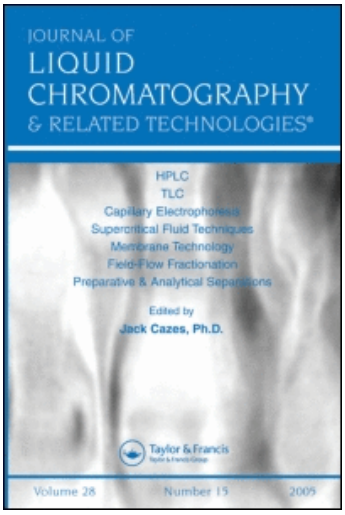

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DETERMINATION OF POTATO GLYCOALKALOIDS USING ISOTACHOPHORESIS AND COMPARISON WITH A HPLC METHOD

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ABSTRACT

A method for the determination of potato glycoalkaloids (PGAs) and their aglycone, solanidine, by capillary isotachophoresis (ITP) was developed. The PGAs were extracted by methanol or by a mixture of methanol : water : acetic acid and then purified by solid phase extraction (SPE). The leading electrolyte consisted of 2 mM HCl solution in 99% methanol and 5 mM $Zn(NO_3)_2$ in 99% methanol served as terminating electrolyte. The average recoveries were 88 - 101% for solanidine and 90 - 103% for α-PGAs at a level of 50 mg/kg. The detection limit of solanidine and α-PGAs was 1 mg/kg and 2 mg/kg, respectively. A comparison of the ITP and a HPLC method for the determination of PGAs in commercial products was carried out. The HPLC determination of PGAs was carried out on a Nucleosil 5-NH_2 column using a mixture of acetonitrile and 20 mM KH_2PO_4 (75 : 25, v/v) as the mobile phase with UV detection at 208 nm.

Comparative studies showed that the methods give similar figures for total glycoalkaloid content (sum of α-solanine and α-chaconine). The HPLC method provided both the separation and quantification of both α-solanine and α-chaconine but this technique under conditions used did not allow the determination of the aglycone solanidine. ITP does not provide the separation of individual glycoalkaloids differing only in sugar moiety such as α-solanine and α-chaconine but does allow the separation and determination of aglycone solanidine and the α-glycoalkaloids in one run.

INTRODUCTION

Potato tubers contain a mixture of the steroidal triglycosides α-solanine and α-chaconine accounting for about 95% of the total glycoalkaloid content. Both possess the same aglycone, solanidine, but differ in their sugar moieties. Because of their known toxicity (gastrointestinal disturbances and neurological disorders), PGAs must be analysed in all new potato varieties before they can be released commercially. Although the exact toxic dose in humans has not been established, potatoes with glycoalkaloid levels not exceeding 200 mg/kg fresh weight are generally regarded as being safe for human consumption.

Methodologies[1-7] for analysis of PGAs and their hydrolysis products have been extensively studied. Among a number of published methods HPLC is increasingly used to analyse both individual glycoalkaloids and hydrolysis products. Typically, an analytical method comprises three major steps, namely extraction of the alkaloids with a suitable solvent (aqueous or non aqueous), removal of any interfering impurities and analysis by either HPLC or GLC.

In this paper the determination of PGAs using a capillary isotachophoretic method being developed at Department of Carbohydrate Chemistry and Technology of Prague's Institute of Chemical Technology is described. The ITP technique is compared with a HPLC method routinely used for glycoalkaloid determination in potato and potato products.

EXPERIMENTAL

Chemicals and reagents

Methanol (AR grade), hexane (AR grade) and acetonitrile (HPLC Far UV grade) were purchased from Koch-Light Ltd. The methanol was demineralized by treatment with strong anion exchanger Amberlite IR 120 (d_p 0.3 - 1.2 mm) and strong cation exchanger Duolite A113 (d_p 0.3 - 1.2 mm) before use. Water was from a Millia 185

TABLE I **Operational electrolyte system**

Parameter	Leading electrolyte	Terminating electrolyte
Solvent	99% methanol	99% methanol
Ion	H^+	Zn^{++}
Concentration (mM)	2	5
Counter ion	Chloride	Nitrate
Concentration (mM)	2	10
Additive	None	None

deioniser. Stock solutions of glycoalkaloids (1 mg/ml) and solanidine (0.5 mg/ml) were prepared by dissolving 5 mg of α-solanine, 5 mg of α-chaconine and 2.5 mg of solanidine (Sigma, USA) respectively, in 5 ml of methanol. The leading electrolyte was prepared by diluting a solution of 1 M hydrochloric acid (BDH, UK) in methanol and the terminating electrolyte by dissolving zinc nitrate hexahydrate AR grade (Sigma, USA) in methanol. The operational electrolyte system is shown in Table I. The HPLC mobile phase was prepared by mixing 20 mM potassium dihydrogenphosphate (AR grade, BDH, UK) buffer solution and acetonitrile (25 : 75, v/v). For SPE sample cleanup Sep-Pak C18 (Millipore, USA) and TechElut NH_2 (500 mg/3 ml syringe; HPLC-Technology, UK) columns were used.

