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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF POTATO GLYCOALKALOIDS

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SUMMARY

Separations of three glycoalkaloids (α-chaconine, β-chaconine, and α-solanine) have been achieved by using three different columns: μBondapak C_{18}, μBondapak NH_2, and a carbohydrate analysis column. These methods have been employed to examine the purity of the potato glycoalkaloids isolated by thin-layer and column chromatographic separations. Also, samples of tubers, peels, blossoms, and sprouts have been analyzed to determine their content of α-chaconine, β-chaconine, and α-solanine by using the carbohydrate and μBondapak NH_2 columns.

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 and/or wild potatoes, the most commonly occurring glycoalkaloids are α-chaconine and α-solanine, although others such as β- and γ-chaconine, β- and γ-solanine, and α- and β-solamarine, commersonine, leptines, and demissine have been isolated as well. These compounds are stress metabolites, arising in tubers, leaves, and blossoms in response to excessive light, wounding, premature harvesting, and other adverse conditions.

The significance of glycoalkaloids in human nutrition is that a high concentration in potatoes may cause poisoning and, in a few cases, death[1–3]. There is also some evidence that these compounds may be teratogenic[4–8].

Methodology for the determination of glycoalkaloids is comprised mainly of wet-chemical methods[9–16]. These methods do not distinguish between different glycoalkaloids but instead give the total amount of glycoalkaloids present. Moreover, these methods are quite time-consuming. Methods for the qualitative and quantitative analysis of individual glycoalkaloids are almost nonexistent. In their liquid chromatographic method, Hunter *et al.*[17] were able to separate several aglycones preparatively. Herb *et al.*[18] developed a gas chromatographic procedure for the separation and quantitative analysis of glycoalkaloids. However, in this method it is necessary to derivatize the glycoalkaloids prior to chromatographic analysis. Our paper presents a method for separating and quantitating α-chaconine, β-chaconine, and α-solanine by high-performance liquid chromatography (HPLC) without derivatization.

EXPERIMENTAL

Apparatus

The HPLC system was comprised of a Waters Assoc. (Milford, Mass., U.S.A.) 6000A pump, a U6K injector, and a differential refractometer along with a Schoeffel (Westwood, N.J., U.S.A.) variable-wavelength UV detector and a Houston Instruments (Austin, Texas, U.S.A.) dual-pen recorder.

Columns. Three columns, a μBondapak C_{18}, a μBondapak NH_2, and a carbohydrate analysis column, were used, all of which were made of precision stainless steel 30 cm × 4 mm I.D., packed with 10-μm packing, and purchased from Waters Assoc.

Mobile phase. With the μBondapak C_{18} column a mixture of tetrahydrofuran–water–acetonitrile (50:30:20) was employed, while for the μBondapak NH_2 column a mixture of tetrahydrofuran–water containing 1.7 g KH_2PO_4 per 500 ml–acetonitrile (50:25:25 and 50:30:20) was utilized. Three solvent systems were used with the carbohydrate analysis column: tetrahydrofuran–water–acetonitrile (56:14:30 and 60:10:30) and acetonitrile–water (85:15).

Reagents

All solvents used were HPLC grade and were obtained from Waters Associates and Fisher Scientific (Pittsburgh, Pa., U.S.A.). All glycoalkaloid standards were obtained from Eugene A. Talley, Eastern Regional Research Center, Agricultural Research Service, USDA, Philadelphia, Pa., U.S.A.

Procedure

All samples and standards were dissolved in tetrahydrofuran–water–acetonitrile (50:30:20). Potato tubers, peels, and sprouts were extracted by using either the binary solvent system developed by Wang *et al.*[13] or a mixture of tetrahydrofuran–water–acetonitrile (50:30:20). Each extraction was carried out on a 50 g sample of material. The extracted material was concentrated on a steam bath almost to dryness and then redissolved in approximately 40 ml of methanol. The glycoalkaloids were precipitated from the methanol solution by adjusting the pH to 10.5 with ammonium hydroxide. The precipitate was collected, dissolved in tetrahydrofuran–water–acetonitrile (50:30:20), and injected into the liquid chromatograph. Before injection, the samples were passed through a 0.45-μm Millipore filter (Waters Assoc.), along with eluents. The flow-rates ranged from 1–2 ml, depending on the chromatographic system employed. Compounds were monitored at either 208, 215, or 225 nm and at 0.4, 0.1, or 0.04 a.u.f.s., depending on the concentration and the presence of other compounds. More details are given in the legends to Figs. 1–6.

