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RAPID QUANTITATIVE ANALYSIS OF SOLASODINE, SOLASODINE GLYCOSIDES AND SOLASODIENE BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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SUMMARY

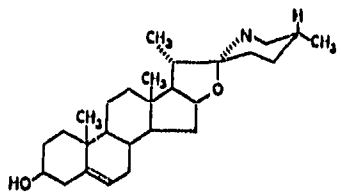
Separation and analysis of solasodine-related species (solasodine, solasonine, solamargine and solasodiene) has been achieved using high-pressure liquid chromatography with low-wavelength UV detection. The chromatographic systems have been applied in kinetic studies of acid hydrolysis of the glycosides and of dehydration of solasodine to the diene, and in examination of methanol leaf extract samples. Errors in sample preparation and quantitative analysis range from 2 to 5%.

INTRODUCTION

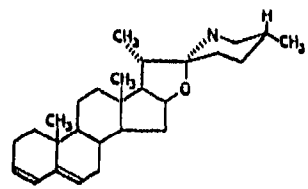
Solasodine is a potential raw material for steroid drug manufacture. It occurs in species of *Solanum* plants as the triglycosides solasonine and solamargine. For our studies related to the production of solasodine from the leaves of *S. aviculare* and *S. laciniatum*, we required a rapid, accurate analytical technique for determination of solasodine-related compounds. In particular, for work on acid hydrolysis of the glycosides and on drying and storage of raw plant material, it was necessary to separate and determine quantitatively solasonine, solamargine, the partial glycosidic forms (di- and mono-glycosides), solasodine and solasodiene. Structures of these compounds are shown in Fig. 1.

Previous studies of solasodine production have relied on chemical analysis techniques, colorimetric, titrimetric and gravimetric[1–9]. These methods are best suited to the determination of total steroid base; gravimetry gives total precipitated base, titrimetric methods give total titrated base, while colorimetry relies on some characteristic of the steroid such as Δ^5 unsaturation or a basic nitrogen. If further separation into aglycone and the various glycosides is required, then a chromatographic separation, either by paper or thin-layer chromatography, is needed before the chemical analysis[10,11]. This leads to considerable increase in the errors involved and in the analysis time. Although published methods rarely quote an observed error, we estimate that titrimetric and colorimetric analysis of plant material involve a standard error of 5–10%; incorporation of chromatography probably increases this to 10–20%, so that results are often presented as semiquantitative.

Aglycones: *Solasodine:*



Solasodiene



Natural triglycosides: *α-Solasonine*

D-Glucose (1β→3)
L-Rhamnose (1α→2) — D-Galactose (1β→3) — Solasodine

α-Solamargine

L-Rhamnose (1α→4)
L-Rhamnose (1α→2) — D-Glucose (1β→3) — Solasodine

Diglycosides: *β_1-Solasonine*

L-Rhamnose (1α→2) — D-Galactose (1β→3) — Solasodine

β_1-Solamargine

L-Rhamnose (1α→2) — D-Glucose (1β→3) — Solasodine

β_2-Solasonine

D-Glucose (1β→3) — D-Galactose (1β→3) — Solasodine

β_2-Solamargine

L-Rhamnose (1α→4) — D-Glucose (1β→3) — Solasodine

Monoglycosides: *γ-Solasonine*

D-Galactose (1β→3) — Solasodine

γ-Solamargine

D-Glucose (1β→3) — Solasodine

Fig. 1. Chemical structures of solasodine-related species.

More recently, gas chromatography has been considered for analysis of steroidal alkaloids. These compounds have low volatility and are thermally unstable. Operation must be in all-glass systems at temperatures near the limit for packing materials. Only the aglycone forms can be analysed directly; glycosides and sugars require permethylation or silylation. The following methods have been published: (a) aglycones: VandenHeuvel *et al.*[12], Weston[13], Osman and Sinden[14]; (b) derivatized glycoalkaloids; Herb *et al.*[15], Roosen-Runge and Schneider[16] and (c) glycoalkaloids indirectly after hydrolysis: Osman and Sinden[14], Siegfried[17]. Only Herb *et al.*[15] indicate the errors and problems involved; at temperatures to 350°, column life was only 100 samples; the average error in analysis of known samples was 7% and in analysis of plant samples the chromatographic procedure gave total glycoalkaloid contents ranging from 35 to 238% of that obtained by a titrimetric method.

It would appear that high-pressure liquid chromatography (HPLC) should overcome the volatility and stability problems of gas chromatography. Liquid chromatography has not previously been applied to the separation of plant steroidal alkaloids, apart from its use for preparatory separations by Hunter *et al.*[18]. Since detection of steroidal alkaloids by UV spectrophotometry requires operation at 210 nm or less, the range of solvents which can be used is limited and the use of solvent gradient programming is unlikely to be appropriate.

PRELIMINARY CONSIDERATIONS

Choice of separation mode

The relevant species are listed and their chromatographic features are summarized in Table I.

