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Determination of glycoalkaloids in potato tubers by reversed-phase high-performance liquid chromatography

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ABSTRACT

A relatively fast and simple method for the determination of the glycoalkaloids α-solanine and α-chaconine in potato tubers is described. The glycoalkaloids are concentrated from potato samples by solid-phase extraction with a disposable C_{18} cartridge column. The recovery, determined by addition of glycoalkaloid standard to a potato sample, was found to be better than 90%. The relative standard deviation of the measured glycoalkaloid levels was less than 4%. For the reversed-phase (RP) HPLC analysis of basic compounds, *e.g.*, glycoalkaloids, too strong an interaction with the residual silanol groups of the stationary phase can be disadvantageous. In this work, acetonitrile–water was used as eluent with an RP-HPLC column with a reduced amount of residual silanol groups. The absence of buffer in the eluent increases the lifetime of the column and makes this system very suitable for the routine determination of glycoalkaloids.

INTRODUCTION

Glycoalkaloids are natural toxins, occurring in all parts of plants of the *Solanum* species [1]. These toxins are considered to form a natural resistance of the plant against parasites and diseases. In the potato plant, high concentrations of glycoalkaloids occur in the peel of the tuber (concentration about 300–600 mg/kg), in the sprouts (about 2000–4000 mg/kg) and in the flowers (3000–5000 mg/kg) [2]. The glycoalkaloid level averaged over the whole potato tuber is about 100 mg/kg. This relatively high level may even increase when the potato tuber experiences a kind of stress situation, *e.g.*, resulting from tuber injury or storing under non-ideal conditions [1,2].

Glycoalkaloids consist of a C_{27}-steroidal alkaloid skeleton (aglycone) to which one or more sugar groups are attached. In cultivated potatoes, α-solanine and α-chaconine, with solanidine as the aglycone, form about 95% of the total glycoalkaloid (TGA) content [2]. Glycoalkaloids are toxic to humans; the lethal dose is considered to be about 3–6 mg per kg body mass [3,4]. Therefore, commercial and especially new potato varieties are routinely screened in our institute for their glycoalkaloid content.

Chromatographic analysis of glycoalkaloids can be performed in a number of ways [4,5]. The intact glycoalkaloids can be analysed by gas chromatography (GC) after derivatization [6]. After hydrolysis of the glycoalkaloids, the aglycone skeleton can be examined by GC without the need for derivatization [7]. The disadvantage of this approach, however, is the high temperature necessary for analysing the compounds, which limits the lifetime of the GC column. For routine determinations of the glycoalkaloids present in potato tubers, high-performance liquid chromatography (HPLC) is probably the method of choice.

For the RP-HPLC of basic compounds, *e.g.*, glycoalkaloids, too strong an interaction between the compounds and the residual silanol groups of the stationary phase can be disadvantageous. This interaction leads to peak tailing and to an

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increase in retention time. The residual amount of silanol groups (the relative acidity of the column packing) greatly influences the retention behaviour of basic compounds [8]. Therefore, the analysis of basic compounds using standard RP-HPLC columns does not always give good results. Most of the HPLC methods described so far require the use of a relatively large amount of buffer in the eluent [9–12]. In this work, better results were obtained with an RP-HPLC column with a reduced amount of residual silanol groups (less acidic column). With this column, analyses for glycoalkaloids did not require the use of buffer in the eluent. The absence of buffer salts increases the lifetime of the column and also prevents the rapid deterioration of seals in the HPLC instrument. Therefore, this system is very suitable for the routine determination of glycoalkaloids in potato tubers.

EXPERIMENTAL

Solid-phase extraction

For concentrating the glycoalkaloids from the potato samples, solid-phase extraction (SPE) with a disposable Sep-Pak C_{18} column (Waters, Milford, MA, USA) was used. The glycoalkaloids were extracted from the potato sample by ion-pair extraction using 0.02 *M* sodium 1-heptanesulphonate (Sigma, St. Louis, MO, USA) in 0.17 *M* acetic acid (Merck, Darmstadt, Germany). For the preparation of solutions, distilled water that had been further purified with a Milli-Q system (Waters) was used. The SPE pretreatment was adapted from that of Carman *et al.* [9]. After thoroughly mixing the sample, insoluble constituents were removed by filtration. The pretreatment of the SPE column consisted of flushing with methanol (analytical-reagent grade; Labscan, Dublin, Ireland) followed by flushing with sodium 1-heptanesulphonate solution. The filtered potato sample was eluted through the SPE column and interfering constituents of the sample were removed by flushing with acetonitrile–water (20:80, v/v). The acetonitrile mixtures were prepared by mixing water or buffer with an azeotrope consisting of acetonitrile–water (83.7:16.3, v/v). The azeotrope mixture was obtained by distillation of HPLC effluents. Finally, the glycoalkaloids were eluted from the Sep-Pak column by applying acetonitrile–buffer (60:40, v/v). The buffer was prepared by dissolving 3 g of $(NH_4)_2HPO_4$ (Merck) in 1 l of water.

