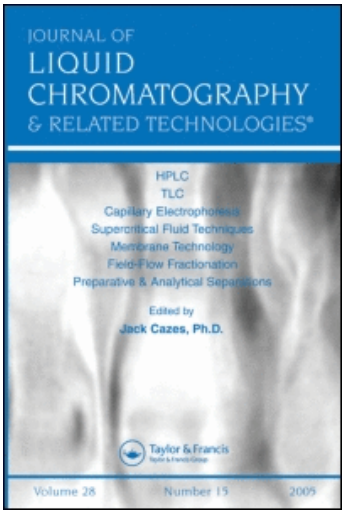

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SEMI-PREPARATIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF POTATO GLYCOALKALOIDS

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ABSTRACT

A semi-preparative high-performance liquid chromatographic method has been developed to separate crude mixtures of the potato glycoalkaloids α-chaconine, α-solanine, commersonine and demissine. Milligram quantities of each substance can be obtained within an 8 hour period. A Zorbax semi-preparative NH_2 column and a solvent system of tetrahydrofuran-water-acetonitrile (55:20:25) were employed for the separation. The flow rate was 1.0 ml/min. Glycoalkaloid separations were monitored using both refractive index and ultraviolet detection (215 nm). Further analyses of these glycoalkaloids were done using analytical high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) to check compound purity and identity.

INTRODUCTION

Potato glycoalkaloids are steroidal alkaloids that are comprised of either a spirosolane or solanidine type aglycone to

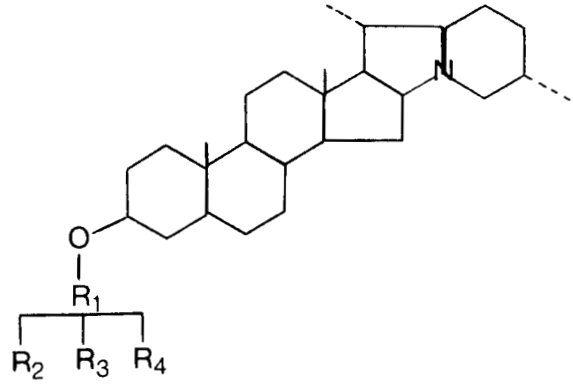
which one to four carbohydrate molecules are attached. Widespread interest has developed in these natural occurring substances because of their toxicity and insecticidal activity. Several cases of glycoalkaloid poisoning have been reported in animals (1) and man (1-4) and, in a few instances, death has occurred from ingestion of large quantities of potato glycoalkaloids (5). Futhermore there is some evidence implicating these compounds as teratogens (6-8). Their possible use as an insecticide, because of their feeding deterrent activity toward the Colorado potato beetle (9-12) and potato leafhopper (13,14), has also created much interest.

Presently, the methods used to purify milligram quantities of potato glycoalkaloids consist of preparative TLC (15) and open column chromatography techniques (16). Both procedures are very time consuming and the glycoalkaloids obtained are not analytically pure. In this paper we describe a method to separate four potato glycoalkaloids (Figure 1) -- commersonine (A), demissine (B), α-chaconine (C) and α-solanine (D) -- using semi-preparative HPLC. This technique is rapid and yields milligram quantities of analytically pure glycoalkaloids.

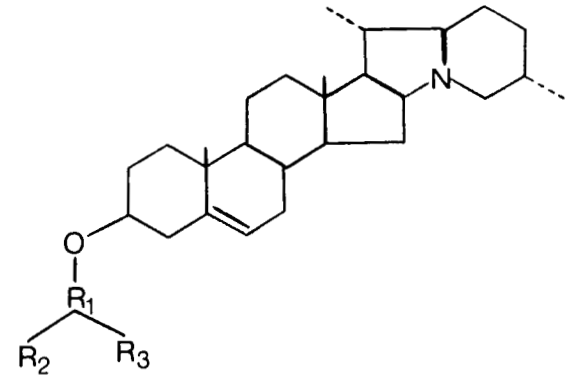
EXPERIMENTAL

Materials

Solvents used for the semi-preparative and analytical high-performance liquid chromatographic separations of glycoalkaloids were HPLC grade (Fisher Scientific Co. Fair Lawn, NJ) while the



	R_1	R_2	R_3	R_4
A	D-GAL	D-GLU	D-GLU	D-GLU
B	D-GAL	D-GLU	D-GLU	D-XYL



	R_1	R_2	R_3
C	D-GLU	L-RHAM	L-RHAM
D	D-GAL	D-GLU	L-RHAM

Figure 1. Potato Glycoalkaloids: Commersonine (A), Demissine (B), α-Chaconine (C) and α-Solanine (D).

solvents employed in the mass extraction of the glycoalkaloids from potato blossoms and thin-layer chromatography were ACS grade (Fisher Scientific Co.).

