

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, α -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 α -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 α -solanine and α -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 Blendor. 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 μ l of methyl iodide in increments of 20, 30, 40, 50, 60, and 100 μ 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 μ 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 μ 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.

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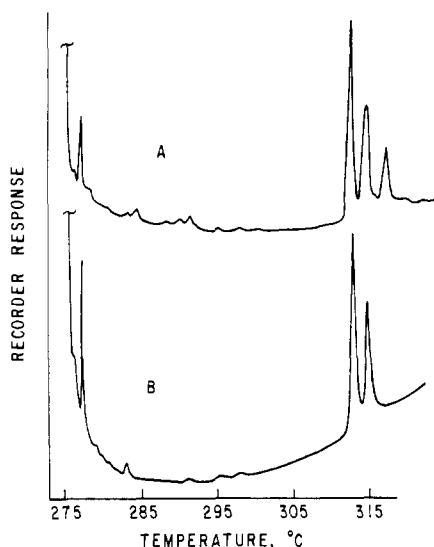


Figure 1. Chromatograms of potato glycoalkaloids showing: (A) incomplete derivatization resulting from insufficient reagent; (B) complete derivatization; column, 120 cm X 2 mm i.d. glass tubing; packing, 3% OV-1 on 100-120 mesh Gas-Chrom Q; helium flow, 55 ml/min; isothermal, 330°.

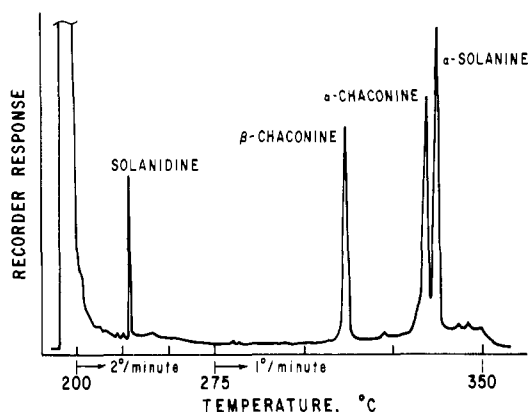


Figure 2. Chromatogram of known mixtures of glycoalkaloids; column, 90 cm X 2 mm i.d. glass tubing; packing, 3% Dexsil 300 on 100-120 mesh Supelcoport; helium flow, 55 ml/min; programmed, 200° isothermal for 2 min, heat 2°/min to 275°, heat 1°/min to 350°.

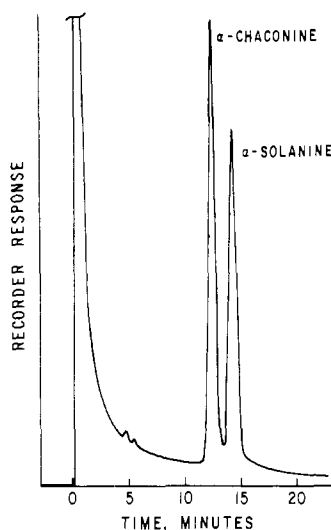


Figure 3. Chromatogram of potato glycoalkaloids. Column and conditions same as Figure 1.

Table I. Relative Retentions and Retention Temperatures of Permethylated Glycoalkaloids

	Rel retention ^a	Retention temp, ^b °C
Solanidine		223
β-Chaconine	0.35	303
α-Chaconine	0.87	324
α-Solanine	1.00 ^c	327
Demissine	2.84	330 ^d
Tomatine	4.23	330 ^d

^a Column, 120 cm X 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°. ^b 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°. ^c All compounds relative to α-solanine; time of elution, 12 min. ^d Heating off at 330°; demissine elutes after 25 min, tomatine after 45 min.

Table II. Quantitative Analysis of Mixtures of α-Solanine and α-Chaconine by GLC

Mixture no.	α-Solanine, %		α-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, ^a 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

^a 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 α-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 α-chaconine and one for α-solanine, addition-

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

Sample	Incubation, days	GLC ^a		α -Chac./ α -sol.	TGA, ^b mg/100 g
		α -Chac., %	α -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	

^a α -Chaconine + α -solanine are considered = 100%. ^b Method of Fitzpatrick and Osman (1974).

al 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, β -chaconine, α -chaconine, and α -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 α -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 α -solanine, of some of the common glycoalkaloids by the isothermal procedure and the retention temperatures when using temperature programming. The

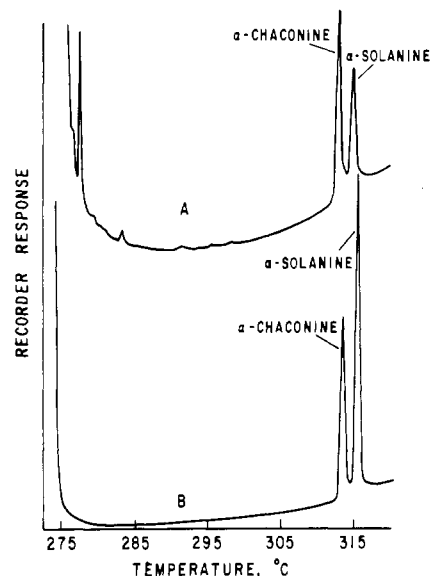


Figure 4. Chromatograms of potato glycoalkaloids: (A) fresh potato slices; (B) 6-day incubated slices. Column and conditions same as Figure 1 except temperature programmed, 2 min isothermal at 275°, heat 1°/min to 330°.