Ion-exchange chromatography cannot be used: the pK_b values which are available show very small differences between compounds. The partial glycosides

TABLE I

CHROMATOGRAPHIC CHARACTERISTICS OF SOLASODINE SPECIES

Species	*MW*	*pK*$_b$*	*Sugars present***	*Number of —OH groups*		*Glycosidic Linkages*		*R*$_s$§
				Axial	*Equatorial*	*Number*	*Types****	
Solasodiene	395	6.20	—	0	0	0	—	—
Solasodine	413	6.30	—	0	1	0	—	15.0
γ-Solasonine	575		D-Ga	1	3	1	eq–eq	10.7
γ-Solamargine	575		D-Gu	0	4	1	eq–eq	10.0
β_2-Solasonine	737		D-Ga,L-Rh	2	4	2	eq–eq;eq–ax	5.7
β_1-Solamargine	721		D-Gu,L-Rh	1	5	2	eq–eq;eq–ax	5.0
β_2-Solamargine	721		D-Gu,L-Rh	1	5	2	eq–eq;eq–ax	5.0
β_1-Solasonine	721		D-Ga,D-Gu	1	6	2	eq–eq;eq–eq	2.5
α-Solamargine	867	6.30	D-Gu,L-Rh,L-Rh	2	6	3	eq–eq;eq–ax;eq–ax	2.3
α-Solasonine	883	6.26	D-Gu,L-Rh,D-Gu	2	7	3	eq–eq;eq–eq;eq–ax	1.3

* Average values taken from Bloom and Briggs[19].

** Ga = galactose, Rh = rhamnose, Gu = glucose.

*** ax = axial, eq = equatorial.

§ Retention times, relative to solanine with R_s = 1.0, from Briggs *et al.*[10,11].

would be expected to have similar pK_b values. Gel permeation could distinguish between aglycones, mono-, di- and triglycosides, but otherwise the small differences between the molecular weights suggests that further separation of individual species would not be possible.

Bonded-phase affinity chromatography was chosen for the separation. The features shown in Table I which are relevant to affinity chromatography do not give such a definite indication of separability as those discussed above. However, they can be used to predict an order of elution. Solasodine is relatively non-polar, is virtually insoluble in water but soluble in alcohols and non-polar solvents such as chloroform and benzene. Solasodiene is less polar, but, because of the presence of sugars in the glycosides, these compounds have a much greater polarity, which increases as more sugars are attached. Axial hydroxyl groups in the sugars D-galactose and L-rhamnose tend to extend above the plane of the molecule and have more effect on polarity than equatorial groups. Relative retention data in paper chromatography[10,11] are also given in Table I, indicating an order of polarities that can be expected to apply, possibly with some minor changes, in other liquid chromatography systems. Because of the low polarity of the molecules to be separated, reversed-phase chromatography was chosen. Examination of reported separations of pharmaceutical steroids and of saccharides[20,21] suggests two possible systems: Waters Assoc. (Milford, Mass., U.S.A.) μBondapak C_{18} packing or equivalent (with methanol–water or acetonitrile–water as the mobile phase) and a packing of intermediate polarity such as that used in the Waters Assoc. Carbohydrate column.

Detection

Plant steroids like solasodine lack chromophoric groups in the common operating range of UV spectrophotometry and absorb only at the low-wavelength end of the UV spectrum. Table II shows experimentally determined UV absorption characteristics for the species of interest. Comparison of the results for solasodiene with the data of Dorfman[22] (Table III) shows good agreement; however, values of λ_{max} and ε_{max} for solasodine differ from Dorfman's values for a Δ^5 double bond, suggesting absorbance by a different chromophore. The absorption spectra were determined in the microcell of the UV detector after separation from other species in the HPLC system, so it is unlikely that the differences are caused by impurities.

Detection of solasodiene is not a problem —good sensitivity can beachieved at 235–240 nm. However, wavelengths less than 210 nm are required for solasodine

TABLE II

EXPERIMENTALLY DETERMINED UV SPECTRA OF SOLASODINE-RELATED SPECIES

Determined in 92% (v/v) methanol, 8% (v/v) water.

Species	λ_{max} *(nm)*	ε_{max} *(relative values)*
Solasodine, aglycone and glycosides	<200*	>0.42*
Solasodiene	228.5	0.98
	234.5	1.00
	241–242**	0.68

* Precise measurements could be made only above 200 nm in aqueous methanol solvents.

