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High-performance thin-layer chromatographic determination of potato glycoalkaloids

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Abstract

A high-performance thin-layer chromatographic (HPTLC) method for the determination of the two main potato glycoalkaloids, α-chaconine and α-solanine, in different parts of the potato plant, is described. Samples were extracted with diluted aqueous acetic acid containing the ion-pairing reagent 1-pentanesulfonic acid, sodium salt. Extracts were purified and analytes fivefold concentrated by a solid-phase extraction (SPE) procedure using a Sep-Pak C_{18} cartridge. The methanol SPE eluate was applied to the HPTLC silica gel 60 F_{254} plates. The separation was performed in chloroform–methanol–2% aqueous NH_4OH (70:30:5) mixture as the mobile phase. Six different detection reagents: two modifications of the Dragendorff's reagent, phosphomolybdic acid, paraformaldehyde–phosphoric acid, Ce(IV)sulfate–sulfuric acid and sulfuric acid–ethanol reagents were applied for quantification by densitometry. Fluorescence enabled detection of 10 ng of each alkaloid.

Keywords: Detection, TLC; Glycoalkaloids; Alkaloids; Solanine; Chaconine

1. Introduction

The toxic steroidal glycoalkaloids are naturally occurring compounds, commonly found in plants of the Solanaceae family (potato, eggplant, tomato, sweet pepper). Under stress conditions (fungi, virus, insects, mechanical injuries) their concentration in plants increases [1].

The most important and known glycoalkaloids, which appear in cultivated potatoes, are α-solanine and α-chaconine. Both are trisaccharide glycosides with a common tertiary amine aglycone solanidine (Fig. 1). The lethal dose is considered to be about 3–6 mg of glycoalkaloids per kg body mass [2]. Taking into account that the glycoalkaloid level over the whole potato tuber may vary in large limits [2,3], screening, especially of new potato varieties is needed.

Determination of toxic glycoalkaloids in potato continuously attracts the attention of analysts [4,5]. A number of high-performance liquid chromatography (HPLC) methods [2,3,6–10] which enable accurate determinations of glycoalkaloids in potato plant materials have been published. Thin-layer chromatography (TLC) with scanning densitometry has also been applied for the same purpose [11].

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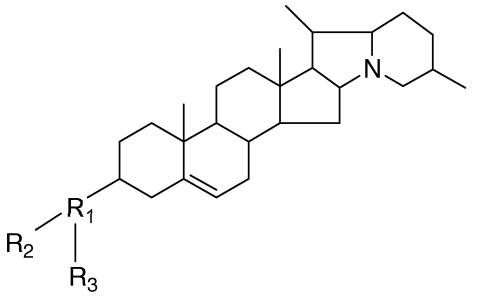

Fig. 1. The structure of glycoalkaloids α-solanine and α-chaconine present in potato. The sugar group for α-solanine consists of R_1=β-D-galactose, R_2=β-D-glucose and R_3=α-L-rhamnose, and R_1=β-D-glucose and R_2=R_3=α-L-rhamnose for α-chaconine.

A solid-phase extraction (SPE) procedure is an important part of the preparation of test solution. Its performance must correspond with the final determination technique. The HPLC technique has different requirements to TLC. For the latter, after elution, the analytes must be dissolved in an appropriate solvent (water is not desired), in a suitable concentration.

Due to their too weak absorption in the UV spectral range it is impossible to detect the potato glycoalkaloids on the plate without derivatisation. The most desired visualisation reagent for the quantitative TLC should be sensitive, specific and giving repeatable results, further it should be stable, simple to prepare and to apply, not to toxic and cheap. Usually a compromise must be accepted. The Carr–Price reagent was successfully applied [11] for the TLC densitometric determination of potato glycoalkalods, but it is unstable and consequently also not cheap. It is also toxic, as chloroform is used as the solvent. Dragendorff's reagent exists in many modifications [12], however, it is not clear which of them was applied for the densitometric determination of glycoalkaloids [11]. The paraformaldehyde–phosphoric acid and the Ce(IV)sulfate–sulfuric acid reagents are known spray reagents for glycoalkaloids [12], but they are not applicable for the quantitative determination, because they deteriorate the layer and give an unequal background.

