CHROM. 3895

THE QUANTITATIVE DETERMINATION OF METHYLENEDIOXY COMPOUNDS BY THIN-LAYER CHROMATOGRAPHY–DIRECT DENSITOMETRY

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(First received November 11th, 1968; revised manuscript received December 3rd, 1968)

SUMMARY

A method for the quantitative analysis of methylenedioxy derivatives is described. The method consists of chromatography on thin-layer plates, application of a chromotropic–sulphuric acid spray reagent and analysis of the resultant characteristic purple spots by direct densitometry. Piperonal, piperine, piperonyl butoxide and safrole were chosen as representative compounds and these could be determined in the gamma range.

The methylenedioxy group is present in a large number of natural and synthetic compounds which have found importance as flavouring agents, perfumes, insecticide synergists and physiologically active agents. Numerous analytical techniques for the determination of members of this general class of compounds exist and these have been mentioned in a recent review which lays special emphasis on chromatographic procedures[1].

As part of a programme for the quantitative analysis of methylenedioxy derivatives in foodstuffs, the use of thin-layer chromatography (TLC) coupled with direct densitometry (DD) was investigated. This combination of techniques appeared attractive because a separation or partial clean-up of a mixture can be achieved on the TLC plate, identification is facilitated by the judicious use of R_F values and quantitation can be achieved directly.

Four representative compounds—piperonal, piperine, piperonyl butoxide and safrole were chosen for study. This paper describes a method that can be employed for the quantitative analysis of these and related compounds in the microgram range.

EXPERIMENTAL

Standard solutions

Piperonyl butoxide (Labelled 100%; Niagara Chemical Division, F.M.C. Corp., Middleport, N.Y.), piperonal (m.p. 36–37°; C.A. Aromatics Corp., Floral Park, N.Y.), safrole (b.p. 232–234°; J. T. Baker Chem. Co., Phillipsburg, N.J.), oil of sassafras

Fritzsche Bros., New York) were all used without further purification at a concentration of 1 mg/ml in benzene. Piperine (m.p. 127–129°; Fritzsche Bros., New York) was used as received at the above concentration in methanol–benzene (1:1).

Thin-layer chromatography

TLC plates (20 × 20 cm) were prepared with a Desaga apparatus at a thickness of 250 μ using Adsorbosil-1 (10 % binder, Applied Science Labs. Inc., State College, Pa.). The finished plates were air-dried for 0.5 h, activated at 90–100° for 1 h, and stored over silica gel. The plates were all used on the day of preparation.

The chromatographic tanks were lined with 3 mm filter paper and were saturated. Solvents were reagent grade and the systems used were: (A) 15 % ethyl acetate in benzene; (B) benzene–*n*-hexane (1:1); (C) 10 % benzene in methanol.

The methylenedioxy compounds were applied onto the TLC plates with lambda micropipettes (1 and 5λ, Drummond Scientific Co., Broomall, Pa.) keeping the spots 1.5–2 cm apart and as small as possible. Spot concentrations were 1, 2, 5, 10 and 20λ. Development in the direction of plate preparation was allowed to proceed till the solvent front reached a marker line 15 cm from the origin. The plates were then air-dried and evenly sprayed with chromotropic–sulphuric acid[2] prepared by the careful addition of concentrated sulphuric acid (15 ml) to a solution of sodium chromotropate (1 g) in water (15 ml). Colour development was achieved by heating the TLC plate at 110-120° for 10–30 min depending on the compounds.

Densitometry

A Photovolt Densitometer (Model 520 M; Photovolt Corp., N.Y.) equipped with a motor-driven TLC stage (2.5 in./min), a Varicord 42B recorder (2.0 in./min) and a Search Unit C was employed. The coloured spots were scanned (transmission densitometry, white light) in a direction at right angles to that of solvent development. The aperture length was adjusted by means of black tape so that it was just long enough to encompass the largest spot in any given run; the width was kept constant at 1 mm. The Search Head was adjusted by means of the levelling screw to just clear the upper surface of the chromatoplate. All readings were taken in a darkened room.

Standard curves

Standard curves were prepared for each plate by plotting the average values of the observed peak areas (peak height × the width at one half the peak height) or observed peak heights *vs.* the amount of material applied. Both square grid and full logarithmic graph paper were used (Figs. 2 and 3).

RESULTS AND DISCUSSION

TLC–DD has been used by a number of workers for the analysis of various compounds[3–5]. DALLAS[6] has recently discussed various factors such as layer thickness and development time, which effect the precision in the densitometry of coloured compounds. While these apply, in general, to colourless materials, one of the prerequisites in this case is the development of reagents which react with the compounds in question to give stable coloured spots with no diffusion of colour to the surrounding adsorbent. The intensity must be reproducible and the absorbance of light by the coloured spot

must show a definite relationship to the quantity of material originally present. In this regard, the use of chromotropic–sulphuric acid was found to be suitable both as a spray and for the preparation of self-indicating impregnated plates[2]. This reagent, which yields clearly defined purple spots on a near colourless background when dilute solutions are spotted, is based on the hydrolysis of the methylenedioxy group to yield formaldehyde and the subsequent reaction of the latter with chromotropic acid to form a characteristic purple complex[7]. Although both detection modes may be employed, only the spray method, which is more generally applicable[2], was used in this study. The reagent has a high degree of specificity (see however refs. 2 and 8) and the resultant chromatograms were stable for 3–5 h when stored in the dark. Normally all densitometric readings were taken immediately after colour development.