RESULTS AND DISCUSSION

Using a μBondapak C_{18} column, it was possible to separate partially β-chaconine from α-chaconine and α-solanine (Fig. 1A), but not α-chaconine from α-solanine. This column with the solvent system tetrahydrofuran–water–acetonitrile (50:30:20) may be useful in separating glycoalkaloids according to the number of carbohydrate residues. α-Chaconine and α-solanine which contain three carbohydrate

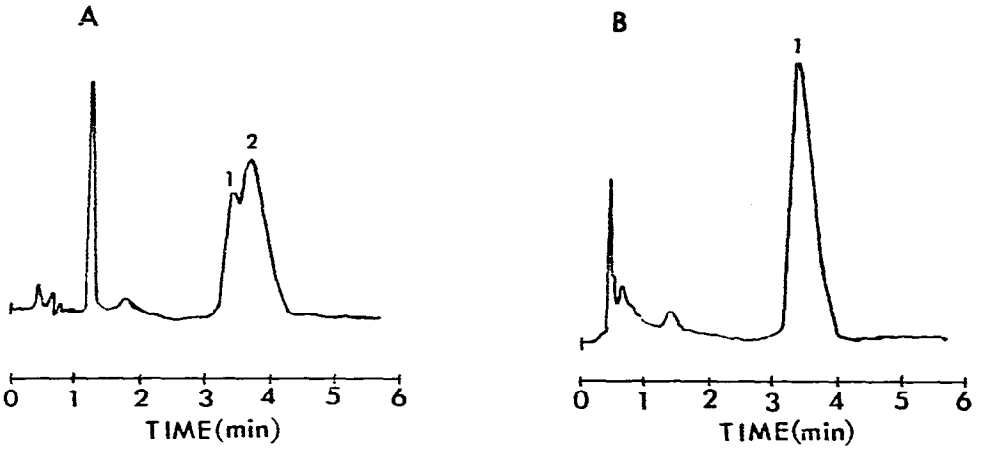

Fig. 1. Chromatogram of a mixture of glycoalkaloids on a μBondapak C_{18} column. Solvent system, tetrahydrofuran–water–acetonitrile (50:30:20); flow-rate, 2 ml/min; detector sensitivity, 0.1 a.u.f.s.; wavelength, 208 nm; chart speed 0.4 in./min. (A) Mixture of 2.5 μg α-chaconine, 5.0 μg β-chaconine, and 2.5 μg α-solanine. (B) Mixture of 5.0 μg α-chaconine and 5.0 μg α-solanine. Peaks: 1 = α-chaconine and α-solanine; 2 = β-chaconine.