** Point of inflection.

TABLE III

UV ABSORPTION OF CHROMOPHORIC GROUPS IN STEROIDS (REF. 22)

Chromophore	λ_{max} *(nm)*	ε_{max} *(relative values in parentheses)*	*Solvent*
Δ^5 Double bond	204	3300 (0.17)	Ethanol
Conjugated $\Delta^{3,5}$ double bond	228	18600 (0.98)	
	235	19000 (1.00)	Isopropanol
	243*	13600 (0.72)	

* Point of inflection.

and its glycosides. Choice of solvents is therefore restricted to high-purity water, acetonitrile, tetrahydrofuran, low-molecular-weight alcohols and hydrocarbons. With these solvents, stable baselines can be obtained using isocratic systems, but the absorbance of the solvents and the low sensitivity of solasodine species makes gradient elution impracticable; the baseline change is so large compared with peak sizes that peak shape and integration are affected. Other problems (non-linear baselines, spurious peaks, inadequate mixing, solvent demixing) which arise with gradient programming are also exaggerated under these conditions.

EQUIPMENT AND REFERENCE COMPOUNDS

The equipment used consisted of Waters Assoc. 6000A solvent delivery system, Waters Assoc. μBondapak C_{18} and Carbohydrate columns (30 cm × 3.9 mm I.D.), Spectra-Physics Valco sample injector, Perkin-Elmer LC55 variable-wavelength UV–VIS detector and Hewlett-Packard 3380S integrator–recorder.

Purified samples of α-solasonine, α-solamargine, solasodine, solasodiene and mixed glycosides were available for development of separation. In addition, mixtures were prepared by partial acid hydrolysis of solasonine and of the mixed glycosides. Pure samples of the β- and γ-glycosides of either solasonine or solamargine were not available and identification of these peaks was made indirectly. Two samples of the acid hydrolysis products were prepared, one early in the hydrolysis and one late in the hydrolysis. Because the diglycoside is formed by the cleavage of only one bond and the monoglycoside by the cleavage of two bonds, the early sample contains more β-form than γ-form and the late sample more γ-form. In addition, the order of retention times is expected to follow the number of sugars present in the glycosidic portion of the molecule (see Table I).

ACHIEVED SEPARATIONS

Because the species are only weakly basic the ionic form is easily produced in acidic media. To achieve reproducible separations it was found necessary to maintain the basic form. This was accomplished by buffering the aqueous portion of the mobile phase to pH 7–7.5 using a 0.01 *M* Tris buffer. Higher pH's should not be used due to increased solubility of silica (the bonded-phase support) in the mobile phase leading to rapid column deterioration. When the mobile phase was buffered, trailing of the recorded peaks was greatly reduced, the separation between

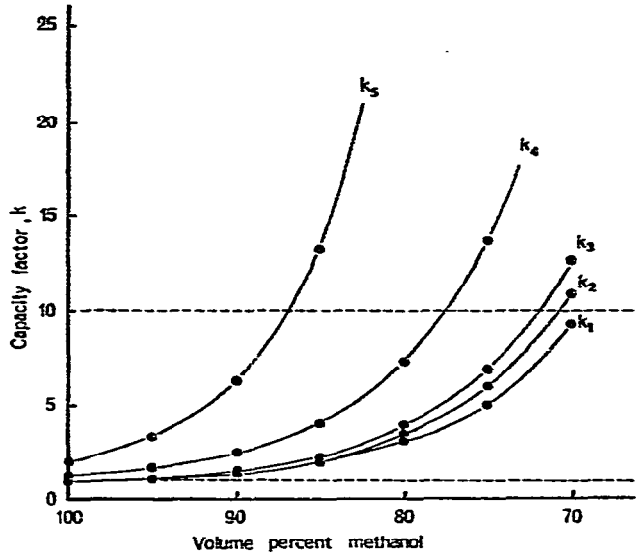


Fig. 2. Effect of solvent composition on capacity factor, k, of solasodine-related species, for μBondapak C_{18} column with methanol–aqueous 0.01 M Tris buffer solvents (2 ml/min, 25°). k_1 = Triglycosides, k_2 = diglycosides, k_3 = monoglycosides, k_4 = solasodine, k_5 = solasodiene.

the peaks improved and peaks were eluted earlier for a given solvent composition. In the following discussion "water" refers to this buffered solution.

Figs. 2, 3 and 4 show the results for the three systems studied. In each case the effect of solvent composition on the capacity factor for the separated peaks is

