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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS ANALYSIS OF α-SOLANINE AND α-CHACONINE IN POTATO PLANTS CULTURED *IN VITRO*

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SUMMARY

An high-performance liquid chromatography (HPLC) method utilizing a μBondapak NH_2 column was developed for the simultaneous measurement of the *cis*-glycoalkaloids α-solanine and α-chaconine present in 1 g of potato tissue. Shoot tissue was extracted twice with methanol and the extract purified on an acetic pretreated silica gel column and a Sep-Pak C_{18} cartridge. Alkaloids were separated by HPLC with a μBondapak NH_2 column and a mobile phase of ethanol–acetonitrile–potassium dihydrogenphosphate (3:2:1). The recoveries of α-solanine and α-chaconine were 91.4 ± 6.5 ($n=3$) and 98.8 ± 12.2% ($n=3$) respectively when spiked to potato shoot material (100 mg/100 g) prior to extraction. The contents of α-solanine and α-chaconine in *in vitro* plantlet material of *Solanum tuberosum* (Danshyaku) were 13.1 ± 1.2 mg/100 g ($n=3$) and 5.2 ± 0.5 mg/100 g ($n=3$) respectively.

INTRODUCTION

Many analytical methods have been reported for glycoalkaloid quantification in potato tissues[1]. These include titrimetry, dye binding colorimetry[2], gas chromatography[3], high-performance liquid chromatography (HPLC)[4–7], radioimmunoassay and enzyme-linked immunosorbent assay[8,9]. These have, however, often required large amounts of material or do not discriminate between α-solanine and α-chaconine.

The *cis*-glycoalkaloids, α-solanine and α-chaconine, account for 95% of the total glycoalkaloids in potato, but differ in structure only in two of their three sugar moieties. This, coupled with the fact that their absorption maxima are 205 nm, which limits the choice of HPLC mobile phase to those with low UV absorptions, makes it difficult to separate them.

Some success in separating these two compounds has been reported. A μBondapak NH_2 column and a tetrahydrofuran–potassium dihydrogenphosphate (1.7 g/100 ml)–acetonitrile (50:25:25) mobile phase were able to separate α-solanine and α-chaconine in standard solutions[4]. However, when potato tuber tissue was analysed, contamination of the α-solanine peak with unknown substances was

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observed. A C_8 or C_{18} column and a solvent system of acetonitrile–water–ethanolamine (55:45:0.1 or 45:55:0.1 respectively) were able to resolve the two *cis*-glycoalkaloids[10] but UV absorption by ethanolamine could sometimes be a problem.

There is an increasing need for a rapid determination method for the above glycoalkaloids. New biotechnological approaches to plant breeding and selection can produce many plantlets through a variety of procedures. Although selections may be for a variety of beneficial traits, *e.g.*, disease resistance, temperature stress resistance, secondary product production, it is necessary to determine alkaloid levels to ensure that the synthetic apparatus has not been perturbed during the selection process. Rapid and accurate measurements are paramount in those projects, *e.g.*, ref. 11, where alkaloid levels are specifically being selected for. Greatest efficiency will be achieved when plant material can be analyzed at the earliest stage possible, so analytical procedures using small tissue samples are also essential.

This paper outlines the development of a new HPLC method to determine α-solanine and α-chaconine in as little as 1 g of potato shoot material.

EXPERIMENTAL

Apparatus

A 880 PU pump, 7125 injector, 870-UV detector (Japan Spectroscopic, Tokyo, Japan) and a Chromatopac C-R3A integrator (Shimadzu, Kyoto, Japan) were used. Two columns, an Hypersil ODS (10 cm × 2.1 mm I.D.) and a μBondapak NH_2 (30 cm × 3.9 mm I.D.) (Waters Assoc., Milford, MA, U.S.A.), were employed.

Mobile phase

Methanol–water–phosphoric acid (95:30:0.1) was used with the Hypersil ODS column, while ethanol–acetonitrile–0.005 *M* potassium dihydrogenphosphate (3:2:1) was used with the μBondapak NH_2 column.

Reagents

All chemicals were of special grade with the exception of acetonitrile which was of HPLC grade, and were obtained from Wako (Osaka, Japan). α-Solanine and α-chaconine were supplied by Sigma (St. Louis, MO, U.S.A.).