The aim of our work was to find a method for the determination of glycoalkaloids in different parts of potato plant by a simple sample preparation, high-performance TLC (HPTLC) separation and detection by a suitable dipping reagent enabling further densitometric quantification.

We modified the paraformaldehyde–phosphoric acid and Ce(IV) sulfate–sulfuric acid spray reagents to make them suitable as dipping detection reagents. We tested also two modifications of Dragendorff's reagent, phosphomolybdic acid reagent and the most simple reagent – concentrated sulfuric acid in ethanol [12,13] for the same purpose.

2. Experimental

2.1. Preparation of samples

The glycoalkaloids were extracted in the same way from all kinds of samples (potato leaf, tuber – cooked and uncooked, skin and sprouts). Extraction solution was 1% acetic acid in Milli-Q water (Waters, Milford, MA, USA) containing the ion-pair reagent 1-pentanesulfonic acid sodium salt (3 g l^{-1}). Sample (usually 1 g) was shaken with 5 ml of extraction solution in a tube. After centrifugation (4500 rpm, 10 min) supernatant was decanted and the residue extracted again with 5 ml of fresh extraction solution and centrifuged. Both extracts were combined and applied to an SPE procedure.

The classical (100 mg) Sep-Pak C_{18} cartridges (Millipore, Milford, MA, USA) were used for clean up and concentration of the analytes. The cartridge was preconditioned with 5 ml of methanol and 5 ml of the extraction solution. About 10 ml of the supernatant was then applied to the cartridge and washed with 5 ml of 40% methanol in water. The analytes were eluted with 2×1 ml of methanol saturated with $(NH_4)_2HPO_4$. The eluate was ready for the application to the plate (5–20 μl). Recovery experiments were made only with potato tubers (uncooked) and leaves with addition of 100 mg kg^{-1} of each alkaloid (six replicates).

2.2. Preparation of standard solutions

Standards of α-solanine and α-chaconine were from Sigma (St. Louis, MO, USA). Separate stock solutions (1 mg ml^{-1} in methanol) were prepared. Application solutions were 0.1 mg ml^{-1} of each alkaloid in methanol, prepared separately and also

together (0.01 mg ml^{-1} for the fluorescence measurements). All solutions were kept in the refrigerator.

2.3. Preparation of standard solution for the determination of glycoalkaloids in potato tuber by densitometry in the visible spectral range

A 200-μl volume of each stock solution (0.2 mg of each alkaloid) was pipetted into a tube and the solvent was carefully evaporated with nitrogen. The residue was dissolved in 10 ml of extract, obtained as described above, from potato with the content of glycoalkaloids under the detection limit. The SPE procedure was performed as described above. Volumes of 2, 4, 6, 8, 10 and 12 μl of the final solution in methanol were applied to the plate (this means 40 to 240 mg kg^{-1} of each alkaloid at application of 10 μl of test solution).

2.4. Thin-layer chromatography

The Chromatoplate was HPTLC silica gel 60 (Merck, Darmstadt, Germany) 10×20 cm, applied without pretreatment for detection in visible spectral range. For the fluorescence detection the plate was developed twice in chloroform–methanol (1:1, v/v) to 8 cm and dried with a hairdryer before application.

The mobile phase [11] consisted of a chloroform–methanol–2% aqueous NH_4OH (70:30:5) solution. All the solvents used were of analytical grade from Merck.

The chromatographic chamber was a twin trough chamber from Camag (Muttenz, Switzerland) of length×width×height (22×6.5×12.5 cm). The chamber was lined on two larger sides with filter paper, 20 ml of the mobile phase was poured into each part and left for at least 30 min. The separation mode was as follows: once ascending, linear. Separation distance and time: 6.0 cm in 20 min.