Apparatus

A single capillary isotachophoregraph IONOSEP 900.1 was used (RECMAN - laboratorní technika, Czech Republic) equipped with a contactless high-frequency conductimeter. The separation was carried out in a PTFE capillary (150 mm x 0.45 mm ID) thermostatted at ambient temperature by a built-in ventilator. The initial driving current of 20 μA was automatically decreased to 5 μA during detection. A 20 μl sample was automatically injected into the isotachophoregraph. ITPgrams were evaluated on-line via a personal computer. The computer-controlled isotachophoregraph uses a software package for both analysis control and ITP data evaluation. Each analysis took 20 - 25 minutes.

HPLC was carried out using a modular chromatograph consisting of PU 4100 Liquid Chromatograph pump, PU 4025 spectrophotometer (both Phillips, UK) and a Gilson autosampler Model 231. The analytical column was Nucleosil 5-NH_2 (250 mm x 4.6 mm ID, Macherey-Nagel, Germany). The mobile phase was run isocratically at 30°C with a flow rate of 1 ml/min (pressure drop 90 bar). The sample (20 μl) was injected by Rheodyne valve. The detection wavelength and the sensitivity were set at 208 nm and 0.02 AUFS respectively. Each analysis took 20 minutes.

Calibration

An external standard calibration method was used for both ITP and HPLC. The α-PGA (equimolar mixture of α-solanine and α-chaconine) and solanidine were injected into the isotachophoregraph at five concentration levels 5 - 25 μg/ml and 2.5 - 12.5 μg/ml, respectively and the relationship evaluated between concentration and step length.

A standard mixture of α-solanine and α-chaconine (1 - 50 μg/ml each) was injected into the HPLC system at five levels. A calibration graph was constructed by measuring the peak heights of the PGAs.

Sample preparation

Potato samples I to III were extracted either sliced and freeze-dried raw or quartered unpeeled and boiled in water for 15 minutes, drained and then freeze-dried. Samples IV to IX were extracted fresh. The varieties used for samples I to IX were Estima (UK), King Edward (UK), Ostara (NL), Eba (NL), Bintje (NL), Korima (Czech Rep.), Lukava (Czech Rep.) and Karim (Czech Rep.) respectively.

Samples with low lipid levels (potato tubers, potato starch, mashed potato) were extracted prior to quantification of the PGAs using the method of Saito *et al.*[6]. The sample (5 g) was homogenised with *ca* 30 ml of methanol for 2 minutes in an ultrasonic bath, followed by filtration through a sinter (S4). The residue was rinsed twice with *ca* 5 ml of methanol and the rinsings were combined with the original filtrate and made up to

50 ml with methanol. An aliquot (5 ml) was mixed with water (8 ml) and applied to a Sep-Pak C18 cartridge (flow rate about 5 ml/min), which was then washed with 5 ml of 40% methanol. In the case of ITP determination of PGAs the cartridge was rinsed with 10 ml of 10 mM acetic acid. The cartridge was then dried with suction using a water pump. The PGAs and solanidine were eluted with 10 ml of methanol, evaporated to dryness using a rotovapor (40°C) and redissolved in 1 ml of methanol. An aliquot (20 μl) of the solution was injected into the isotachophoregraph and/or the chromatograph. Samples with high lipid levels (potato crisps, chips and snacks) were treated in two different ways.

i) The sample (5 g) was mixed with 100 ml of hexane and boiled for 30 minutes, the hexane removed by decantation and the defatting procedure repeated with fresh hexane (100 ml). After decantation, the remaining hexane was removed on a rotovapor under vacuum. The defatted sample was extracted with 30 ml of a mixture of methanol : water : acetic acid (94 : 6 : 1, v/v/v) for 2 minutes in an ultrasonic bath. After filtration the residue was rinsed twice with *ca* 5 ml of the mixture, the rinsings were combined with the original filtrate and made up to a volume of 50 ml. The procedure then followed that used for low lipid content samples.

ii) The method of Saito *et al.*[6] was used for treatment of high fat content samples. A 5 g sample was treated in a manner described for low lipid content samples. The residue after SPE on Sep-Pak C18 treatment was dissolved in 1 ml of methanol and the solution mixed with 19 ml of acetonitrile. The mixture was applied to a TechElut NH_2 cartridge (flow rate about 5 ml/min), washed with 5 ml of acetonitrile and the PGAs and solanidine eluted with 10 ml of methanol. The eluate was evaporated to dryness using a rotovapor (40°C) and redissolved in 1 ml of methanol. An aliquot (20 μl) of the solution was injected into the isotachophoregraph and/or the chromatograph.