Crude glycoalkaloid mixtures, used to obtain the individual compounds by semi-preparative HPLC, were isolated using the procedure of Bushway et al. (17). α-Chaconine and α-solanine were extracted from Katahdin potato blossoms and a mixture of commersonine and demissine were obtained from blossoms of Solanum demissum Lindl.

Potato glycoalkaloid standards were a gift from Eugene A. Talley, Eastern Regional Research Center, USDA, Philadelphia, PA and Steve L. Sinden, Beltsville Agricultural Research Center, USDA, Beltsville, MD.

The TLC plates employed in this study were HP-KF high-performance silica gel plates 10x10 cm, 200 μ thickness (Whatman Inc., Clifton, NJ).

Apparatus

The HPLC system consisted of a Waters Assoc. 6000 A pump, a U6K injector, a differential refractometer R 401, a Schoeffel variable-wavelength UV detector (Westwood, NJ) and a Houston Instruments dual pen recorder (Austin, TX). The semi-preparative column (25 cm x 9.4 mm I.D.) was a Zorbax NH_2 (DuPont de Nemours and Co., Wilmington. DE). Operating conditions were: mobile phase, tetrahydrofuran-water-acetonitrile (55:20:25); flow rate,

1.0 ml/min; column temperature, ambient; wavelength, 215 nm; refractive index setting, 16X; attenuation, 0.4 a.u.f.s.; and chart speed, 0.4 in/min.

The analytical column (30 cm x 4 mm I.D.) was a Carbohydrate analysis column (Waters Assoc.). Operating conditions were: mobile phase, tetrahydrofuran-water-acetonitrile (53:17:30); flow rate, 1.5 ml/min; column temperature, ambient; wavelength, 215 nm; attenuation, 0.04 a.u.f.s.; and chart speed, 0.4 in/min.

Methods

Semi-preparative HPLC separations: Two glycoalkaloid solutions were prepared using the mixtures obtained from the mass extraction of the potato blossoms. One solution contained primarily α-chaconine and α-solanine at a total glycoalkaloid concentration of 8 mg/ml, while the other consisted mostly of commersonine and demissine at a total concentration of 1.5 mg/ml. Tetrahydrofuran-water-acetonitrile (50:30:20) was used to dissolve the crude mixtures of glycoalkaloids. Before injecting 500 µl of the mixtures, the solutions were filtered through a 0.45 µm Millipore organic filter (Waters Assoc.). The individual glycoalkaloids were collected and concentrated using rotary evaporation.

Analytical HPLC separations: Analytical HPLC separations were performed using the procedure of Bushway et al. (18).

TLC separations: The thin-layer method employed was that of McCollum and Sinden (19).

RESULTS AND DISCUSSION

The chromatograms of the semi-preparative separations of the potato glycoalkaloids are shown in Figures 2 (α-chaconine and α-solanine) and 3 (commersonine and demissine). Total elution time when all compounds are considered in the crude mixtures varies from 24 min. for the α-chaconine and α-solanine mixture to 30 min. for commersonine and demissine. If one includes only α-chaconine and α-solanine in the mixture than the elution time is 20 min. compared to 24 for only commmersonine and demissine (Figures

