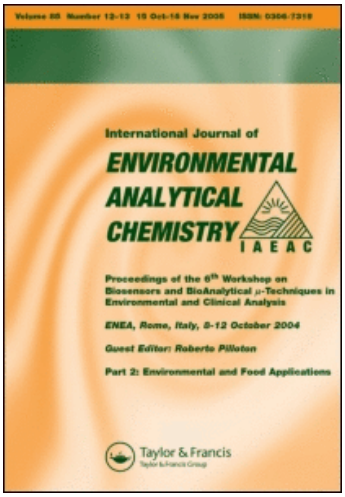

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Extraction and determination of the potato glycoalkaloid α-solanine in soil

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The toxic glycoalkaloids α-solanine and α-chaconine are produced in all parts of the potato plant, and post-harvest potato tubers may represent a source of soil and water contamination. A new method was developed for extraction and purification of α-solanine in soil samples. Soil samples were extracted with $THF:H_2O:ACN:CH_3COOH$ (50 : 30 : 20 : 1) and the extract purified by SPE before HPLC determination of α-solanine. The limit of detection was 2.4 mg of α-solanine kg^{-1} soil. The new procedure was used for determination of α-solanine in spiked soils with varying content of organic matter and texture. Recovery for soil samples spiked with α-solanine 1 h before extraction was 61–68% for soils low in organic carbon (< 2.2% C), and to 47% for soil high in organic carbon. Similar recoveries were obtained for α-chaconine. The reproducibility of the method shown by the relative standard deviation varied from 1.7 to 10.1%, depending on the soil type. No decrease in extractable α-solanine was observed until day 17 for soil samples spiked with pure α-solanine kept at 5°C, while the content in samples spiked with potato materials showed a faster decline. This indicates that the degradation and/or ageing processes proceed relatively slowly for glycoalkaloids in soil matrices. This is the first method reported for determination of potato glycoalkaloids in soil.

Keywords: *Solanum tuberosum*; Glycoalkaloids; α-Solanine; Soil; Extraction; SPE

1. Introduction

Numerous plants produce toxins as protection against fungi, insects, and animals, and as allelochemicals in the competition with other species. A large group of such natural toxins are the alkaloids counting very toxic compounds such as strychnine, morphine, cytisine, and atropine. According to Raffauf [1], more than 10,000 different alkaloids are distributed among 300 plant families, including *Solanaceae* with about 2600 species. *Solanaceae* counts several important crop plants such as eggplant (*Solanum melongena* L.), chilli pepper (*Capsicum sp.* L.), tomato (*Lycopersicon esculentum* Mill.), and potato

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Table 1. Selected properties of α-solanine.

Compound	α-solanine
CAS no.	20562-02-1
Molecular formula	$C_{45}H_{73}NO_{15}$
Molar mass	868.07 g mol^{-1}
Water solubility	3 mg L^{-1} [a]
log K_{OW}	2.0[a]
log K_{OC}	4.3[a]
pK_a	6.66[b]
LD_{50} (mice)	32.3 mg kg^{-1} [c]

[a]Calculated with EPIwin v3.11, [4].
[b][5].
[c][6].

(*Solanum tuberosum* L.), all containing different types of steroid alkaloids, some of which are conjugated to carbohydrates to form glycoalkaloids as in potato plants. In tubers from commercial potato cultivars, more than 95% of the glycoalkaloids are made up of α-solanine and α-chaconine [2]. The concentration of glycoalkaloids varies widely among cultivars; e.g. Friedman *et al*. [3] found concentrations in the range of 7–187 mg kg^{-1} for eight different cultivars. Selected properties of α-solanine are presented in table 1.

α-Solanine and α-chaconine contain a polar (carbohydrate) and a non-polar (steroidal) part (figure 1), and hence are expected to have surfactant properties. The solubility of α-solanine in water is low, and the estimated octanol–water (K_{OW}) and soil organic (K_{OC}) partitioning coefficients reflect a relatively high affinity of the compound for sorption to natural organic matter. α-Solanine has a pK_a-value of 6.66, and therefore both the cationic and uncharged form will be present in most agricultural soils and seepage water having pH in the range 5–7. Both glycoalkaloids are quite resistant to acidic conditions and hydrolyses only slowly, but α-chaconine hydrolyses at a higher pH than α-solanine. Experiments have shown that more than 96 and 99% of α-chaconine was still present after 1 h at HCl concentrations of 0.25 M (60°C) and 1 M (38°C), respectively [7, 8].