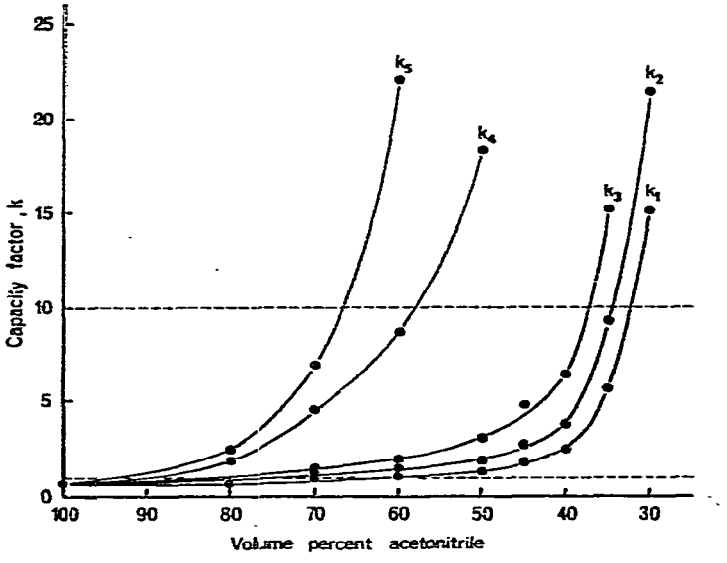


Fig. 3. Effect of solvent composition on capacity factor, k, of solasodine-related species for μBondapak C_{18} column with acetonitrile–aqueous 0.01 M Tris buffer solvents (2 ml/min, 25°. k_1 = Triglycosides, k_2 = diglycosides, k_3 = monoglycosides, k_4 = solasodine, k_5 = solasodiene.

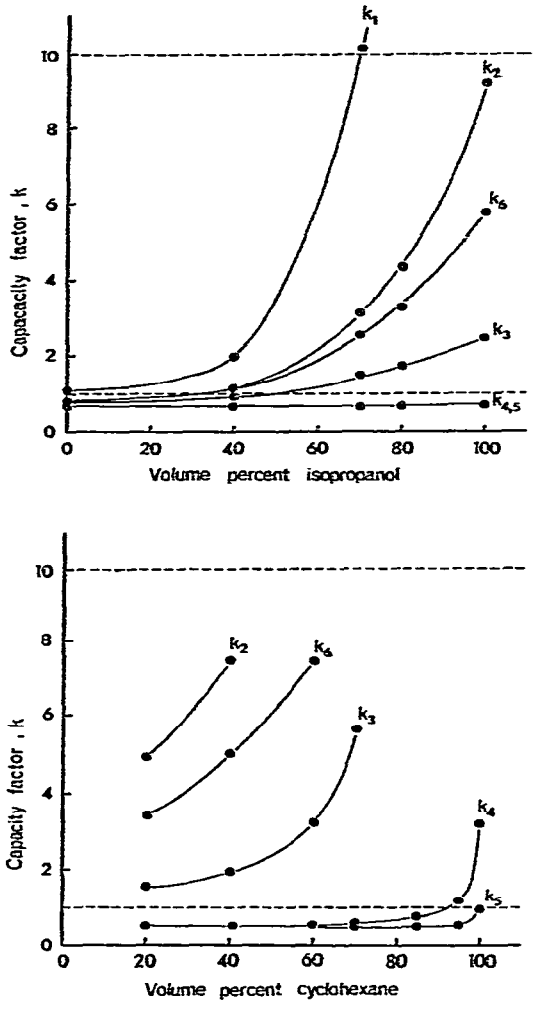
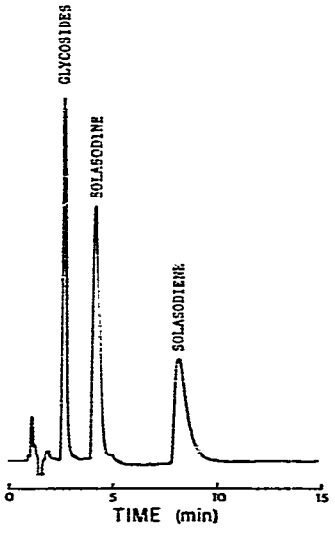


Fig. 4. Effect of solvent composition on capacity factor, k, of solasodine-related species for Carbohydrate column. (a) Methanol–isopropanol solvents (2 ml/min, 40°); (b) isopropanol–cyclohexane solvents (2 ml/min, 60°). k_1 = α-solasonine, k_2 = β-solasonine, k_3 = γ-solasonine, k_4 = solasodine, k_5 = solasodiene, k_6 = α-solamargine.

shown. The horizontal lines represent $k = 1$ and $k = 10$. For a practical separation the capacity factors of the separated peaks should be within these limits. Each of these systems has advantages and disadvantages and its suitability depends on the required application.

Using the first system (85–90% (v/v) methanol in water; mobile phase with μBondapak C_{18} columns), a sample may be easily separated into three peaks corresponding to solasodine glycosides, solasodine aglycone and solasodiene (see Fig. 5). With a higher water content in the solvent (75% (v/v) methanol; see Fig. 6) the glycoside peak can be separated into mono-, di- and triglycosidic species, with no further distinction possible between those glycosides derived from solasonine and

those derived from solamargine. Although the specified theoretical plate count of the column is high (minimum of 10,000 plates/m for an acenaphthene sample with acetonitrile–water mobile phase) the theoretical plate count for the peaks of solasodine-related species using methanol–water as mobile phase was found to be much lower (approximately 2000 plates/m).



