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Note

A method for the estimation of anthraquinones using densitometric thin-layer chromatography

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Numerous methods for the colorimetric estimation of anthraquinones in drugs have been published[1–5]. Lemli[6] devised a method for the densitometric estimation of the aglycones of some glycosides and later Dequeker *et al.*[7] estimated dianthrone aglycones by densitometry on paper chromatograms. Furuya *et al.*[8] separated the trimethylsilyl ethers of anthraquinones by gas–liquid chromatography and suggested their use for quantitative purposes.

An application of densitometric thin-layer chromatography in the estimation of anthraquinones has recently been reported by Rai *et al.*[9]. This report describes an improved method for the estimation of anthracene-derived constituents —chrysophanol, emodin, rhein and aloe emodin— in plant drugs using alizarin as an internal standard and with concentrations expressed as w/w in the original plant material.

EXPERIMENTAL AND RESULTS

The extracts were prepared by oxidative hydrolysis of the dry plant material, evaporated to dryness under reduced pressure and taken up in 2 ml chloroform containing alizarin (0.02% w/v). The extracts and standard solutions (5 μl) were applied on precoated silica gel N-HR (Camlab) 0.002-mm plates and developed to 15 cm with benzene–ethyl formate–formic acid (75:25:2). The plates were air dried, sprayed with a solution of 5% KOH in methanol (w/v) and re-dried in hot air. The spot characteristics for standard compounds obtained are listed in Table I.

A mixture of all standard solutions was used each time on all plates in six varying dilutions. The amount of alizarin is fixed in all dilutions, and for each 5 μl of sample there is 1 μg alizarin. Chrysophanol, emodin, rhein and aloe emodin were used in the concentrations 0.5, 1.0, 2.5, 5.0, 7.5, and 10.0 μg/5 μl.

A sprayed plate was left aside for 15 min to allow the spot intensities to stabilize (Fig. 1), and then measured in transmission mode using the "Vitatron TLD 100 Universal Densitometer", and using filter 525, which produced optimal light absorption to the standard solutions (Fig. 2). The spots were scanned under the following conditions: Lamp, tungsten; mode log—; C, 4; damping, 2; filter, 525; span, 890; circular aperture, 0.25 mm; strike length, 10 cm; scan speed, 3 cm/min; integrator reading, 6; and recorder speed, 10 cm/min. A typical densitometric trace is

TABLE I

SPOT CHARACTERISTICS OF STANDARD COMPOUNDS

Compound	*Colour (daylight)*	hR_F
Chrysophanol	Orange-pink	73.3
Alizarin	Purple	43.3
Emodin	Orange-pink	34.6
Rhein	Orange-pink	28.6
Aloe emodin	Orange-pink	24.6

shown in Fig. 3. The sinusoidal scanning produces for each spot a peak on the recorder chart. The integrated area under a peak, corrected for the base line drift, quantifies the concentration of the spot.

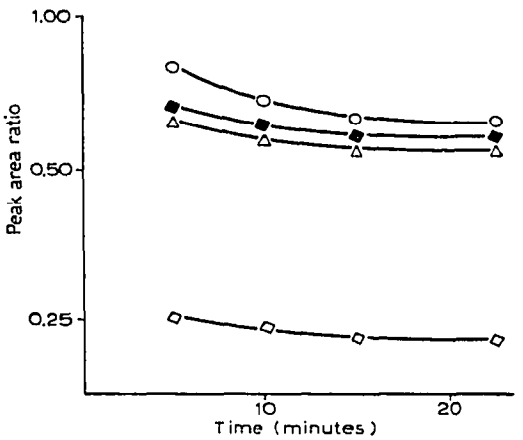

Fig. 1. The effect of time on the ratios of chrysophanol, aloe emodin, emodin and rhein to internal standard alizarin. ○, Alizarin-chrysophanol; ◆, alizarin-aloe emodin; △, alizarin-emodin; ◇, alizarin-rhein.