A detailed review of the toxicity of the glycoalkaloids is found in Friedman [9]. The two main effects are an inhibition of the two cholinesterases, acetyl- and butyrylcholinesterase, and cell disruption caused by a complex formation with the cell membrane. Several other effects in animals have been reported including the ability to induce spina bifida, anecephaly, embryotoxicity and teratogenicity [9]. Morris and Lee [10] estimated that an oral intake of 2–5 mg of potato glycoalkaloids per kilogram of body weight was toxic, and 3–6 mg of potato glycoalkaloids per kilogram of body weight was lethal for humans. Studies have shown that potato glycoalkaloids are toxic to fungi, insects, and snails, for example [11–14], while in other studies no toxic effects were seen towards potato pathogens [13–15]. α-Solanine and α-chaconine can be metabolized by fungi to the corresponding β- or γ-compounds or further to the aglycone, solanidine. One strain of the potato pathogen *Gibberella pulicaris* metabolized both α-solanine and α-chaconine within 24 and 2 h, respectively. Only α-chaconine was metabolized to the aglycone and even further to two unknown metabolites [16]. Another strain in the same study [16] metabolized only α-chaconine, which was also found in a study of three strains of filamentous fungi [17].

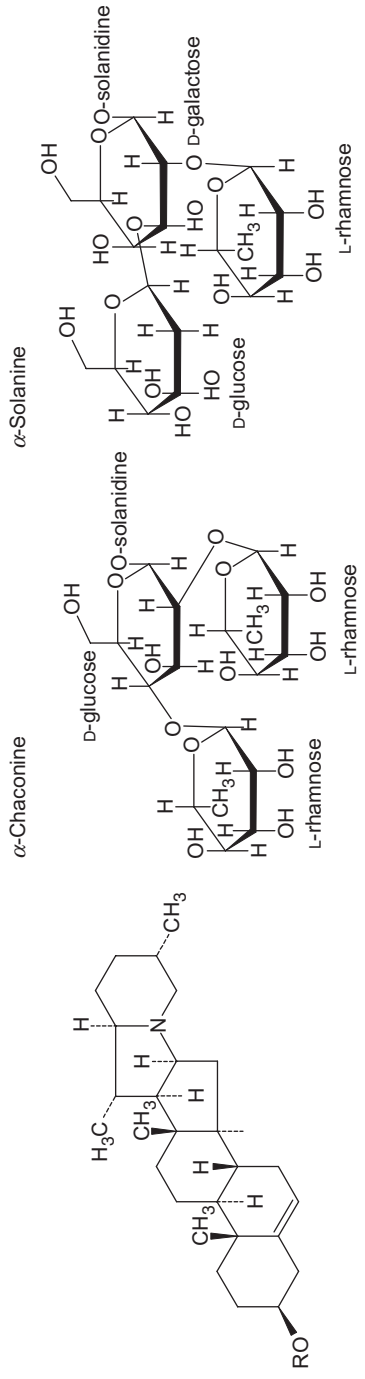

Figure 1. Chemical structures for the glycoalkaloids, α-chaconine and α-solanine.

Potato glycoalkaloids are only sparingly soluble in aqueous solutions at $pH \geq 7$. Hence, solvents to extract the glycoalkaloids are organic, acidic, or both [15]. Heat during extraction should be avoided, since a combination of heat and acid might cause hydrolysis. Solid-phase extraction (SPE) is the most common sample clean-up technique for determination of potato glycoalkaloids in plant material, and HPLC with UV-VIS detection is the preferred method of analysis due to its ability to distinguish between the two glycoalkaloids without prior derivatization as needed when determination is carried out by GC [15].

Several classes of secondary metabolites can be released from the plant [18], and alkaloids from *Cinchona* plants have also been detected in the soil environment [19]. To our best knowledge, no experiments concerning the release of glycoalkaloids from potato plants or any methods to extract and determine the amount of potato glycoalkaloids in soil have been published.