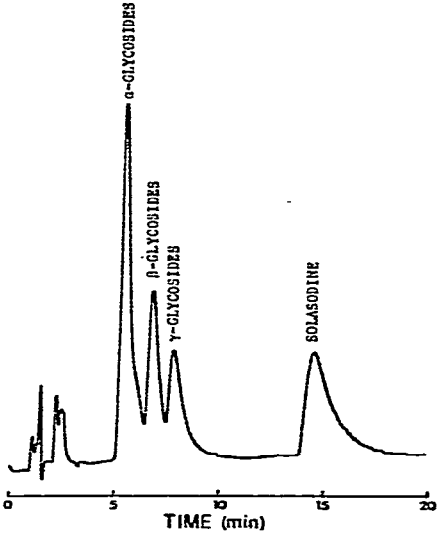


Fig. 5. Separation of glycosides, solasodine and solasodiene on μBondapak C_{18} using aqueous methanol solvent. Column: 30 cm × 3.9 mm I.D.; solvent: methanol–aqueous 0.01 *M* Tris buffer (88:12); flow-rate: 2 ml/min; temperature: 25°; detection: UV, 205 nm.

Fig. 6. Separation of solasonine glycosides and solasodine on μBondapak C_{18} using aqueous methanol solvents. Column: 30 cm × 3.9 mm I.D.; solvent: methanol–aqueous 0.01 *M* Tris buffer (75:25); flow-rate: 2 ml/min; temperature: 25°; detection: UV, 205 nm.

Both the capacity factor and the number of theoretical plates were found to be changed markedly by a change in either column temperature or mobile phase flow-rate. An increase in column temperature from 20 to 90° caused a decrease in k by almost 75% and increased N by 300% (both based on the α-solasonine peak) but made only minor changes to selectivity between peaks. Increased temperature also lowers column back-pressure by lowering mobile phase viscosity; for a 20 to 90° increase the back-pressure decreased by about 60%. However, operation at increased column temperatures has two major disadvantages: acceleration of the reaction of column contaminants with the column packing and increased solubility of the silica packing in aqueous solution. Both act to reduce the life of the column. An increase of flow-rate from 1 to 10 ml/min at 80° caused the number of theoretical plates to decrease by 70% but left retention volumes and selectivity unchanged.

It is interesting to compare the results obtained when acetonitrile–water replaced the methanol–water mobile phase. Solvent composition has a much greater effect on the capacity factor (Fig. 3) than was the case with methanol–water mixtures (Fig. 2). The values of k increase more quickly as the solvent composition is changed. This makes it impossible to obtain separation of solasodine, tri-, di- and monoglyco-

sides and the aglycone on the same chromatogram if k is to be kept between 1 and 10. Selectivity between peaks is greater for the acetonitrile–water case and, combined with a higher number of theoretical plates (400 plates/m), leads to improved resolution between peaks. Chromatograms are included for the separation of solasodine and solasodiene (Fig. 7; 70% (v/v) acetonitrile) and of the glycosides (Fig. 8; 40% (v/v) acetonitrile). Marginal separation between α-solasonine and α-solamargine can be obtained at higher water contents (30% (v/v) acetonitrile).

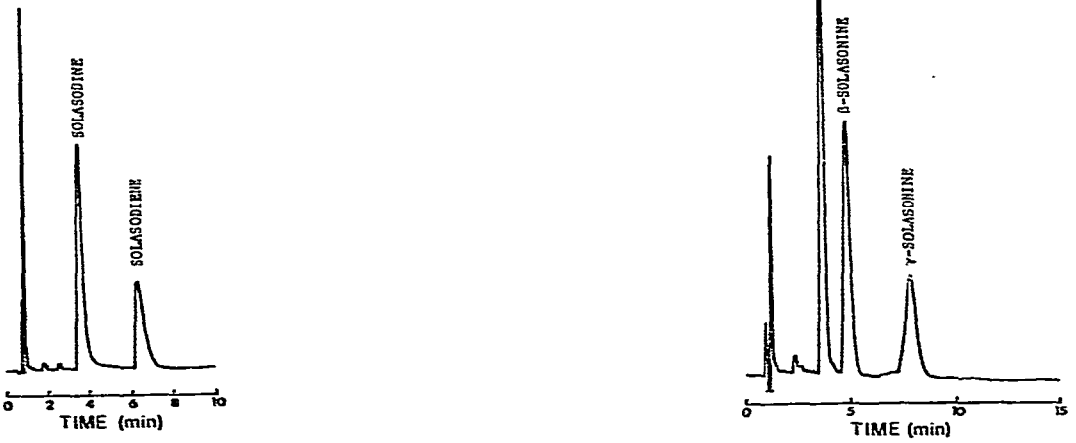


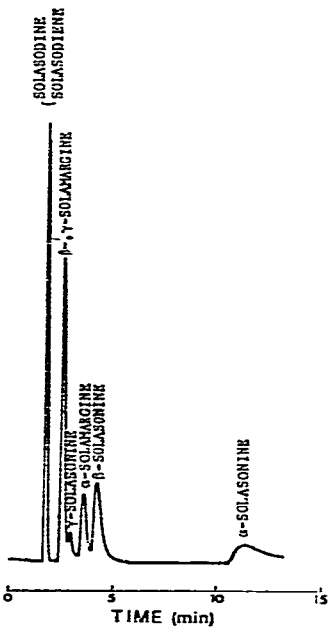
Fig. 7. Separation of solasodine and solasodiene on μBondapak C_{18} using aqueous acetonitrile solvent. Column: 30 cm × 3.9 mm I.D.; solvent: acetonitrile–aqueous 0.01 *M* Tris buffer (70:30); flow-rate: 4 ml/min; temperature: 25°; detection: UV, 205 nm.

