Reversed-Phase High-Performance Liquid Chromatographic Separation of Potato Glycoalkaloids and Hydrolysis Products on Acidic Columns

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As part of a program of potato improvement, improved procedures were developed for reversed-phase high-performance liquid chromatographic (HPLC) separation of the alkaloids present in commercial potatoes: the two major glycoalkaloids, α-chaconine and α-solanine; hydrolysis products or metabolites derived from these two compounds, the so-called β- and γ-chaconines and solanines; and their aglycon, solanidine. For comparison, we also included the glycoside solasonine, present in wild potatoes, and its aglycon solasodine. Column "acidity", caused by active silanol sites on the packing's surface, was found to strongly influence chromatographic separation of *Solanum* alkaloids. Ten commercially available HPLC columns with reversed-phase packings in a total of 13 configurations were evaluated for relative acidity, with the aid of two standards: diphenhydramine and 5-(*p*-methylphenyl)-5-phenylhydantoin. Resolution of the glycoalkaloids improved with increasing acidity. The peaks associated with the glycoalkaloids lacked the tailing and excessive retention usually observed with basic compounds on acidic columns. The aglycons, however, required a nonacidic column for optimum chromatography. These studies facilitated choosing conditions to optimize the separation and quantitative estimation of *Solanum* alkaloids, both in pure form and in potato extracts, and proved the usefulness of acidic columns in certain applications. The possible value of these findings to food safety and plant physiology is discussed.

INTRODUCTION

Glycoalkaloids, natural components of plants in the *Solanum* family, are potentially toxic (McMillan and Thompson, 1979; Willimott, 1933). As part of a multidisciplinary program of potato improvement, we previously used extraction and HPLC procedures to measure the α-chaconine and α-solanine content in different parts of the potato plant, in commercial and experimental potato varieties, and in processed potato products (Friedman and Dao, 1992). The ratio of these two major potato glycoalkaloids varied widely. This finding has implications for food safety and plant physiology since we also found that (a) α-chaconine is more toxic than α-solanine to frog embryos and induces greater amounts of the hepatic enzyme ornithine decarboxylase in rats; (b) the aglycons solanidine and solasodine are less toxic than the glycosides from which they are derived; and (c) the carbohydrate part of the glycoside molecules appears to be paramount in influencing physiological and toxicological activities (Blankemeyer et al., 1992; Caldwell et al., 1991; Friedman et al., 1991, 1992). Since biological potencies are strongly influenced by chemical structures, knowledge of the composition of alkaloid-containing diets is paramount to evaluate safety.

Our major objective is to decrease the activity of potato enzymes involved in the biosynthesis of the more toxic of the potato alkaloids (Stapleton et al., 1991, 1992). This objective requires accurate analytical methods for glycoalkaloids, metabolites, aglycons, and biosynthetic intermediates, to measure the biosynthesis and distribution of these compounds in new potato varieties and in animal fluids and tissues after ingestion. As part of this effort, the objective of this study was to find optimum conditions for the separation of mixtures of α-chaconine, α-solanine, β-chaconines, β-solanines, γ-chaconine, γ-solanine, and the aglycons, both in pure form and as extracted from the potato.

As column chemistry appeared to play an important role in the chromatography of our compounds, we carried out a methodical search for appropriate columns. One of the major differences between reversed-phase packings from different manufacturers is their degree of acidity. This property is caused by the highly polar and retentive silanol (SiOH) sites remaining on silica-based packing following derivatization with C18 or C8 hydrophobic groups. Basic compounds react with silanols in a cation-exchange mode, creating a second retention mechanism to the primary hydrophobic mode. This mixed-mode retention frequently causes tailing and excessive elution times with basic samples (Stadalius, 1988). Variations in acidity appeared to be responsible for the diverse chromatographic results we observed with different columns. To better understand this effect, we examined relative acidities of a number of reversed-phase columns by comparing the chromatography of diphenhydramine (DPH), which is very sensitive to residual silanols, to that of 5-(*p*-methylphenyl)-5-phenylhydantoin (MPPH), which is relatively unaffected by them (Dalrup and Kardel, 1984). High levels of silanols cause increased retention and tailing peaks for DPH yet have little effect on MPPH. The retention of DPH relative to MPPH and the quality of the peak shape of the DPH band provided a basis for comparing column acidities. Once the columns were ranked according to the relative retention time of DPH to MPPH, column acidity and performance were correlated.