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 α -chaconine and α -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 (Kuć, 1964). However, it was not reported whether both α -chaconine and α -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 α -chaconine to α -solanine had, in some cases, changed significantly. These changes are shown in Table IV and Figure 4. The ratio of α -chaconine to α -solanine was not always consistent; for example, samples 1 and 2 are the same variety, but the increase in α -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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Quantitating the Recovery of Thiamine (Vitamin B₁) from Decalso in the Thiochrome Method for the Determination of Thiamine

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In the determination of thiamine (vitamin B₁) by the thiochrome method, purification of sample extracts on the cation exchanger, Decalso, is usually necessary. This purification step, as usually carried out, can be a major source of error because recovery of thiamine from Decalso is low (about 90%) and varies $\pm 8.5\%$ from the mean recovery value. A study of the variables involved showed that eluent temperature and volume are

important factors affecting thiamine recovery. Determination of the quantitative relationship between these factors and thiamine recovery led to a minor modification of the purification step that gave nearly quantitative (97.5% average) recovery of thiamine from Decalso and thus substantially enhances the precision and accuracy of the thiochrome method for the determination of thiamine.

In a study at the Western Regional Research Laboratory, on the effect of air pollutants on nutrients in food, we have determined thiamine in hundreds of samples, principally vegetables, by the thiochrome method and, in the process, have critically reexamined some aspects of this widely used procedure.

Purification of the sample extract, with an artificial silicate cation exchanger or its commercially available equivalent, Decalso, is generally recommended in the thiochrome method (Freed, 1966; Strohecker and Henning, 1966). Because omission of this step left fluorescent impurities in our sample extracts, we also had to routinely include the Decalso purification step in our determination of thiamine in fruits and vegetables.

If sample extracts are treated with Decalso, the thiamine standards also should be treated (Freed, 1966; Strohecker and Henning, 1966). However, during 5 months in which we used recommended procedures, our recoveries of thiamine were low and inconsistent (Table I). Mean recovery (89.4%, Table I) was substantially less than the 92-96% recovery deemed satisfactory (Freed, 1966) and the wide range (16.7% of mean recovery value) and large standard deviation indicated that the Decalso purification step was a major source of error.

Previous investigators too have found that recovery of thiamine from Decalso may be incomplete and irregular. Jowett (1940) reported only 68% recovery over a wide range of concentrations, Harris and Wang (1941) recommended omission of the absorption step because it caused variable loss of thiamine, and Bechtel and Hollenbeck (1958) reported recoveries ranging from 84 to 100%.

Low recovery that is consistent may be acceptable in a

quantitative method but with inconsistent recovery, precision and accuracy suffer in proportion to the magnitude of the inconsistency. We, therefore, investigated the Decalso purification step to determine if the variables could be manipulated to improve recovery of thiamine. The variables tested were thiamine/Decalso ratio, regeneration of Decalso, and temperature and volume of eluent.

MATERIALS AND METHODS

Reagents. Decalso (Thiochrome Decalso, Permutit T, Fisher Scientific Co. No. T-97) was used as received except in one test with regenerated Decalso (Table II). Decalso was regenerated (or reactivated), after one cycle of thiamine adsorption and elution with acid-KCl, as described by Freed (1966). Water and isobutyl alcohol were redistilled in glass. A stock thiamine solution (100 μg of thiamine hydrochloride/ml of 0.01 N HCl) prepared from a dry reference standard was appropriately diluted for either direct fluorometric determination or for application to Decalso columns. For direct fluorometric determinations the stock was diluted with acid-KCl reagent. For application to Decalso columns, the stock was diluted so that the resulting solution contained the equivalent of 5.0 ml of 2.5 M sodium acetate per 100 ml and was about 0.075 N in HCl.

Thiamine in pinto beans was determined by extracting the ground beans with hot 0.1 N HCl, treatment with a dual enzyme mixture consisting of takadiastase and papain in sodium acetate buffer, filtration, Decalso treatment, etc. as described by Freed (1966).

All other reagents were prepared as described by Freed (1966).

Apparatus. The borosilicate glass columns consisted of an 8 mm o.d. \times 14.5 cm tube having a capillary tube (3.5 cm long \times 6 mm o.d. \times 0.5 mm i.d.) sealed to one end and a reservoir (35 mm o.d. \times 11.7 cm long) sealed to the other (top) end. Maizel-Gerson reaction flasks used were Wilkins-Anderson (1972) Catalog No. 15845-00. Fluores-

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