After the potato harvest, approximately 0.1–0.5 $kg\,m^{-2}$ of tubers are left in the field. These tubers, often having been damaged by machinery, are exposed to sunlight and cold weather, and damaged from attacks by animals and pests. All these factors usually increase the glycoalkaloid concentration in the tubers. In tubers exposed to daylight for 21 days, the content increased 3.6–10 times depending on the cultivar [20]. Mechanical injuries doubled the content [21] and pests caused a 50% increase [22]. Glycoalkaloids and other constituents may leak from the tubers to the soil from which leaching to surface or groundwater may take place; in addition, the soil glycoalkaloids may affect soil organisms and future crops. The increased content of glycoalkaloids in the tubers left after harvest may significantly increase the risk of environmental or health effects. Hence, a method for determination of glycoalkaloids in soil is needed.

In this work, we report a method for extraction, clean-up, and HPLC determination of α-solanine and α-chaconine in soil samples, and the method is tested on soil samples spiked with pure α-solanine or potato matrices.

2. Experimental

2.1 *Materials*

For use in the method development, α-solanine was extracted and isolated from potato sprouts by the method of Bushway *et al.* [23]. Isolation and purification of α-solanine were done according to Bushway [24]. Potato tubers were placed in daylight for sprouting. The sprouts were collected after about 1 month and freeze-dried. Eighty grams of freeze-dried sprouts containing 31.6 mg of α-solanine g^{-1} yielded 0.59 g of α-solanine as a powder-like white solid. This corresponds to a 23% yield. The identity and purity of the extracted α-solanine were verified against α-solanine obtained from Sigma-Aldrich (>95% purity, Sigma-Aldrich, Milwaukee, WI) using C- and H-NMR, FT-IR, HPLC, and TLC. Except for trace amounts of α-chaconine, no impurities were detected. Stock solutions of α-solanine were prepared in 0.1 M KH_2PO_4.

Four arable soils were selected to cover a range in texture and soil organic matter. Soil I is a topsoil (0–30 cm) from Tybjerg, middle of Zealand, Denmark, and Soil II is a topsoil (0–25 cm) from eastern Zealand. Soil III is a topsoil (0–25 cm) and soil IV a subsoil (35–70 cm); both were sampled near Grindsted, Jutland. Soil I is a sandy loam,

soil II is a sandy clay loam, and Soils III and IV are sandy soils. Soils I, III, and IV were all appropriate for potato crop growth. The soils were air-dried, and the fine earth fraction was obtained after passing through a 2-mm sieve. Soil III was used throughout the method development. The spike solution (α-solanine in 5 mM HCl) was added to a small portion of the soil and mixed. The rest of the soil was divided into smaller portions, and added one by one to the spiked portion with mixing in-between. The addition of small amounts of 5 mM HCl to the soil decreased the soil pH by less than <0.1 pH unit.

2.2 *Chemicals and reagents*

C18 SPE columns (500 mg 3 mL^{-1}, IST, Argonaut Technologies, CA or DSC-18, Supelco, PA) were used for solid-phase extraction and clean-up. Water was purified in a Milli-Q Reagent Grade Water System, and all solvents were of HPLC grade. All chemicals used were of analytical grade or higher.

2.3 *Determination with HPLC*

HPLC with UV/VIS detector (Merck Hitachi L-4200 UV-VIS, D-6000 Interface, L-6200 Intelligent Pump, 655 A-40 Autosampler) was used for isocratic determination of α-solanine in the extracts. The compounds were separated using a Purospher RP-18E column (5 µm, 125 × 4 mm) guarded by a LiChrospher 100 RP-8 guard column, both from Agilent. The oven temperature was 40°C. The eluent was 60% ACN in 0.01 M phosphate buffer (K_2HPO_4 and KH_2PO_4, pH 7.6) applied at a constant flow of 1.0 mL min^{-1}. The wavelength of the detector was set at 198 nm, and the injection volume was 30 µL. Examples of chromatograms for standards and spiked soil samples can be seen in figure 2. Due to the co-extracted compounds from the soil, the baseline