Fig. 8. Separation of solasonine glycosides on μBondapak C_{18} using aqueous acetonitrile solvent. Column: 30 cm × 3.9 mm I.D.; solvent: acetonitrile–aqueous 0.01 *M* Tris buffer (40:60); flow-rate: 2 ml/min; temperature: 25°; detection: UV, 205 nm.

The μBondapak C_{18} packing gives limited separation between the solasodine glycosides. Only differences of a whole sugar unit can be resolved, the exception being slight separation between α-solasonine and α-solamargine in some cases. Since the μBondapak C_{18} packing is very non-polar it will attract preferentially the non-polar end of the solasodine species. The differences between the solasodine species are all at the polar end of the molecule; such differences would be expected to affect only slightly the adsorption characteristics of the non-polar end.

The Waters Carbohydrate analysis column is expected to show some advantage in separating glycosides, since it contains a more polar packing. The polar end of the molecule will be preferentially adsorbed, making the adsorption characteristics much more sensitive to changes in the glycosidic sugars. The observed variations of capacity factor with solvent composition for methanol–isopropanol and isopropanol–

cyclohexane mixtures are shown in Fig. 4. The glycosidic species from solasonine are easily separated as are α-solasonine and α-solamargine (Fig. 9) but solasodine and solasodiene are unretained, except when extreme solvent concentrations (greater than 90% (v/v) cyclohexane) are used. At such solvent compositions the peaks show a pronounced trailing edge (Fig. 10), showing that the column is being operated beyond its solvent polarity limits.



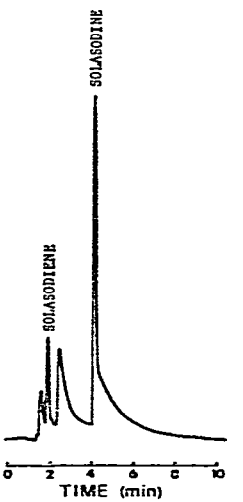


Fig. 9. Separation of solasodine glycosides on μBondapak C_{18} Carbohydrate using methanol–isopropanol solvents. Column: 30 cm × 3.9 mm I.D.; Solvent: isopropanol–methanol (70:30); flow-rate: 2 ml/min; temperature: 40°; detection: UV, 205 nm.

Fig. 10. Separation of solasodine and solasodiene on μBondapak C_{18} Carbohydrate using isopropanol–cyclohexane solvents. Column: 30 cm × 3.9 mm I.D.; solvent: cyclohexane; flow-rate: 2 ml/min; temperature: 60°; detection: UV, 205 nm.

The positions of β- and γ-forms of solamargine are not shown in Fig. 4; they appeared unresolved between k_3 and k_4 on the plots (see Fig. 9). It will be noted that the Carbohydrate column behaves as a normal-phase column, eluting the most polar compounds last (*cf.* the reversed-phase μBondapak C_{18} column which elutes the most polar compounds first). This was found to be important if neutralization salts were present in an acid-hydrolysed mixture of glycosides. With the Carbohydrate column these salts appeared on the chromatogram as a large undefined peak covering the α-solasonine peak, but with the μBondapak C_{18} column these salts were unretained and eluted before the glycoside peaks.

The number of theoretical plates for the solasodine species peaks on the Carbohydrate column was similar to that of the μBondapak C_{18} column even though the specified minimum plate count is only 2000 plates/m compared with 10,000 plates/m for the μBondapak C_{18} column. A disadvantage with the use of the

Carbohydrate column is that it is much more susceptible to irreversible contamination and has a much shorter life than the μBondapak C_{18} column.

In none of the systems studied was it possible to separate β_1- and β_2-glycosidic forms.

APPLICATIONS

The above separations have been used to study acid hydrolysis of the triglycosides, acid-induced conversion of solasodine to solasodiene and the levels of glycosides and aglycone in leaf extracts. Choice of analysis system and preparation of the samples for analysis will be briefly discussed to illustrate possible applications of the separations.

