

## Separation of Potato Glycoalkaloids by Gas Chromatography

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Permethylated derivatives of potato glycoalkaloids have been separated by gas chromatography. These derivatives are shown to be readily prepared and chromatographed on the usual high-temperature liquid coatings OV-1 and Dexsil 300. Qualitative and quantitative results

are given for several varieties of fresh potatoes and after incubation of tuber slices at 20°. The procedure is reproducible and reliable and shows promise of development into a quantitative method.

Glycoalkaloids, a class of nitrogen-containing steroidal glycosides, are found in potato tissues. These compounds have been implicated during the last 75 years as being responsible for a number of maladies and, in some cases, death. However, very little is known about the individual glycoalkaloids from the standpoint of their concentration in potatoes or their relative toxicity.

In 1826,  $\alpha$ -solanine was the first glycoalkaloid to be reported as a natural constituent of potatoes (Baup, 1826) and it was considered to be the only one present until 1954 when  $\alpha$ -chaconine was found (Kuhn and Löw, 1954). These two are the predominant potato glycoalkaloids. More recently, other glycoalkaloids have been reported in potatoes as normally present, induced genetically, or caused by various conditions of stress.

Many procedures have been proposed for estimation of total glycoalkaloids but they were primarily developed for the analysis of  $\alpha$ -solanine and  $\alpha$ -chaconine and are of limited value for the analysis of other glycoalkaloids. An exception is the recent procedure (Fitzpatrick and Osman, 1974) which is based on the titration of the steroidal nitrogen present in all glycoalkaloids. Paper and thin-layer chromatographic procedures have been used for the separation of individual glycoalkaloids but these procedures are usually not satisfactory for quantitation.

The use of gas chromatography has not been reported probably because of the difficulty in chromatographing a compound of this high molecular weight and low volatility (solanine,  $C_{45}H_{73}NO_{15}$ , mol wt 868; demissine,  $C_{50}H_{83}NO_{21}$ , mol wt 1034). A method has been developed in this laboratory for the permethylation of glycoalkaloids to improve their volatility and their subsequent qualitative and quantitative analysis by a gas chromatographic procedure.

### EXPERIMENTAL SECTION

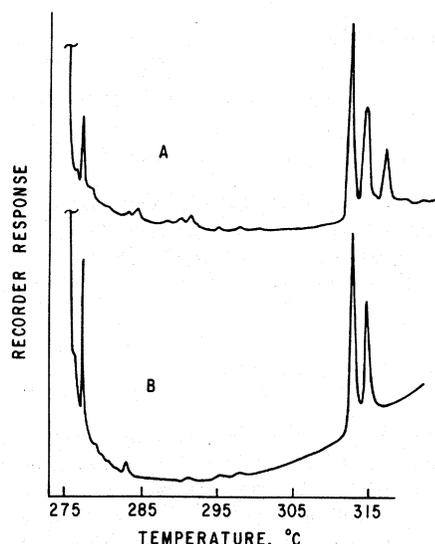
**Apparatus.** Separations were made employing a Varian Aerograph Model 2100 gas chromatograph equipped with a flame ionization detector. Two columns were utilized: (1) a 90 cm  $\times$  2 mm glass tubing packed with 3% Dexsil 300 on 100-120 mesh Supelcoport, and (2) a 120 cm  $\times$  2 mm glass tubing packed with 3% OV-1 on 100-120 mesh Gas-Chrom Q. Operating conditions were varied depending on the separation required.

**Reagents.** Sodium hydride (2.0 g) (50% oil dispersion), K&K Laboratories, Inc., Plainview, N.Y., was added to 30 ml of dimethyl sulfoxide ( $Me_2SO$ ), ACS certified, Fisher Scientific Co., Fairlawn, N.J., and stirred for 3 hr under a blanket of  $N_2$  and protection from moisture. Reagent can be stored for 2 months under proper storage conditions (protection from air and moisture, stored in cold). **Caution:** Proper care should be exercised in handling sodium hydride.

