

# Improved High-Performance Liquid Chromatographic Method for the Analysis of Potato (*Solanum tuberosum*) Glycoalkaloids

Everard J. Edwards and Andrew H. Cobb\*

Department of Life Sciences, The Nottingham Trent University, Clifton Lane,  
Nottingham NG11 8NS, United Kingdom

An improved high-performance liquid chromatographic (HPLC) method for the determination of the potato glycoalkaloids,  $\alpha$ -solanine and  $\alpha$ -chaconine, is described. The glycoalkaloids were extracted with aqueous acetic acid, concentrated, and cleaned up by solid phase extraction using  $C_{18}$  cartridge columns and separated using a reverse phase  $C_{18}$  HPLC column. Recoveries averaged  $93\% \pm 1.3$  for  $\alpha$ -solanine and  $99\% \pm 3.1$  for  $\alpha$ -chaconine. Comparisons were made at each step with existing published methods. We conclude that trifunctional endcapped  $C_{18}$  SPE cartridges were more reliable than traditional monofunctional non-endcapped cartridges and that low carbon load  $C_{18}$  HPLC columns provided an improved separation of glycoalkaloids when compared to columns with a high carbon loading. An acidic extraction medium excluding organic solvents is recommended.

**Keywords:**  $\alpha$ -Chaconine; glycoalkaloids; HPLC; potato;  $\alpha$ -solanine; *Solanum tuberosum*

## INTRODUCTION

Glycoalkaloids are naturally occurring toxins found in all parts of the potato plant (*Solanum tuberosum* L.). The principal glycoalkaloids in potato tubers are  $\alpha$ -solanine and  $\alpha$ -chaconine, both glucosylated forms of the steroidal alkaloid, solanidine (Figure 1). Together they comprise approximately 95% of total potato glycoalkaloids (TGA) (Olsson, 1989).

Potato tubers typically contain about 20–60 mg of TGA  $100\text{ g}^{-1}$  freeze-dried matter (FDM) (Griffiths *et al.*, 1994), equivalent to 4–12 mg of TGA  $100\text{ g}^{-1}$  fresh weight (fwt). At these concentrations glycoalkaloids are considered to enhance potato flavor. However, at concentrations in excess of 20 mg  $100\text{ g}^{-1}$  fwt they impart a bitter taste and can cause gastroenteritic symptoms, coma, and even death. The toxic dose is considered to be approximately 2–5 mg  $\text{kg}^{-1}$  body mass, whereas the lethal dose is probably only 3–6 mg  $\text{kg}^{-1}$  (Morris and Lee, 1984).

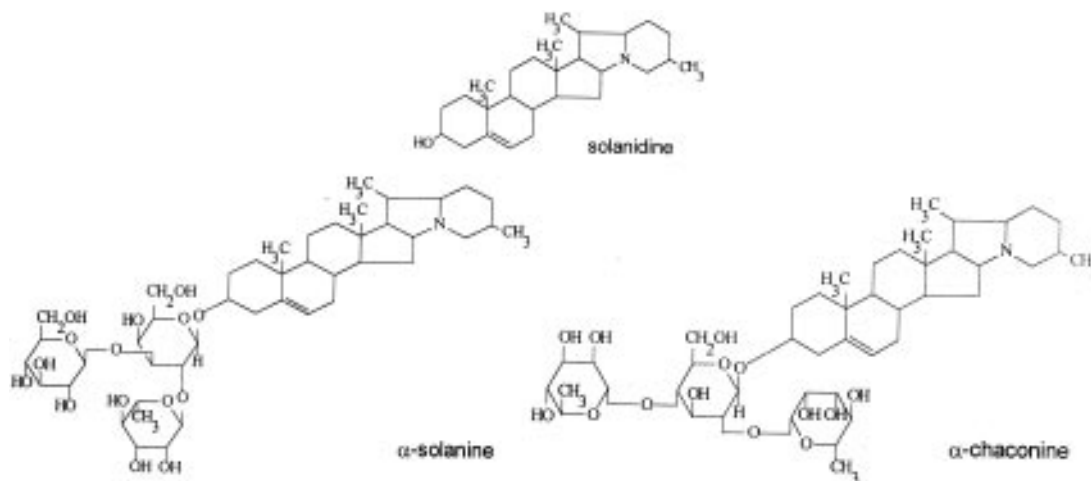
Approximately 30 deaths, more than 2000 cases of poisoning, and numerous livestock losses have been reported as caused by consumption of potatoes with high concentrations of glycoalkaloids (Morris and Lee, 1984). Indeed, as recently as 1993 a Japanese woman was taken ill after using a folk remedy prepared from potatoes containing 15-fold normal TGA content (Gonomori *et al.*, 1993).