MATERIALS AND METHODS

Materials. Solvents were of HPLC grade. Ammonium phosphate (monobasic) was obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). It was recrystallized from methanol before use. A high-glycoalkaloid variety (NDA 1725) potato was

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obtained from the Potato Breeding Program, University of Idaho, Aberdeen, ID. The α-chaconine, α-solanine, solanidine, solasodine, diphenhydramine, and 5-(p-methylphenyl)-5-phenylhydantoin were obtained from Sigma Chemical Co. (St. Louis, MO). Solasonine was obtained from Research Plus, Inc. (Bayonne and Denville, NJ). The β_1-chaconine, β_2-chaconine, γ-chaconine, β_2-solanine, and γ-solanine were prepared by partial acid hydrolysis of α-chaconine and α-solanine, respectively (Zitnak and Filadelfi-Keszi, 1988; Coxon, 1984; Filadelfi and Zitnak, 1982, 1983; Swain et al., 1978). These compounds were characterized by thin-layer chromatography and mass spectrometry (Friedman et al., 1992b).

Instrumentation. A Beckman Model 334 liquid chromatograph with a 427 integrator and a 165 UV–visible variable-wavelength detector was used (Friedman and Levin, 1989). We tested the following columns: two Resolve C18, 5 μm, 3.9 × 300 mm columns (Waters Chromatography Division, Milford, MA); two Ultrasphere C18, 5 μm, 4.6 × 250 mm columns (Beckman Instruments, San Ramon, CA); five Pecosphere C18, 3 μm, 4.6 × 83 mm columns, four with standard and one with reduced activity packing (Perkin-Elmer Corp., Norwalk, CT); and one Supelcosil C18 deactivated base, 3 μm, 4.6 × 150 mm column (Supelco Inc., Bellefonte, PA). The shorter Pecosphere columns were tested both individually and coupled in series to produce a longer column comparable to the others. Testing different lengths of column packing helped us understand the effect of this factor on resolution. Three coupled-column arrangements were made, increasing the total number of columns tested from 10 to 13.

Methods. Fresh potatoes were rinsed and patted dry, cubed, and immediately frozen in liquid nitrogen. Samples were then lyophilized. Samples were weighed before and after for moisture determination. The dried potato was then ground on a Wiley mill so that it passed through a 1-mm screen.

The extraction technique was adapted from that of Bushway et al. (1986). A 20-mL sample of 5:3:2:0.1 tetrahydrofuran–water–acetonitrile–acetic acid was blended with 1 g of potato powder for 10 min in a Sorvall Omni-mixer (Newtown, CT). This mixture was then centrifuged for 10 min at 3000 rpm and filtered through a 0.45-μm membrane with a prefilter. The residue was resuspended in 20 mL of extracting solvent and treated as above. The combined filtrates were evaporated to about 4 mL on a Buchi rotovapor (Buchi Laboratories, Zurich, Switzerland) and transferred to a centrifuge tube; the flask was rinsed three times with 10 mL of 0.02 M heptanesulfonic acid–1% acetic acid. This solution was centrifuged for 10 min at 3000 rpm and then filtered through a 0.45-μm membrane plus prefilter. The filtrate was brought up to a volume of 50 mL with water, giving a final concentration of 20 mg/mL.

The extract was further purified using solid-phase extraction. The solid-phase extraction tube, a 6-mL Supelclean ENVI-18 (Supelco), was conditioned with 2 mL of methanol followed by 2 mL of water. Five milliliters of the extract was applied. The tube was then washed with 5 mL of water, 5 mL of 50 mM ammonium bicarbonate, 10 mL of 50:50 methanol–50 mM ammonium bicarbonate, and finally 10 mL of water. The alkaloids were eluted with 3 mL of 80:20 methanol–50 mM HCl. The sample was dried on the rotovapor, rinsed once with water to remove traces of HCl, and dried again. The residue was then taken up in 1 mL of 50% methanol–water and 0.01% acetic acid for injection into the HPLC column.

The chromatograph was set to deliver solvent at 1 mL/min at 0.1 absorbance units full scale (AUFS). Peaks were detected by UV absorbance at 200 nm. The solvent eluent for glycoalkaloid separation consisted of 35% acetonitrile and 100 mM ammonium phosphate (monobasic) adjusted to pH 3.5 with phosphoric acid. The eluent for detection of solanidine and solasodine in the column survey was 60% acetonitrile and 50 mM ammonium phosphate (monobasic) adjusted to pH 3.5. Optimized conditions for the detection of the two aglycons on the Supelcosil column were 60% acetonitrile and 10 mM ammonium phosphate (monobasic) at pH 2.5.

