Modification of the Rapid High-Performance Liquid Chromatographic Method for the Determination of Potato Glycoalkaloids

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A comprehensive evaluation of Carman et al.'s (1984) rapid high-performance liquid chromatographic analysis of potato glycoalkaloids has led to some modifications. Heptanesulfonic acid extracts approximately 81% of the glycoalkaloids from fresh tubers when compared to the accepted organic solvent methanol/chloroform/acetic acid (67:33:1). Glycoalkaloids in dehydrated potatoes are still best extracted with tetrahydrofuran/water/acetonitrile/glacial acetic acid (50:30:20:1). Samples extracted with heptanesulfonic acid must be cleaned up within 3–4 h after extraction. Those extracted in organic solvents are stable indefinitely. C_{18} Sep-Pak's can be used six times before they are not effective. Acetonitrile/water (50:50) elutes the glycoalkaloids more effectively from the C_{18} Sep-Pak than acetonitrile/buffer (50:50). Glycoalkaloid separations on an amino column can be cleaned up on C_{18} Sep-Pak's.

INTRODUCTION

Potato glycoalkaloids belong to a class of compounds with a steroidal base, the aglycone, to which are attached one to four sugar molecules. In cultivated tubers, the most commonly occurring glycoalkaloids are α-chaconine and α-solanine and their metabolites. These alkaloids are stress metabolites, arising in tubers, in response to excessive light, wounding, premature harvesting, and other adverse conditions (Sinden and Webb, 1974).

Potato breeders and toxicologists spend much time investigating potato glycoalkaloids because of their known acute toxicity (Jellema et al., 1980; Willimott, 1933; McMillan and Thompson, 1979), their possible chronic toxicity (Mun et al., 1975; Keeler et al., 1975; Keeler et al., 1976), and their characteristic bitter flavor (Sinden et al., 1974; Filadelphi, 1980). Furthermore, new potato varieties are checked for glycoalkaloid levels since there is a guideline stating that new potato varieties should not contain more than 20 mg of glycoalkaloid/100 g of fresh weight (Bomer and Mattis, 1924).

Past HPLC methods for glycoalkaloid analyses (Bushway et al., 1979; Morris and Lee, 1981) contained an ammonium precepitation clean-up step that is very time consuming. Recently Carman et al. (1984) have developed a rapid procedure whereby extracted glycoalkaloids were cleaned up with C_{18} Sep-Paks. However, after an extensive evaluation, there are some changes that can be made to improve this method.

This paper describes the modifications that will make the method of Carman et al. (1984) more effective and versatile for potato glycoalkaloid analyses.

MATERIALS AND METHODS

Samples. Raw tuber samples—Russet Burbank, Sheppardy, Bel Rus, Katahdin and Variety No. 398—were obtained from Arrostook State Farm, Presque Isle, ME. Four freeze-dried experimental varieties were used. Two were from the International Potato Center, Lima, Peru, and the others were from Alaska. Potato chips were purchased locally.

Reagents. All solvents were obtained from Fisher Scientific Co., Medford, MA. Chloroform, methanol, tetrahydrofuran, and acetonitrile were ACS-certified grade while the glacial acetic acid was ACS reagent grade. Water was glass distilled. HPLC grade solvents were used for HPLC analyses. 1-Heptanesulfonic acid was purchased from Sigma Chemical Co., St. Louis, MO. Sodium bisulfite was bought from Fisher Scientific Co. Glycoalkaloid standards, α-chaconine and α-solanine, were isolated by using the procedure of Bushway (1983). Both compounds had a purity of 98.0%. C_{18} Sep-Pak's were obtained from Water's Associates, Milford, MA.

Sample Extraction. Three methods were used in this study. First, Carman et al.'s (1984) procedure was employed on both raw and dried samples. For the fresh tubers, 100 g were extracted in 120 mL of 0.02 M 1-heptanesulfonic acid (containing 1% glacial acetic acid) in a Waring blender for 3 min at a high speed followed by filtration through coarse filter paper. As for the dried samples, 10 g was extracted. Potato chips could not be done by using Carman et al.'s method because of the fat.