Application to the plate by Linomat IV (Camag) was as follows: 15 mm from the lower edge, 15 mm from the left edge, 8 mm band width (4 mm for fluorescence measurements), speed 12 s μl^{-1}.

2.4.1. Detection

(1) Molybdatophosphoric acid reagent: 4 ml conc. HCl was mixed with 100 ml of 5% phosphomolybdic acid in ethanol (95%). The reagent was stable in the dark for 1 week. The developed and dried plate was dipped for 2 s into the reagent in the dipping device (Camag), then dried with a hairdryer and heated about 5 min at 120°C on the plate heater (Camag).

(2) Paraformaldehyde reagent: 7 mg paraformaldehyde was dissolved in 15 ml 85% H_3PO_4 with heating and cold diluted with acetone to 100 ml. Undiluted and also diluted reagent was stable when kept in refrigerator for 2 weeks. Detection was made as above, except that only 1 min of heating at 120°C was needed for measurements in visible spectral range. Longer heating (5 min) was needed for fluorescence detection. Fixation of fluorescence with dipping into a mixture of liquid paraffin–*n*-hexane (1:2) for 1 s and drying.

(3) Ce(IV) sulfate reagent: saturated solution in conc. H_2SO_4–water (1:1, v/v). For application as the dipping reagent it was diluted with methanol tenfold immediately before use. The diluted reagent was stable for 2 days in the refrigerator. The plate was heated for 2 min at 120°C.

(4) Dragendorff's reagents: the acetic acid and tartaric acid modification [12]. Reagents were stable, heating of plates was not necessary, only careful drying with a hairdryer was enough.

(5) Concentrated sulfuric acid in ethanol: 2 ml of acid in 100 ml of absolute ethanol (95%) as dipping reagent. Ten minutes of heating the plate at 120°C after dipping. Fixation of fluorescence as with the paraformaldehyde reagent (2).

2.4.2. Scanning and image processing

Quantitative evaluation of the developed HPTLC plates was performed densitometrically using the Camag TLC scanner II equipped with a built-in 12-bit A/D converter, and controlled by an external personal computer via an RS232 interface. Absorbance/transmittance mode: the monochromator bandwidth was 30 nm at the following wavelengths (a tungsten source): 660 nm for molybdatophosphoric acid reagent, 535 nm for paraformaldehyde reagent, 540 nm for Ce(IV) sulfate and 505 nm [11] for the Dragendorff's reagents, slit dimensions settings of length 6 mm and width 0.6 mm, and a scanning rate of 10 mm s^{-1}. Fluorescence mode: a Hg lamp was used as light source, bandwidth was 10

nm, slit length 4 mm and slit width 1.2 mm (macro), filter K400.

All the plates were scanned measured about 30 min after detection. The automatic scanning was controlled by the QTLC-pack (KIBK-IFC, 1990) software.

A Camag Video Documentation System in conjunction with the Reprostar 3 was used for imaging and archiving the thin-layer chromatograms. The objects were captured by means of a highly sensitive video camera 3×1/2 in. (1 in.=2.54 cm) CCD camera, Model HV-C20 (Hitachi, Denshi, Japan). A special digitizing board (frame grabber) assists rapid processing via the personal computer system. Image acquisition, processing and archiving were controlled

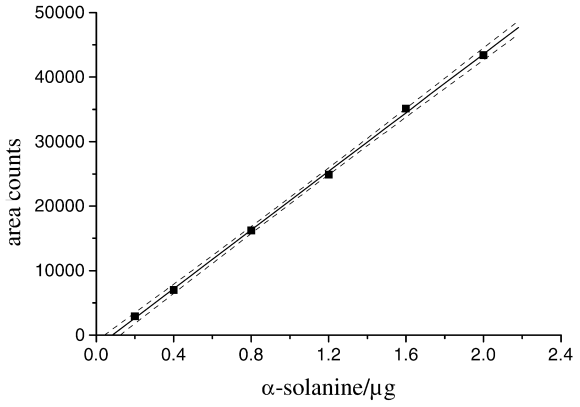

Fig. 3. Calibration curve with 95% confidence limits – Ce(IV) sulfate–sulfuric acid reagent (n=6).