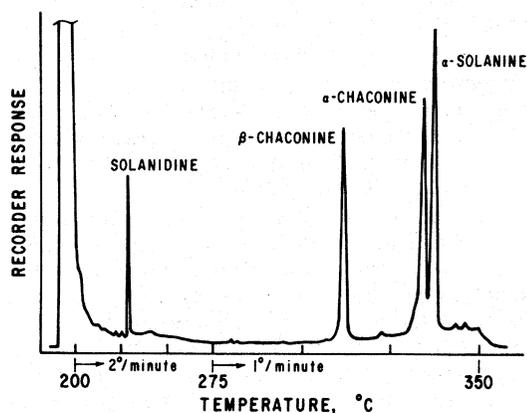
**Procedures. Extraction.** Glycoalkaloids were extracted from potatoes by a modification of the procedure reported by Wang et al. (1972). A representative 20-g portion of potato solids was extracted twice with 100 ml of methanol-chloroform (2:1) mixture while grinding for 5 min in a Waring Blender. The bisolvent layers were separated by adding 100 ml of 0.8% aqueous  $Na_2SO_4$  solution. The glycoalkaloids are found in the methanol layer along with free sugars, amino acids, browning reaction products, and  $Na_2SO_4$ . This volume of potato extract (ca. 200 ml) is measured to permit calculation to the original potato sample. Known aliquots are taken for determination of total glycoalkaloids (TGA) and estimation of the individual compounds by gas-liquid chromatography (GLC).

**Determination of TGA.** A 25-ml aliquot of the potato extract is hydrolyzed with 2 N  $H_2SO_4$ . The recovered aglycones are subjected to a nonaqueous titration of the nitrogen group, common to all glycoalkaloids, by the method of Fitzpatrick and Osman (1974).

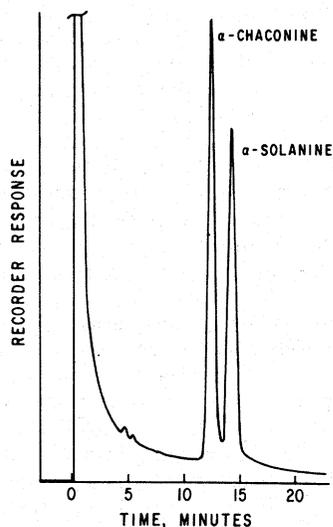
**Permethylation for GLC Separation.** The permethylation is a modification of the procedure of Hakomori (1964). The size of the aliquot of potato extract taken for permethylation is adjusted according to the amount of glycoalkaloids present. Typical aliquots are: 25 ml for 0 to 20 mg/100 g fresh weight, 10 ml for 20 to 40 mg/100 g fresh weight, and 5 ml for 40 to 60 mg/100 g fresh weight. Remove excess  $Na_2SO_4$  from the aliquot by taking to dryness in a stream of  $N_2$  or by removal of the methanol-water at reduced pressure in a rotary evaporator. Redissolve the residue which contains the glycoalkaloids in absolute methanol (the residue from a 25-ml aliquot would be redissolved in 20 ml of methanol). Filter off  $Na_2SO_4$  and transfer the filtrate to a 60 mm  $\times$  17 mm screw cap vial. The methanol and moisture are removed completely with a stream of  $N_2$ . When dry, add 1.5 ml of  $Me_2SO$  to the vial and dissolve the sample (ultrasonication is advantageous). Add 2 ml of the sodium hydride reagent and shake frequently during the reaction period of 30 to 40 min. Then add 300  $\mu$ l of methyl iodide in increments of 20, 30, 40, 50, 60, and 100  $\mu$ l, cooling and shaking after each addition. Let stand for 20 min with occasional shaking and then add slowly 3 ml of water to stop the reaction. While in the vial, extract the permethylated glycoalkaloids from the reaction mixture with two 1.5-ml portions of benzene. The extracts are transferred with a small pipet or syringe to a 35 mm  $\times$  12 mm vial. Remove the benzene after each extraction by passing a stream of  $N_2$  over the surface and dissolve the residue in absolute methanol to appropriate volume. A small amount of oil from the reagent and carried through the procedure is insoluble in the methanol. Typical dilutions are 50  $\mu$ l of methanol for glycoalkaloids ranging from 0 to 20 mg/100 g fresh potato; for concentrations of 20 to 40 mg/100 g, dilute with 100  $\mu$ l of methanol. **Note:** Precautions should be taken during the permethylation reaction to ensure absence of moisture; remove cap from vial only long enough to make required additions.



**Figure 1.** Chromatograms of potato glycoalkaloids showing: (A) incomplete derivatization resulting from insufficient reagent; (B) complete derivatization; column, 120 cm  $\times$  2 mm i.d. glass tubing; packing, 3% OV-1 on 100-120 mesh Gas-Chrom Q; helium flow, 55 ml/min; isothermal, 330°.



**Figure 2.** Chromatogram of known mixtures of glycoalkaloids; column, 90 cm  $\times$  2 mm i.d. glass tubing; packing, 3% Dexsil 300 on 100-120 mesh Supelcoport; helium flow, 55 ml/min; programmed, 200° isothermal for 2 min, heat 2°/min to 275°, heat 1°/min to 350°.