Due to the possible occurrence of irregular shaped TLC spots such as crescents etc., it was decided to scan the entire coloured spot. Because of a gradation in size (approx. 2.5–7 mm diam.) caused by the twenty-fold concentration range, the slit length was adjusted to just encompass the largest spot in any given run while the slit width was kept constant at 1 mm. The direction of scan was perpendicular to the direction of solvent flow. This procedure was the most convenient and rapid one as it avoided the necessity of and errors in plate repositioning. In addition the analysis of unknown mixtures was facilitated because, by placing several spots of an unknown beside those of the standard, the plate could be scanned entirely in one pass allowing for the construction of a standard curve and the concomitant analysis of the unknown. Fig. 1 illustrates the results of scanning each of the methylenedioxy compounds used in this study.

Each plate was scanned up to ten times in this manner and both peak heights and peak areas were measured and the average values were plotted directly on square grid and full logarithmic graph paper (Figs. 1 and 3). Although the plots of peak area *vs.* concentration approached linearity more closely than those of the peak height plots,

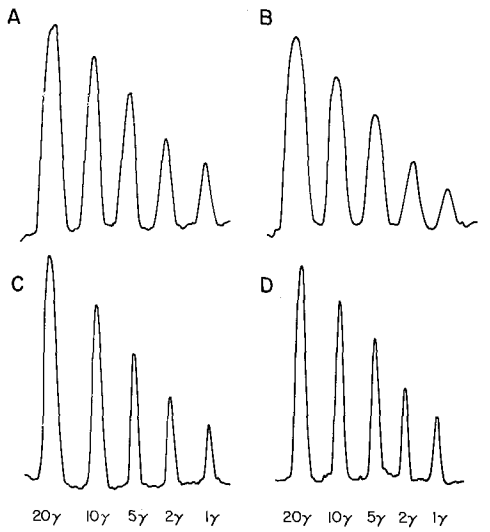

Fig. 1. Densitometric profiles of (A) piperine (plate development in solvent C); (B) safrole (solvent B); (C) piperonal (solvent B); (D) piperonyl butoxide (solvent A).

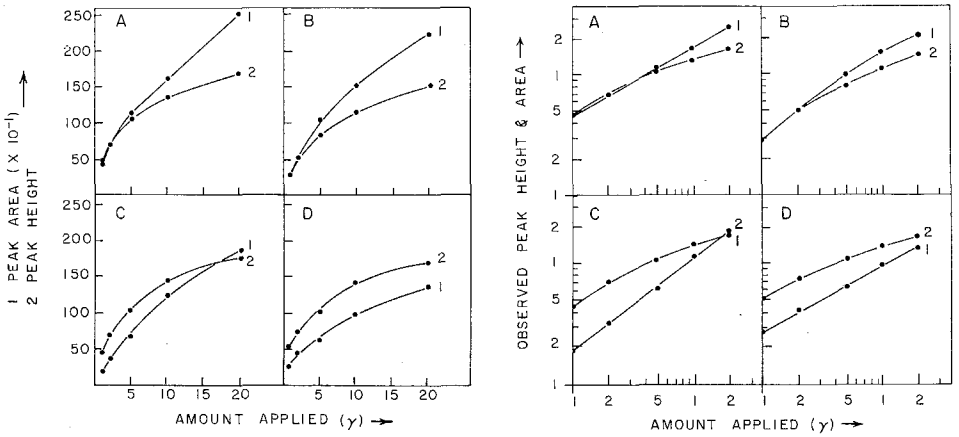

Fig. 2. Standard curves of methylenedioxy derivatives plotted on square grid paper. (A) = Piperine; (B) = safrole; (C) = piperonal; (D) = piperonyl butoxide.

Fig. 3. Standard curves of methylenedioxy derivatives plotted on full logarithmic paper. (A) = Piperine; (B) = safrole; (C) = piperonal; (D) = piperonyl butoxide.

the latter are easier to prepare and there is less error involved in actual measurements. The relative standard deviations pertinent to the determination of the standard curves for the four compounds ranged from 2–4 % for the peak height plots and from 3–7 % for the peak area plots. In practice the choice of which standard curve to employ (square grid or logarithmic plot) would depend on the compound in question.

As an example of this procedure a sample of commercially available oil of sassafras was analysed for its safrole content. Five spots of the standard safrole solution (1–20λ) together with two spots of the essential oil solution (5 and 10λ) were applied onto a TLC plate and the method was carried out as described above. A value of 85 % ($\pm$ 2%) safrole was obtained using the peak height plot on square grid graph paper. Gas chromatographic analysis of this oil indicated a safrole content of 87 % as determined by disc integration.

In general TLC–DD appears to be of potential use for the quantitative analysis of methylenedioxy derivatives. However, care must be exercised in regard to plate preparation, sample spotting and plate spraying. The procedure, as outlined above, is useful up to a range of 20γ. Larger quantities may be applied to extend the standard curve but varying degrees of colour diffusion[2], depending on the compound in question and the developing solvent will probably limit the useful range to less than 100γ.

ACKNOWLEDGEMENTS

The author wishes to thank Mr. T. B. HAND for technical assistance, Dr. M. R. SAHASRABUDHE for helpful discussions and Fritzsche Bros. and C.A. Aromatics for gifts of chemicals.

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