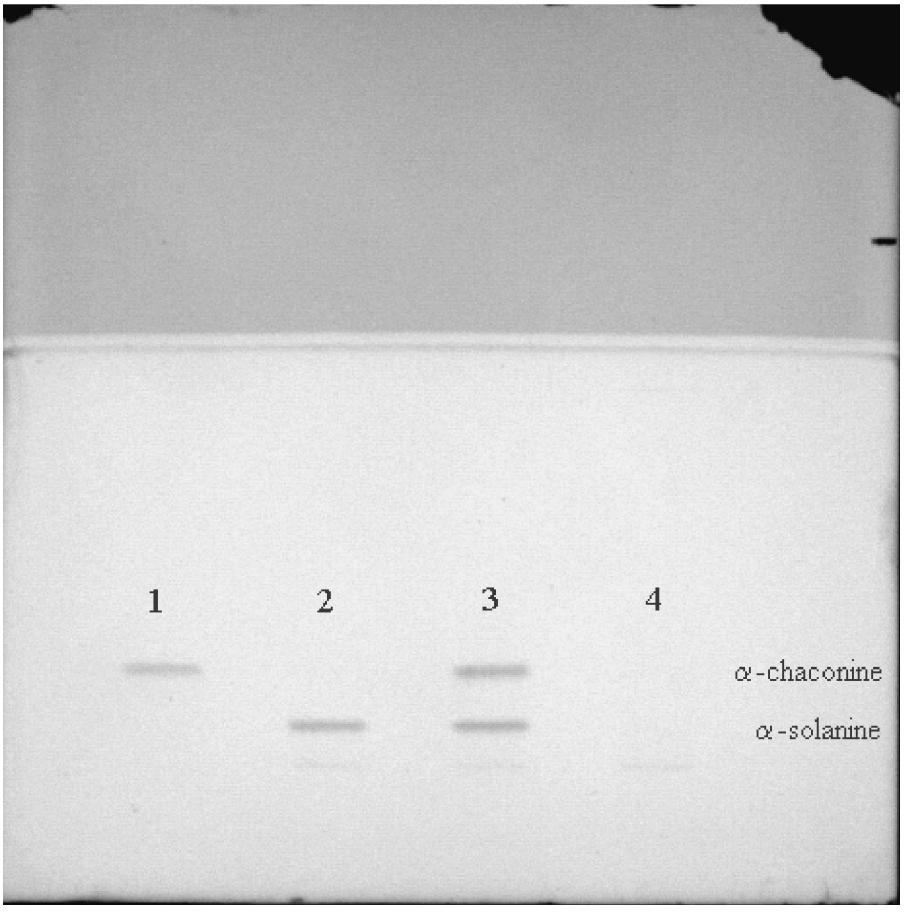

Fig. 2. CCD image presenting the separation of α-solanine and α-chaconine using Ce(IV) sulfate as a detection reagent. (1) 0.5 μg of α-chaconine, (2) 0.5 μg of α-solanine, (3) 100 mg kg^{-1} of each alkaloid added to the potato tuber extract before SPE, (4) SPE cleaned extract equivalent to 5 mg of potato tuber.