Conditions for determining the acidity of the columns were as described by Dalrup and Kardel (1984). Briefly, flow rate was 1 mL/min and detection at 220 nm. The mobile phase consisted of 156 g of acetonitrile and 340 g of buffer. The buffer contained 6.66 g of potassium phosphate (monobasic)/L, adjusted to pH 2.3 with phosphoric acid. MPPH and DPH were chromatographed together on this system, and the retention time of DPH relative to that of MPPH (RRT = $t_{R\ DPH}/t_{R\ MPPH}$) was recorded.

Peak shape, the width of a peak relative to its retention, is described by the equation for theoretical plates, $N = 5.54(t_R/w)^2$, where N is the number of theoretical plates, t_R the retention time, and w the width of peak at half-height (Snyder and Kirkland, 1979). N takes into account the normal broadening of peaks expected as retention time increases. Good peak shapes will have narrow peak widths relative to retention and thus larger values for N.

The capacity factor, k', is a measure of the ability of the column packing material to retain a specific compound. It is the ratio of moles in the stationary phase to moles in the mobile phase and is calculated by the equation $k' = (t_R - t_0)/t_0$, where t_R is the retention time of the compound of interest and t_0 is the retention time of unretained compounds (Snyder and Kirkland, 1979). The capacity factor should be the same for any given packing regardless of column geometry.

Band resolution was calculated with the equation $Rs = t_2 - t_1/(w_1 + w_2)$, where t_1 and t_2 represent the retention times of the two peaks and w_1 and w_2 represent the peaks' width at baseline measured from tangents to their slopes (Snyder and Kirkland, 1979). Resolution of the system was equal to the resolution of the least resolved pair. A resolution of at least 1.0 was desirable.

RESULTS

Figure 1 shows the structures of the compounds evaluated in this study. Figure 2 depicts a chromatogram of each of the glycoalkaloids separated on the Resolve T12031 column. The peaks are well separated and have good peak shape, and the elution times are quite acceptable. We found UV response to be linear in the range tested, from 0.2 to 4 μg. Figure 3 is a chromatogram of a potato sample. The chromatogram has no inteferences in the region of interest. Spiking the potato meal before extraction with 0.5 mg each of α-solanine and α-chaconine/g of dry potato meal resulted in recoveries between 90 and 100%. Figure 4 is a chromatogram of an extract of potato roots. This sample naturally contained β_1-chaconine, β_2-chaconine, and β_2-solanine, as well as much larger levels of α-solanine and α-chaconine. A more concentrated sample than would be optimum for integration of α-solanine and α-chaconine had to be applied to the column, to bring the β- and γ-glycoalkaloids into a desirable region for integration. The large peaks due to α-solanine and α-chaconine did not interfere with integration of the smaller peaks. To ensure that the β- and γ-glycoalkaloids were not artifacts of the purification techniques, 0.5 mg each of α-solanine and β-chaconine was dissolved in 0.02 M heptanesulfonic acid–0.01% acetic acid and purified by solid-phase extraction. The drying technique on the rotovapor was particularly suscept because dilute acid could hydrolyze glycoalkaloids in the presence of heat. No hydrolysis was detected. Only the chromatograms of the sprouts contained any peaks for β- or γ-glycoalkaloids. Tubers did not contain these peaks, indicating that hydrolysis of the glycoalkaloids did not occur during normal extraction conditions.

Figure 5 is a chromatogram of 0.5 μg each of solanidine and solasodine on the Supelcosil column. Eluent was 60% acetonitrile and 10 mM ammonium phosphate (monobasic). Peak shape for solanidine was sharper when the eluent contained 50 mM ammonium phosphate, as in our column screenings; however, the separation of solanidine and solasodine was better when the eluent contained 10 mM ammonium phosphate. Both solanidine and solasodine were detectable at levels as low as 0.02 μg, and UV response was linear from 0.1 to 4.0 μg. At higher levels the peaks became too large to integrate accurately.

The spiking of potato samples with 0.5 mg of solanidine/g of dried potato meal yielded about a 70% recovery overall.