Plant material

In vitro Solanum tuberosum (Danshyaku) plantlets were obtained from the National Center for Seeds and Seedlings and propagated by single node cutting in MS (Murashige and Skoog) salts[12] and vitamins, 3% sucrose and 0.15% gellan gum at 20°C under a 4000-Lux fluorescence light source.

Extraction of α-solanine and α-chaconine

A 1-g amount of shoot tissue was chopped into small pieces and 5 ml methanol were added prior to homogenation. The homogenized sample was filtered under suction using Toyo No. 5C filter-paper and the residue reextracted as above. The filtrates were pooled and made up to 10 ml with methanol.

Purification of the extract and alkaloid content determination

A 2-ml volume of extract solution was concentrated to dryness by rotary evaporation at 50°C, redissolved in 2 ml methanol and purified on a silica gel column. The silica gel was washed twice with methanol–water–acetic acid (70:30:0.15), three times with methanol and, after drying, activated at 140°C for 24 h. A 3.5-g amount of the treated silica gel was suspended in 20 ml methanol, and packed into a glass column to a height of 8 cm. The packed column was washed with methanol–water–acetic acid (10 ml:0.3 ml:5 μl) and then 15 ml methanol. The 2-ml sample was loaded and allowed to drain until the surface of the gel was just covered. A 1-ml methanol rinse of the sample flask was also applied in this way. The column was left for 15 min and then washed with 12 ml methanol at a flow-rate of *ca.* 0.5 ml/min. Alkaloids were eluted with 20 ml methanol–water–acetic acid (80:20:0.1). The eluate was concentrated almost to dryness, dissolved in 10 ml water and purified on a Sep-Pak C_{18} cartridge column, which was washed first with 10 ml methanol, then 10 ml 1% acetic acid and finally 20 ml water. The 10-ml sample was loaded and the column washed with 2 ml water and then 5 ml 30% methanol. Alkaloids were eluted with 5 ml methanol. The eluate was concentrated to dryness and dissolved in 1 ml ethanol–acetonitrile–0.005 *M* potassium dihydrogenphosphate (3:2:1). An aliquot was then applied to the HPLC column.

RESULTS AND DISCUSSION

Extraction of α-solanine and α-chaconine from young potato plantlets

The poor solubility of α-solanine and α-chaconine limits the choice of extraction solvent, however aqueous acetic acid solutions and aqueous ethanol or methanol mixtures with, or without, the addition of 5% acetic acid are most commonly used[8]. The methanol extraction procedure of Kajiwara *et al.*[13] was used in this study.

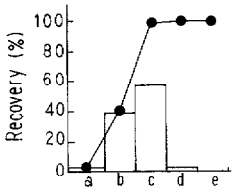
Purification by silica gel column chromatography

When α-solanine and α-chaconine (in standard solution) were loaded onto a silica gel column they were bound tightly and elution was poor. If the column was pretreated with a mixture of 10 ml methanol, 0–0.3 ml water and 0–5.0 μl acetic acid prior to elution with 20 ml of methanol–water–acetic acid (80:20:0.1), recovery was

TABLE I

EFFECT OF PRETREATMENT ON RECOVERY OF α-SOLANINE FROM A SILICA GEL COLUMN

Solvent systems for pre-treatment of silica gel column			*Recovery of α-solanine (%)*
Methanol (ml)	*Water (ml)*	*Acetic acid (μl)*	
10	0.0	0.0	41
10	0.3	0.0	70
10	0.3	1.0	85
10	0.3	2.5	92
10	0.3	5.0	100



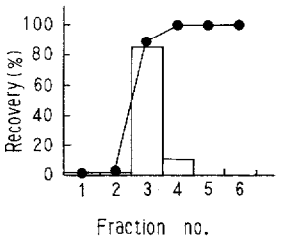


Fig. 1. Stepwise elution of α-solanine from a silica gel column. An 150-μg amount of α-solanine was loaded onto a pre-treated silica gel column. Eluents were used in the following order: a, 12 ml methanol; b, 10 ml methanol–water–acetic acid (99:1:0.005); c, 10 ml (95:5:0.025); d, 10 ml (80:20:0.1); e, 10 ml (70:30:0.15). Each column in the figure represents recovery of α-solanine from a fraction, and the closed circles represent total recovery.