The concentration of tuber glycoalkaloids increases in response to a number of factors, including physical injury (either from harvesting and sorting or herbivory), fungal attack, poor growing conditions, climate, and, perhaps most importantly, inadequate storage conditions. It has long been known that exposure of tubers to light can rapidly cause a large increase in TGA concentrations (Conner, 1937). This can occur during growth, harvesting, transport, storage, and at point of sale. Therefore light exposure is probably the most important commercial factor influencing TGA content in potato tubers. Breeding with wild *Solanum* biotypes for new varieties can also result in unacceptably high TGA levels (Sanford *et al.*, 1992).

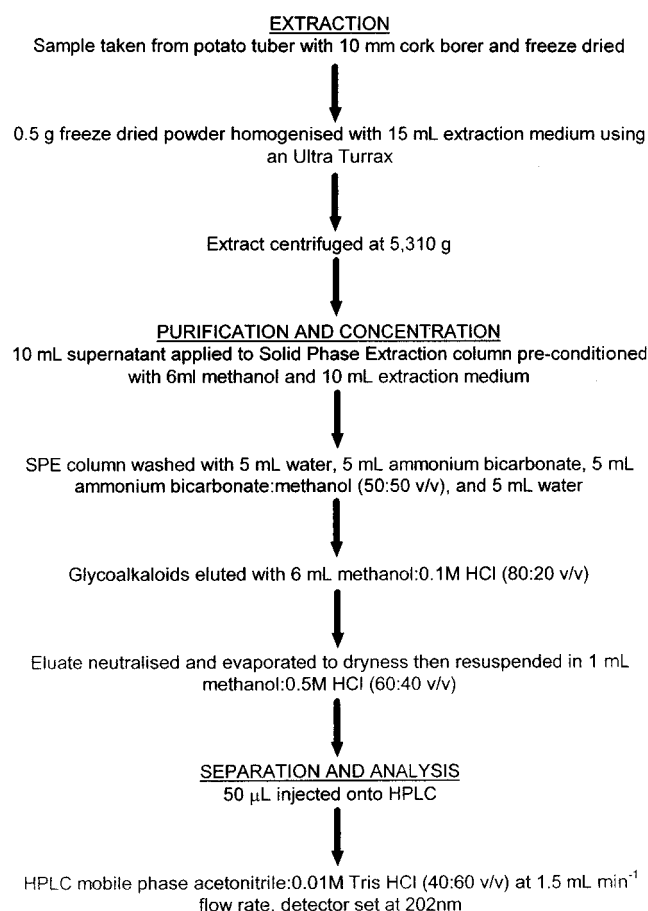
Consideration of the above demonstrates the importance of accurate and reliable methods of analysis for

tuber TGA. Modern commercial practice also necessitates that these methods be rapid and inexpensive. There are many methods for TGA analysis reported in the literature including mass spectrometry (Chen *et al.*, 1994), isotachyphoresis (Kvasnicka *et al.*, 1994), thin layer chromatographic scanning (Ferreira *et al.*, 1993), various colorimetric methods, gas chromatography (Lawson *et al.*, 1992), countercurrent chromatography (Fukuhara and Kubo, 1991), high-pressure liquid chromatography (HPLC), and enzyme immunoassays. Each method has relative advantages and disadvantages, such as lack of sensitivity, a need for derivitization, expensive chemicals, or excessively long preparatory steps. Currently the most realistic methods utilized are HPLC and immunoassays. The latter offers the possibility of a sensitive, simple, rapid, and relatively cheap detection method. However, polyclonal antibodies reported in the literature show a lack of specificity or immunological response (Morgan *et al.*, 1983; Plhak and Sporns, 1992; Plhak and Sporns, 1994) and the monoclonals that have been raised to date, although specific to solanidine-based compounds, cannot distinguish between individual glycoalkaloids, making them unsuitable for some studies (Plhak and Sporns, 1994; Stanker *et al.*, 1994).