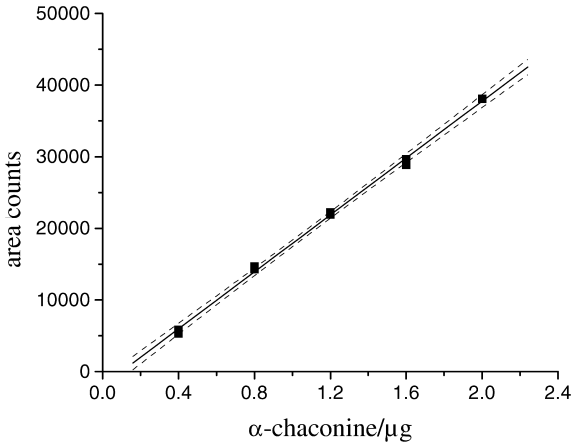

Fig. 4. Calibration curve with 95% confidence limits – paraformaldehyde–phosphoric acid reagent (visible spectral range, $n=6$).

via Video Store 2.30, a high-performance documentation software running under Windows 95. All images were taken from the distance 56 cm by aperture 1.4 using attachment lens +2 Dpt for optimum adaption to the size of the object and camera was set to the manual mode. In the case of fluorescence measurements, images were captured using a UV filter under exposure to the 366 nm UV light. The best image was observed when the camera integration period was 4 frames, which means the integration time was 160 ms.

3. Results and discussion

With all visualisation reagents α-solanine at R_F 0.13 and α-chaconine at R_F 0.25 appeared (Fig. 2). Colours of the bands were as follows: orange on yellowish to brownish coloured background for the Dragendorff's reagents, blue on light yellow background for phosphomolybdic acid, pink on white (and blue fluorescence under UV lamp at 366 nm) for the paraldehyde reagent, dark violet on white for Ce(IV) sulfate and brown for sulfuric acid reagent (and blue fluorescence under UV lamp at 366 nm). The sensitivity of all reagents in visible spectral range was similar. The limit of detection was between 0.1 and 0.2 μg and the linear range from 0.2 to about 2 μg of each alkaloid. We observed the same sensitivity (equal areas for equal applications) for both alkaloids using paraldehyde or Ce(IV) sulfate or Dragendorff's reagent and somewhere greater for α-chaconine using phosphomolybdic acid

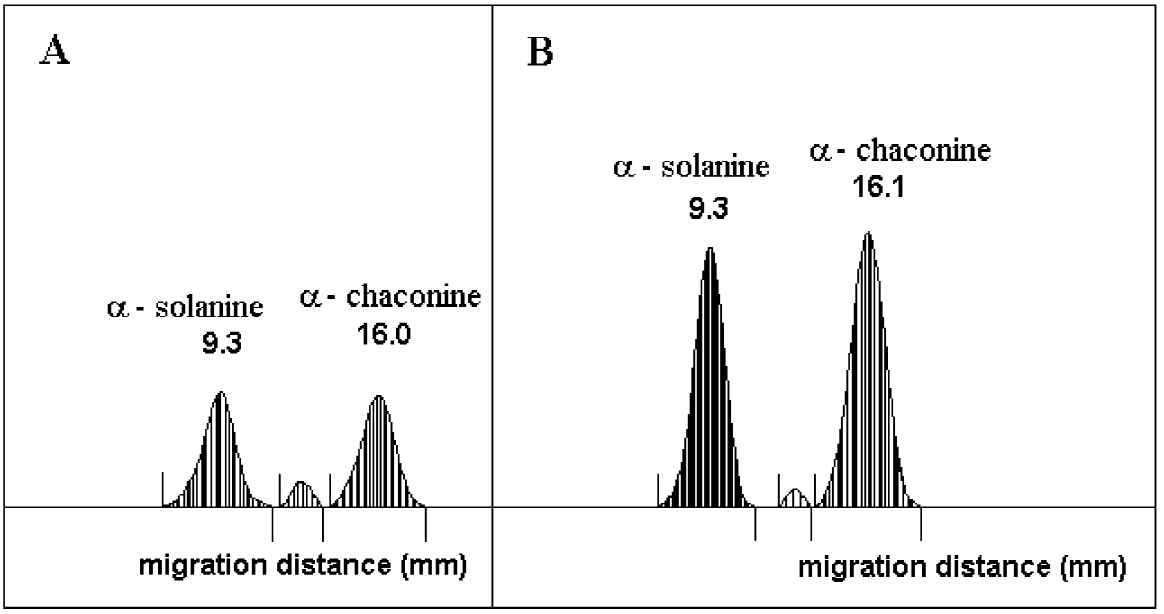

Fig. 5. Densitograms obtained by fluorescence measurement before (A) and after fixation (B) with paraffin in *n*-hexane (paraldehyde reagent).