HPLC of glycoalkaloids

HPLC was performed using a Gilson (Middleton, WI, USA) Model 305 pump, a manometer, a Marathon (Spark, Emmen, Netherlands) autosampler with a 20-μl loop, an Applied Biosystems (San Jose, CA, USA) Model 757 UV detector operating at 202 nm and a Spectra-Physics (San Jose, CA, USA) SP 4600 integrator. Initially, a RoSil C_8 (3 μm) column (150 × 4.6 mm I.D.) (Bio-Rad, Richmond, CA, USA) with a Nucleosil 120-5 C_8 precolumn (30 × 4 mm I.D.) (Machery–Nagel, Düren, Germany) was used. The eluent consisted of acetonitrile–3 g/l $(NH_4)_2HPO_4$ buffer (45:55, v/v). However, the chromatographic conditions severely reduced the lifetime of this column system. Better results were obtained when using a Nucleosil 5 C_{18}-AB (5 μm) column (250 × 4 mm I.D.) with a Nucleosil 100-5 C_{18}-AB precolumn (30 × 4 mm I.D.) (Machery–Nagel). With this column system, glycoalkaloids were separated with acetonitrile–water (60:40, v/v) as the eluent at a flow-rate of 1 ml/min. For the determination of the glycoalkaloids, standards of α-solanine (purity >99%; Roth, Karlsruhe, Germany) and α-chaconine (purity >95%, Roth) dissolved in acetonitrile–buffer (60:40, v/v) were used. Quantitative analysis of the glycoalkaloid level was performed by using external standard solutions, which were directly injected into the chromatographic system.

RESULTS AND DISCUSSION

Fig. 1 shows the general structure of the glycoalkaloids α-solanine and α-chaconine present in potato tubers. For HPLC, the glycoalkaloids have to be concentrated from the sample. Further, they must be separated from interfering constituents present in the potato tuber. Fig. 2 illustrates the sample clean-up resulting from SPE. For this experiment, 10 ml of potato tuber extract were eluted through the SPE column, which was then flushed with 5 ml of water or 5 ml of acetonitrile–water (20:80, v/v). Final-

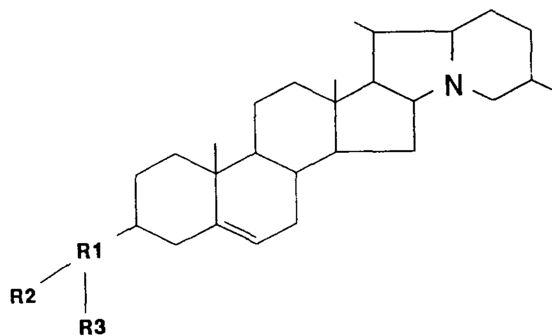

Fig. 1. General structure of the glycoalkaloids α-solanine and α-chaconine present in potato tubers. The sugar group consists of R1 = β-D-galactose, R2 = β-D-glucose and R3 = α-L-rhamnose for α-solanine and R1 = β-D-glucose and R2 = R3 = α-L-rhamnose for α-chaconine.

ly, the column was eluted with 3 ml of acetonitrile–buffer (60:40, v/v) and the eluate was subjected to HPLC.

As can be seen from Fig. 2, the glycoalkaloids can be efficiently separated from other constituents by flushing the SPE column with acetonitrile–water (20:80, v/v). Almost no sample clean-up was achieved by rinsing the column with water. This experiment indicates that complex chromatograms would be obtained without sample pretreatment, which would prevent the accurate determination of the glycoalkaloids. The relatively strong interaction between the SPE column and the glycoalkaloids results in an efficient sample clean-up, but also indicates that complete elution of the glycoalkaloids necessitates a relatively strong eluent. Table I gives the measured peak areas of α-solanine and α-chaconine after HPLC of a potato tuber sample in relation to the composition of the SPE eluent used. For this experiment, the SPE eluents consisted of mixtures of acetonitrile (ACN) with water or with buffer.

