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Note

High-performance liquid chromatographic method for the determination of diosgenin in plants

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Diosgenin is one of the most important and versatile starting materials for the manufacture of steroid drugs, including oral contraceptives. The steroid drug industry in India relies almost entirely on diosgenin as the base material, the chief sources being the yams of the different Dioscorea spp. New sources of diosgenin, such as *Costus speciosus*[1,2] and *Kallstroemia pubescens*[3–5], are being developed for commercial exploitation. Whether it is a Dioscorea tuber or a plant sample to be screened for the presence of diosgenin, or whether it is a cell mass from tissue culture or a plant part from a breeding programme, the diosgenin content must be determined by an accurate and reproducible method. Several procedures are available for the determination of diosgenin, such as gravimetric[6–11], spectrophotometric[12,13], gas–liquid chromatographic (GLC)[14], densitometric thin-layer chromatographic (TLC)[15,16] and IR spectrometric[17] methods. The gravimetric method is widely used on account of its simplicity and inexpensive apparatus. However, it has some disadvantages, *e.g.*, it gives the total sapogenin content and not only diosgenin. The other methods mentioned above also have disadvantages[18]. The GLC method developed by Cooke[14], although it works well with certain Dioscorea spp. where there is no interference from other substances, is unsatisfactory when such interfering compounds are present.

High-performance liquid chromatographic (HPLC) techniques have been reported for determination of various materials, including glycyrrhizin[19,20], cholesterol autoxidation products[21], potato glycoalkaloids[22] and monoterpenes[23]. The separation of diosgenin by HPLC has also been reported[24]. This paper reports an HPLC method for the determination of diosgenin in some plant species.

EXPERIMENTAL

Apparatus

The liquid chromatograph consisted of a Waters Assoc. Model 6000A pump, U6K injector, Model 440 absorbance detector with a fixed wavelength of 254 nm and an R-401 differential refractometer connected to an Omniscribe recorder. The chromatograph contained a Waters Assoc. 30 cm × 3.9 mm I.D. stainless-steel column packed with a μPorasil microparticulate (10 μm) silica gel column. The mobile phase was light petroleum (b.p. 60–80°C)–isopropanol (12:1), the flow-rate 0.8 ml/min, the pressure 125 p.s.i., the temperature 20°C, the attenuation 8 × and the recorder chart speed 1 cm/min.

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Chemicals and reagents

All solvents used were glass-distilled. The diosgenin used for the preparation of the calibration graph was isolated from *Kallstroemia pubescens* and purified by chromatography [m.p. 206–207°C; $[\alpha]_D$ −119° ($CHCl_3$)].

Preparation of standards

A set of ten standard solutions were prepared containing 2.26–0.226 mg/ml of diosgenin and were stored at ambient temperature.

Extraction procedure

Plant samples were dried, either in air or at 110°C in an air oven, and then powdered. Each of the samples (500 mg) was placed in a 25-ml conical flask and hydrolysed with 2.5 *N* hydrochloric acid (7 ml) for 4 h by keeping the flask partially immersed in boiling water, keeping the mouth loosely closed with a stopper. The contents of the flask were cooled, filtered quantitatively using a Whatman No. 41 filter-paper and washed until the residue was free from acid. The washed residue was dried at 105°C and then extracted with light petroleum (b.p. 60–80°C) in a Soxhlet extractor for 4 h. The solvent was evaporated and the residue was dissolved in HPLC-grade light petroleum–isopropanol (12:1) and filtered into a measuring flask using a sample clarification kit (Millipore, Bedford, MA, U.S.A.) consisting of a 10-ml syringe, Swinney filter holder and Millipore filters (0.5 μm). The solution was then made up to a suitable concentration by dilution with the same solvent in a volumetric flask. A 20-μl volume of the solution was injected into the chromatograph with a 25-μl Hamilton syringe.

RESULTS AND DISCUSSION

As diosgenin does not exhibit any UV absorption that would be helpful for detection with the 254-nm UV detector, a differential refractometer was used. With a 30 cm × 3.9 mm I.D. column and a mobile phase flow-rate of 0.8 ml/min the retention time was 347 ± 5 sec for diosgenin based on 20 measurements. The retention time was very sensitive to the light petroleum–isopropanol mobile phase composition. Differences in retention time between batches existed, indicating a slight variation in the composition of the mobile phase from time to time. An internal standard was not incorporated in the assay procedure, because the recoveries were consistent and almost identical with the theoretical recovery.

Of all column and mobile phase combinations tried, the μPorasil column with light petroleum–isopropanol (12:1) proved to be the best because there were no interfering peaks. The line obtained by plotting peak height against concentration was always straight. Calibration graphs were constructed every time a new series of plant material was extracted. A calibration graph covering the range from 2.26 to 0.226 mg/ml of diosgenin is shown in Fig. 1.

For each analysis 500 mg of dried plant sample was used and the method was found to be quantitative and reproducible. The same results were obtained when more than 500 mg of plant material was used. However, less than 500 mg of plant sample gave a slightly low value. Ten samples of *Kallstroemia pubescens*, two samples of *Dioscorea prazeri* and one sample each of *Dioscorea floribunda*, *Dioscorea deltoidea*

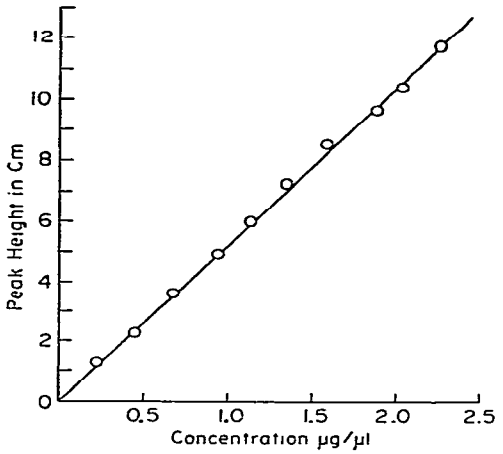

Fig. 1. Calibration graph for diosgenin.