There are a great many published HPLC methods due to the proven ability of HPLC to separate and quantify potato glycoalkaloids. In addition, there is a wide availability of HPLC equipment and these methods have the advantage of speed and ease of use. The majority of reported HPLC methods use reverse phase (RP)  $C_{18}$  or  $\text{NH}_2$  sorbents with a mobile phase consisting of acetonitrile and a biological buffer, commonly phosphate. However, extraction techniques and reported recoveries vary greatly. Indeed, with over 400 papers published in the past 25 years (Plhak and Sporns, 1994) there is much contradiction and confusion in the literature on TGA analysis. The main objective of this study has been to rationalize and consolidate these methods and produce a concise, reproducible method for the routine extraction and quantification of potato glycoalkaloids.



**Figure 1.** Chemical structures of the major potato alkaloids.



**Figure 2.** Flow diagram of method described in the text.

## MATERIALS AND METHODS

**Plant Material.** Potato tubers (cv. King Edward) were bought from a local supermarket, washed, and used on the day of purchase. Following a detailed evaluation and verification of the available published methods, the scheme illustrated in Figure 2 was adopted for all further studies. Each step is now described in detail.

**TGA Extraction.** A 10 mm diameter cork borer was used to take samples vertically through fresh tubers, avoiding the apex, base, and eyes. These were cut in half and either (a) ground fresh in 15 mL of extraction medium (0.02 M heptane-sulfonic acid in 1% aqueous acetic acid (v/v) with 1 mg/mL sodium bisulfite) using a mortar and pestle or (b) frozen in 10 mL of 0.1% aqueous poly(vinylpyrrolidone) (w/v) to  $-70^{\circ}\text{C}$ , lyophilized, and ground to a powder with a glass rod. Five

hundred milligrams of the powder was mixed with 15 mL of extraction medium.

The extract was homogenized with an Ultra Turrax TP18/10 with a 10N probe (Fisons, Loughborough, UK), for 30 s and then centrifuged at 5310g in a MSE Chilspin (Fisons) at  $4^{\circ}\text{C}$  for 15 min. Samples were kept on ice throughout the procedure. All solutions were prepared with ultrapure water from an Elga Maxima (Elga Ltd., High Wycombe, UK) and all chemicals were AnalaR grade unless otherwise stated.

**Purification and Concentration.** Ten milliliters of the supernatant was applied to a solid phase extraction (SPE) column. A comparison was made between four commercially available SPE sorbents; Sep-Pak C<sub>18</sub> (Waters, Watford, UK), Isolute monofunctional non-encapped C<sub>18</sub> (MF-NE), Isolute trifunctional non-encapped C<sub>18</sub> (TF-NE), and Isolute trifunctional endcapped C<sub>18</sub> (TF-EC) (all from Jones Chromatography, Hengoed, UK). Four weights of the TF-EC sorbent, 200, 500, 1000, and 2000 mg, were tested to determine optimal recoveries of spiked samples.

The columns were activated with 5–10 mL of methanol, according to sorbent volume, and equilibrated with 10 mL of extraction medium. Potato extract was applied to the column and interfering constituents were removed with a protocol adapted from Friedman and Levin (1992). This consisted of a series of washes: 5 mL of water, 5 mL of 0.05 M ammonium bicarbonate, 5 mL of methanol–ammonium bicarbonate (50:50 v/v), and 5 mL of water. All washings were collected and tested for the presence of glycoalkaloids. The glycoalkaloids were finally eluted with 4–8 mL of methanol–0.1 M hydrochloric acid (80:20 v/v). The eluent was neutralized with 15  $\mu$ L of 2 M NaOH/mL and dried under vacuum with a Jouan RC 10.22 centrifugal evaporator (Tring, UK). The residue was resuspended with 1 mL of methanol–0.5 M HCl (60:40 v/v) and 50  $\mu$ L samples were analyzed by HPLC.

**Separation and Analysis.** HPLC of TGA was performed using a System Gold 126 pump, 166 variable wavelength UV detector, and 507 autosampler (all Beckman Instruments, High Wycombe, UK) controlled with a Viglen SL personal computer (Alpertown, UK) using System Gold chromatography software (Beckman Instruments).