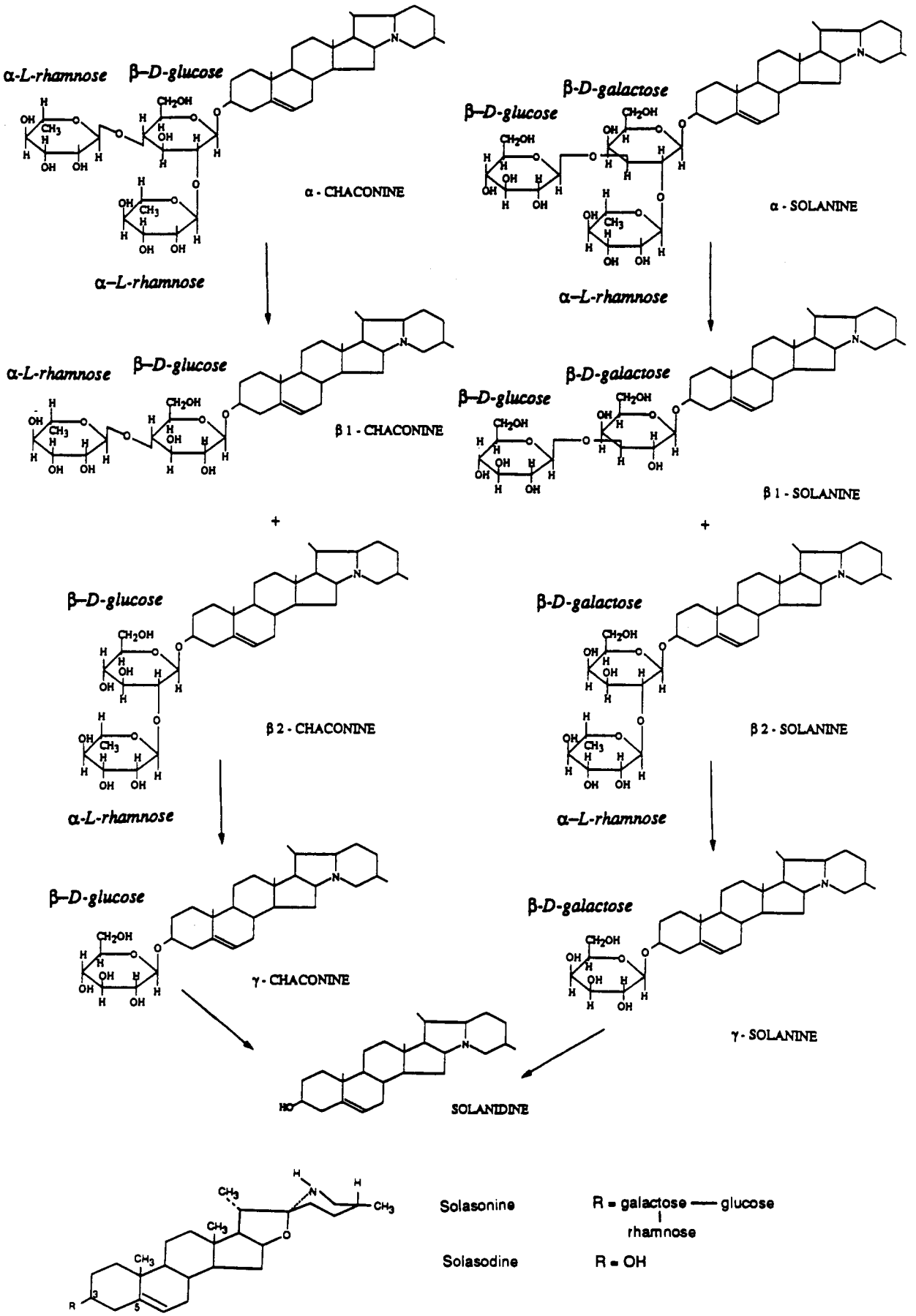

Figure 1. Structures of compounds evaluated in this study. β_1-Solanine was omitted from the study because we were unable to isolate it from the hydrolysis mixture.

There appeared to be a significant loss during solid-phase extraction. We were able to omit the solid-phase extraction step and inject the extract directly into the chromatograph (Figure 6), increasing the recovery to 90%. Many of the

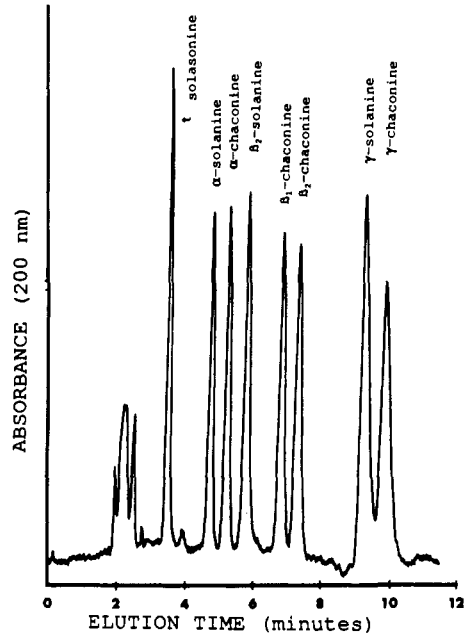

Figure 2. Chromatogram of approximately 1 μg of each of the glycoalkaloids. Column: Resolve C18, 5 μm, 3.9 × 300 mm, lot T12031. Eluent: 35% acetonitrile and 100 mM ammonium phosphate (monobasic) at pH 3.5. Detector: UV spectrophotometer, 200 nm, 0.1 AUFS.

