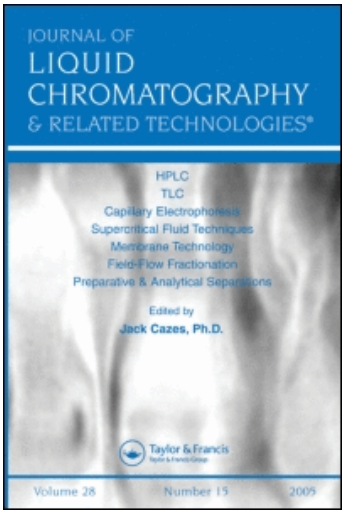

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DETERMINATION OF SOLASODINE IN FRUITS OF SOLANUM KHASIANUM BY A COMBINATION OF CHROMATOFUGE AND HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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ABSTRACT

An extract of Solanum khasianum fruits was fractionated with a chromatofuge. The fraction containing solasodine was applied to a HPLC column and the effluent was monitored by UV. The peak height gave an accurate measure of the amount present. The efficiency and load capacity of the chromatofuge, as well as the accuracy and precision of the HPLC method were determined.

INTRODUCTION

We have so far used the chromatofuge (1) only for the preparative separation of amino acids (2). When an instrument specifically manufactured for centrifugal preparative chromatography**

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**Hitachi Centrifugal Chromatograph. Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

became available, we were interested in testing its applicability to the analysis of solasodine in fruits of Solanum khasianum. This is a problem in connection with a project in which our laboratory collaborates with another laboratory in Israel.

Earlier methods for the quantitative analysis of solasodine in plant extracts involved preliminary purification by chromatography on adsorption (3) and ion-exchange (4) columns or by thin-layer chromatography (TLC) (5). For the estimation of the alkaloid concentration, other investigators have used colorimetry (6), titrimetry (7), planimetry (8), and gravimetry (9).

Because our earlier procedure for isolating steroidal alkaloids by preparative high-pressure liquid chromatography (HPLC) (10) is too time-consuming for routine analyses, we developed a rapid HPLC technique for their separation (11). In combination with the rapid isolation by the chromatofuge, this new HPLC technique has proved to be a fast and reliable method for the routine quantitative assay of plant extracts.

METHODS

Extraction

Ten lyophilized S. khasianum fruits, weighing 20.58 g, were powdered in a mortar and then boiled under reflux in 1 liter of 2 N hydrochloric acid for 2 hrs. The cooled hydrolyzate was made alkaline to litmus by the addition of 5 N sodium hydroxide solution. Solasodine was extracted with 3 portions of 500 ml dichloromethane, which were washed by extraction with a 1-liter

portion of water. The combined extracts were dried over sodium sulfate and evaporated under reduced pressure. The residue was divided into 20 equal portions of "Solanum extract".

Chromatofuge

The chromatofuge was a Model CLC-5 Centrifugal Chromatograph (NSA Hitachi Scientific Instruments, Mountain View, Calif.) with a 3-mm spacer. With the rotor spinning at 400 rpm, it was packed by first pouring 4 g dry silica (SilicAR, Mallinckrodt, 100-200 mesh) into the chamber, then increasing the speed to 600 rpm and pouring a slurry of 50 g silica gel (Woelm, "less than 0.08 mm") in 150 ml absolute ethanol continuously into the chamber. The eluent was delivered by means of a solvent pump (Model 110, Altex, Berkeley, Calif.) directly into the chamber without using the solvent distributor. The fraction collector was kept at 8 min/fraction, while the speed of rotation was varied and the pumping rate was adjusted accordingly to prevent flooding or drying of the adsorbent.

HPLC

The HPLC apparatus consisted of a 300 x 4.6-mm i.d. stainless steel tube, packed with Zorbax-Sil (6 μm, Du Pont de Nemours, Wilmington, Del.), preceded by a 50-mm precolumn, packed with Porasil A (37-75 μm, Waters Associates, Milford, Mass.). An Altex Model 110 pump was connected to the column through a sample injection valve (Model 7125, Rheodyne, Berkeley, Calif.) with a 500-μl loop volume. The detector was a Hitachi variable-wavelength spectrometer (Model 155, Altex), equipped with a flow-cell having a

10-mm pathlength and a 20-µl volume, which was set at 212 nm, 0.1 AUFS. The signal from the detector was fed into a single-channel recorder (Model 335, Linear, Irvine, Calif.), which was set at 10 mV.

The eluent was a mixture of n-hexane, methanol, and acetone (18:1:1) ("Distilled-in-Glass" quality, Burdick & Jackson, Muskegon, Mich.), which was delivered at a flow-rate of 3 ml/min.

Microgram quantities of solasodine (K & K Labs Div., ICN Pharmaceuticals, Plainview, NY.) in dichloromethane solution were injected into the HPLC apparatus and the detector response was recorded. Solasodine was eluted about 5 min after injection. A plot of peak heights vs. amounts of solasodine injected was linear from 0 to 500 µg. This calibration was used for all subsequent experiments.

Thin-Layer Chromatography

Precoated Silica Gel G plates (250 µm, Analtech, Newark, Del.) were developed with n-hexane-ethyl acetate (1:1), then sprayed with 50% aqueous sulfuric acid and heated (12).

RESULTS AND DISCUSSION

Efficiency of the Chromatofuge

In addition to UV-absorbing carotenoid pigments, which are removed in the earliest fractions from the chromatofuge, the "Solanum extract" may be expected to contain certain cyclic poly-isoprenoids, which could conceivably interfere with the assay. In order to test the efficiency of the chromatofuge in removing

such compounds, a mixture of 1 mg each of solasodine and of the following "contaminants" was prepared: stigmasterol, α-amyrin, diosgenin, tomatidine, cycloartenol, and 3β-hydroxy-5,16-pregnadien-20-one. The mixture was dissolved in 7 ml dichloromethane and introduced into the chromatofuge, which had previously been flushed with 100 ml of 25% ethanol in n-pentane (Eastman, Rochester, N.Y.). The fraction collector was kept at 8 min/fraction, while pumping rate and speed of rotation were adjusted as shown in Table 1.