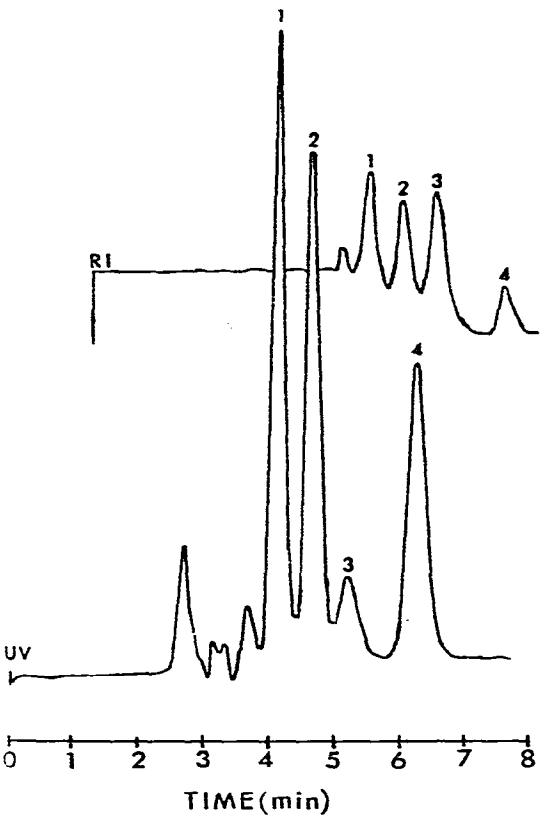

Fig. 2. Chromatogram of a mixture of glycoalkaloids on a μBondapak NH_2 column. Solvent system, tetrahydrofuran–water containing 1.70 g KH_2PO_4 per 500 ml–acetonitrile (50:25:25); flow-rate, 1.0 ml/min; detector sensitivity, 0.1 a.u.f.s.; wavelength, 208 nm; refractometer sensitivity, 4×; chart speed, 0.4 in./min. Mixture of 2.5 μg β-chaconine, 1.9 μg α-chaconine, 2.3 μg α-solanine and 3.1 μg tomatine. Peaks: 1 = β-chaconine; 2 = α-chaconine; 3 = tomatine; 4 = α-solanine.

residues, do not separate, whereas β-chaconine which contains two carbohydrate residues is partially separated from them (100% separation of β-chaconine from the other two glycoalkaloids can be accomplished without separating α-chaconine and α-solanine by lowering the percentage of tetrahydrofuran). Furthermore, tomatine, a glycoalkaloid found in tomatoes containing four carbohydrate moieties, is eluted much earlier than the glycoalkaloids with two and three carbohydrate residues. To substantiate this observation, one should try demissine and/or commersonine, which contain four carbohydrate residues and have aglycones very similar to solanidine.