Fig. 2. Isocratic elution of α-solanine from a silica gel column with methanol–water–acetic acid (80:20:0.1). The loaded column was washed with methanol (fraction 1) and eluted (fractions 2–6). Each fraction was 5 ml. Other conditions as in Fig. 1.

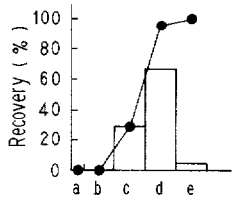
improved. Recovery percentages for α-solanine are shown in Table I. Maximum elution efficiency was achieved when a pretreatment with 10 ml methanol, 0.3 ml water and 5 μl acetic acid was included.

The most effective elution solvent was then investigated. An 150-μg amount of α-solanine in 1 ml methanol was loaded onto a pretreated column. The column was washed with 12 ml methanol and eluted with mixtures of methanol, water and acetic acid in the ratios and order 99:1:0.005; 95:5:0.25; 80:20:0.1 and 70:30:0.15. The elution profile is shown in Fig. 1. The second and third solutions eluted most α-solanine but total recovery was achieved only after addition of the fourth (80:20:0.1) mixture.

An isocratic elution with the 80:20:0.1 mixture was carried out to try to simplify the elution procedure. Fig. 2 shows that three 5-ml elution fractions were effective in the elution of 100% of the α-solanine. A total volume of 20 ml was utilized for elution in later experiments to allow for variability in column conditions.

Purification on a Sep-Pak C_{18} cartridge

Further purification was carried out on a Sep-Pak C_{18} cartridge. Elution of a standard amount of α-solanine was performed in steps using the water–methanol



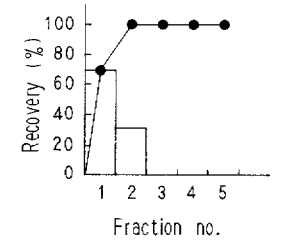


Fig. 3. Stepwise elution of α-solanine from Sep-Pak C_{18}. An 150-μg amount of α-solanine was loaded onto a pre-washed Sep-Pak C_{18} cartridge column. Eluents were used in the following order: a, 2 ml water; b, 10 ml 30% methanol; c, 10 ml 50% methanol; d, 10 ml 75% methanol; e, 10 ml methanol.

Fig. 4. Isocratic elution of α-solanine from Sep-Pak C_{18}. The loaded column was washed with water and 30% methanol, then eluted with methanol. Each methanol fraction was 1 ml. Other conditions as in Fig. 3.

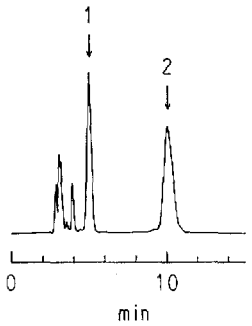


Fig. 5. Chromatogram of α-chaconine and α-solanine standard solution. HPLC conditions: column: μBondapak NH_2 (30 cm × 3.9 mm); mobile phase, ethanol–acetonitrile–0.005 *M* potassium dihydrogenphosphate (3:2:1); detector sensitivity, 64 mV a.u.f.s. Peaks: 1 = α-chaconine; 2 = α-solanine.

mixtures outlined in Fig. 3. α-Solanine was eluted with solutions c, d and e (50, 75 and 100% methanol respectively). It was decided to use a 30% methanol wash of the Sep-Pak cartridge followed by 100% methanol to elute the α-solanine. A 2-ml volume of 100% methanol was effective in totally removing the α-solanine from the Sep-Pak cartridge (Fig. 4).

Similar investigations were carried out with α-chaconine standard solutions, and the silica gel column and Sep-Pak C_{18} procedures were found to be equally effective in purification and recovery.

HPLC conditions

Resolution of α-solanine and α-chaconine was first attempted using an ODS column and mobile phase which included tetrahydrofuran or ethanolamine. It was not possible to separate the glycoalkaloids however, because of the instability of the baseline. A methanol–water–phosphoric acid mobile phase was also unable to resolve the two glycoalkaloids, with retention times of 4.4 and 4.9 min for α-chaconine and α-solanine respectively (data not presented). A modification of Bushway's method[4] utilizing μBondapak NH_2 was then used. Ethanol was substituted for tetrahydrofuran in the mobile phase because of its low UV absorption. This column and a mobile phase of ethanol–acetonitrile–0.005 *M* potassium dihydrogenphosphate (3:2:1) was able to separate α-solanine and α-chaconine (Fig. 5). Retention times of

TABLE II

EFFECT OF COMPOSITION OF MOBILE PHASE ON THE RETENTION OF α-SOLANINE AND α-CHACONINE

HPLC conditions: column μBondapak NH_2 (30 cm × 3.9 mm I.D.); flow-rate, 1.5 ml/min; wavelength, 205 nm.