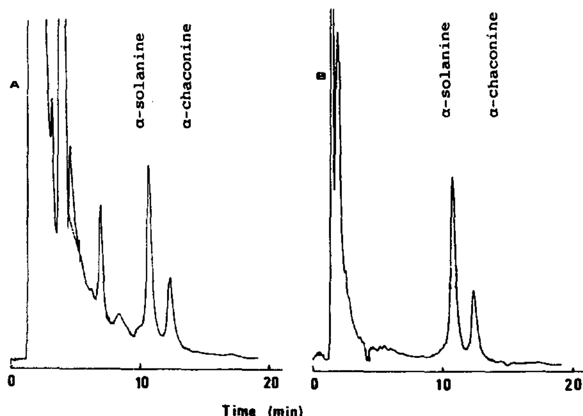

Fig. 2. Illustration of sample clean-up resulting from solid-phase extraction. (A) Washing SPE column with 5 ml of water; (B) washing SPE column with 5 ml of acetonitrile–water (20:80, v/v). The chromatograms were obtained with the RoSil C_8 column.

From Table I, it is clear that elution with a mixture of acetonitrile and buffer gives the highest recovery for the glycoalkaloids. The presence of buffer reduces the interaction between the glycoalkaloids and the silanol groups on the SPE packing. In this way, almost all of the glycoalkaloids are eluted from the SPE column in the first step. Only very small amounts of glycoalkaloids were recovered when using acetonitrile–water. To verify that the glycoalkaloids are completely eluted with acetonitrile–buffer (60:40, v/v), a known amount of glycoalkaloid standard was added to a potato tuber sample in which the glycoalkaloid level was known. For this experiment, the glycoalkaloid standard was dissolved in sodium 1-heptanesulphonate and 1, 2 or 5 ml of this standard solution was added to the potato sample. The samples were then treated and analysed as described above. In Table II are given the calculated recoveries after addition of the standard to the potato sample, which originally contained 72 mg/kg of α-solanine and 46 mg/kg of α-chaconine. As can be seen, the recovery of the glycoalkaloids is generally more than 90% under the SPE conditions used.

Initially, HPLC of glycoalkaloids was performed with a Rosil C_8 column. However, this column contained a relatively large amount of residual silanol groups on the silica packing. This type of column is routinely prepared by attaching C_8 chains to the silica packing by reaction with silanol groups [8,13]. However, because of steric hindrance usually only 30–40% of the silanols present on the packing are consumed in this reaction [8]. Even after end-capping, a relatively large number of silanol groups remain on the silica surface, which complicates the analysis of basic compounds. Because of the acidic character of the silanols, an ion-exchange process can occur between silanols and basic compounds, which can result in peak tailing and excessive retention times [8]. The retention mechanism of

TABLE I

PEAK AREAS OF α-SOLANINE AND α-CHACONINE WITH THREE DIFFERENT SPE ELUENTS

Average results of two individual analyses. Samples analysed with a RoSil column.

Compound	Eluent		
	ACN–water (45:55, v/v)	ACN–water (83.7:16.3, v/v)	ACN–buffer (60:40, v/v)
1st elution, 2 ml			
α-Solanine	0.54	1.8	10.8
α-Chaconine	0.25	1.8	8.4
2nd elution, 1 ml			
α-Solanine	0.75	1.9	0.75
α-Chaconine	0.45	3.0	0.81

the glycoalkaloids on standard RP-HPLC columns thus involves both hydrophobic interaction with the alkyl chain and ion-exchange interaction with residual silanol groups on the silica packing. The interaction with the silanol groups can be suppressed by addition of buffer to the eluent. In our experiments, a high-pH buffer was used, which prevents the protonation of the glycoalkaloids and thus interferes with the ion-exchange process. In this way, analyses were performed with an eluent consisting of acetonitrile–$(NH_4)_2HPO_4$ buffer (45:55, v/v). The relatively high pH of the buffer solution (*ca.* 8) severely reduces the lifetime of the column, however, probably because of dissolution of the silica packing. Another approach to prevent silanol interaction is to use a low-pH eluent (pH $<$ 2.5), which prevents the ionization of the silanol groups [8]. RP-HPLC of glycoalkaloids with a low-pH buffer was recently described by Friedman and Levin [12]. They found an increased resolution with increasing acidity of the column. However, these findings are confusing because the columns were tested using a relatively large amount of buffer in the eluent, which masks the acidity of the column to a great extent. Further, a drawback of this approach is deterioration of the column because stripping of the alkyl ligands

TABLE II

RECOVERIES OF α-SOLANINE AND α-CHACONINE AFTER ADDITION OF GLYCOALKALOID STANDARD TO A POTATO TUBER SAMPLE

Average results of two individual analyses, samples analysed with a Nucleosil column.

Compound	Standard solution added (ml)	Recovery (%)		
		1st elution, 2 ml	2nd elution, 1 ml	Total
α-Solanine	1	74	13	87
	2	83	7	90
	5	81	9	90
α-Chaconine	1	83	14	97
	2	89	10	99
	5	86	19	105

from the silica surface may occur [8,13]. In conclusion, the determination of basic compounds with standard RP-HPLC columns often requires the use of low- or high-pH eluents, thus limiting the lifetime of the column.