Although this system does not separate α-chaconine from α-solanine, the

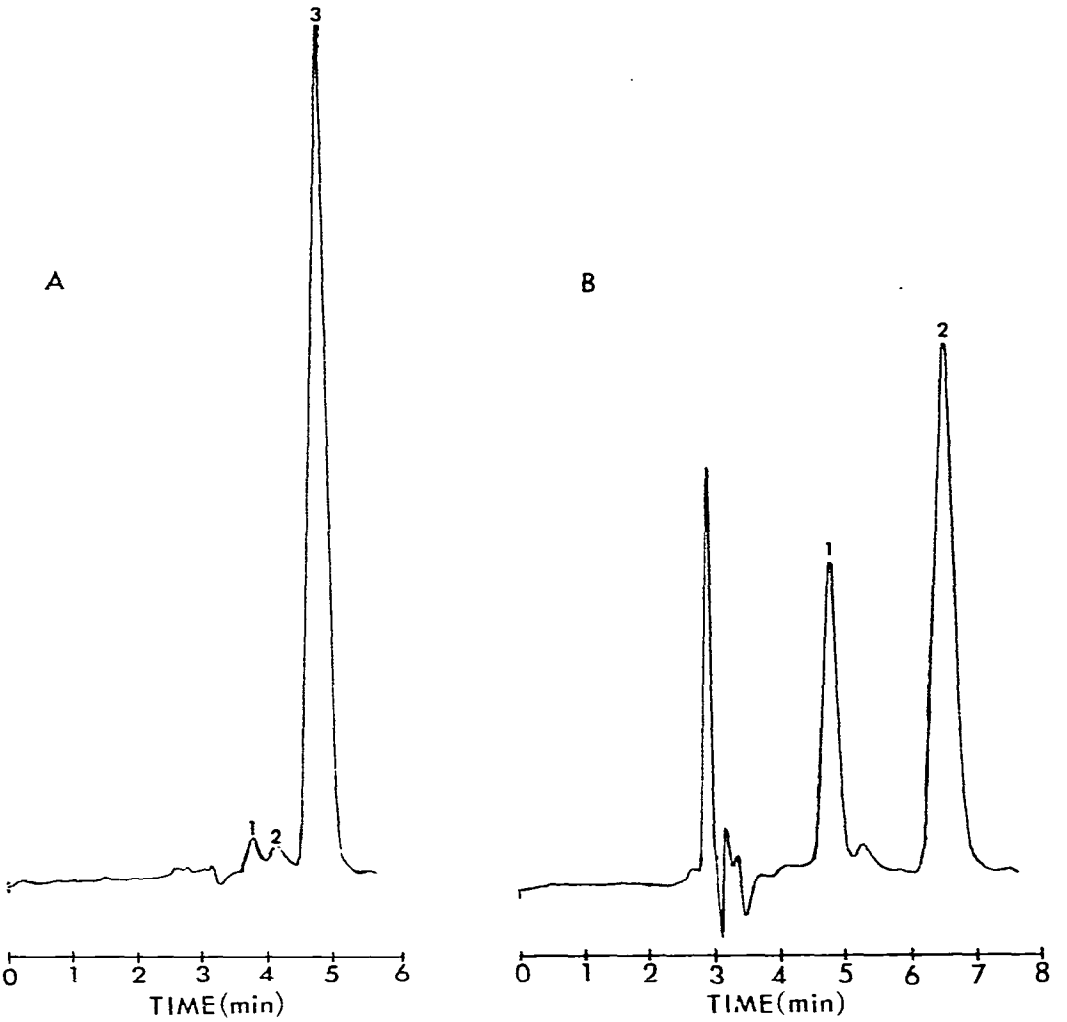

Fig. 3. Chromatogram of (A) α-solanine isolated from a thin-layer chromatogram and (B) a mixture of glycoalkaloids isolated from potato blossoms. Solvent system for A, tetrahydrofuran–water containing 1.70 g KH_2PO_4 per 500 ml–acetonitrile (50:30:20); solvent system for B, as in Fig. 2. All other conditions as listed in Fig. 2. Peaks for A: 1 = α-chaconine; 2 = unknown; 3 = α-solanine. B: 1 = α-chaconine; 2 = α-solanine.

method could be used for rapid screening of potatoes for their glycoalkaloid content, since 95% of the glycoalkaloids in potatoes consist of α-solanine and α-chaconine[12]. Fig. 1B shows a mixture of α-solanine and α-chaconine, extracted from potato blossoms. As can be seen, there is only a single peak, but the concentration can be determined because the response factors are the same for both compounds and a a linear relationship exists between peak height and area *vs.* concentration.

It was possible to separate β-chaconine, α-chaconine, and α-solanine by using a μBondapak NH_2 column in the reversed-phase mode (Fig. 2). This method is very rapid, the complete analysis being accomplished in less than 7 min, and has been very useful for checking the purity of glycoalkaloids isolated by thin-layer chromatography (TLC) (Fig. 3A) along with the qualitative and quantitative analysis of glycoalkaloids from potato blossoms (Fig. 3B). In Fig. 3A it can be seen that glycoalkaloids isolated by TLC may contain several impurities. The HPLC procedure can be modified slightly to enable semi-preparative separation. This is useful in obtaining high-purity standards and is preferable to the more time-consuming TLC. For quantitative analysis, it is helpful that the peak height is directly proportional to concentration, both in UV absorption and in the refractive index. When potato tuber extracts were analyzed by this system, α-chaconine but not α-solanine was contaminated by other compounds.

Of all the column and solvent combinations tried, the carbohydrate analysis column with tetrahydrofuran–water–acetonitrile (56:14:30) proved to be best for analyzing samples of potato tubers, peels and sprouts, because this combination provided separation without interfering peaks. The line obtained by plotting peak height *vs.* concentration was always straight, but did not pass through the origin at 0.4 a.u.f.s. The eluates from the liquid chromatograph were also analyzed by TLC. The thin-layer chromatograms indicated single components in each zone with retention times corresponding to α-chaconine and α-solanine. Also, absorbance ratios were