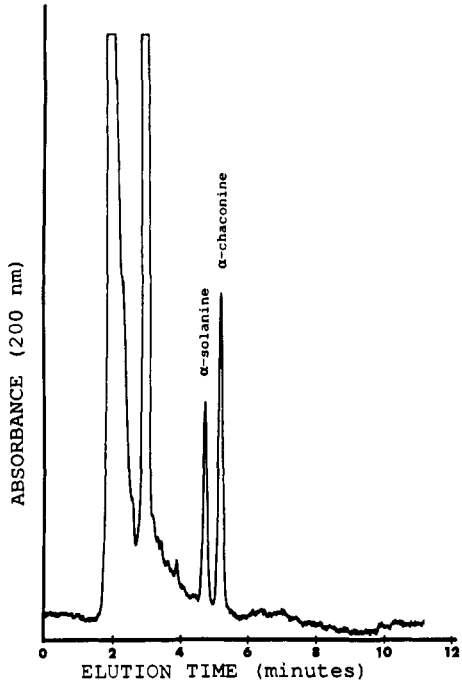

Figure 3. Chromatogram of a purified extract containing 2 mg of potato tuber solids from a high-glycoalkaloid potato variety (NDA 1725). The sample contains 0.7 μg of α-solanine and 1.1 μg of α-chaconine. Column: Resolve C18, 5 μm, 3.9 × 300 mm, lot T12031. Eluent: 35% acetonitrile and 100 mM ammonium phosphate (monobasic) at pH 3.5. Detector: UV spectrophotometer, 200 nm, 0.1 AUFS.

potentially intefering compounds eluted with the solvent from (unlabeled peaks at the beginning of each chromatogram).

Table I summarizes the results of the acidity and performance testing of the columns. The columns are ranked in order of relative retention time of DPH to MPPH ($t_{R\ DPH}/t_{R\ MPPH}$), which corresponds to column acidity. The columns with base deactivation or exhaustive end-capping of silanol groups placed near the top of the list, and those

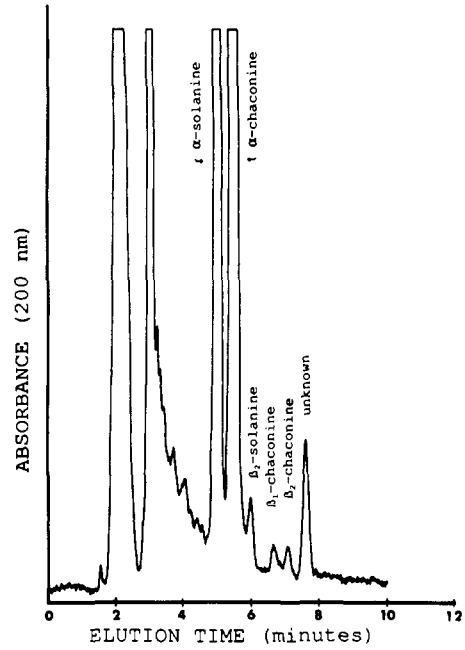

Figure 4. Chromatogram of a purified extract containing 2 mg of potato root solids from a high-glycoalkaloid potato variety (NDA 1725). The sample contains 12 μg of α-solanine, 13 μg of α-chaconine, 0.4 μg of β_2-solanine, 0.1 μg of β_1-chaconine, and 0.2 μg of β_2-chaconine. Column: Resolve C18, 5 μm, 3.9 × 300 mm, lot T12031. Eluent: 35% acetonitrile and 100 mM ammonium phosphate (monobasic) at pH 3.5. Detector: UV spectrophotometer, 200 nm, 0.1 AUFS.