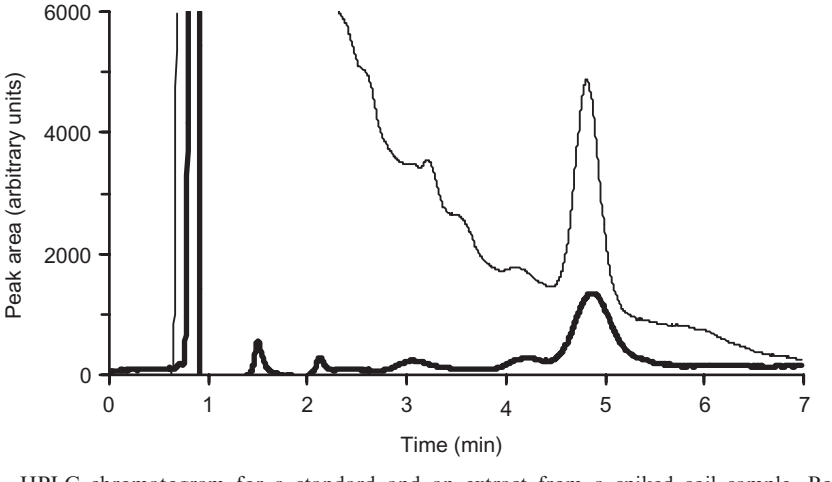

Figure 2. HPLC chromatogram for a standard and an extract from a spiked soil sample. Bold line: α-solanine standard with an α-solanine concentration of 5 mg L^{-1}. Thin line: spiked soil sample. α-Solanine is eluted at 4.9 min; any α-chaconine would be eluted after 6.5 min.

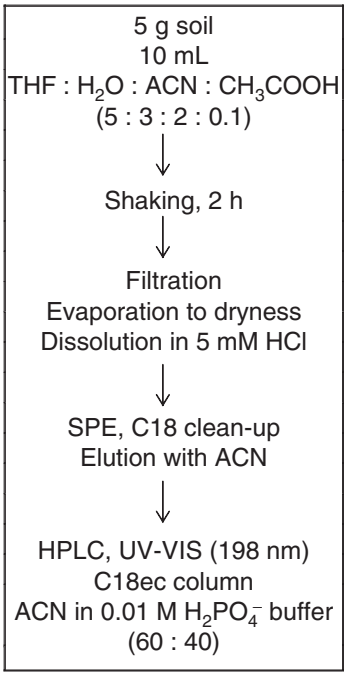

Figure 3. Procedure for extraction, clean-up, and determination of glycoalkaloids in soil.

for spiked samples is more uneven than for the standards, but no interfering peaks from the soil matrix were observed.

2.4 *Final method*

Five grams of soil samples were weighed out into 50-mL glass centrifuge tubes capped with Teflon lids (figure 3). Ten millilitres of THF : H_2O : ACN : CH_3COOH (50 : 30 : 20 : 1) was added, and the tubes were shaken for 2 h at room temperature in a reciprocal shaker at 200 strokes min^{-1}. After filtration (Whatman No. 1), the filtrate was quantitatively transferred to 50-mL glass beakers and evaporated to dryness using a sand bath at 60°C. The residue was dissolved in 30 mL of 5 mM HCl. The walls of the beakers were scraped with a transfer pipette to dissolve as much residue as possible. The solution was quantitatively transferred to a C18 SPE column conditioned with 3 mL of ACN and 3 mL of 5 mM HCl. The column was rinsed with 3 mL of 20% ACN, and α-solanine was eluted with 3 mL of 60% ACN in 0.01 M phosphate buffer and stored in the dark at 5°C until analysis by HPLC.

2.5 *Recovery and reproducibility*

To test the recovery, soil III was spiked with α-solanine to obtain five different concentrations between 5 and 100 mg of α-solanine kg^{-1} soil. One hour after spiking, the soil was extracted in triplicate according to the procedure above. To compare the

recoveries for different soil types and to determine the reproducibility of the method, soils I–IV were spiked to obtain a concentration of 13.2 mg of α-solanine kg^{-1} soil, and three to six samples of each were extracted. To determine the applicability of the method to α-chaconine as well, a mixture of the two glycoalkaloids were added to soil III (66 mg kg^{-1} and 30 mg kg^{-1} for α-solanine and α-chaconine, respectively) and the recoveries were determined by extraction of four replicates.

2.6 *Ageing*

The effect of a longer contact time between α-solanine and soil material was tested. After spiking 100 g of soil III with α-solanine to obtain a soil concentration of 88.4 mg kg^{-1} and a moisture content of 10%, the soil was placed in the dark at 5°C to minimize microbial degradation. After 0, 7, 17, and 36 days, three subsamples were taken, and the content of α-solanine was determined as described above.