**Figure 3.** Chromatogram of potato glycoalkaloids. Column and conditions same as Figure 1.

**Table I. Relative Retentions and Retention Temperatures of Permethylated Glycoalkaloids**

	Rel retention <sup>a</sup>	Retention temp, <sup>b</sup> °C
Solanidine		223
$\beta$ -Chaconine	0.35	303
$\alpha$ -Chaconine	0.87	324
$\alpha$ -Solanine	1.00 <sup>c</sup>	327
Demissine	2.84	330 <sup>d</sup>
Tomatine	4.23	330 <sup>d</sup>

<sup>a</sup> Column, 120 cm  $\times$  2 mm i.d. glass tubing; packing, 3% OV-1 on 100-120 mesh Gas-Chrom Q; He flow, 55 ml/min; column temperature, isothermal 330°. <sup>b</sup> Same column and conditions as above except column temperature; hold 2 min at 200°, heat 2°/min to 275°, and then at 1°/min to 330°. <sup>c</sup> All compounds relative to  $\alpha$ -solanine; time of elution, 12 min. <sup>d</sup> Heating off at 330°; demissine elutes after 25 min, tomatine after 45 min.

**Table II. Quantitative Analysis of Mixtures of  $\alpha$ -Solanine and  $\alpha$ -Chaconine by GLC**

Mixture no.	$\alpha$ -Solanine, %		$\alpha$ -Chaconine, %	
	Known	Found	Known	Found
1	19.8	21.8	80.2	78.2
2	39.5	36.8	60.5	63.2
3	49.3	48.1	50.7	51.9
4	59.0	58.7	41.0	41.3
5	78.3	77.5	21.7	22.5

**Table III. Comparison of TGA of Fresh Potatoes by Titration and GLC**

Sample	TGA, <sup>a</sup> mg/100 g	GLC, mg/100 g
1	5.0	7.9
2	4.8	6.5
3	4.0	2.7
4	4.0	1.4
5	2.4	5.7
6	4.0	3.8

<sup>a</sup> Method of Fitzpatrick and Osman (1974).

## RESULTS AND DISCUSSION

The permethylated derivative of the glycoalkaloids is preferred over the trimethylsilyl derivative because of the greater volatility and lower molecular weight of the former. The permethylation procedure was modified to enable the reaction to be carried out on small samples with a minimum number of transfers. The derivatization is reproducible within a few percent, resulting in complete permethylation as determined by NMR.

The methylation procedure was first calculated to contain enough reagent during the reaction to methylate up to 5 mg of glycoalkaloids. It was effective for that amount of purified  $\alpha$ -solanine standard and effective also for most fresh potato extracts containing up to this amount of TGA. However, certain fresh potato extracts and extracts from potato slices which were incubated for several days had material present that apparently reacted with the reagent and larger quantities of reagent were required. Figure 1 is a chromatogram of glycoalkaloids showing the effect of either a too small amount of reagent or too large a sample size. Instead of one peak on the chromatogram representing  $\alpha$ -chaconine and one for  $\alpha$ -solanine, addition-

**Table IV. Changes in Glycoalkaloid Content of Potato Slices during Incubation at 20°**

Sample	Incubation days	GLC <sup>a</sup>		$\alpha$ -Chac./ $\alpha$ -sol.	TGA, <sup>b</sup> mg/100 g
		$\alpha$ -Chac., %	$\alpha$ -Sol., %		
1	0	52.0	48.0	1.1	5.0
	4	53.2	46.8	1.1	
	7	46.8	53.2	0.9	55.0
2	0	61.1	38.9	1.6	4.8
	6	38.2	47.2	0.8	55.6
3	0	68.9	31.1	2.2	8.8
	4	65.6	34.4	1.9	
	7	68.8	31.2	2.2	55.0
4	0	57.1	42.9	1.3	4.0
	6	53.2	46.8	1.1	42.4
5	0	74.2	25.8	2.9	4.0
	7	39.8	60.2	0.7	33.6
6	0	56.4	43.6	1.3	2.4
	6	39.5	60.5	0.7	33.6
7	0	74.2	25.8	2.9	
	4	69.2	30.8	2.2	
	7	55.6	44.4	1.3	

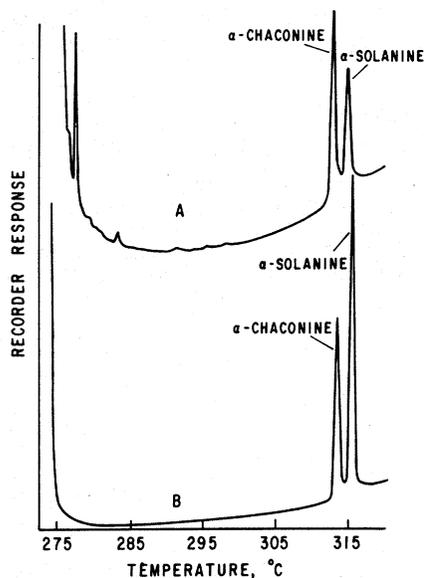
<sup>a</sup>  $\alpha$ -Chaconine +  $\alpha$ -solanine are considered = 100%. <sup>b</sup> Method of Fitzpatrick and Osman (1974).

all peaks are present which are thought to be partially methylated glycoalkaloids. Also shown is a chromatogram of the same sample using a larger amount of reagent where the extraneous peaks are not present.