Acid hydrolysis of glycosides and acid-induced solasodiene formation

The substrate (pure samples of α-solasonine, α-solamargine or solasodine, 0.5–5.0 × 10^{-3} *M*) was reacted with acid (0.5–4 *N* HCl) in aqueous alcohol solvent and the reaction mixture neutralized with aqueous ammonia solution before evaporation of the solvent to yield a solid residue. For analysis this residue was dissolved in methanol for injection into the HPLC system. The major problem with the analysis for the reaction products in the residue is the presence of ammonium chloride. The greater the initial acid strength the larger the quantity of ammonium chloride. Because only small amounts of pure substrates were available, the reaction was carried out in small glass vials containing 1–2 ml of solution, at each sampling one vial being removed. Only small amounts of alkaloids (0.2–4.0 mg) are present, so separation from the neutralization salts (0.03–0.4 g) before analysis is difficult. The method developed to reduce the amount of ammonium chloride present involved dissolving the residue in a two-phase system of 0.1 *M* aqueous ammonia–butan-1-ol. The alkaloid species are quantitatively transferred (better than 99%) into the butan-1-ol phase while the neutralization salts are preferentially transferred into the aqueous phase. Because butan-1-ol has a low UV absorption at 205 nm, the butan-1-ol phase (the upper layer) may be injected directly into the HPLC system for analysis. The sample does not leave the reaction vial at any stage and consequently manipulative losses are avoided.

The analysis system chosen was the μBondapak C_{18} column with buffered aqueous methanol solvents. The Carbohydrate column could not be used because of the presence of the ammonium chloride. (Other possible neutralization salts gave a similar problem.) Aqueous methanol was chosen rather than aqueous acetonitrile because tri-, di- and monoglycosides and the aglycone could be analysed on the same chromatogram (compare Figs. 2 and 3). In addition, special HPLC acetonitrile is needed, whereas AR grade methanol was found to be satisfactory.

Leaf extracts

The alkaloids are extracted from the plant material using methanol. This extract solution will generally contain the triglycosides, α-solasonine and α-solamargine. Depending on the treatment of the plant material prior to extraction, partial glycosides and to a lesser extent the solasodine aglycone itself may be present. In addition there will be a large amount of co-extracted impurities which need to be

separated from the alkaloids before analysis. To purify the extract samples to protect the packing from contamination and improve the separation, Waters Seppak C_{18} cartridges were used. These contain Bondapak C_{18} packing and enabled both more polar and less polar impurities to be removed before analysis. The following procedure was found to be quantitative: the methanol extract (5–10 ml) was diluted with water to 45% (v/v) methanol, then passed through the cartridge. The retained alkaloids were then eluted with pure methanol (5–8 ml), the solvent removed and the residue made up to the required volume (1 or 2 ml) with pure methanol before injection into the HPLC system. (There appeared to be a batch to batch variation in the performance of the Seppak cartridges. It is suggested that adsorption characteristics of the cartridges be checked prior to use.)

For the analysis a μBondapak C_{18} column with aqueous methanol solvent was chosen as before. The μBondapak C_{18} column was used because the Seppak C_{18} cartridges are designed to protect this type of packing from contamination. Also the μBondapak C_{18} packing is less susceptible to irreversible contamination than the Carbohydrate packing. If distinction between solasonine and solamargine is required then it is necessary to use the Carbohydrate column. Aqueous methanol was preferred over aqueous acetonitrile for the same reasons as above.

QUANTITATION AND ERROR IN ANALYSIS

To make the results quantitative two types of calibration are possible: either absolute calibration of each peak by means of an external sample or relative calibration using normalization of the peaks with correction factors. The latter requires measurement of the detector response factor for each component. The detector response factor is the molar quantity of a component in the sample divided by the area of its peak. For n components each with response factor R and recorded area A:

$$\text{percentage component } j = \frac{100 A_j R_j}{\sum_{i=1}^{n} A_i R_i}$$

Response factors were obtained for the species α-solasonine, α-solamargine, solasodine and solasodiene. The values for R (relative to solasodine) are given in Table IV. Each factor includes an estimate of the standard error in brackets. Although the experimental error in these factors is large (because only small volumes (8 ml) of standard solutions could be prepared), there is no indication that, over the range 200–215 nm, solasodine, α-solamargine or α-solasonine have different absorbances on a molar basis. To verify this, the values of R for the sugars D-glucose, D-galactose and L-rhamnose were measured at 200 and 205 nm (see Table IV). If the absorbances of the sugar moieties of α-solasonine or α-solamargine are calculated by summation of individual absorbances then the results indicate negligible difference between aglycone and glycoside at 205 nm ($< 0.5\%$). This confirms that at 205 nm a relative response factor of 1.00 applies for α-solasonine, α-solamargine and solasodine. For β- and γ-glycosides, whose absorbance would be expected to lie between that of the relevant triglycoside and solasodine, a relative response factor of 1.00 may also be assumed. This conclusion was substantiated during the acid

TABLE IV

RESPONSE FACTORS (RELATIVE TO SOLASODINE) FOR SOLASODINE-RELATED SPECIES

Species	*Wavelength (nm)* *			
	200	*205*	*210*	*215*
Solasodine	1.00 (1.9)	1.00 (1.7)	1.00 (1.6)	1.00 (3.5)
α-Solasonine	1.01 (1.8)	1.00 (1.3)	1.00 (1.9)	1.02 (2.2)
α-Solamargine	1.00 (1.7)	0.98 (1.9)	0.99 (2.0)	1.02 (2.4)
Solasodiene	1.62 (3.3)	0.86 (2.3)	0.26 (2.9)	0.047 (2.3)
D-Glucose**	300	1120		
D-Galactose**	92	500		
L-Rhamnose**	110	420		

* Percentage error in each response factor is given in parentheses.
** AR grade chemicals.

hydrolysis experiments; throughout any one experimental run the total peak area of the alkaloid species remained constant.