RESULTS AND DISCUSSION

The ITP analysis is based on the fact that both PGAs and solanidine are weak bases and migrate towards the cathode under certain conditions. For this migration an acidic

electrolyte system was used and several cations were examined as leading and/or terminating ion (hydroxonium, potassium, sodium, β-alanine, etc.). Water, methanol and water-methanol mixture were tested as electrolyte solvents. The best electrolyte system is described in Table I. The effective mobilities of α-PGA and solanidine are sufficiently different to give a good separation, but α-solanine and α-chaconine are not separated due to their having a common aglycone, solanidine, bearing a positive charge and also having practically the same molecular weight (867 and 851, respectively).

Results of qualitative and quantitative ITP calibration analyses are summarised in Table II. The relationship between concentration and step length was linear in the case of both the PGAs and solanidine. Similarly in the case of HPLC calibration analyses a linearity between glycoalkaloids (both α-chaconine and α-solanine) concentration (1 - 50 μg/ml) and peak height was confirmed (r = 0.998).

The calibration results obtained showed that both α-solanine and α-chaconine gave almost the same step height and step length and thus either could be used for ITP calibration analyses. Figure 1 shows an isotachophoregram of a standard mixture of solanidine (7.5 μg/ml) and of α-PGAs (15 μg/ml).

The ITP detection limit corresponding to a step length of 50 counts was 0.5 mg/ml and 1 mg/ml for solanidine and α-PGAs, respectively. A similar detection limit was achieved by the HPLC technique. A chromatogram of a standard mixture of β_2-chaconine, α-chaconine and α-solanine (15 ppm each) is shown in Figure 2.

TABLE II **ITP calibration results**

Species	RSH (-)	Intercept (counts^{+})	Slope (counts μg^{-1}.ml)	Correlation coefficient	Range (μg/ml)
hydroxonium	0.0	-	-	-	-
solanidine	65.7	25	42.5	0.999	2.5 - 12.5
α-solanine	82.0	28	26.8	0.999	5 - 25
α-chaconine	81.5	30	27.4	0.997	5 - 25
α-PGAs^{++}	82.5	30	27.0	0.998	5 - 25
zinc	100.0	-	-	-	-

$^{+}$ - 1 count = 50 ms (sampling frequency 20 Hz)

$^{++}$ - equimolar mixture of α-solanine and α-chaconine

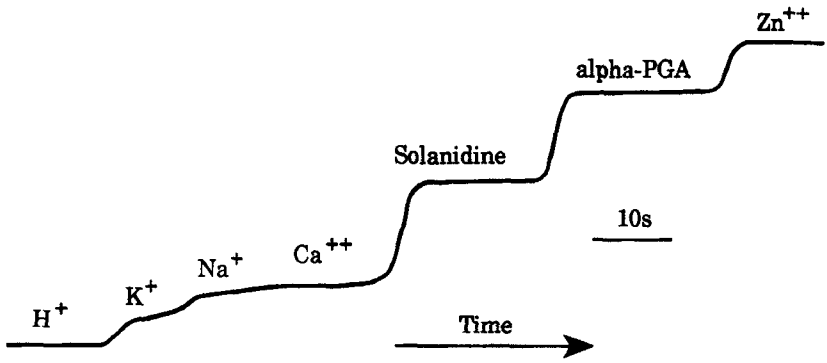

FIGURE 1 **Isotachophoregram of standard mixture of solanidine (7.5 μg/ml) and α-PGAs (15 μg/ml) - for analysis conditions see text**