TABLE 1

Efficiency of the Isolation of Solasodine by Chromatography

Fraction No.	Volume ml	Pump rate ml/min	Spin rate rpm	Solvent composition % ethanol in n-pentane	Fraction composition
1	14	5.5	350	25	0
2	20	3.0	350	25	0
3	15	3.0	350	25	contaminants
4	15	3.5	350	25	contaminants
5	16	4.5	350	25	contaminants
6	16	4.5	350	50	0
7	17	4.5	450	50	0
8	28	4.5	450	50	0
9	27	4.5	500	50	solasodine
10	28	4.5	500	50	solasodine
11	28	4.5	500	50	solasodine
12	28	4.5	500	50	solasodine
13	30	4.5	500	50	solasodine
14	30	4.5	500	50	solasodine
15	30	5.5	500	50	solasodine
16	30	5.5	500	100	solasodine
17	30	5.5	500	100	solasodine
18	46	5.5	500	100	0
19	36	5.5	500	100	0

Each fraction was concentrated under a stream of nitrogen and examined by TLC. The individual "contaminants" were detected by their fluorescence under UV light (366 nm). As shown in Table 1, the "contaminants" were eluted in Fractions 3-5, whereas solasodine was found in Fractions 9-17. Stigmasterol and cycloartenol were eluted ahead of diosgenin and 3β-hydroxy-5,16-pregnadien-20-one, but no attempt was made to increase resolution.

Precision

The precision of the method was tested by both HPLC assay (see above) and gravimetry. Five samples of solasodine, each containing 20 mg in 2 ml dichloromethane, were introduced into the chromatofuge, which was eluted essentially as described in the foregoing section, i.e., with 50 ml 25% and 50 ml 50% ethanol in n-pentane and then with 300 ml ethanol, but the entire ethanol eluate was pooled. A 1%-portion was used for the HPLC assay and the rest for gravimetry. Table 2 shows the results. Although the amounts recovered were higher by gravimetry, the precision of the HPLC assay was greater. The higher amounts found by gravimetry are probably the result of contamination by silica, which is slightly soluble in ethanol.

Accuracy

The accuracy of the method was tested by recovery experiments. Nine equal portions of the "Solanum extract" (see above) were introduced into the chromatofuge. Varying amounts of solasodine had been added to six of them and the total amounts of solasodine were then determined by HPLC in each of the following

TABLE 2

Recovery of 20-mg Portions of Solasodine from the Chromatofuge

Experiment No.	Recovery by HPLC mg	HPLC %	Gravimetry mg	Gravimetry %
1	19.44	97.2	20.6	103.0
2	19.90	99.5	20.0	100.0
3	19.44	97.2	19.5	97.5
4	19.58	97.9	20.0	100.0
5	19.90	99.5	21.5	107.5
Mean	19.65		20.3	
S.D.	± 0.22		± 0.79	

4 fractions from the chromatofuge: 100 ml of 25% and 100 ml of 50% ethanol in n-pentane, 250 ml and 50 ml of 100% ethanol. No solasodine was found in the first and last fraction. The amounts of solasodine in Fractions 2 and 3 were added to give the results shown in Table 3. The asterisks in the Table designate experiments in which the chromatofuge was reused after one sample had already been analyzed. For the remaining experiments the chromatofuge was always freshly packed, as described under Chromatofuge above. Reusing the chromatofuge saves time but decreases the recovery somewhat. In contrast, the HPLC column can be reused for a long time, provided the forecolumn is periodically changed.

TABLE 3

Accuracy of the Chromatofuge-HPLC Combination in Solasodine Recovery

Experiment No.	Added mg	Recovered mg	Percent Recovery
1	0	1.52	100.0
2	0	1.50	98.7
3	0	1.52	100.0
4	1.50	3.05	100.0
5*	1.50	2.82	93.4
6	10.00	9.80	85.1
7*	10.00	9.10	79.0
8	18.75	19.82	97.8
9*	18.75	19.05	94.0

* Chromatofuge packing reused.

Load Capacity

The load capacity of the chromatofuge depends on the size of the spacer, other conditions being kept constant. The largest spacer available is 10 mm high. A determination of the load capacity is meaningless if the recovery is unspecified. The percent recovery will decrease with increasing load.

To test the load capacity with the 3-mm spacer, 500 mg of cholesterol was "spiked" with 51,700 cpm cholesterol-4-^{14}C. After it had passed through the chromatofuge, the radioactivity of the eluted cholesterol was determined. This showed a recovery of 98.8%. When the experiment was repeated with 1.00g of cholesterol, the recovery dropped to 90%.

Conclusions

The chromatofuge-HPLC combination is suitable for the determination of solasodine in a plant extract. However, the amount of solasodine in the plant is not accurately determined by this method because the extraction is not quantitative. We have used an adaptation of conventional methods for the extraction of S. khasianum fruits. This includes the hydrolysis of the glycoalkaloids by boiling the plant material in 2 N hydrochloric acid for 2 hrs (7). None of the hydrolytic methods presently available gives quantitative yields of steroidal alkaloids. Hydrochloric acid converts some of the solasodine into solasodiene, which is always found in the forefractions from the chromatofuge. It should be emphasized that our method does not address the problem of glycoalkaloid hydrolysis.

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