Eight RP columns, six C<sub>18</sub> and two C<sub>8</sub>, were evaluated (Table 1) using a method adapted from that of Jonker *et al.* (1992). The mobile phase was acetonitrile–0.01 M Tris·HCl buffer (40:60 v/v) adjusted to pH 7.8 with HCl. This was filtered through a 0.22  $\mu$ m filter and degassed for 20 min under reduced pressure. The HPLC flow rate was 1.5 mL min<sup>-1</sup>, and the detector was set at 202 nm. The method was calibrated using  $\alpha$ -solanine and  $\alpha$ -chaconine standards (purity >99% Fluka, Gillingham, UK) dissolved in methanol–0.5 M HCl (60:40 v/v). Recovery was estimated using internal standards of 0.25 mg per sample with four spiked and four control samples taken from the same tuber.

**Table 1. HPLC Columns Used**

sorbent	particle size ( $\mu\text{m}$ )	C <sub>18</sub> /C <sub>8</sub>	manufacturer	notes
$\mu$ Bondapak	5	C <sub>18</sub>	Fisons, UK	
Spherisorb	5	C <sub>18</sub>	Fisons, UK	
Techsphere 80	5	C <sub>18</sub>	HPLC Technology, Macclesfield, UK	low carbon
Techsphere 80	5	C <sub>8</sub>	HPLC Technology, UK	low carbon
Techsphere BDS	5	C <sub>18</sub>	HPLC Technology, UK	
Ultrasphere	5	C <sub>18</sub>	Beckman Instruments, UK	
Zorbax	5	C <sub>18</sub>	Fisons, UK	
Zorbax	5	C <sub>8</sub>	Fisons, UK	

**Table 2. SPE Sorbent Type Recoveries<sup>a</sup>**

sorbent	mean recovery (%)	
	solanine	chaconine
Sep-Pak	26 $\pm$ 1.3	30 $\pm$ 1.4
monofunctional non-encapped	31 $\pm$ 1.5	35 $\pm$ 1.6
trifunctional non-encapped	85 $\pm$ 2.5	86 $\pm$ 2.4
trifunctional encapped	99 $\pm$ 1.8	101 $\pm$ 3.2

<sup>a</sup> Results are the mean percentage recovery of standards  $\pm$  standard errors, where  $n = 4$ .

**Table 3. SPE Sorbent Volume Recoveries<sup>a</sup>**

sorbent weight (mg)	mean recovery (%)	
	solanine	chaconine
200	54 $\pm$ 1.9	59 $\pm$ 1.6
500	68 $\pm$ 1.6	64 $\pm$ 1.1
1000	93 $\pm$ 1.3	99 $\pm$ 3.1
2000	71 $\pm$ 3.3	59 $\pm$ 5.5

<sup>a</sup> Sorbent used was TF-EC. Results are the mean percentage recovery of standards from spiked samples  $\pm$  standard errors, where  $n = 4$ .

## RESULTS

The use of freeze-dried powder gave recoveries with no significant difference from those from fresh tissue (data not shown). The highest recovery of 0.5 mg/mL standards was achieved using Isolute TF-EC columns (Table 2), with virtually 100% recovery, whereas Sep-Pak columns gave only 25–30%. None of the other SPE columns used gave recoveries as high as those obtained with the TF-EC.

Initially, 200 mg columns were used but it was found that breakthrough of sample occurred for potato samples spiked with standards. It was, therefore, necessary to increase the amount of sorbent in the columns. One gram of sorbent weight was found to give the best recovery (Table 3). It is probable that the 2 g columns gave a lower recovery due to irreversible adsorption of TGA.

