two months (7 samples)

No. sample	Date of collection	Concentration / (mg kg^{-1})[a]
1	July 17th	α-chaconine = 0.343 α-solanine = 0.194 demissidine = 0.000686 solasodine = 0.000948
2	July 24th	α-chaconine = 0.2506 α-solanine = 0.2465 demissidine = 0.0004693 solasodine = 0.0005513
3	July 31th	α-chaconine = 0.1708 α-solanine = 0.1719 demissidine = 0.00000479 solasodine = 0.0004542
4	August 7th	α-chaconine = 0.1081 α-solanine = 0.09945 desmissidine = 0.006933 solasodine = 0.005057
5	August 14th	α-chaconine = 0.6677 α-solanine = 0.6239 demissidine = 0.05074 solasodine = 0.04071
6	August 21th	α-chaconine = 0.5209 α-solanine = 0.7812 demissidine = n.d. solasodine = 0.0000994
7	August 27th	α-chaconine = 0.3745 α-solanine = 0.3616 demissidine = 0.02454 solasodine = 0.01915

[a]All samples were prepared in triplicate with RSD% lower than 6.7.

The accurate mass assigned to the $[M + H]^+$ ions of each of the four analytes in soil samples in each acquired mass spectrum was measured. Fourteen mass spectra of each analyte were selected to provide a set of mass measurement data acquired at Orbitrap mass resolving powers of 30,000 FWHM and scan cycle times 0.25 s. Fourteen mass measurements for $[M + H]^+$ of each analyte in soil samples by using Orbitrap MS at a mass resolving power of 30,000 FWHM were carried out. The mean of the measured mass and mass error are shown in Table 3. Also in this case, results are excellent (mass error ranged from –1.705617 to –0.071880 ppm).

Conclusion

In conclusion, with this research, we set up a new method for the separation and quantification of glycoalkaloids and aglycons in soil samples.

The levels of the detected glycoalkaloids and aglycons are low, but the method developed provided high selectivity and efficiency, as confirmed by the mass error always lower than 2 ppm. This study represents a simple, fast and reliable method for the quantification of these compounds in potato

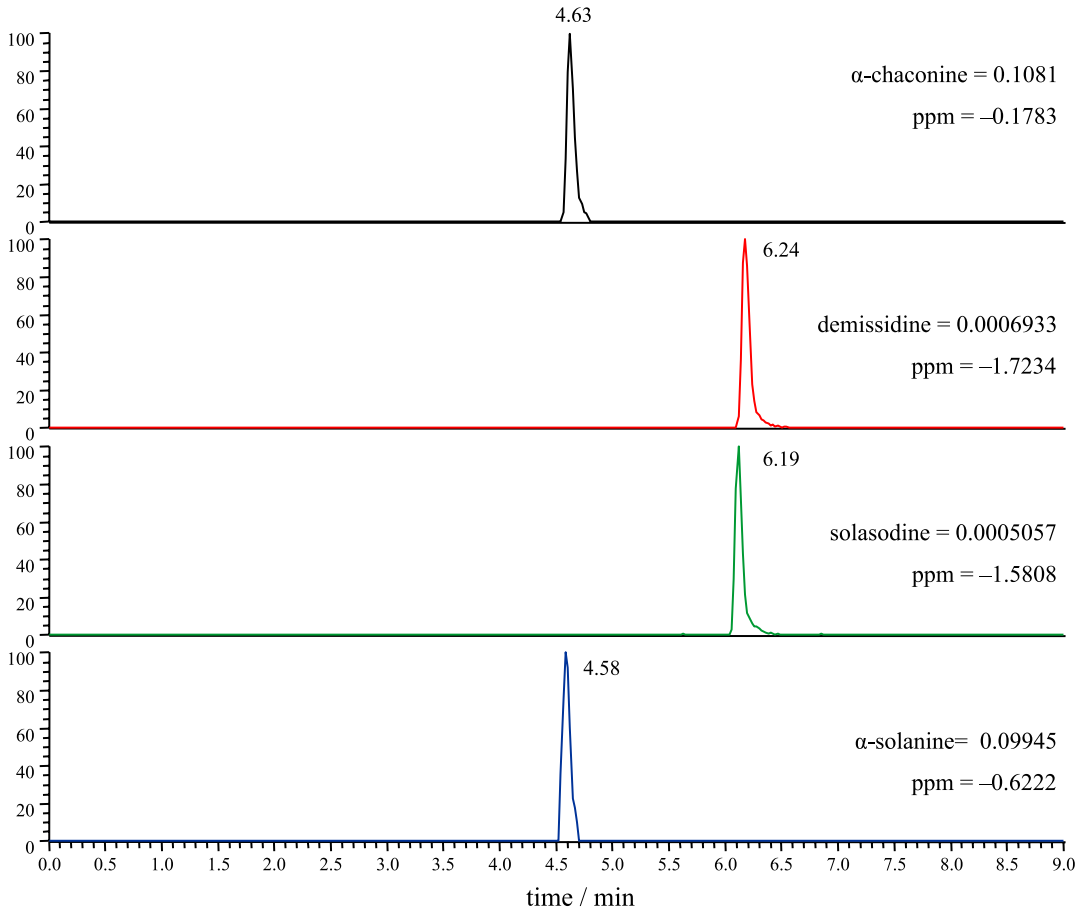

Figure 2. Soil sample collected on August 7th, contained the four analytes at different concentration. Mass error in ppm is shown for each one.

Table 3. Summary of results from 14 mass measurements for $[M + H]^+$ of each analyte in soil samples by using Orbitrap MS at a mass resolving power of 30,000 FWHM.

Compound	Theoretical *m/z* of $[M + H]^+$ ion	Mean measured *m/z* (n = 14)	Mass error / ppm
α-chaconine	852,510932	852,510825	–0.125512
α-solanine	868,505846	868,505784	–0.071880
demissidine	400,357940	400,357257	–1.705617
solasodine	414,337205	414,336594	–1.475679

upper soils. The developed method can be really useful for laboratory involved in the routine screening of these compounds for regulatory monitoring purposes.

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