The second technique was that of Bushway et al. (1985) and was used for freeze-dried and potato chip samples. A 10-g sample was extracted for 10 min in a Waring blender at medium speed with 130 mL of tetrahydrofuran/water/acetonitrile/glacial acetic acid (50:30:20:1, v/v). Extracts were vacuum filtered and brought to a 250-mL volume with extracting solvent. A 100-mL aliquot (placed in a 500-mL round-bottom flask) was rotary evaporated to 5 mL, followed by the addition of 3–10-mL aliquots of 1-heptanesulfonic acid. Each aliquot was sonicated for 1 min and transferred to a 40-mL centrifuge tube that was brought to the 40-mL volume with acid before centrifuging at 38000g for 10 min. A 10-mL aliquot of supernatant was removed and cleaned up by using the procedure of Carman et al. (1984). For potato chips (because of the fat) the 40-mL supernatant was placed in a 125-mL separatory funnel and allowed to set for 5–10 min while the fat rose to the top. A 10-mL aliquot was removed for cleanup.

The final extraction technique was a modified Wang et al. (1972) method in which acetic acid was added to the methanol/chloroform solvent system. This method was used for fresh tubers and was basically the same as the second extraction procedure except for the solvent, the amount of tuber extracted (50 g), and the size of the round-bottom flask (250 mL).

Sample Cleanup. All samples were cleaned up by using the procedure of Carman et al. (1984). The method consisted of conditioning a C_{18} Sep-Pak with 3 mL of HPLC-grade methanol followed by 5 mL of 1-heptanesulfonic acid. After which time, a 10-mL portion of sample was passed through the C_{18} cartridge. The Sep-Pak was then eluted with 5 mL of acetonitrile/water (20:80). The C_{18} column was dried by vacuum before being eluted with

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Table I. Comparison of Glycoalkaloid Methods in Fresh Tuber (mg of Glycoalkaloid/100 g of Tuber)[a]

variety	TGA value	
	Carman	modified
Russet Burbank	4.51	5.50
Sheppardy	13.05	16.07
Bel Rus	1.09	1.47
Katahdin	6.53	7.89
≈398	13.43	16.17

av percent found: 80.6
range percent found: 74.1–83.1

[a] Average of triplicate analyses. Value based on a comparison with modified method.

Table II. Comparison of Glycoalkaloid Methods in Dried Tubers (mg of Glycoalkaloid/20 g of Dried Product)

sample	TGA value	
	Carman	modified
freeze-dried	23.30	27.20
freeze-dried	10.80	13.91
freeze-dried	6.19	7.34
freeze-dried	8.00	11.78
oven-dried	3.23	8.96

av percent found: 70.3
range percent found: 36.0–85.7

[a] Average of duplicate analyses. [b] Values based on a comparison with modified method.

2 mL of acetonitrile/water (50:50). A 5-μL portion of this 2-mL solution was injected into the HPLC.

All samples were quantified using the HPLC procedure of Bushway et al. (1979) in which an amino column was employed. Since 1979 we have switched to the IBM carbohydrate column (Danbury, CT) in place of the Water's carbohydrate column.

RESULTS AND DISCUSSION

Recently, Carman et al. (1984) developed a rapid potato glycoalkaloid method that has two advantages over other HPLC methods (Bushway et al., 1979; Morris and Lee, 1981)—a simple extraction and quick cleanup. Such simplicity has decreased the analysis time of HPLC methods by 90%. However, in an attempt to use this procedure with our previously developed HPLC method (Bushway et al., 1979) led us to make some modifications that will improve the method. Four of the changes were concerned with the extraction while the others had to do with the cleanup.