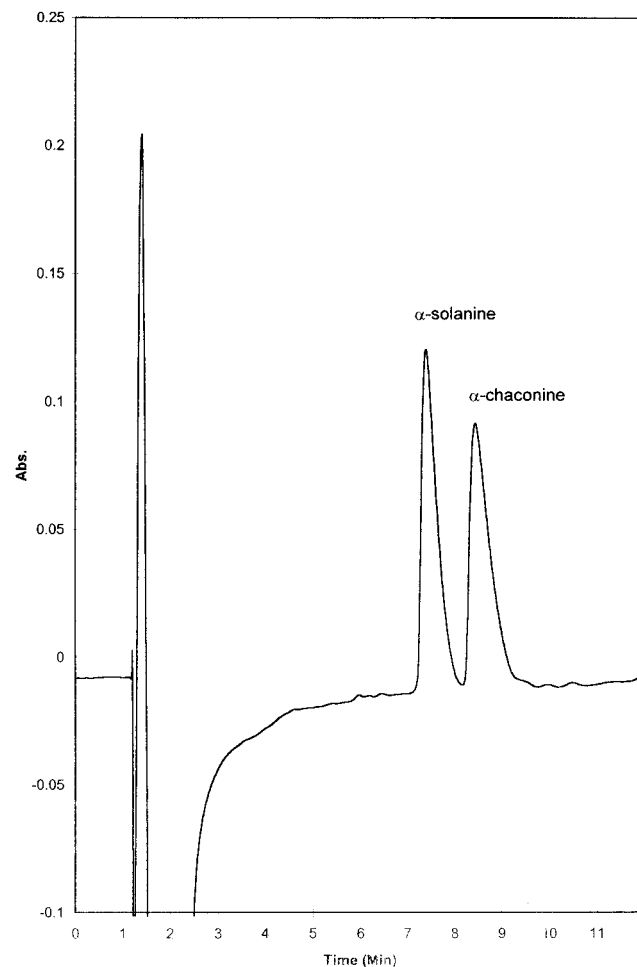
Glycoalkaloids were not detected in any of the washes used to elute unwanted components from the SPE columns.

The degree of separation of  $\alpha$ -solanine and  $\alpha$ -chaconine by HPLC was dependent on both column choice and the composition of the mobile phase. Two sorbents,  $\mu$ Bondapak and Techsphere BDS, gave no separation of the standards. Zorbax C<sub>18</sub> produced uneven peaks with excessively long retention times. Only three columns gave greater than 90% separation with 0.5 mg/mL standards: Techsphere 80 C<sub>18</sub> and the two C<sub>8</sub> sorbents (Table 4). Asymmetry of the eluted peaks varied greatly between the columns, with the C<sub>8</sub> sorbents giving the least tailing and Spherisorb the most. Techsphere 80 was subject to the least band broadening, had short retention times, and was used for all subsequent work (Figure 3).

**Table 4. Separation, Peak Shape, and Retention of Glycoalkaloids by Representative C<sub>8</sub> and C<sub>18</sub> HPLC Sorbents<sup>a</sup>**

sorbent	separation	tailing		peak width (min)		retention time (min)	
		S	C	S	C	S	C
		Techsphere 80 C <sub>18</sub>	0.93	3.0	3.5	1.0	1.3
Zorbax C <sub>8</sub>	0.96	2.0	2.4	2.0	2.4	12.1	14.2

<sup>a</sup> Separation calculated as  $R_s = 2(t_2 - t_1)/(w_1 + w_2)$ , where  $R_s$  = separation,  $t$  = retention time, and  $w$  = peak width at baseline; peak asymmetry calculated as  $A_{sf} = b/a$ , where  $A_{sf}$  = asymmetry,  $b$  = rear baseline segment at 10% peak height, and  $a$  = front baseline segment at 10% peak height. Zorbax C<sub>8</sub> results with a mobile phase of 55:45 Tris:acetonitrile.

**Figure 3.** Chromatogram of 0.25 mg/mL standards.

## DISCUSSION

When analyzing potato tubers for TGA, it is important to consider the sampling procedure. Almost all TGA found in freshly harvested, nongreened, potatoes are found in the outer layers (Kozukue *et al.*, 1987), and small and immature tubers have greater TGA concen-

trations (Hellenas, 1995). The results obtained by any sampling method are consequently affected by the ratio of the surface area of peel to volume of sample. The effect of this is greatest when using whole tubers but can be minimized by using half cores taken through the center of the potato.

Since tuber tissues are approximately 80% water by weight, the freeze-drying of samples provides a 5-fold concentration of TGA. It also allows samples to be stored for longer periods of time prior to analysis. In this study, no differences were found in TGA content between fresh and lyophilized potato samples. Considering these advantages, freeze-dried tissues were used throughout this research project.