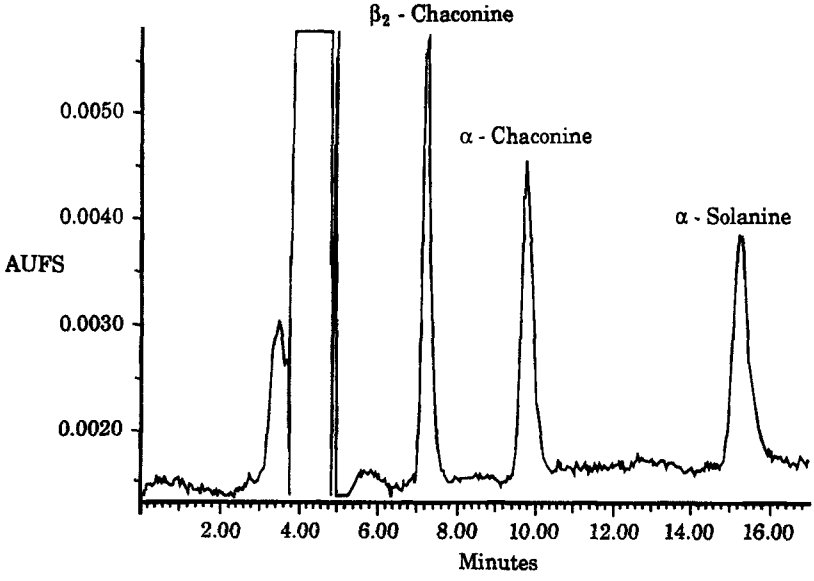

FIGURE 2 **Chromatogram of standard mixture of β_2-chaconine, α-chaconine and α-solanine (15 μg/ml each) - analysis condition see in text.**

Two different sample treatments (see in Sample preparation) of samples with high lipid content were compared and the results obtained showed that both techniques gave almost the same values of glycoalkaloid content (9 samples; correl. coeff. 0.995). When the determination of lipid content is carried out simultaneously with PGAs determination the technique based on sample defatting by hexane prior to PGAs extraction is recommended. In other cases the sample treatment based on two step SPE cleanup on Sep-Pak C18 and NH_2 cartridges is considered more suitable because it is less labour intensive and uses less solvent. These two SPE cleanup steps are necessary in the case of HPLC but not for ITP although it was found that ITP does need the second cleanup step by Tech Elut NH_2 in the case of high lipid containing samples; this makes ITP determination of PGAs simpler than HPLC.

The method developed using ITP for determination of PGAs was compared with the HPLC method and the results obtained are summarised in Table III. The data were handled by regression analysis to evaluate the relationship between ITP and HPLC values of α-PGA content. The following equation was obtained:

$$\text{ITP} = 1.077 * \text{HPLC} - 0.7 \quad (r = 0.989)$$

From this equation it is clear that the ITP technique gave a slightly higher figure for α-PGA content than that from HPLC analysis. The relatively high correlation coefficient confirms the linearity between these two techniques. On the basis of experiments described here it is possible to state that both techniques give similar values of α-PGA content in the samples of potato and potato products.

Although both the techniques described here give comparable total values, their particular strengths lie in their ability to measure individual components within the total glycoalkaloid mixture. It should be noted however that a more rapid technique, ELISA, has found considerable use in the measurement of total glycoalkaloid content of potato and its products.

The PGA content, as shown in Table III, varied from 16.6 to 169.9 mg/kg in the raw tissue of the commercial varieties, reducing to 47.0, 42.4 and 32.5% respectively by

TABLE III **Comparison of HPLC and ITP methods on PGAs (sum of α-solanine and α-chaconine) content**

SAMPLE	HPLC			ITP	
	(mg/kg) α-chaconine	(mg/kg) α-solanine	(mg/kg) α-PGAs	(mg/kg) α-PGAs	(mg/kg) Solanidine
Raw Potato I	63.8	18.4	82.2	91.4	-
Cooked Potato I	30	8.6	38.6	35.4	-
Raw Potato II	70	38	108.0	131.6	6.5
Cooked Potato II	29.7	14.9	44.6	40.7	-
Raw Potato III	125.7	44.2	169.9	182.6	-
Cooked Potato III	40.8	12.3	53.1	46.2	-
Raw Potato IV	33.5	19	52.5	44.4	2.5
Raw Potato V	29.4	12.7	42.1	33.4	-
Raw Potato VI	27.8	12	39.8	44.7	-
Raw Potato VII	39.8	20.4	60.2	55.4	-
Raw Potato VIII	9.2	7.4	16.6	28.1	-
Raw Potato IX	33.6	16	49.6	55.8	-
Snacks I	2.1	6.9	8.0	5.3	-
Snacks II	3	2.2	5.2	9.8	-
Dried Potato I	1.5	1.3	2.8	3.7	-
Dried Potato II	2	1.9	3.9	8.0	-
Unpeeled Crisps I	3.3	1.8	5.1	3.3	-
Unpeeled Crisps II	6.4	2.3	8.7	10.2	1.1
Unpeeled Crisps III	15.6	5.7	21.3	27.2	-
Unpeeled Crisps IV	10.5	6	16.5	15.4	-
Peeled Crisps I	4.1	2.2	6.3	9.6	-
Peeled Crisps II	2.9	1.3	4.2	5.2	-
Chips	2.3	1.6	3.9	2.8	-
Potato Starch I	1.1	0.8	1.9	2.2	-
Potato Starch II	0.5	0.4	0.9	0.89	-