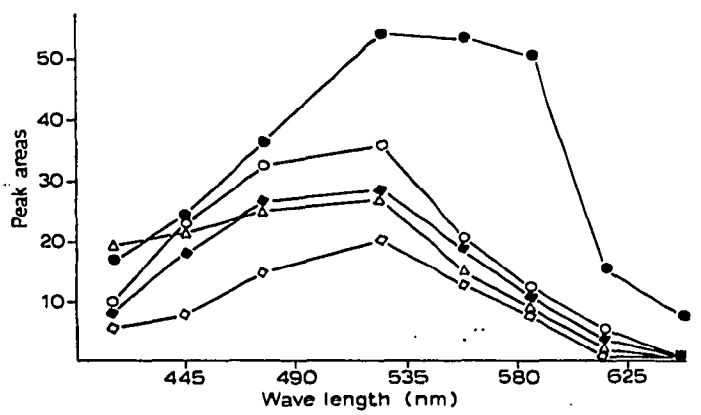

Fig. 2. The effect of filter wavelength on light absorption. ●, Alizarin; ○, chrysophanol; ◆, aloe emodin; △, emodin; ◇, rhein.

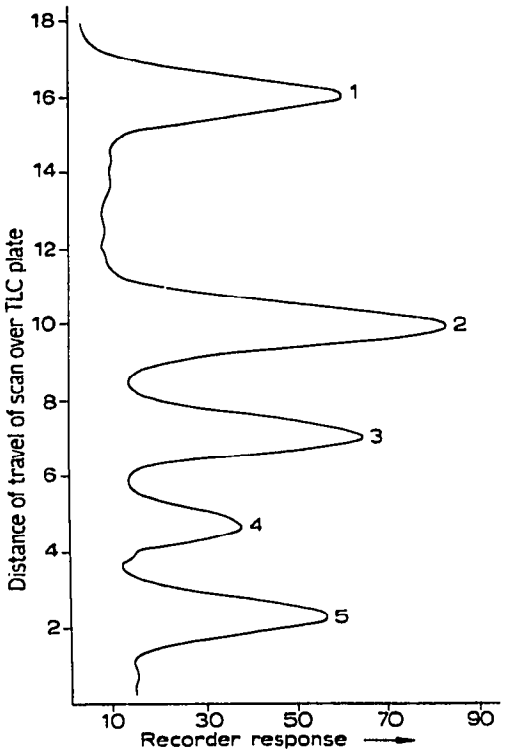

Fig. 3. A densitometric trace of TLC separation of (1) chrysophanol (hR_F = 73.3), (2) alizarin (43.3), (3) emodin (34.6), (4) rhein (28.6), and (5) aloe emodin (24.6). A mixture of 5 μg of each of the named anthraquinones was applied. For the operating conditions, see the text.

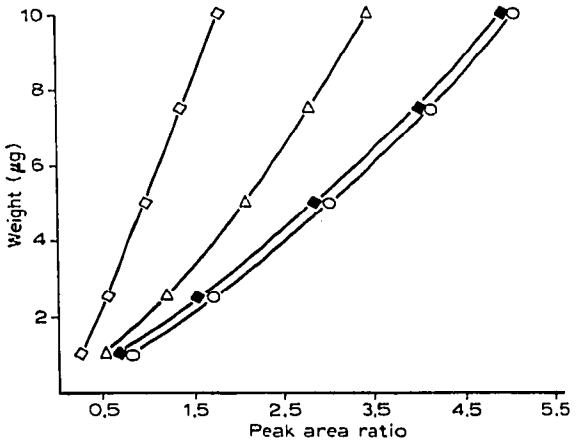

Fig. 4. The relationship between the quantity of chrysophanol (○), aloe emodin (◆), emodin (△), rhein (◇), and their absorption ratios to internal standard.

Calibration curves are plotted for the ratios of peak areas obtained using a fixed amount of alizarin (1.0 μg) and varying amounts of the standard compounds: chrysophanol, emodin, rhein, and aloe emodin. The compounds in the sample extract, which have been made up to contain the same fixed amount of alizarin, may therefore be determined by reference to the calibration figures. Fig. 4 illustrates the normal pattern of the curves obtained. Determination on standard solutions differed from the known value by $\pm$ 4%.

DISCUSSION

Peak areas for a spot, containing the same amount of substance and scanned under identical conditions were found to vary from plate to plate. This variation in integrated peak areas does not permit the adoption of standard concentration (weight μg/peak area) curves for reference compounds. It was necessary therefore to develop this method in which calibration curves for reference compounds are prepared for each plate, thus overcoming the existing variation in peak area response.

Chrysophanol, emodin, rhein, and aloe emodin are common constituents of certain purgative plant drugs. This method allows each compound to be estimated (with reference to the pure compound) and its concentration expressed in terms of w/w dry weight of plant material.

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