reagent. The modification of Dragendorff's reagent with tartaric acid was better than the acetic acid modification: the colour of the bands was more stable, but the background slowly coloured darker than the bands (in about 2 h, depending also on the way of drying the plate) and thereafter the bands were not suitable for densitometric quantification. Experience and skill is needed to apply this reagent or maybe a better modification and/or conditions must be found. Its advantage is its stability and good selectivity when analysing potato leaves. Phosphomolybdic acid reagent prepared in different ways is a rather general reagent and therefore less selective. Although the chromatographic separation of peaks enabled determination of glycoalkaloids in potato tuber, this reagent was less suitable for the determination of α-solanine in leaves, because of an adjacent peak. On the contrary, the Ce(IV) sulfate reagent was suitable for the determination of this alkaloid in leaves and less suitable for the determination of α-chaconine in leaves. All reagents were suitable for the determination of glycoalkaloids in fresh and cooked potato tuber from about 20 to 240 mg kg^{-1} of each (or more if less test solution is applied) by densitometry in the visible spectral range (Figs. 3 and 4). The chromatograms (densitograms)

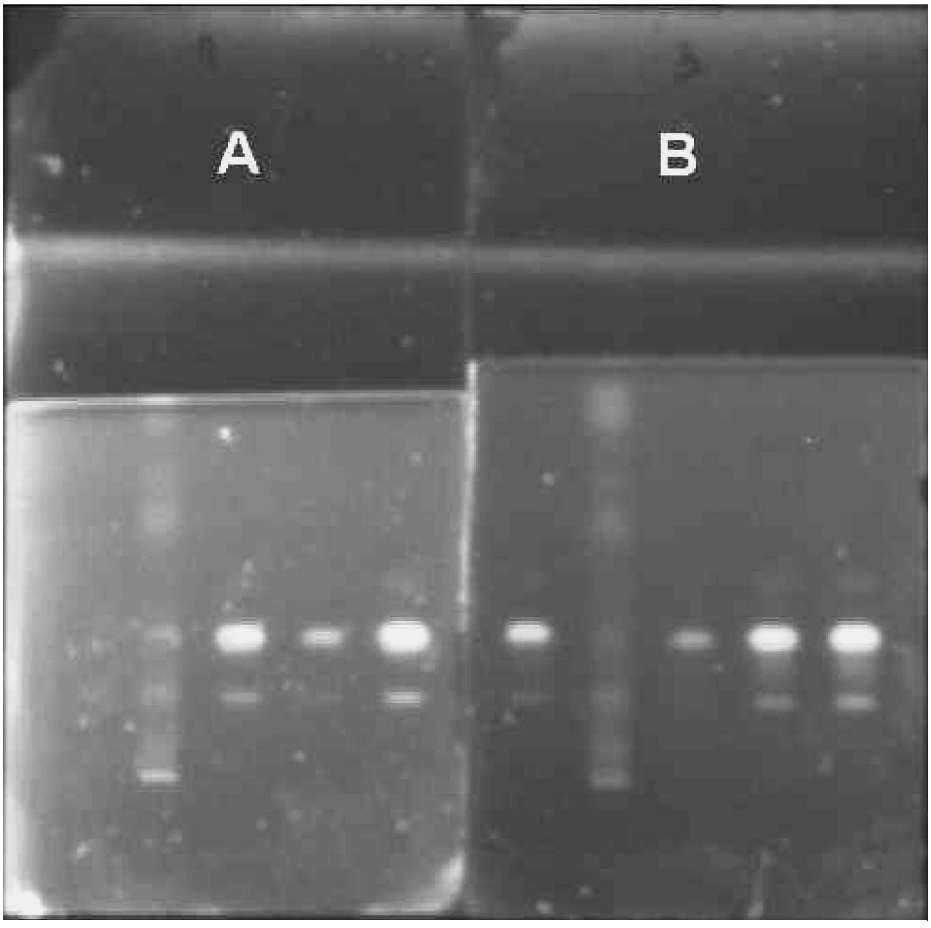

Fig. 6. CCD images of two plates before fixation of fluorescence at 366 nm. (A) Paraformaldehyde–phosphoric acid reagent. Lanes: 1=aliquot corresponding to 10 mg fresh potato tuber, 2–4=standards 300, 100 and 500 ng of α-chaconine and 30, 10 and 50 ng of α-solanine. (B) Sulfuric acid–ethanol reagent. Lanes: 2=aliquot corresponding to 10 mg of fresh potato tuber, 1, 3, 4, 5=standards 300, 100, 500 and 700 ng of α-chaconine and 30, 10, 50 and 70 ng of α-solanine.

showed no interference. The supernatant of cooked potato was turbid, but the eluate after the SPE was clear.