First, it was observed that a correction factor should be employed when analyzing the glycoalkaloid content of raw tubers. It was shown (Table I) that extraction with 1-heptanesulfonic acid was not as effective as the modified Wang et al. (1972) solvent in extracting glycoalkaloids from raw tubers. A comparison of solvents using five different varieties was made (Table I). Using the average, the acid system of Carman et al. was only 80% as efficient as the modified solvent. Since the range for the five different tuber samples was small (74.1–83.1) with the Carman et al. extraction, it appears as if one could use the average of 80% as a built-in correction factor for the method of Carman et al. (1984) when analyzing the glycoalkaloid content of raw potatoes. If one feels uneasy using a correction factor, then the modified solvent extraction can be employed in conjunction with the Carman et al. cleanup.

Second, totally using the rapid procedure for dried products is not possible because of the variation observed in TGA values of the Carman et al. method compared to the accepted tetrahydrofuran (THF) technique (Bushway et al., 1985). The variability for dried samples ranged from 36.0 to 85.7% when compared to the THF extraction (Table II). Thus, a correction factor cannot be employed. So when dried products are analyzed for glycoalkaloids, the THF extraction should be employed along with the cleanup of Carman et al. (1984). It is slightly slower having to use the THF extraction, but not nearly as lengthy as the ammonium precipitation cleanup.

Next, samples containing fat were difficult to impossible to extract with the sulfonic acid solution. To eliminate this problem the THF extraction was employed along with a separatory funnel that helped in fat removal after centrifugation.

Finally, it was shown that the glycoalkaloids were not stable for more than a few hours in the sulfonic acid solution, at which time the sugars on the glycoalkaloids begin to hydrolyze to form the lower glycosides. This makes long storage of extracted samples impossible.

As for the cleanup procedure of the rapid glycoalkaloid method, it was concluded after extensive studies that each C_{18} Sep-Pak could be reused six times with fresh potatoes. For samples like potato chips and dried products, the C_{18} Sep-Paks were good for at least four samples.

Elution of samples from the C_{18} Sep-Paks were best with acetonitrile/water (50:50) instead of the acetonitrile/buffer (50:50) employed by Carman et al. (1984). Both raw and dried samples including potato chips were eluted from the Sep-Paks with the two solvent systems, and the acetonitrile/water yielded higher glycoalkaloid levels by 5–10%.

Peak broadening caused by solvent effect was observed when glycoalkaloid samples dissolved in acetonitrile/water were injected into the carbohydrate system for glycoalkaloid analysis. To prevent broadening, samples can either be taken to dryness after Sep-Pak elution and brought to volume with THF/water/acetonitrile (50:30:20) or 1 mL of THF can be added to 1 mL of the eluted sample. If one has sufficient sensitivity, the addition of THF is the simpler solution.

Two comparisons of ammonium-precipitated samples with the Carman et al. (1984) cleanup were made. A raw potato sample had a glycoalkaloid value of 4.55 mg/100 g of fresh tuber weight when precipitated and 4.84 mg/100 g of fresh tuber when Carman et al.'s cleanup was used. A potato chip sample yielded a glycoalkaloid level of 4.14 mg/100 g of product when precipitated and 5.24 mg/100 g of product (not precipitated). These results indicate that the much simpler cleanup step of Carman et al. (1984) gives better results than the ammonium precipitation. This is understandable since there are less analytical steps in the Sep-Pak cleanup.

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Rapid High-Performance Liquid Chromatographic Determination of the Potato Glycoalkaloids α-Solanine and α-Chaconine

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A method for the determination of the glycoalkaloids α-solanine and α-chaconine in fresh potato tubers and processed potato products is described. The glycoalkaloids are extracted with dilute aqueous acetic acid in the presence of an ion-pairing reagent. The crude extract is prepared for analysis by ion-pair column chromatography on a commercially available disposable C-18 cartridge column. Recoveries of added α-solanine and α-chaconine averaged 90% and 96%, respectively, with a coefficient of variation of approximately 4.5%. An assay time of 20 min and the possibility of preparing up to eight samples concurrently for analysis facilitate the assay of large numbers of samples in a short time. Survey data are presented for fresh potato samples collected from the three major potato growing areas in the U.S. and for commercially prepared frozen potato skins collected from one of these areas.

