Journal of Chromatography, 95 (1974) 250–253

CHROM. 7450

Note

Simultaneous estimation of sterol and steroidal sapogenin in plant extracts by densitometric thin-layer chromatography

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(Received January 31st, 1974)

Various methods have been published for the routine estimation of sterols including colorimetry[1], gravimetric digitonide precipitation[2], IR spectometry[3] and GLC[4]. Steroidal sapogenin has been estimated by direct gravimetry[5,6], colorimetry[7–9], IR spectrometry[10–12], GLC[13] and densitometric TLC[14–16]. The chromatographic methods have the advantages of high sensitivity and specificity and require only small amounts of sample. In the published densitometric methods[14–16] the amount of sapogenin is estimated by comparison with an external sapogenin standard. The present development involves the use of an internal standard to obviate the problems associated with accurate sample application[17] and the need for relatively complex apparatus. It also allows the simultaneous estimation of both sterol and steroidal sapogenin in a crude extract.

Lanosterol was selected as internal standard. It was adequately resolved from sterol and sapogenin in a 10-cm run on 250-μm silica gel G plates developed in *n*-hexane–acetone (4:1). The plates were air-dried for 10 min, sprayed with a saturated solution of antimony trichloride in chloroform, and heated in a forced air oven at

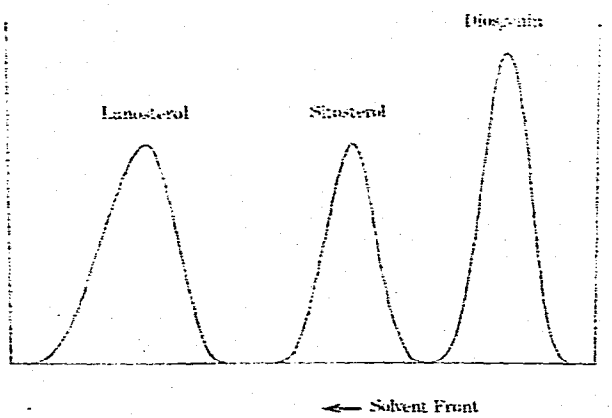

Fig. 1. A densitometric trace of TLC separation of lanosterol (internal standard), sitosterol, and diosgenin. Conditions: log-mode; filter, 477 nm; aperture, 0.25-mm circle; strike length, 10 mm; scan speed, 3 cm/min; chart speed, 20 cm/min; integrator rate, 7.

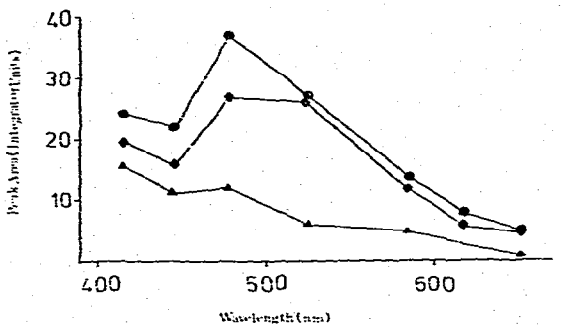

Fig. 2. The effect of filter wavelength on light absorption by sterol, sapogenin, and internal standard spots. ◆—◆, Sitosterol; ●—●, diosgenin; ▲—▲, lanosterol.

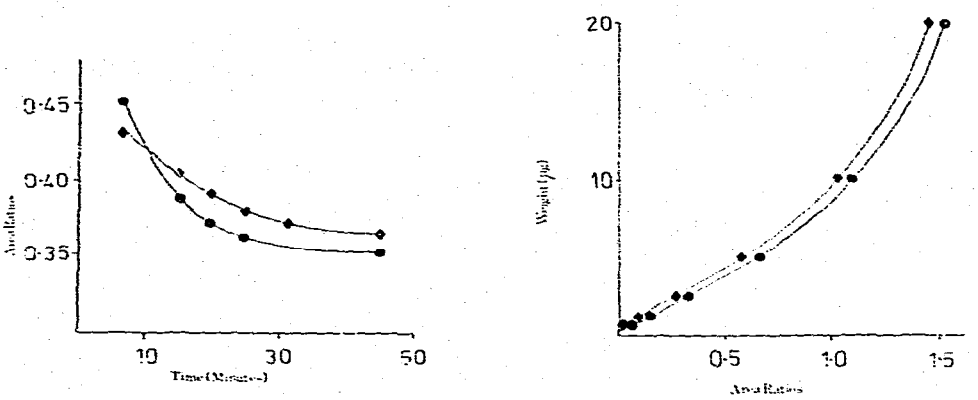

Fig. 3. The effect of time on the ratios of sterol and sapogenin response to that of the internal standard. ●—●, Diosgenin; ◆—◆, sitosterol.

Fig. 4. The relationship between the quantities of sterol and sapogenin and their absorption ratios to the internal standard. ●—●, Diosgenin; ◆—◆, sitosterol.

100° for 8 min (Table 1). The plates were scanned in transmission on a Vitatron Model TLD 100 flying spot scanner and Fig. 1 shows a typical trace. The optimal wavelength for maximum absorption of the three compounds was 477 nm (Fig. 2). The spot intensity faded relatively rapidly at first but it was found that the ratio of the response of the sterol and sapogenin to that of the internal standard stabilised after about 30 min (Fig. 3). Measurements were therefore routinely made 40 min after removal of the plate from the oven. The relationships between sterol and sapogenin concentrations and their absorption ratios to the internal standard are shown in Fig. 4. As the response was not linear, and also varied to some extent from plate to plate, a separate calibration graph was used for each plate.

In the routine procedure adopted the test samples were taken up in 3 ml of chloroform containing 6.67 μg/10 μl lanosterol. Six test samples and six reference samples, each of 10 μl, containing varying ratios of sterol and sapogenin to internal

TABLE I

R_F AND COLOUR RESPONSE OF STANDARDS

Compound	R_F	*Colour response*
Lanosterol	0.53	Brown
Sitosterol	0.43	Purple
Diosgenin	0.35	Red

TABLE II

COMPOSITION OF REFERENCE SAMPLES APPLIED TO PLATES IN THE ROUTINE PROCEDURE

Concentration (μg/10 μl)		
Diosgenin	*Sitosterol*	*Internal standard*
0.5	0.5	6.67
1.0	1.0	6.67
2.5	2.5	6.67
5.0	5.0	6.67
10.0	10.0	6.67
20.0	20.0	6.67

TABLE III

PRECISION OF SIX REPLICATE ESTIMATIONS OF A TEST EXTRACT

Plate	*Standard deviation (%)*	
	Diosgenin	*Sitosterol*
1	1.4	1.5
2	0.9	0.5
3	1.4	1.2
Mean	1.2	1.1

standard (Table II) were applied to each 20 × 20 cm plate and the results were calculated in terms of sitosterol and diosgenin.

The precision of the procedure was calculated from six replicate estimations of a single test solution on each of three plates. Table III indicates that the total error involved was comparable to that found previously (0.95%) for sapogenin using the more lengthy and complex external standard method[16].

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