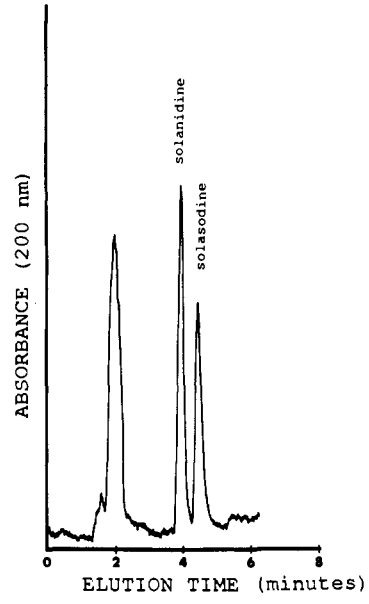

Figure 5. Chromatogram of 0.5 μg each of solanidine and solasodine. Column: Supelcosil C18-DB, 3 μm, 4.6 × 150 mm. Eluent: 60% acetonitrile and 10 mM ammonium phosphate (monobasic) at pH 2.5. Detector: UV spectrophotometer, 200 nm, 0.1 AUFS.

without adequate or any end-capping positioned nearer the bottom. Peak shape for DPH, although also influenced by overall column efficiency, was also an indicator of column acidity.

The ranking of the columns corresponds roughly to the resolution of the system, indicating an association between acidity and resolution. The Pearson correlation coefficient (Denenberg, 1976) for the relationship between RRT_{DPH} and resolution of the β-chaconine pair was calculated to be 0.716, which is significant at the 0.6% level. The coefficient for the relationship between RRT_{DPH} and the

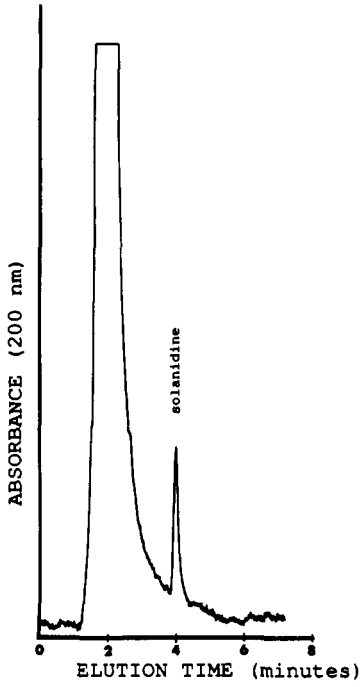

Figure 6. Chromatogram of 0.4 μg of potato tuber solids spiked with 0.2 μg of solanidine. The extracted sample was not subjected to solid-phase extraction. Column: Supelcosil C18-DB, 3 μm, 4.6 × 150 mm. Eluent: 60% acetonitrile and 10 mM ammonium phosphate (monobasic) at pH 2.5. Detector: UV spectrophotometer, 200 nm, 0.1 AUFS.

γ-solanine and γ-chaconine pair was calculated to be 0.583, which is significant at the 3.7% level. This means there are only 0.6 and 3.7% chances, respectively, that correlations this high could have occurred randomly. Comparison of these coefficients suggests that the resolution of the β_1- and β_2-chaconine pair is more dependent on column acidity than is resolution of the γ-solanine and γ-chaconine pair. This may be due to the fact that the more polar β-chaconines interact more with the silica than the γ-glycoalkaloids.

As expected, the efficiency of the column, represented by N_{MPPH}, strongly influenced band resolution by improving peak shape. Calculated Pearson correlation coefficients for the relationship between N_{MPPH} and the resolution of our two pairs are 0.539 for the β-chaconine pair, significant at the 5.8% level, and 0.593 for the γ-chaconine and solanine pair, significant at the 3.3% level.

$N_{\gamma\text{-chaconine}}$ values parallel N_{MPPH} values more closely than N_{DPH} values, indicating that column efficiency is more important than column acidity for achieving a good peak shape for γ-chaconine. Thus, the unique selectivity of acidic columns may be utilized without compromising good peak shape. In contrast, $N_{solanidine}$ parallels N_{DPH} more closely than N_{MPPH}. Column acidity affects peak shape for solanidine negatively; therefore, a nonacidic column is preferable for this compound.

The performance of the Pecosphere 3CR column is surprising since it is the exception to many of the above observations. It was the only column to produce very poor peak shapes for γ-chaconine relative to MPPH, as well as poor glycoalkaloid resolution, despite a relatively high acidic ranking. The capacity factor for γ-chaconine was low relative to the capacity factors for DPH and solanidine. The glycoalkaloids appear to be interacting with the silanols less effectively than either DPH or solanidine. Because both DPH and solanidine are smaller in size than the glycoalkaloids, we suspect steric hindrance may be limiting access to the silanols. Since β-chaconines are especially dependent on silanols for good separation, the postulated steric effect would explain the poorer than expected resolution of the two isomeric chaconines relative to the column's acidity ranking. The manufacturer has discontinued production of this packing due to batch-to-batch irreproducibility and plans to release an improved version (private communication).