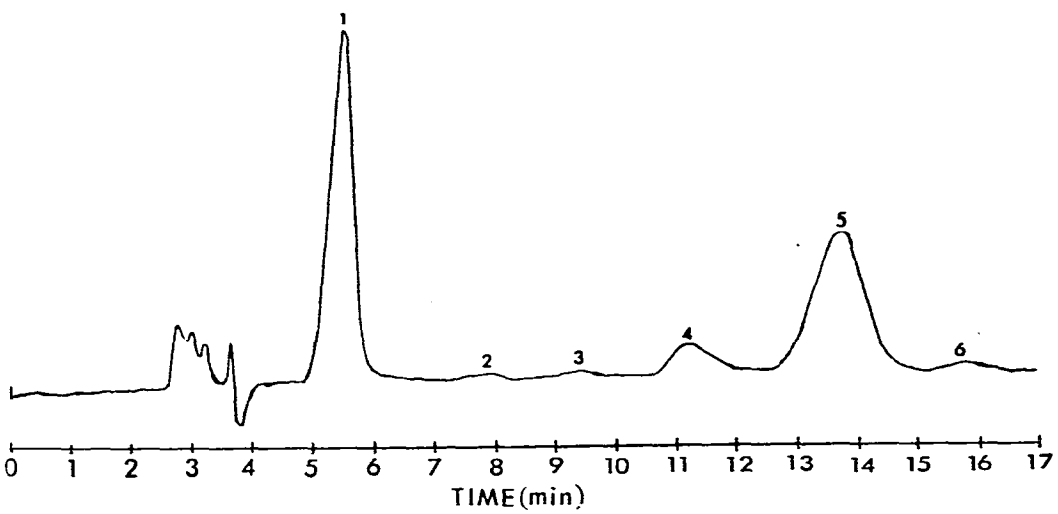

Fig. 4. Chromatogram of glycoalkaloids from an extract of Katahdin sprouts. Solvent system, tetrahydrofuran–water–acetonitrile (56:14:30); flow-rate, 1 ml/min; detector sensitivity, 0.4 a.u.f.s.; wavelength, 208 nm; chart speed, 0.4 in./min. Peaks: 1 = α-chaconine; 2, 3, 4, 6 = unknown; 5 = α-solanine.

determined at 215, 225, 235, and 245 nm which further verified the peaks as α-chaconine and α-solanine. A sprout extract from Katahdin potatoes is shown in Fig. 4. The chromatogram shows four unknown peaks and α-chaconine and α-solanine. The unidentified peaks appear to be other glycoalkaloids, since they decrease in intensity when monitored at 215 and 225 nm. Quantitative analysis of α-chaconine and α-