There is a wide range of extraction solvents employed in published methods. Most are based on a weak solution of acetic acid with the addition of other solvents or salts. Methanol (Jonker *et al.*, 1992) or tetrahydrofuran (THF) (Bushway *et al.*, 1986) is commonly added. However, as glycoalkaloids are probably stored within the aqueous phase of the potato cell and are readily soluble in dilute acid, the use of these solvents is unnecessary. Furthermore, they require removal before any SPE step as many organic solvents prevent full adsorption of TGA onto C<sub>18</sub> SPE sorbents. The additional steps required for the removal of such solvents further contribute to a reduced recovery. The use of heptanesulfonic acid as an ion-pair reagent (Carmen *et al.*, 1986) enhances complete adsorption of TGA and was therefore used in these investigations. Sodium bisulfite was used to reduce oxidation of the extract (Hellenas, 1986).

A number of published methods involve an extraction step using methanol (e.g., Kvasnicka, 1994). However, the present study found potato glycoalkaloids to be virtually insoluble in cold methanol and so recovery from these methods can be at best uncertain, even at the low concentrations present in most potato tubers.

Virtually every recent paper describing a clean-up method for TGA advocates the use of Sep-Pak C<sub>18</sub> SPE cartridges. However, we have observed extremely variable results between batches and tested three other cartridge types for reproducibility of recoveries. The Isolute sorbent equivalent to Sep-Pak C<sub>18</sub> (MF-NE) gave recoveries similar to Sep-Pak, but the TF-EC sorbent gave the highest recovery and good reproducibility. Unfortunately, the low selectivity of endcapped C<sub>18</sub> sorbents necessitated a large sorbent weight due to the high number of compounds in the extract capable of binding to the column. No significant batch to batch variation has yet been observed with these cartridges, and therefore, these cartridges have been adopted for all subsequent work.

While Friedman and Levin (1992) did not detect any hydrolysis of TGA when evaporating to dryness in the presence of acid, this investigation found that drying samples in dilute HCl gave extremely variable recoveries. Friedman *et al.* (1993, 1995) demonstrated that 50% of  $\alpha$ -chaconine is hydrolyzed in 0.2 N HCl at 50 °C and that the presence of methanol increases the rate of hydrolysis. Neutralizing the SPE eluate prevented this hydrolysis, and the excess NaOH had no detectable adverse effect on the glycoalkaloid extract. The surplus alkali did, however, make it necessary to resuspend the samples in a stronger acid than was used for the SPE elution.

The composition of the mobile phase used for HPLC was important in ensuring full separation of  $\alpha$ -solanine

and  $\alpha$ -chaconine. A number of published methods have used acid phosphate buffers (Hellenas, 1986). However, full separation could not be attained with the HPLC columns reported above. Jonker *et al.* (1992) proposed the use of Tris-HCl at near-neutral pH. Good separation was achieved on the Techsphere 80 column using this mobile phase.

Separation and retention of the analytes was increased by raising the buffer pH. However, this resulted in reduced sensitivity of the system due to the glycoalkaloids being less soluble. No TGA was detected above pH 8 due to precipitation upon injection onto the column. The optimum pH was found to be 7.6–7.8. Reducing the amount of acetonitrile in the mobile phase also improved separation. Using 40% acetonitrile, 60% buffer gave maximum separation, although glycoalkaloids are insoluble at lower acetonitrile ratios.

Detection of potato glycoalkaloids requires the use of UV as there is no chromophore within the solanidine molecule. This limits sensitivity of detection, requiring the use of relatively high sample volumes. Spectra of  $\alpha$ -solanine and  $\alpha$ -chaconine in the adopted mobile phase show maxima at 202 and 203 nm, respectively. Therefore, 202 nm was used for quantification of TGA. This also means that mobile phases containing THF are unsuitable as it has an UV cutoff of 212 nm.

The method described in this paper addresses a number of problems with HPLC analysis of potato glycoalkaloids and utilizes a new SPE sorbent to improve sample recoveries resulting in a reliable routine assay of use to both researchers and the potato industry. It is being used in this laboratory to investigate the response of potato tuber TGA concentrations to environmental conditions during storage.

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