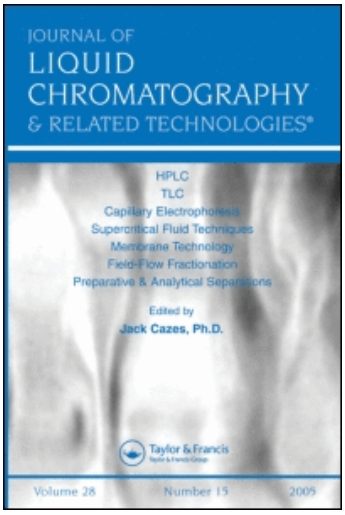

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High-Performance Liquid Chromatographic Determination of the Metabolites of the Potato Glycoalkaloids, α-Chaconine and α-Solanine, in Potato Tubers and Potato Products

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF THE METABOLITES OF THE POTATO GLYCOALKALOIDS, α-CHACONINE AND α-SOLANINE, IN POTATO TUBERS AND POTATO PRODUCTS

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ABSTRACT

A high-performance liquid chromatographic method has been developed to separate and quantify the metabolites, γ-chaconine, β_1- and β_2-chaconine, γ-solanine and β_2-solanine, of the potato glycoalkaloids α-chaconine and α-solanine in potatoes and potato products. A carbohydrate analysis column and a solvent system of tetrahydrofuran-water-acetonitrile (55:8:37) were employed for the separation. Flow rate was 1.1 ml/min and the compounds were monitored at 215 nm. β_2-chaconine (0.63 mg to 29.75 mg/100 g dried weight) was present in all samples whereas the other glycosides of α-chaconine were only detectable in the animal feed products. It appears that some of the animal feeds may contain trace amounts of γ-solanine and an unknown which maybe β_1-solanine. Limit of detection for all glycosides was 0.05 μg/μl. Elution time for all the lower glycosides of α-chaconine was 8 min versus 16 min for the α-solanine group. These metabolic compounds were confirmed using thin-layer chromatography.

INTRODUCTION

The majority of glycoalkaloids found in commercial potato varieties or tubers in breeding programs are of the solanidine series which are comprised of α-chaconine and α-solanine and their

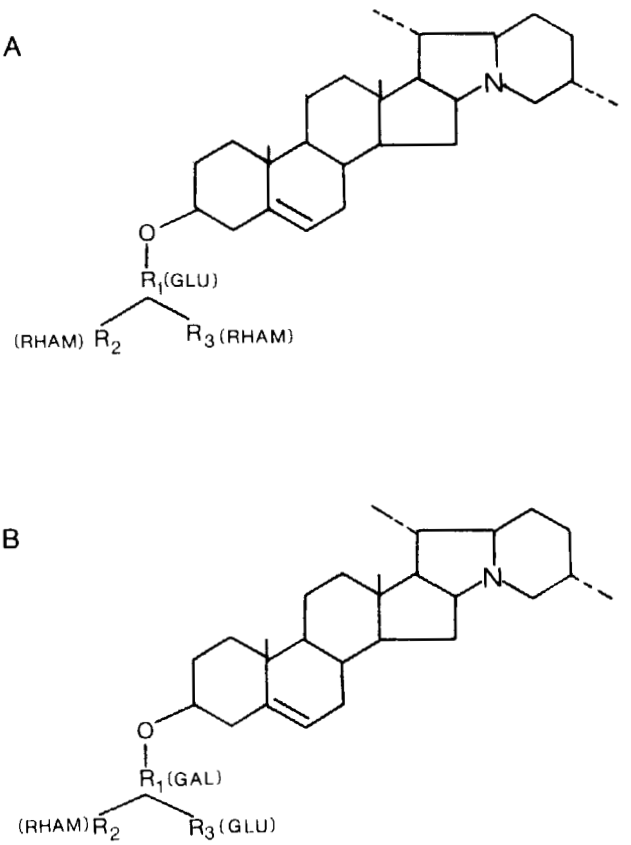

Figure 1. Metabolites of α-Chaconine (A) and α-Solanine (B), γ-Chaconine (R_1), β_1-Chaconine ($R_1 + R_2$), β_2-Chaconine ($R_1 + R_3$), γ-Solanine (R_1), β_1-Solanine ($R_1 + R_2$) and β_2-Solanine ($R_1 + R_3$).

metabolites (Figure 1). Because of their known acute toxicity (1-3), their possible chronic toxicity (4-6), and their characteristic bitter flavor (7,8), glycoalkaloids must be analyzed in all new potato varieties before they can be released commercially.

Of all the possible methods-colorimetric (9,10), titrimetric (11), thin-layer chromatographic (12,13), gas chromatographic

(14,15) and high-performance liquid chromatographic (15-20)-available to quantify total or individual glycoalkaloids, HPLC is the most applicable to quantify these metabolites because it is fast, accurate, reproducible and can be used to determine both individual and total glycoalkaloids. Analysis of the individual glycoalkaloids is most important since they may vary in their degree of bitterness and toxicity (7,8).