Dexsil 300 columns were first utilized because of their heat stability and thus low bleed at the elevated temperatures that were believed to be necessary for the elution of glycoalkaloids. However, after several days of use, peaks were observed on the chromatogram which were determined to be the result of artifacts. Similar peaks could be induced by employing higher than necessary injection port temperatures. It has been stated that silane groups of silanized supports will bleed off above 350° (Analabs Catalog No. 17, 1974). Therefore, Dexsil 300 on an unsilanized support was tried but it had excessive adsorption of the glycoalkaloids and could not be used. Metal columns were also detrimental for these compounds and best results were obtained on glass columns.

A typical chromatogram of a mixture of permethylated solanidine,  $\beta$ -chaconine,  $\alpha$ -chaconine, and  $\alpha$ -solanine on a 3% Dexsil 300 column is shown in Figure 2. Components are all readily separated. When analysis of solanidine is not required, the starting temperature can be raised and the time of analysis reduced. Similar chromatograms were obtained on 3% OV-1 columns but at lower temperatures. With the OV-1 columns, it was found that the glycoalkaloids could be eluted at temperatures 25 to 30° lower than on the Dexsil 300 columns and with equivalent separation but at a reduced sensitivity because of greater bleed. However, the OV-1 columns usually were effective for 4 to 6 weeks before either the separation became poor or the peaks of the artifacts appeared.

In an effort to decrease further the time required for analysis, several isothermal runs were made at various temperatures. It was found that at 330°, Figure 3, an analysis could be completed in 15 min when the aglycones or glycoalkaloids with greater retention than  $\alpha$ -solanine, such as demissine and tomatine, were not present or not important in the analysis. In Table I are listed the retention times, relative to  $\alpha$ -solanine, of some of the common glycoalkaloids by the isothermal procedure and the retention temperatures when using temperature programming. The



**Figure 4.** Chromatograms of potato glycoalkaloids: (A) fresh potato slices; (B) 6-day incubated slices. Column and conditions same as Figure 1 except temperature programmed, 2 min isothermal at 275°, heat 1°/min to 330°.

isothermal procedure was used for most of the quantitative data reported. It was feared that the column would not last long at these elevated temperatures, but by maintaining the column at this temperature only when necessary and accumulating a number of samples to be analyzed consecutively, it was found that approximately 100 analyses could be made before the column deteriorated.

For quantitation studies, highly purified samples of  $\alpha$ -chaconine and  $\alpha$ -solanine were mixed to give a range of concentrations of each. The mixtures were permethylated and extracted, and the area percent calculated after GLC was compared to the known mixtures. The results are given in Table II. The results are quite acceptable estimates of each component and are probably better than could be obtained by thin-layer chromatography (TLC) or paper chromatography. From these data, a standard curve was drawn to estimate the amounts of the alkaloids in other samples.

Total glycoalkaloid values by the TGA method and the GLC procedure with some potato samples were compared. The results are given in Table III. Except for sample 6, these samples were not determined for TGA by GLC with quantitation in mind. However, sufficient data were available to make calculations. The agreements, while not the best, are acceptable for most practical purposes but the advantage with the GLC procedure is that the amount of each glycoalkaloid can be determined.

It is known that the TGA of potato tuber slices increases five- to tenfold on incubation at 20° over a period of 4 to 6 days (Kuĉ, 1964). However, it was not reported whether both  $\alpha$ -chaconine and  $\alpha$ -solanine increased proportionally or whether one or the other was favored. To determine the changes that took place, several varieties of tubers were sliced in half and two slices, each 3 mm thick, were cut from the facing halves. One slice was analyzed immediately and the other was incubated for 6 days. TGA analysis showed that there was an increase of 7 to 9 times that of the original value. The GLC analysis showed that, in addition to the increase in TGA, the ratio of  $\alpha$ -chaconine to  $\alpha$ -solanine had, in some cases, changed significantly. These changes are shown in Table IV and Figure 4. The ratio of  $\alpha$ -chaconine to  $\alpha$ -solanine was not always consistent; for example, samples 1 and 2 are the same variety, but the increase in  $\alpha$ -solanine was small in sample 1

but rather large in sample 2. The reason for this is not yet apparent and these differences will be studied more thoroughly.

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