Potato tubers of the cultivar Sava were placed in daylight at room temperature for about 3 weeks to induce the α-solanine production [20]. Potato tubers were finely grated by a juicer (Braun MP-80 Juicer), separating the potato juice and the potato pulp. A mixture of juice and pulp corresponding to the proportions of juice and pulp in the tubers was prepared. The content of α-solanine in the juice and in the mixture was determined according to AOAC [25]. Soil III was spiked with juice or mixture, respectively, in the proportions 1 : 5 (potato material : soil), hereby obtaining a concentration of α-solanine of 23–26 mg kg^{-1}. One hundred grams of spiked soil was placed into 500-mL bottles (triplicate) and placed in the dark at 5°C. After 0, 6, 12, and 26 days, subsamples were taken, and the content of α-solanine was determined.

3. Results and discussion

3.1 *Method optimization*

3.1.1 Extraction solution. Four different solutions were initially tested for their ability to extract α-solanine from soil. Using an aqueous solution of 5% acetic acid, a reagent which had previously been used for extraction of α-solanine from potato tubers [25], no α-solanine was extracted. Using a solution of THF : water : ACN (50 : 30 : 20), a reagent which had previously been used for extraction of α-solanine from freeze-dried potato blossoms [23], a recovery of 34% was obtained. A similar solution with an addition of acetic acid (50 : 30 : 20 : 1) was previously used by Bushway *et al.* [26] for extraction from freeze-dried or dehydrated potatoes. Using this solution, the recovery increased to 53%. A solution of THF solely did not extract any α-solanine. Of the four tested solutions, the best extractant was THF : water : ACN : CH_3COOH (50 : 30 : 20 : 1), and this extractant was used in the further work.

3.1.2 Soil : solution ratio. A number of soil : solution ratios were tested to determine the ratio resulting in the highest recovery. The tested ratios were 1 : 1, 1 : 2, 1 : 3, and 1 : 5. The best recoveries were obtained for 1 : 1 or 1 : 2. For further work, a ratio of 1 : 2

Table 2. Recoveries for 17 different apolar SPE-columns spiked with 10 mL of 5 mM HCl-solution containing 25.4 mg of α-solanine L^{-1}.[a]

SPE-column	Recovery (%)	SPE-column	Recovery (%)
C-2	105	DSC-18 (Supelco)	103
C-2 (end-capped)	103	PH	106
C-4	105	101 (200 mg)	97
C-6	103	ENV+ (200 mg)	86
C-8	102	ENV+	10
C-8 (end-capped)	106	SDB-L (Phenomenex)	105
C-18	99	RP-select B (Merck)	105
C-18 (end-capped)	100	CN (Merck)	104
MFC-18	107		

[a]All columns contain 500 mg of packing material and are from IST unless otherwise stated. All recoveries are based on a single measurement.

(5 g soil + 10 mL extraction solution) was selected, as this ratio and amount gave a satisfactory amount of filtrate for further analysis.

3.1.3 Extraction time. To determine the optimal extraction time for high extraction efficiency, samples were shaken for 2, 16, 48, and 72 h. Only minor improvements in recovery were obtained using a longer extraction time than 2 h, so an extraction time of 2 h was found to be sufficient.

3.1.4 SPE column. The recoveries of 17 different apolar SPE columns were tested to find a suitable SPE column for the clean-up process. The columns were spiked with 10 mL of 5 mM HCl-solution containing 25.4 mg of α-solanine L^{-1}. Most of the columns showed good recoveries around 100% (table 2), and the recovery did not depend on the formulation of the packing material, including the length of the alkyl chains attached to the sorbent particles. Only the ENV + sorbent showed low recoveries. The C18 column, IST or the similar DSC-18, Supelco was used in the further development of the method.

3.1.5 Matrix effect. In order to discover any matrix interferences compared with pure standards, two sets of standards were made: one with α-solanine dissolved in 0.1 M KH_2PO_4 and eluent, and one with 0.1 M KH_2PO_4 and eluent containing soil extracts. The extracts were made as described in the final method. For all standard curves, the correlation coefficient R^2 was at least 0.999 in the concentration range of 5–200 mg of α-solanine L^{-1}. There was no significant difference between the slopes of the standard curves prepared in eluent and in eluent containing soil extracts at a 5% significance level. Hence, pure standards were used for preparation of standard curves.