Although there have been several HPLC methods developed for determining glycoalkaloids (15-20), this is the first method that can separate all the lower glycosides of α-chaconine and α-solanine in the same sample.

EXPERIMENTAL

Materials

Solvents used for the extraction and for TLC of the glycoalkaloids were ACS grade (Fisher Scientific Co. Fair Lawn, NJ) while those employed in the HPLC separations and to dissolve the glycoalkaloids were HPLC grade (Fisher Scientific Co.).

The glycoalkaloids, α-chaconine and α-solanine, were obtained from the procedure of Bushway and Storch (21) while β_2-chaconine was a gift from Eugene A. Talley, Eastern Regional Research Center, USDA, Philadelphia, PA. The other glycosides were obtained from acid hydrolysis of α-chaconine and α-solanine employing the procedure of Filadelfi (8).

TLC plates were HP-KF high-performance silica gel plates 10 x 10 cm; 200 μ thickness (Whatman Inc. Clifton, NJ).

Apparatus

The HPLC system consisted of a Waters Assoc. (Milford, MA), 6000 A pump, a U6K injector, a Schoeffel variable-wavelength UV detector (Westwood, NJ) and a Houston Instruments dual pen recorder (Austin, TX).

Methods

Extraction: The procedure used to extract the tubers and potato products was that of Bushway et al. (17) with one modification. The extracting solution was methanol-water-acetic acid (92:5:3).

HPLC separations: In order to establish the conditions needed to separate the metabolic degradation products, α-chaconine (9 mg/25 ml) and α-solanine (25 mg/25 ml) were acid hydrolyzed (8) which yielded γ-chaconine and γ-solanine, β_1-chaconine, β_2-chaconine, β_2-solanine and possibly a minute amount of β_1-solanine. Operating conditions were: column, Carbohydrate analysis column (Waters Assoc.); mobile phase, tetrahydrofuran-water-acetonitrile (55:8:37); flow rate, 1.1 ml/min; column temperature, ambient; wavelength, 215 nm; attenuation, 0.04 a.u.f.s.; and chart speed, 0.4 in/min. Before injecting 5 µl of each sample, they were filtered through a 0.45 µm Millipore organic filter (Waters Assoc.).

TLC separation: The thin-layer chromatographic method employed was that of McCollum and Sinden (22).

RESULTS AND DISCUSSION

A chromatogram of the separation of the metabolites of α-chaconine and α-solanine is shown in Figure 2. Elution time of

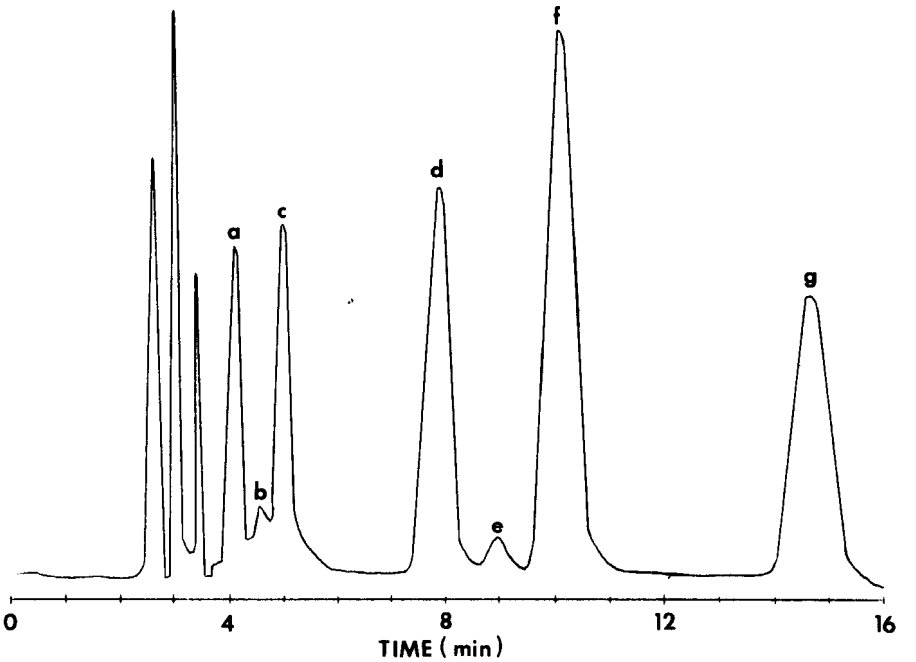

Figure 2. Chromatogram of the Metabolites of α-Chaconine and α-Solanine. Peaks: a, γ-Chaconine; b, γ-Solanine; c, β_2-Chaconine; d, β_1-Chaconine; e, unknown; f, α-Chaconine; g, β_2-Solanine.

the lower glycosides of α-chaconine was 8 min except in the presence of α-chaconine, in which case the elution time was 11 min (Figure 2). The elution time for degradation products of α-solanine was 16 min, not including α-solanine (Figure 2). α-Solanine can best be analyzed using a previously developed method (17) since it elutes very slowly in this solvent system. The retention time for the glycosides of α-solanine can be decreased by increasing the water content of the mobile phase if the metabolites of α-chaconine are not present. As can be seen in Figure 2,

TABLE 1

Level of Metabolic Degradation Products of α-Chaconine and α-Solanine in Potatoes and Potato Products.