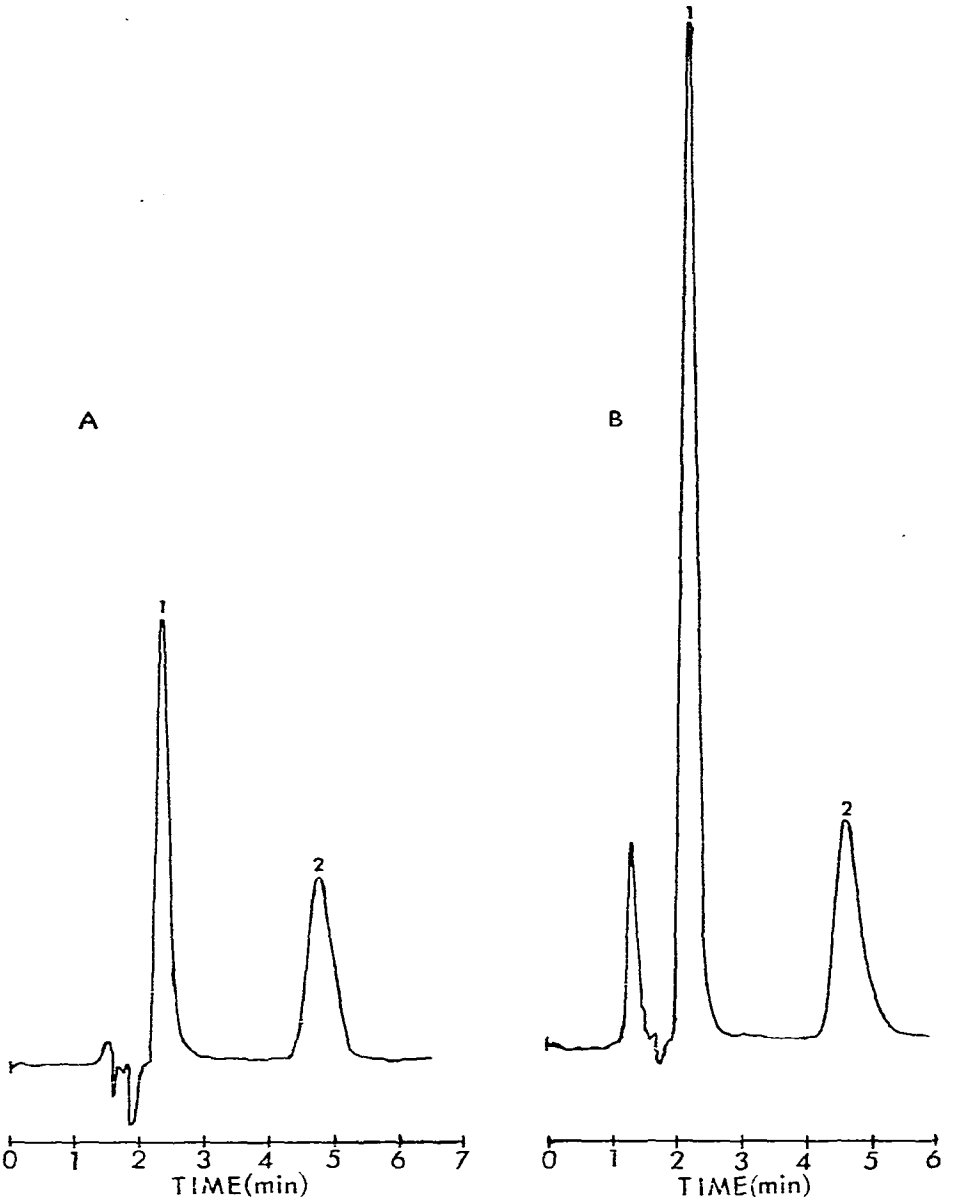

Fig. 5. Chromatogram of α-chaconine and α-solanine present in mixed dried peels (A) and in Lenape potatoes (B). Solvent system, tetrahydrofuran–water–acetonitrile (56:14:30); flow-rate, 2 ml/min; detector sensitivity, 0.04 a.u.f.s.; wavelength, 215 nm; chart speed, 0.4 in./min. Peaks: 1 = α-chaconine; 2 = α-solanine.

solanine indicated that these sprouts contained 157.8 mg of α-chaconine and 131.9 mg of α-solanine per 100 g (wet weight) of sprouts.

Since the potato tubers and peels did not produce any interfering peaks, it was possible to increase the flow-rate from 1 to 2 ml/min. The potato peel sample was in the form of a dried product that is used for cattle feed. When two different varieties of this feed were analyzed for glycoalkaloids by HPLC, it was found that one contained 20.1 mg of α-chaconine and 6.6 mg of α-solanine, while the other consisted of 8.9 mg of α-chaconine and 3.0 mg of α-solanine per 50 g of dried feed (Fig. 5A).

This method has also been used to analyze potatoes for glycoalkaloids (Fig. 5B). The results of analyses of six different 50-g samples of the Lenape variety are given in Table I. Statistical analyses on these six replications indicate that the percent coefficient of variation was 11.1% for α-chaconine and 10.4% for α-solanine. Preliminary studies on other potato varieties such as Kennebec, Russet Burbank, Superior, and Katahdin have indicated that the method is sufficiently sensitive and that there is no interference.

TABLE I

α-CHACONINE AND α-SOLANINE CONTENT IN LENAPE POTATOES

Sample number	*α-Chaconine (mg per 100 g*	*α-Solanine (mg per 100 g*
1	27.8	17.8
2	25.4	14.1
3	32.4	17.6
4	32.6	15.4
5	25.7	14.6
6	26.7	17.6
Average	28.4	16.2
S.D.	3.3	1.7
C.V. (%)	11.1	10.4

Recovery experiments were not performed, because the extraction procedure used was similar to the one developed by Fitzpatrick and Osman[15], known to give a 95% recovery. The analysis of potato tubers and peels for glycoalkaloids was performed at 215 nm instead of 208 nm, since the baseline was more stable and the sensitivity was sufficient. The limit of detection for each glycoalkaloid was determined to be 5–15 ppm.

Further investigation with other solvent systems and the carbohydrate analysis column has demonstrated that a small increase in sensitivity may be obtained by using acetonitrile–water (85:15) as eluent, since the UV cut-off of these solvents permits monitoring at 200 nm. The chromatogram in Fig. 6 shows the separation of a standard mixture of α-chaconine and α-solanine with this eluent.