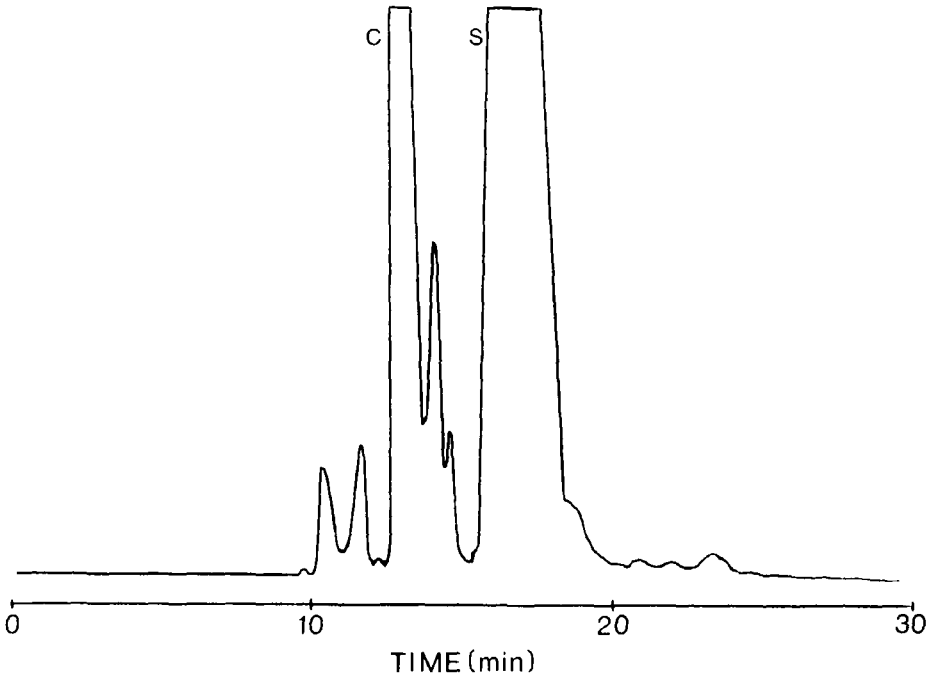


Figure 2. Semi-preparative HPLC Chromatogram of α-Chaconine (C) and α-Solanine (S). Obtained by Mass Extraction of Katahdin Blossoms.

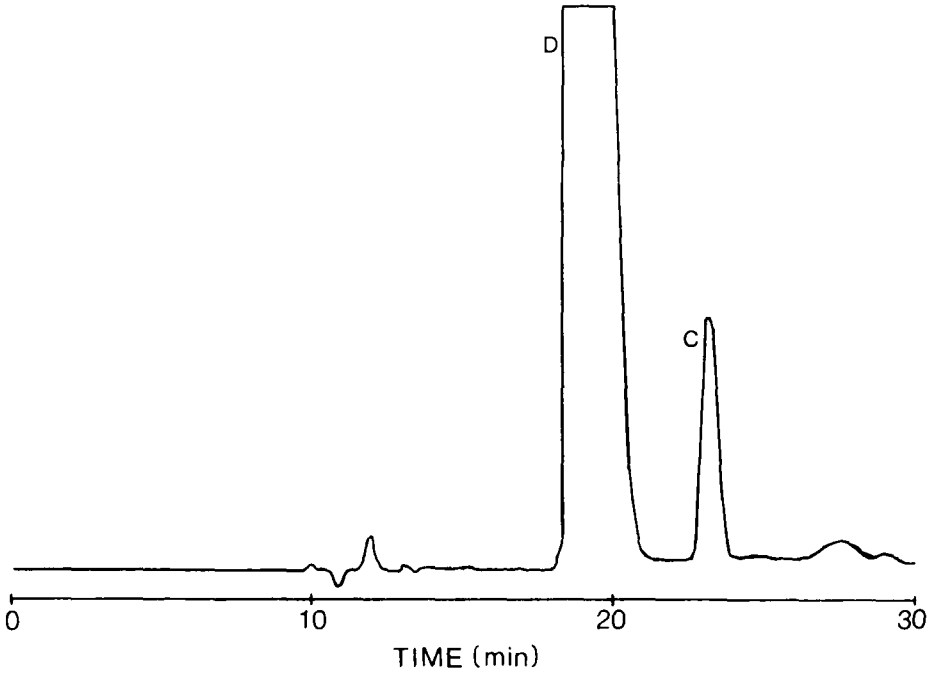


Figure 3. Semi-preparative HPLC Chromatogram of Demissine (D) and Commersonine (C). Obtained by Mass Extraction of Solanum demissum Blossoms.

2 and 3). By staggering injections, it is possible to collect 30 -60 mg each of α-chaconine and α-solanine in an 8 hour period. As for demissine and commersonine, one can obtain approximately 13 mg of demissine and 3 mg of commersonine. Of course the amount of each glycoalkaloid collected will depend upon the quantity of each in the starting crude mixtures. This can vary with the species of plant used and the number of crystallization steps since each glycoalkaloid has a different solubility product. As can be seen (Figures 2 and 3), there are other compounds in these mixtures es-

pecially in the α-chaconine and α-solanine fraction. We are presently trying to identify these substances which are most likely glycoalkaloids.