Sample	mg/100 g dried weight				
	γ-Chaconine	β_2-Chaconine	β_1-Chaconine	γ-Solanine	β_2-Solanine
Potato Waste Animal Feed #1	1.76	15.25	1.30	ND*	ND*
Potato Waste Animal Feed #2	8.00	29.75	1.49	ND	ND
Potato Waste Animal Feed #3	2.60	22.00	1.75	ND	ND
Potato Waste Animal Feed #4	0.49	4.75	0.82	ND	ND
Potato Waste Animal Feed #5	0.70	13.00	1.30	ND	ND
Potato Peel Waste	ND	10.18	ND	ND	ND
Russet Burbank Potatoes	ND	0.63	ND	ND	ND
Norchip Potatoes	ND	1.00	ND	ND	ND
Kennebec Potatoes	ND	0.73	ND	ND	ND

ND=none detected at a detection limit of 0.05 μg/μl.

this time is needed for complete separation of all the metabolites. When samples containing α-solanine are analyzed using this procedure, the injections can be staggered so that α-solanine does not interfere with the other glycoalkaloids.

Several potato varieties and potato products were analyzed for their content of metabolic glycosides and the results are presented in Table 1. Some of the feed products appear to have a trace of γ-solanine and an unknown compound (Figure 3) that maybe β_1-solanine (based on acid hydrolysis of α-solanine standard). We are presently trying to identify this substance. All samples

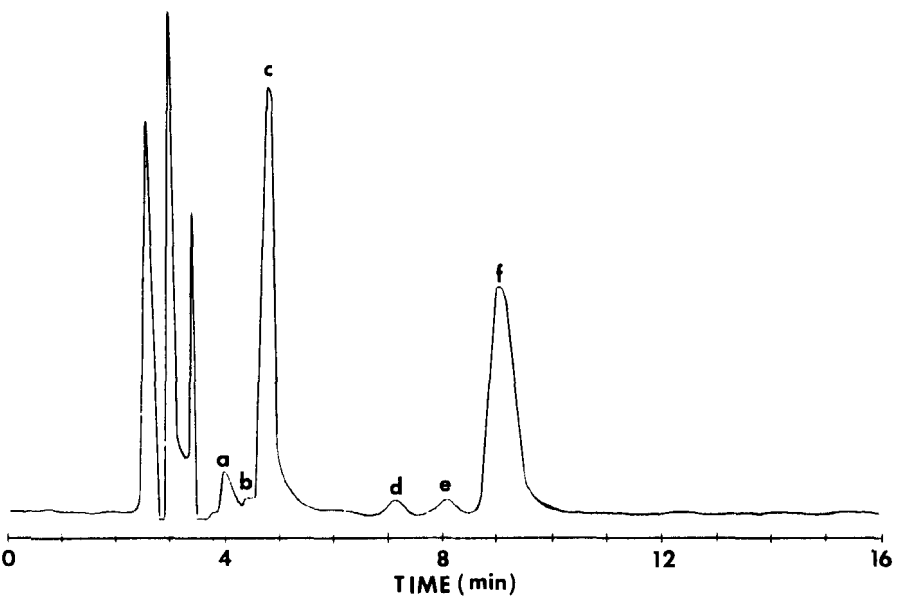

Figure 3. Chromatogram of a Potato Waste Animal Feed Product. Peaks: a, γ-Chaconine; b, γ-Solanine; c, β_2-Chaconine; d, β_1-Chaconine; e, unknown; f, α-Chaconine.

had at least one detectable degradation product of α-chaconine. The potato waste animal feed samples contained all three metabolites of α-chaconine while commercial tuber varieties had low levels of β_2-chaconine. Large quantities of these degradation glycoalkaloids were found in some of these animal feed products (Figure 3 and Table 1). The presence of these high levels of individual glycoalkaloids could cause bitter flavor which could preclude the use of the product as an animal feed. Also high amounts of glycoalkaloids could have toxic effects on animals and/or people consuming the animals. Further research is necessary to determine if and to what extent the bitterness and toxicity problems exist.

The amount of β_2-chaconine in samples was calculated using a standard curve. However, γ- and β_1-chaconine standards had not been isolated at the time of this study so a response factor was determined using the quantity of solanidine present in these two lower glycosides and comparing this with β_2-chaconine. Such a calculation can be performed since solanidine is the only portion of the molecule that will absorb UV light at 215 nm. The identity of these lower glycosides were confirmed using TLC.

The reproducibility of this method was tested on a potato animal feed sample #5 (Table 1). Five subsamples of the product which contained γ-, β_1- and β_2-chaconine were extracted and the per cent coefficients of variation for three glycoalkaloids were 3.72, 3.28 and 7.59%. All coefficients of variation were below 10 per cent indicating that the method is reproducible.

This HPLC procedure offers a quick, accurate and reproducible method for plant breeders and toxicologists to determine the metabolites of α-chaconine and α-solanine in potatoes and potato products.

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