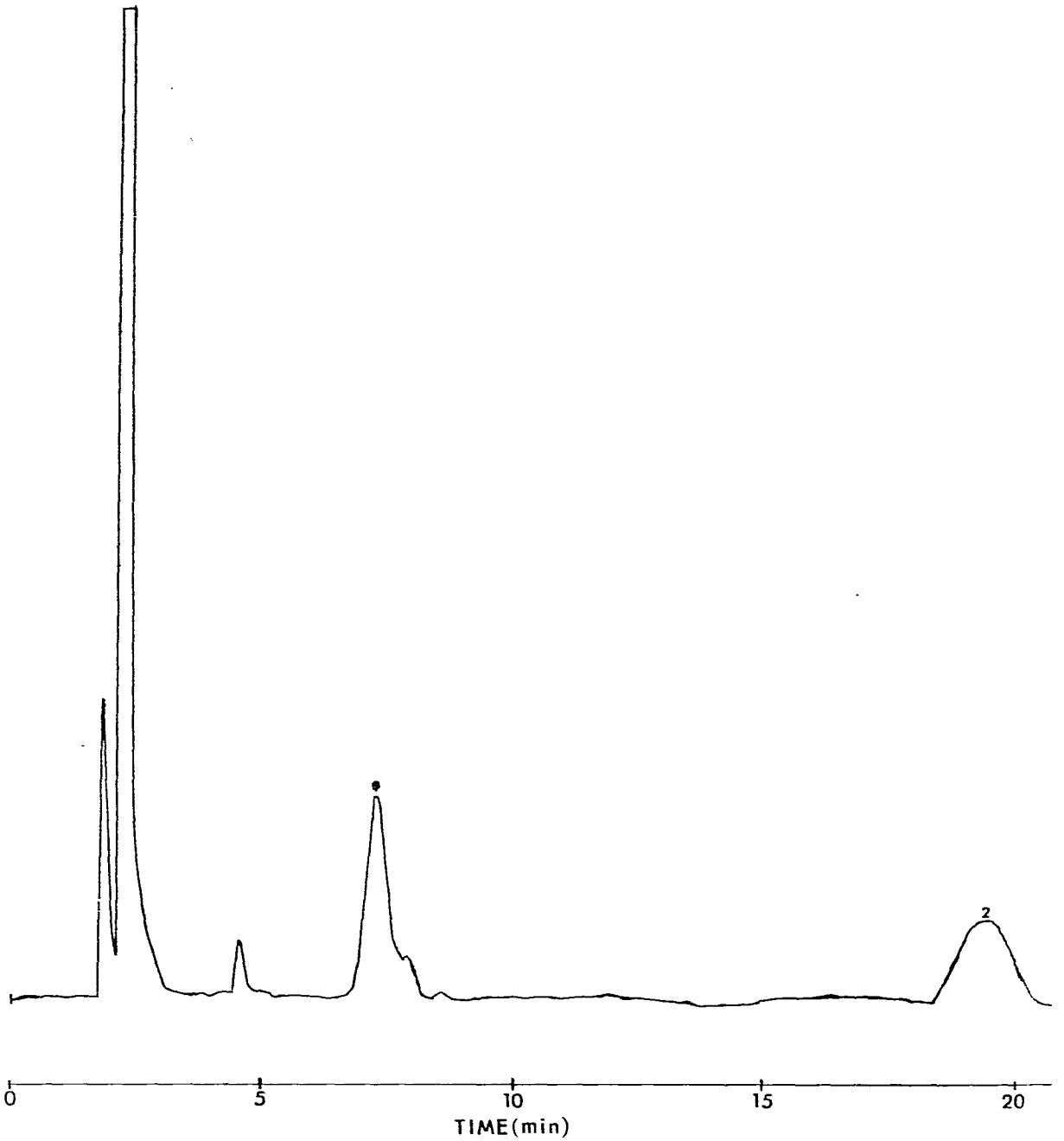

Fig. 6. Chromatogram of a mixture of α-chaconine and α-solanine. Solvent system, acetonitrile-water (85:15); flow-rate, 1.5 ml/min; detector sensitivity, 0.04 a.u.f.s.; wavelength, 200 nm; chart speed, 0.4 in./min. The mixture contained 1 μg/ml of each glycoalkaloid. Peaks: 1 = α-chaconine; 2 = α-solanine.

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