In order to ascertain the purity and to check the identity of each glycoalkaloid isolated, analytical HPLC and TLC were employed. The results are presented in Figure 4 for the analysis by TLC and Figures 5 and 6 for the evaluation of each by analytical HPLC. A trace amount of α-chaconine was shown to be present by TLC (Figure 4) in the semi-preparative fraction of α-solanine and vice versa (Figure 4). Also the commersonine fraction contained

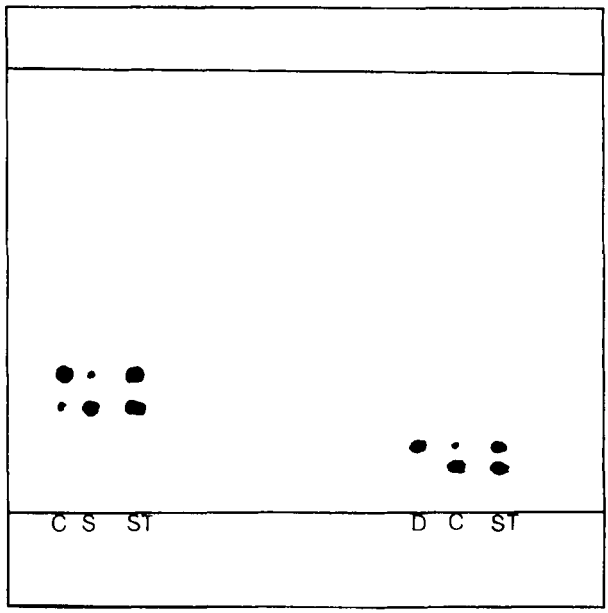


Figure 4. TLC Chromatogram of α-Chaconine (C), α-Solanine (S), Demissine (D) and Commersonine (C). Fractions Collected From the Semi-preparative Separations. ST, Standards.