Ethanol–acetonitrile–0.005 M KH_2PO_4	*Retention time (min)*	
	α-Solanine	*α-Chaconine*
1:2:1	3.6	2.4
2:2:1	5.1	2.8
3:2:1	7.3	3.2

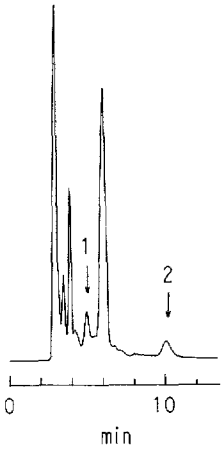


Fig. 6. Chromatogram of the eluate from Sep Pak C_{18} of an extract from young *S. tuberosum* plantlets first separated on a silica gel column. This figure represents sample 2 in Table IV.

α-solanine and α-chaconine could be increased by increasing the proportion of ethanol in the mobile phase (Table II).

The contents of α-solanine and α-chaconine in potato plantlet material could also be determined using this column and mobile phase provided the silica gel and Sep-Pak C_{18} chromatography purification procedures outlined earlier were followed (Fig. 6). Previous methods of extracting from large amounts of plant material precipitated alkaloids from methanol extracts by ammonium hydroxide addition[4,6]. This, however, could not be used in the present studies as only 1 g of material was used and insufficient precipitation occurred. The two clean-up procedures of the extracts eliminated the need for such a precipitation. Sensitivity was also increased because the substitution of tetrahydrofuran by ethanol in the HPLC mobile phase allowed the use of a detection wavelength of 205 nm rather than the 215 nm utilized with a tetrahydrofuran-containing mobile phase[7]. The former wavelength corresponds to the maximum absorption of these alkaloids.

TABLE III

RECOVERIES OF α-SOLANINE (100 mg/100 g) AND α-CHACONINE (100 mg/100 g), SPIKED TO YOUNG *S. TUBEROSUM* PLANTLETS

Sample No.	*Recovery (%)*	
	α-Solanine	*α-Chaconine*
1	97.3	90.7
2	84.5	112.8
3	92.4	93.0
Av. ± S.D.	91.4 ± 6.5	98.8 ± 12.2

TABLE IV

α-SOLANINE AND α-CHACONINE OF YOUNG *S. TUBEROSUM* PLANTLETS

Sample No.	*α-Solanine (mg/100 g)*	*α-Chaconine (mg/100 g)*
1	14.3	5.4
2	13.1	5.6
3	11.9	4.6
Av. ± S.D.	13.1 ± 1.2	5.2 ± 0.5

Analysis of α-solanine and α-chaconine in in vitro potato plantlets

Table III shows recoveries of α-solanine and α-chaconine from *Solanum tuberosum* (Danshyaku) plantlets cultured *in vitro*, each spiked at 100 mg/100 g. Recovery was 91.4 ± 6.5 ($n=3$) and 98.8 ±12.2% ($n=3$) for α-solanine and α-chaconine respectively. No binding of glycoalkaloids to plant components appears to occur. The detection limits were 2 mg/100 g for α-solanine and 1 mg/100 g for α-chaconine.

Table IV shows the glycoalkaloid content in young *in vitro* plantlets. The α-solanine content was 13.1 ± 1.2 mg/100 g and that of α-chaconine was 5.2 ± 0.5 mg/100 g. Previous reports have noted glycoalkaloids contents in the range of 10–100 mg/100 g but comparison with the data presented here is difficult. As culture conditions play an important role in the accumulation of glycoalkaloids, it is appropriate only to make comparisons when more data are collected from material cultured *in vitro*. Nonetheless, this work is an important step towards the rapid screening of *in vitro* material since these compounds can be determined in tissue samples as small as 1 g.

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