Because N_{MPPH} and RRT_{DPH} both significantly affect component resolution, Pearson correlation coefficients were calculated between the product of N_{MPPH} and RRT_{DPH} and the resolution of the two pairs. The value for $Rs_{\beta 1\text{-},\beta 2\text{-chaconine}}$ vs the product $N_{MPPH} \times RRT_{DPH}$ was calculated to be 0.803, significant at the 0.1% level. The value for $Rs_{\gamma\text{-solanine},\gamma\text{-chaconine}}$ vs the product $N_{MPPH} \times RRT_{DPH}$ was calculated to be 0.779, significant at the 0.2% level. These are better correlations than the correlations to either N_{MPPH} or RRT_{DPH} alone. Table II ranks the columns in order of increasing $N_{MPPH} \times RRT_{DPH}$ values. The resolution values fall into ascending order, even more so than when RRT_{DPH} values are ranked (Table I). Thus, attention to both column efficiency and acidity is important when selecting a column for optimal resolution.

DISCUSSION

HPLC separation of the potato glycoalkaloids α-chaconine and α-solanine and the corresponding hydrolysis products (metabolites) β- and γ-chaconines and solanines has proven difficult. Previous studies (Bushway, 1982; Kozukue and Mizuno, 1986), while achieving good separations of the glycoalkaloids, have had drawbacks such as the use of tetrahydrofuran at low UV detection or the use of the less efficient and short-lived polar bonded-phase columns. In trying to reproduce these results, we ran into problems with unstable baselines, high background absorbance, and poor peak shape. The tetrahydrofuran had to be double-distilled and degassed to minimize these problems, but they were still not eliminated. Tetrahydrofuran can be difficult to work with at low UV detection because of its relatively high UV cutoff of 212 nm and because it frequently contains impurities. The peroxides which form over time in the ultrapure reagent also intefere in low UV detection. The HPLC grade, unstabilized, tetrahydrofuran we purchased commercially was extremely high in peroxides. In addition to the above considerations, formation of peroxides may present a safety hazard to the investigator.

The reversed-phase method described by Carman et al. (1986) avoided these problems by using acetonitrile and water on a C8 column. These authors separated α-chaconine and α-solanine but not the hydrolysis products. Nonetheless, their method has the following advantages: low UV background absorbance, improved solvent composition, and more stable and efficient columns. We set out to improve upon this method so as to include the β- and γ-metabolites and the aglycons in the chromatographic separation.

In trying to repeat the reversed-phase chromatography, we noticed marked differences in the separations depending on which manufacturer's columns we used, all of them C18 or C8. We suspected that the silanol activity of the packing was strongly influencing the chromatography. Silanols, the underivatized polar surfaces of silica, are implicated in causing broad and tailing peaks and excessive retention when basic compounds such as alkaloids are chromatographed. To better understand the role of silanols in the chromatography of our polar and basic samples, we assembled a number of reversed-phase columns and ranked them according to silanol activity or

Table I. Chromatographic Parameters of Columns

column, lot no.	DPH RRT[a]	DPH k'[b]	DPH N[c]	solanidine k'	solanidine N	MPPH N	γ-C[d] k'	γ-C N	Rs[e] β-C[f]	Rs γ[g]
Ultrasphere, 9UE3457	0.5	2.2	4700	0.7	6800	14 300	1.0	7700	0	1.0
Supelcosil, DB 112623AA	0.7	4.1	2200	1.1	5000	13 800	1.3	7600	0	0.9
Ultrasphere, 5UE946N	1.0	6.2	3900	2.5	4800	11 600	2.6	6300	0.3	1.0
Pecosphere 1, M938	1.4	5.3	3100	4.4	4100	9 400	4.0	6200	1.1	0.2
Pecosphere 2, M938	1.5	6.8	2900	4.1	4200	8 400	3.9	4600	0.9	0.3
Pecosphere 1+2	1.5	6.2	7700	3.4	5300	20 700	3.8	7300	1.2	0.7
Pecosphere 1+2+3	1.8	7.3	1100	5.2	700	32 000	3.8	15500	1.5	1.3
Pecosphere 3CR, unknown	2.0	13.0	300	7.5	400	9 500	2.6	2700	0.1	0.7
Pecosphere 3, M989	2.2	15.1	300	7.9	400	11 800	4.7	5100	1.0	1.1
Resolve, T11581	2.3	15.2	2400	5.6	2500	17 100	4.0	8000	1.2	1.3
Pecosphere 3+4	2.9	14.0	300	8.9	400	21 600	3.7	11700	1.5	1.7
Resolve, T12031	3.0	17.3	1400	9.0	1600	20 000	4.5	8400	1.4	1.7
Pecosphere 4, M989	3.1	21.2	200	10.1	200	11 000	4.4	5300	1.2	1.2