Glycoalkaloids are toxic, naturally occurring compounds commonly found in plants that are members of the Solanaceae or "Nightshade" family. Well-known members of this family are the potato, eggplant, tomato, capiscum, nightshade, and thorn apple. Of the more than 10 different glycoalkaloids found in these plants α-solanine and α-chaconine account for more than 95% of the glycoalkaloids found in the tuber (edible portion) of the potato plant (Guseva and Paseshnichenko, 1957). The generally accepted safe upper limit for glycoalkaloids in potato tubers in the U.S. is 20 mg of total glycoalkaloids/100 g of tuber. However, some researchers feel that the safe upper limit is much lower, 6 mg/100 g (Morris and Lee, 1984).

A number of analytical methods have been reported for the determination of individual and total glycoalkaloids in potato products. These methods commonly employ extraction by aqueous/organic or bisolvent systems followed by precipitation of the glycoalkaloids with aqueous base. The determinative steps involve thin-layer chromatography (Cadle et al., 1978), gas–liquid chromatography (Herb et al., 1975), high-performance liquid chromatography (HPLC) (Bushway et al., 1979; Morris and Lee, 1981), colorimetry (Wang et al., 1972), or titrimetry (Fitzpatrick and Osman, 1974). These methods can suffer from one or more of the following problems: large volumes of expensive or hazardous solvents, nonspecificity, and long analysis times. In contrast, the method described here is rapid (20-min assay time), simple, and economical. In addition, with minor changes, the proposed method accommodates samples of widely varying size and has a range of detection for each glycoalkaloid (α-solanine and α-chaconine) from 0.2 to 20 mg/100 g. As many as eight samples can be prepared for determination by HPLC at one time.

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EXPERIMENTAL SECTION

Materials. α-Solanine, α-chaconine, and 1-heptanesulfonic acid were purchased from Sigma Chemical Co. (St. Louis, MO). The standards were assumed to be 98% pure (Bushway, 1983) and were not further purified or tested. All other solvents and reagents were ACS reagent grade except for acetonitrile, which was HPLC (UV) grade purchased from Mallinckrodt Chemical Co. (Paris, KY). Distilled water was used for the preparation of the extracting solution, and HPLC-grade water was used for the preparation of the HPLC mobile phase.

Fresh potatoes for method development and recovery studies were purchased from local supermarkets. Fresh potatoes for the survey were collected by Food and Drug Administration (FDA) investigators from packers, growers, or shippers located in the potato-growing areas of Texas, Idaho, and Maine. Commercially prepared frozen potato skins were collected from shippers in Maine. Fresh potatoes and frozen potato skins were shipped within several days after collection. Fresh potato samples were shipped at ambient temperature, and frozen potato skin samples were packed in dry ice for shipment. Upon receipt, the fresh samples were stored at 4 °C, and the frozen samples were stored at −8 °C until analyzed. All samples were analyzed within 30 days of receipt.

Apparatus. The C-18 cartridge columns and Sep-Pak column manifold system were obtained from Waters Associates, Inc, (Milford, MA).

An Altex/Beckman, Inc. (Berkeley, CA), Model 322 MP system equipped with an Altex/Beckman, Inc., Model 210 injection valve fitted with a 20-μL injection loop was used for HPLC separations. An Altex/Beckman 4.6 mm × 25 cm HPLC column packed with 5-μm octyl (C-8) spherical silica was used for all separations, which were performed at a flow rate of 1.0 mL/min and ambient temperature.

A Hitachi, Ltd. (Tokyo, Japan), Model 100-40 UV/visible variable-wavelength detector was operated at 202 nm and fitted with an Altex/Beckman flow cell. Detector