- = not detected

the cooking procedure which presumably leached the remainder from the tissue into the cooking water. It is difficult to make direct comparison with data from other publications due to differences in cooking conditions, although, smaller but significant losses have been observed from boiled whole tubers. Bushway and Ponnampalam[8] measured changes in commercial whole unpeeled tubers after boiling in water and found the PGAs reduced to 71% of the original level (161 mg/kg). Similarly Maga[9] demonstrated a reduction to 74% from a level of 43 mg/kg in the raw unpeeled whole tuber.

From the data presented here, the ratio of α-chaconine to α-solanine varied between 1.24 and 3.49 and did not appear to be affected by cooking. Processed material

contained much lower levels ranging from 2.8 to 21.3 mg/kg with the ratio of the individual glycoalkaloids also lower in the range 0.3 to 2.78. The higher levels of glycoalkaloids found in crisps made from unpeeled tubers (upto 21.3 mg/kg) when compared with the lower levels in those made from peeled tubers (upto 6.3 mg/kg) is consistent with the fact that 90% of the glycoalkaloid is known to reside in the peel of the tuber.

The incidence of the aglycone solanidine in only 3 samples with a PGA content in excess of 10 mg/kg is interesting to note because if its presence was due to degradation of the PGAs during extraction then some would be expected in all samples, especially in those with high PGA content. Since this is not the case, its occurrence is presumably due to genetic factors relevant to those particular varieties.

CONCLUSIONS

The results show that the ITP technique is suitable for the determination of α-PGA in potato and products such as potato crisps, snacks or in dried potato products. The ITP method gives a slightly higher figure ($< 10\%$) of α-PGA than HPLC. By this technique it is not possible under the given analysis conditions to determine an individual α-PGA such as α-chaconine or α-solanine as in the case of HPLC. However ITP has some advantages in comparison with HPLC such as lower chemical consumption (only ca 1 ml of both leading and terminating electrolytes per analysis) resulting in very low running costs and the ability to determine the solanidine together with α-PGA. One step SPE cleanup of high lipid content samples by C18 cartridge is another benefit since HPLC needs an additional cleanup step using a NH_2 cartridge. Preliminary experiments on the separation of glycoalkaloids differing in the number of sugar molecules bounded to the aglycone (using a mixture of solanidine, β_2-chaconine and α-chaconine or products of the hydrolysis of tomatine) demonstrated the potential value of ITP.

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REFERENCES

1. R.J. Bushway. High performance liquid chromatographic separation of potato glycoalkaloids using radial compressed amino column. J. Chromatogr., 247, 180 - 183 (1982).

2. M.A. Filadelfi and A. Zitnak. A simple TLC standard for identification of potato glycoalkaloids. Can. Inst. Food Sci. Technol. J., 16, 151 - 153 (1983).

3. K.E. Hellenäs. A simplified procedure for quantification of potato glycoalkaloids in tuber extracts by HPLC; comparison with ELISA and a colorimetric method. J. Sci. Food Agric., 37, 776 - 782 (1986).

4. R.J. Bushway, J.L. Bureau and J. King. Modification of the rapid HPLC method for the determination of potato glycoalkaloids. J. Agric. Food Chem., 34, 277 - 279 (1986).

5. K. Kobayashi, A.D. Powell, M. Toyoda and Y. Saito. HPLC method for simultaneous analysis of α-solanine and α-chaconine in potato plants cultured *in vitro*. J. Chromatogr., 462, 357 - 364 (1989).

6. K. Saito, M. Horie, Y. Hoshino, N. Nose and H. Nakazawa. HPLC determination of glycoalkaloids in potato products. J. Chromatogr., 508, 141 - 147 (1990).

7. M.R.A. Morgan, R. McNerney, J.A. Matthew, D.T. Coxon and H.W-S. Chan. An enzyme-linked immunosorbent assay for total glycoalkaloids in potato tubers. J. Sci. Food Agric., 34, 593 - 598 (1983).

8. R.J. Bushway and R. Ponnampalam. α-chaconine and α-solanine content of potato products and their stability during several modes of cooking. J. Agric. Food Chem., 29, 814 - 817 (1981).

9. J.A. Maga. Potato glycoalkaloids. CRC Crit. Rev. in Food Sci. and Nutr., 371 - 405 (1980).

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