[a] Relative retention time (RRT) of diphenhydramine (DPH) to 5-(*p*-methylphenyl)-5-phenylhydantoin (MPPH). [b] k', capacity factor. See text for definition. [c] N, number of theoretical plates. See text for definition. [d] C, chaconine. [e] Rs, resolution. See text for definition. [f] β_1- and β_2-chaconine pair. [g] γ-Chaconine and γ-solanine pair.

Table II. Values for the Product of the Retention Time (RRT) for DPH and the Number of Theoretical Plates (N) for MPPH and Resolutions of the β_1- and β_2-Chaconine Pair (Rs β-C) and the γ-Chaconine and γ-Solanine Pair (Rs γ)

column, lot no.	$RRT_{DPH} \times N_{MPPH}$	Rs β-C	Rs γ
Ultrasphere, 9UE3457	7 150	0	1.0
Supelcosil, DB 112623AA	9 660	0	0.9
Ultrasphere, 5UE946N	11 600	0.3	1.0
Pecosphere 2, M938	12 600	0.9	0.3
Pecosphere 1, M938	13 160	1.1	0.2
Pecosphere 3CR, unknown	19 000	0.1	0.7
Pecosphere 3, M989	25 960	1.0	1.1
Pecosphere 1+2	31 050	1.2	0.7
Pecosphere 4, M989	34 100	1.2	1.2
Resolve, T11581	39 330	1.2	1.3
Pecosphere 1+2+3	57 600	1.5	1.3
Resolve, T12031	60 000	1.4	1.7
Pecosphere 3+4	62 640	1.5	1.7

relative acidity as described by Dalrup and Kardel (1984) and Stadalius (1988).

Dalrup and Kardel's procedure for determining the relative acidity of reversed-phase columns is based on the retention time of DPH relative to that of MPPH. Comparison of the columns' relative retention times and the peak shapes of DPH is the basis for our ranking of the columns for overall acidity.

Although many column manufacturers recommend using fully end-capped and/or base-deactivated packings for reversed-phase chromatography of basic compounds, we found a negative correlation between those columns and resolution of the potato glycoalkaloids. Table I shows that the resolution of glycoalkaloids increases and peak shape is not negatively affected by increasing column acidity. The special selectivity acidic columns offer may provide a good chromatogram from an otherwise unresolvable sample.

These studies facilitated choosing chromatographic conditions for the separation of potato glycoalkaloids and aglycons and developed a rational approach of column selection based on physicochemical properties of column packings. The acidity concept may be used to make a rational choice of column selection for specific needs.

CONCLUSIONS

The concepts and HPLC conditions developed in this study for the analysis of complex mixtures of *Solanum* alkaloids are an improvement over previous methods in terms of sensitivity, simplicity, and efficiency. Separation of the α-, β-, and γ-glycoalkaloids is excellent. The chromatograms have good baselines, show no interferences, and are reproducible. Although one of our objectives was to develop a single assay for both glycoalkaloids and aglycons, we found that column and eluent requirements prevented realizing this objective. The glycoalkaloids chromatographed best on a very acidic column, such as one chosen from the bottom of Table I, whereas the aglycons required both a nonacidic column, such as one from the top of Table I, and a less polar eluent. The described methods may also find application in the analysis of related natural and synthetic compounds with basic properties. Studies of their applicability to biosynthetic intermediates in plants and to the distribution of the glycoalkaloids in animal tissues and fluids are in progress.

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LITERATURE CITED

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Registry No. Resolve C18, 92879-65-7; Ultrasphere C18, 94699-49-7; Pecosphere C18, 142368-61-4; Supelcosil C18, 82197-84-0; α-chaconine, 20562-03-2; β_1-chaconine, 472-51-5; β_2-chaconine, 469-14-7; γ-chaconine, 511-36-4; solanidine, 80-78-4; γ-solanine, 511-37-5; β_2-solanine, 61877-94-9; β_1-solanine, 142287-76-1; α-solanine, 20562-02-1; solasonine, 19121-58-5; solasodine, 126-17-0.