Thus at 205 nm absolute calibration of the solasodine species may be achieved by the use of only two calibration curves: one for solasodine (which applies to all the glycosidic forms as well as the aglycone) and one for solasodiene.

When applying quantitative HPLC analysis to unknown samples, errors can arise from three different sources: (a) error in preparing the experimental samples for quantitative analysis; (b) error in obtaining reproducible results from the HPLC system; and (c) error in preparing standard solutions of pure compounds either for calculation of response factors or for calculation of calibration curves. An estimate of the magnitude of these errors in each of the applications discussed above is given in Table V. If it is assumed that the three sources of error are independent then an

TABLE V

ESTIMATED STANDARD PERCENTAGE ERRORS IN THE HPLC ANALYSIS OF UNKNOWN SAMPLES

Source of error	*Application*		
	Analysis of reaction products from glycoside hydrolysis	*Analysis of reaction products from solasodiene formation*	*Analysis of leaf extract samples*
Preparation of sample*	1.3–1.5	1.4	1.3–1.8
Calibration of results**	1.0–1.3	2.0–2.5	1.0–1.3
Analysis on HPLC***			
Absolute calibration	2.6–3.4	2.6–3.4	2.6–3.4
Relative calibration	0.6–0.9	0.6–0.9	0.6–0.9

* Based on reported errors in use of standard laboratory equipment; does not include manipulative losses.
** Very dependent on the volume and concentration of the standard solution prepared; based on ≈1 mg/ml in 8 ml solvent; assumes $R = 1$ with no error for applications which do not include analysis for solasodiene.
*** Obtained experimentally; refers to the error in the result from a single injection.

estimate of the overall error in the result from a single injection can be made by summing the relevant variances calculated from the standard errors in the table.

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REFERENCES

1 J. Birner, *J. Pharm. Sci.*, 58 (1969) 258.
2 J. E. Lancaster and J. D. Mann, *N.Z. J. Agr. Res.*, 18 (1975) 139.
3 R. Hardman and T. G. Williams, *J. Pharm. Pharmacol.*, 23 (1971) 231 S.
4 R. Hardman and T. G. Williams, *Planta Med.*, 29 (1976) 66.
5 V. V. Panina and T. B. Pimonova, *Khim.-Farm. Zh.*, 6 (1972) 46.
6 V. V. Panina, G. S. Glyzina, O. P. Avilova, A. K. Prashchurovich and L. M. Kogan, *Khim.-Farm. Zh.*, 10 (1976) 135.
7 L. Telek, *J. Pharm. Sci.*, 66 (1977) 699.
8 J. D. Mackenzie and P. Gregory, *Amer. Potato. J.*, 56 (1979) 27.
9 S. B. Mahato, B K Saikia, N P. Sahu and R. Chakravarti, *J. Inst. Chem., Calcutta*, 47 (1975) 237.
10 L. H. Briggs, R. C. Cambie and J. L. Hoare, *J. Chem. Soc.*, (1961) 4645.
11 L. H. Briggs, R. C. Cambie and J. L. Hoare, *J. Chem. Soc.*, (1963) 2848.
12 W. J. VandenHeuvel, E. C. Horning, Y. Sato and N. Ikekawa, *J. Org. Chem.*, 26 (1961) 628.
13 R. J. Weston, *J. Appl. Chem. Biotechnol.*, 26 (1976) 657.
14 S. F. Osman and S. L. Sinden, *J. Agr. Food Chem.*, 25 (1977) 955.
15 S. F. Herb, T. J. Fitzpatrick and S. F. Osman, *J. Agr. Food Chem.*, 23 (1975) 520.
16 C. Roosen-Runge and E. Schneider, *Z. Lebensm.-Unters.-Forsch.*, 164 (1977) 96.
17 R. Siegried, *Z. Lebensm.-Unters.-Forsch.*, 162 (1976) 253.
18 I. R. Hunter, M. K. Walden, J. R. Wagner and E. Heftmann, *J. Chromatogr.*, 119 (1976) 223.
19 H. Bloom and L. H. Briggs, *J. Chem. Soc.*, (1952) 3591.
20 Waters Associates Inc., *Publication N68*, 1976.
21 Waters Associates Inc., *Publications DS 049F, AH338*, 1974.
22 L. Dorfman, *Chem. Rev.*, 53 (1953) 47.