3.1.6 Extraction efficiency and reproducibility. An extraction efficiency of $63 \pm 8\%$ was found for concentrations between 5 and 100 mg of α-solanine kg^{-1} soil for soils with a low organic C content ($<2.2\%$); concentrations lower than 5 mg kg^{-1} did result in lower recoveries. Comparing the efficiencies found for the four soils (figure 4),

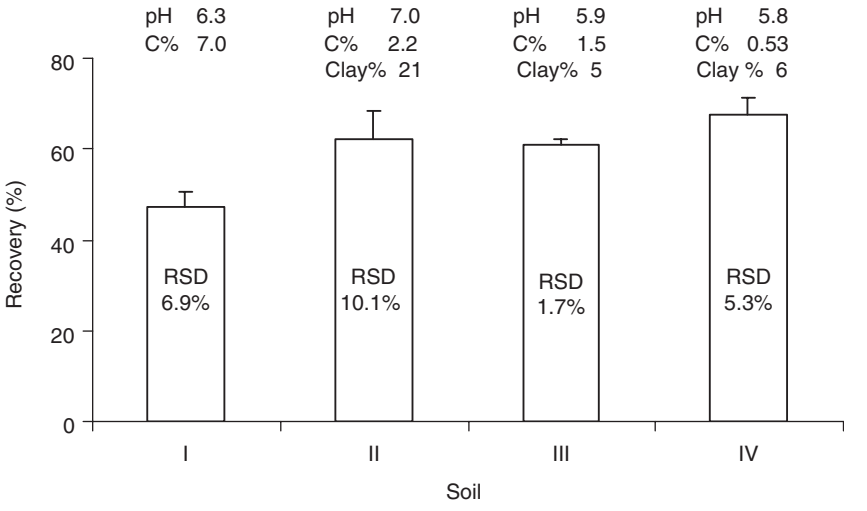

Figure 4. Recovery of α-solanine from four soils spiked with 13.2 mg of α-solanine kg^{-1} soil 1 h before extraction. Bars represent the standard deviation. $n = 5$, 6, 3, and 6 for soils I, II, III, and IV, respectively. The relative standard deviation (RSD) shows the reproducibility. The soil pH was determined in water, total content of carbon in organic matter by dry combustion, and content of clay by sedimentation.

a lower recovery ($47 \pm 3\%$) is observed for the soil with a higher organic C content (soil I). The lower recovery may be a result of an irreversible sorption or a reaction with the soil organic matter. For determination of spiked α-solanine in various potato products, recoveries between 82.4 and 99.7% have been reported [27, 28]. No reports of extraction of α-solanine or other glycoalkaloids from soil have been found. For determination of pesticides in soil or surface waters, recoveries in similar ranges have been reported; e.g. Polati *et al.* [29] found recoveries in the range of 60–106% for extraction and determination of sulfonylurea herbicides in drinking and canal water, and Hernández-Borges *et al.* [30] found recoveries in the range of 50–84% for five triaolopyrimidine sulfonanilide pesticides in soil. The lower recoveries obtained for the soil material might be a result of a sorption of α-solanine to the soil organic matter, which can also be supported by the lower efficiency found for soil I with the higher organic C content.

The reproducibility of the method was determined by calculating the relative standard deviation (RSD) for the extraction efficiency for each soil. The relative standard deviations are in the range of 1.7–10.1%; only the RSD from soil III exceeds 7%.

The extraction of a mixture of both glycoalkaloids showed recoveries of $72 \pm 3\%$ and $69 \pm 2\%$ for α-solanine and α-chaconine, respectively. No difference is seen between the recoveries of the individual glycoalkaloids, and the method can hence be applied to both glycoalkaloids.