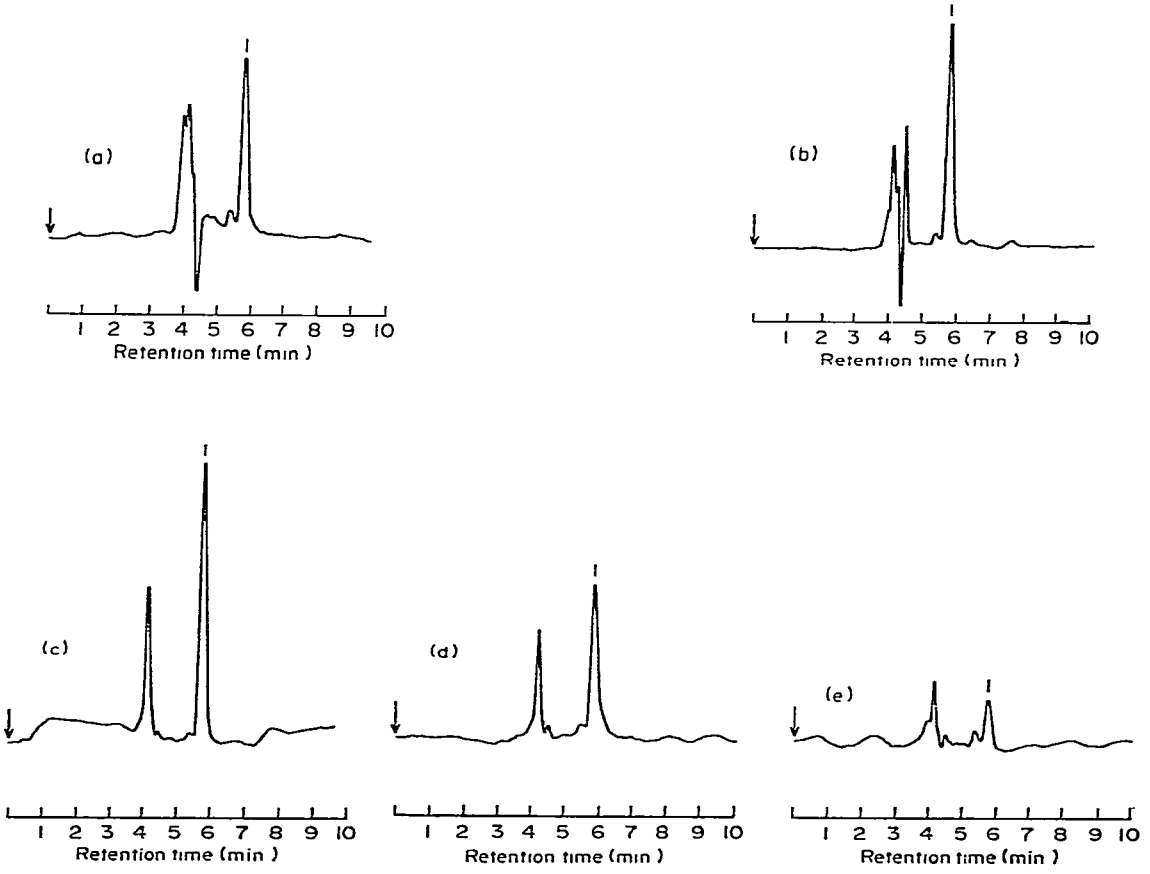

Fig. 2. Representative chromatograms of (a) *Kallstroemia pubescens*; (b) *Dioscorea floribunda*; (c) *Dioscorea deltoidea*; (d) *Dioscorea prazeri*; (e) *Costus speciosus*. Peak 1 = diosgenin.

and *Costus speciosus* were assayed by the proposed HPLC method. Chromatograms of one sample of each species are shown in Fig. 2.

Attempts were made to combine the hydrolysis and extraction stages by heating the plant sample with 2.5 *N* hydrochloric acid and *n*-hexane and working up in the usual way, but much too low values were obtained even after heating for 6 h so these attempts were abandoned.

The results obtained by HPLC were checked against the usual gravimetric determination by using aliquots from a larger sample. A comparison of the values obtained is shown in Table I. As would be expected, the results obtained by HPLC were found lower as diosgenin alone is determined, whereas in the gravimetric method other constituents present in the diosgenin extract are also determined.

TABLE I

COMPARISON OF RESULTS OBTAINED FOR SAMPLES OF *KALLSTROEMIA PUBESCENS* (KP), *DIOSCOREA FLORIBUNDA* (DF), *DIOSCOREA DELTOIDEA* (DD), *DIOSCOREA PRAZERI* (DP) AND *COSTUS SPECIOSUS* (CS) BY HPLC AND GRAVIMETRIC METHODS

Sample	*Diosgenin (%)*	
	HPLC method	*Gravimetric method*
KP-1	0.93	1.04
KP-2	1.14	1.27
KP-3	1.05	1.19
KP-4	0.88	1.01
KP-5	0.88	1.02
KP-6	0.93	1.12
KP-7	1.04	1.08
KP-8	1.06	1.11
KP-9	1.05	1.09
KP-10	1.04	1.21
DF-1	1.42	1.63
DD-1	3.48	3.70
DP-1	0.62	0.65
DP-2	1.92	2.08
CS-1	0.34	0.39

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