By measurement of fluorescence (possible for paraformaldehyde–phosphoric acid and sulfuric acid–ethanol reagent) lower detection limits were obtained. The fluorescence was stable and was additionally enhanced by dipping the plate into paraffin–*n*-hexane mixture (Fig. 5). The aliquot of the test solution of fresh potato tuber should not exceed 5 μl (equivalent to 2.5 mg of fresh potato tuber) in this case because of interferences (Fig. 6). The limit of detection about 4 mg kg^{-1} of each alkaloid in fresh potato tuber was achieved with sulfuric acid–ethanol reagent.

Repeatability of densitometric determination expressed with relative standard deviation (RSD) for 1 μg of each alkaloid (six replicates on the same plate) in the visible spectral range (paraldehyde reagent) was 3–5%.

The average recovery from fresh potato tuber at 100 mg kg^{-1} (six replicates) was 86% (RSD 5.9%) for α-solanine and 92% (RSD 6.9%) for α-chaconine. The chromatograms (densitograms) showed no interferences. We tried to use the extraction solution without ion-pairing reagent [11], however in this case there was not enough retention on the SPE cartridge to collect the analytes into methanol.

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References

[1] S.F. Osman, R.M. Zacharius, E.B. Kalan, T.J. Fitzpatrick, S. Krulick, J. Food Protect. 42 (1979) 502.
[2] R.J. Houben, K. Brunt, J. Chromatogr. A 661 (1994) 169.
[3] K.E. Hellenäs, C. Branzell, H. Johnsson, P. Slanina, J. Sci. Food Agric. 67 (1995) 125.
[4] J.M. Betz, J. AOAC Int. 80 (1997) 126.
[5] J.M. Betz, J. AOAC Int. 81 (1998) 137.
[6] K.E. Hellenäs, C. Branzell, J. AOAC Int. 80 (1997) 549.
[7] A.S. Carman, S.S. Kuan, G.M. Ware, O.J. Francis, G.P. Kirschenheuter, J. Agric. Food Chem. 34 (1986) 279.
[8] K. Saito, M. Horie, Y. Hoshimo, N. Nose, H. Nakazawa, J. Chromatogr. 508 (1990) 141.
[9] L.E. Rodriguez-Saona, R.E. Wrolstad, C. Perreira, J. Food Sci. 64 (1999) 445.
[10] M. Friedman, C.E. Levin, J. Agric. Food Chem. 40 (1992) 2157A.
[11] F. Ferreira, P. Moyna, J. Chromatogr. A 653 (1993) 380.
[12] J. Sherma, in: G. Zweig, J. Sherma (Eds.), Handbook of Chromatography, Vol. II, CRC Press, Cleveland, OH, 1972.
[13] H. Jork, W. Funk, W. Fischer, H. Wimmer, Dunnschichtchromatographie, Band 1a, VCH, Weinheim, 1989.