3.1.7 Detection limit. The detection limit (DL) for the HPLC analysis, i.e. the concentration where the signal is different from the background signal at a significance level of 5%, was calculated as $DL = t(5\%, \text{one-sided}, n-1) \cdot s_c$, where s_c is the standard

deviation of n measurements at a concentration close to DL. The limit of detection for the method (LOD) is the concentration in the soil, where the probability that the signal is not being detected is 5%, i.e. LOD = 2 × DL/recovery. This procedure for the detection limit is a slight modification of the IUPAC recommendations, because the standard deviation at the lower concentration limit is determined from a standard solution with a low concentration and not from a blank solution [31]. The recovery is also included to give a conservative estimate of the real limit of detection. From nine measurements at a soil concentration of 1.0 mg kg^{-1} and a recovery of 29%, DL was found to be 0.35 mg kg^{-1}, and the LOD was 2.4 mg kg^{-1}. The detection limit may be further improved by increasing the amount of soil used for the extraction or by decreasing the volume of eluent used for the release of glycoalkaloids from the SPE column; later tests have shown a possibility of reducing the eluent volume from 3 ml to less than 1 ml.

3.2 *Application*

3.2.1 Ageing. Determination of α-solanine in the same spiked soil (soil III) at contact times up to 36 days showed that recovery declined slightly over time. For the samples, which were spiked with α-solanine, no significant decline was observed until day 17 (figure 5). As the soil was not sterilized, the decrease in extractable α-solanine

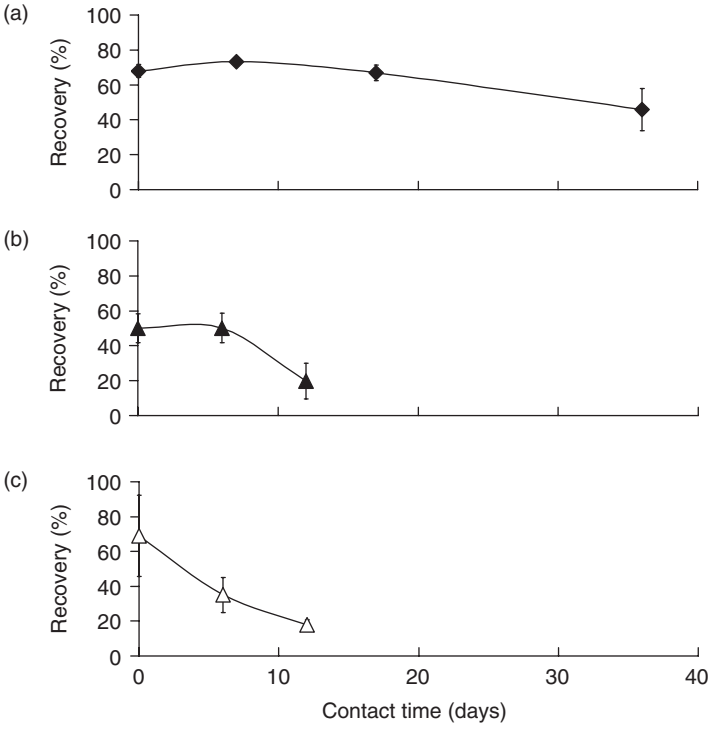

Figure 5. Change in recovery of α-solanine from soil III depending on the time of contact between α-solanine and the soil kept in the dark at 5°C. Bars represent the standard deviation; $n = 3$. Spiking materials: α-solanine in 5 mM HCl (a), potato juice (b), and potato mixture (c).

after day 17 may be due to degradation as well as to a stronger bonding or ageing with time.

For the samples spiked with α-solanine in potato matrices, the decline proceeded faster: within the first week for the samples spiked with the mixture and within the second week for samples spiked with the juice. At day 26, a small amount of α-solanine is still present (<17–19% of the initial added), though too little for quantification. The faster decline is probably due to degradation – either by microorganisms, which will be stimulated by the easily available nutrients from the potato matrices or from enzymes in the potato matrices.

The results demonstrate that the dissipation of α-solanine is not very fast. Irrespective of the spiking matrix, more than 35% of the spiked α-solanine is still extractable after 1 week, and even 1 month after application, α-solanine is still detectable.

4. Conclusion

A method to extract and determine α-solanine in soil has been established. Results show the method to be applicable to α-chaconine, too. An acceptable recovery of the method is obtained. α-Solanine is probably sorbed by soil organic matter, since the extraction efficiency decreases with increasing organic matter content in the soil. The detection limit of the method is 2.4 mg of α-solanine kg^{-1} soil. α-Solanine can still be detected in the soil 1 month after application; and hence if leaching of α-solanine from the plant parts takes place, there is a potential to find α-solanine in potato fields due to the relatively slow dissipation.

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