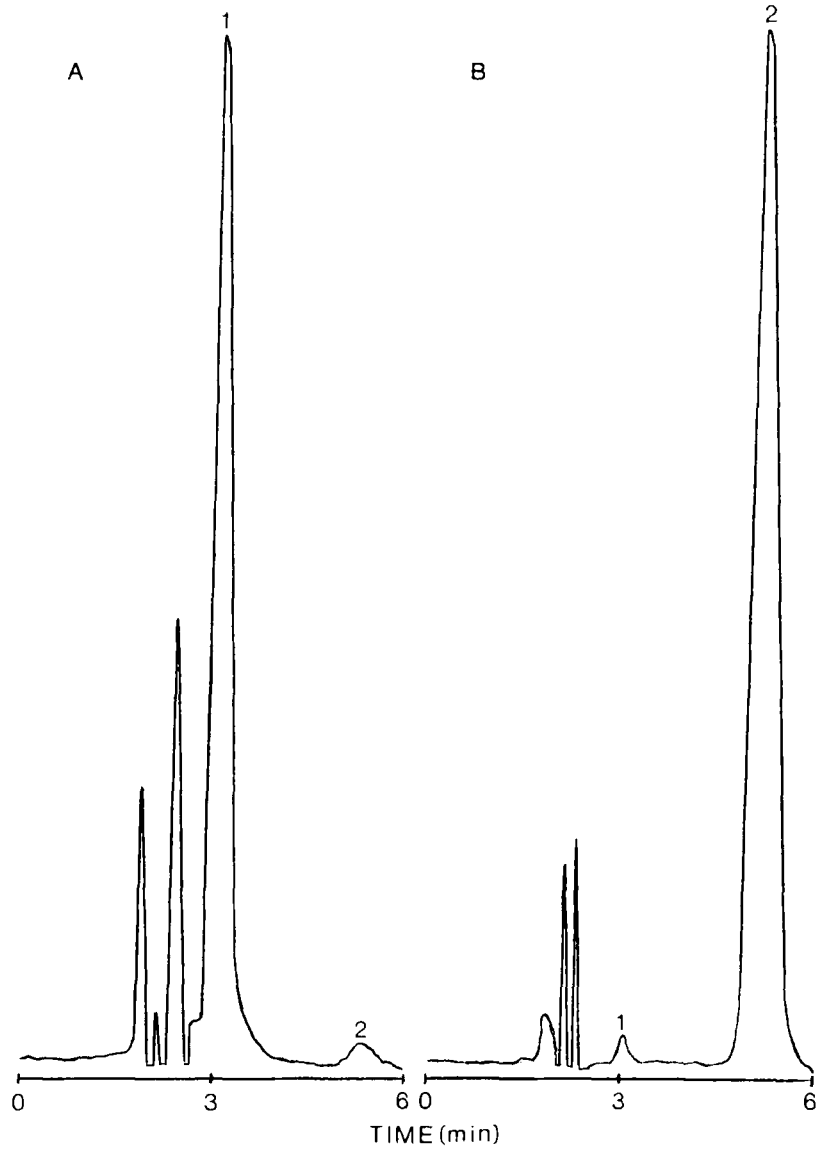


Figure 5. Analytical HPLC Chromatogram of α-Chaconine (A) and α-Solanine (B). Fractions Collected From the Semi-preparative Separations. Peak 1, α-Chaconine; Peak 2, α-Solanine.

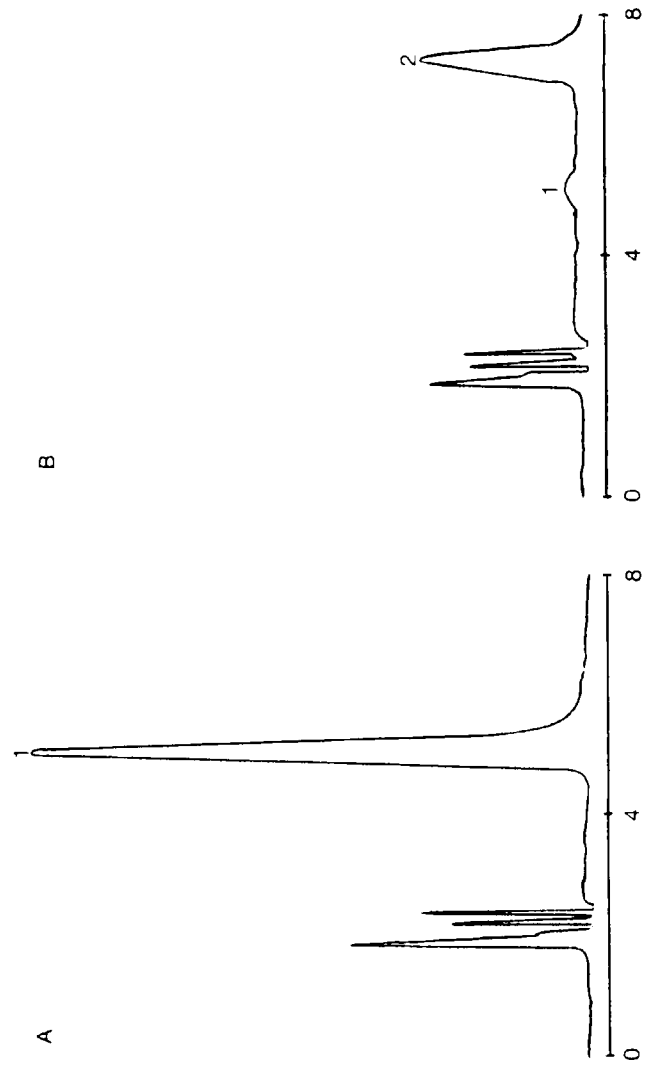


Figure 6. Analytical HPLC Chromatogram of Demissine (A) and Commersonine (B). Fractions Collected From the Semi-preparative Separations. Peak 1, Demissine; Peak 2, Commersonine.

a small amount of demissine (Figure 4). To quantify these contaminants, analytical HPLC was used (Figures 5 and 6). The trace amounts of α-solanine and α-chaconine observed in each fraction were 1.3% and 1.2%, respectively, while demissine was at a level of 1.0% in the commersonine fraction. Cross contamination can be alleviated by fine tuning the technique for collecting fractions.

This semi-preparative HPLC method offers a rapid means of obtaining potato glycoalkaloids that are analytically pure (98.7%-99%) and in sufficient quantity to do biochemical and toxicological research.

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