The determination of glycoalkaloids by using polymer-based RP-HPLC columns (*e.g.*, with a polystyrene–divinylbenzene packing) with an acetonitrile–water eluent only gave good results for standards; for potato tuber samples, no good separation between the glycoalkaloids and the interfering compounds was obtained. The total lack of silanol groups on the polymer packing made this type of column insufficiently selective for application to glycoalkaloids. The results indicated that a relatively small amount of silanol groups may be necessary for the determination of basic compounds.

We concluded that the determination of glycoalkaloids requires a column with a reduced amount of silanol groups. The residual amount of silanols present on the column packing should be large enough to distinguish between glycoalkaloids and interfering constituents, but on the other hand low enough to be able to elute the glycoalkaloids without using buffers in the eluent. Good baseline resolution was obtained when using acetonitrile–water (60:40, v/v) as the eluent with a Nucleosil C_{18}-AB column (see Fig. 3). The absence of buffer in the eluent increases the lifetime of the column and diminishes problems caused by rapid wearing out of seals present in the pump and in the injector.

The standard deviation of the measured glycoalkaloid level was determined by repeatedly analysing a sample on one day and on several days. These analyses resulted in a relative standard deviation that was better than 4% for a sample containing 200 mg/kg of glycoalkaloids. Further, the glycoalkaloid levels determined with the described HPLC method were compared with those obtained with a spectrophotometric method. This method consisted of bisolvent extraction of glycoalkaloids from the sample followed by reaction with a mixture of phosphoric acid and paraformaldehyde and measurement of the absorbance at 600 nm [14,15]. As can be seen in Fig. 4, a good correlation was found between the two methods for 64 different potato tuber samples. The deviation from the ideal line (dashed line in Fig. 4) is most pronounced for a high glycoalkaloid level. This slight deviation is

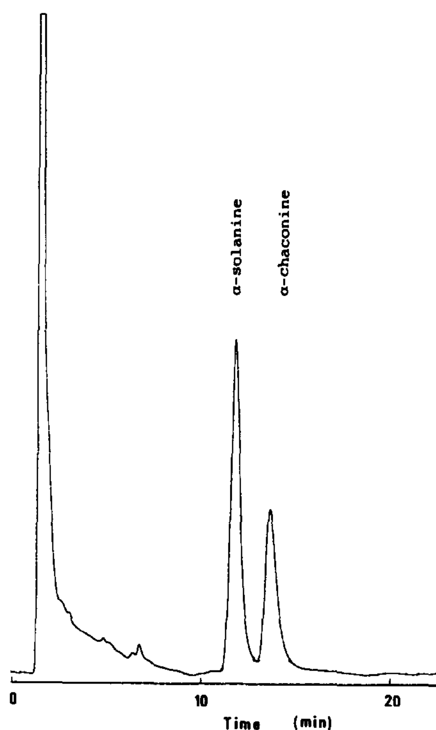

Fig. 3. Example of glycoalkaloid determination with an RP-HPLC column especially suitable for basic compounds. A Nucleosil 5 C_{18}-AB column was used with acetonitrile–water (60:40, v/v) as the eluent.

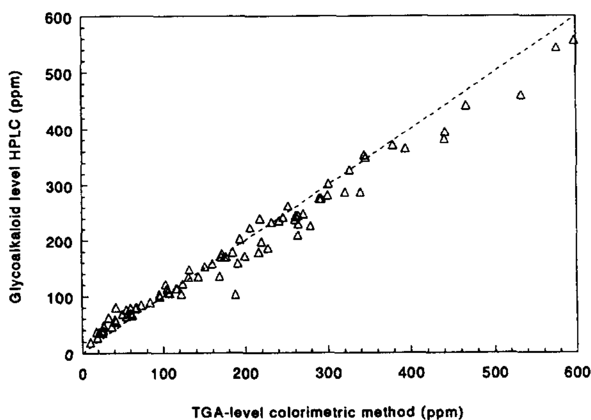

Fig. 4. Comparison between glycoalkaloid levels determined by the described HPLC method and a spectrophotometric method. TGA = total glycoalkaloids. For the 64 potato tuber samples, a correlation coefficient of 0.989 was found.

probably caused by the fact that the HPLC method only determines the glycoalkaloids α-solanine and α-chaconine, whereas the spectrophotometric method determines the total glycoalkaloid level. The high correlation coefficient (0.989) found between the two methods, however, confirms the accuracy of the method of analysis.

In conclusion, the relatively simple and fast method described for the determination of glycoalkaloids in potato tubers has the advantages of high precision and accuracy. Further, the chromatographic conditions with a column especially suitable for basic compounds are advantageous because of the long lifetime of the column. These conditions make routine determinations of glycoalkaloids possible.

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