Two improved methods for the separation of 2,4-dinitrophenylhydrazones of carbonyl compounds

During our work the need was felt for a rapid method for the determination of certain carbonyl compounds. We therefore applied the method evolved by HORNER AND KIRMSE[1], which was later modified by GASPARIČ AND VEČEŘA[2]. To accelerate this method the apparatus for centrifugal chromatography, described by PAVLÍČEK AND DEYL[3], was used. At the same time we separated these substances on chromatoplates using the modified technique described by MOTTIER[4].

Standards of the 2,4-dinitrophenylhydrazones of the carbonyl compounds were prepared from aqueous solutions of pure substances by precipitation with 0.5 % solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid.

1. *Separation on chromatoplates*

The chromatographic separation was carried out on a layer of aluminium oxide (degree of activity II–III), which was prepared by coating a glass plate with a layer of 0.8–0.9 mm thickness, according to the procedure described by MOTTIER[4]. Development by the ascending method was employed, the glass plate being inclined at an angle of 15–20° to the horizontal plane. Two different solvent systems were chosen as mobile phases, ether (only once distilled and not dried) and the mixture benzene–hexane (1 : 1). Using a layer of 25 cm length, the time for development is about 15 minutes. Samples in the form of a 2 % solution in chloroform were applied by the following procedure: 0.1 ml was spotted for analysis, or 0.5–0.75 ml as a 4 cm long line for preparative purposes.

The R_F values are given in Table I and the appearance of the chromatogram is shown in Fig. 1.

2. *Centrifugal separation*

Chromatography in a centrifugal field was carried out using Whatman No. 3 paper, impregnated with a 25 % solution of dimethylformamide in ethanol. The chromatographic paper was dried at room temperature by a stream of cold air. Samples of the 2,4-dinitrophenylhydrazones were spotted on the start, which was about 1.5 cm from the centre of rotation. Aliquots of the sample of 10–20 μl were applied on the start in the form of a 2% solution in chloroform.

The chromatograms were developed with cyclohexane, and the tank was saturated with both the mobile and the stationary phases; when a paper disc of 20 cm diameter was used at 20° and 750 r.p.m. the developing time was about 30 min. The overflowing technique was applied with great success for the separation of formaldehyde and acetaldehyde (*i.e.* until the indicating spot of acetone 2,4-dinitrophenylhydrazone reaches the front of the paper, see Fig. 2). This separation takes 50 minutes on an average.

Only those 2,4-dinitrophenylhydrazones were separated whose R_F values have

TABLE I

R_F VALUES OF SOME 2,4-DINITROPHENYLHYDRAZONES OF CARBONYL COMPOUNDS, USING THE CHROMATOPLATE TECHNIQUE

Carbonyl compound	*Solvent system*	
	Ether	*Benzene–hexane (1:1)*
Formaldehyde	0.71	0.36
Acetaldehyde	0.87	0.38
Pentanal	0.90	0.76
Furfural, *cis*	0.86	diffuse
Furfural, *trans*	0.76	zone
Benzaldehyde	diffuse zone	0.40
Acetone	0.86	0.34
Methyl ethyl ketone	0.98	0.45
Cyclohexanone	0.93	0.70
Glyoxal	0.49	0.70
Methylglyoxal	0.46	0.67
Lævulic acid	0.00	0.00

been determined by the ascending technique and partially the descending technique[1,2]. The R_F values obtained with centrifugal chromatography are given in Table II.

Rapid and accurate marking of the front is of great importance in the determination of R_F values. Fig. 3 shows also a typical example of the separation of 2,4-dinitrophenylhydrazones by centrifugally accelerated chromatography. It can be seen

TABLE II

R_F VALUES OF SOME 2,4-DINITROPHENYLHYDRAZONES OF CARBONYL COMPOUNDS, USING DESCENDING AND CENTRIFUGAL DEVELOPMENT

Carbonyl compound	*Descending development*		*Centrifugal development**
	HORNER AND KIRMSE[1]	GAŠPARIČ AND VEČEŘA[2]	
Formaldehyde	0.17	0.20	0.45
Acetaldehyde	0.27	0.32	0.63
Pentanal	—	—	0.92
Heptanal	0.83	0.82	0.94
Furfural, *cis*	—	0.26	0.61
Furfural, *trans*	—	0.12	0.40
Acetone	0.43	0.48	0.67
Methyl ethyl ketone	0.56	0.66	0.83
Cyclohexanone	0.70	0.77	0.93
Laevulic acid	—	—	0.05
Acetoacetic acid ethyl ester	—	—	0.50

* Whatman No. 3 paper.

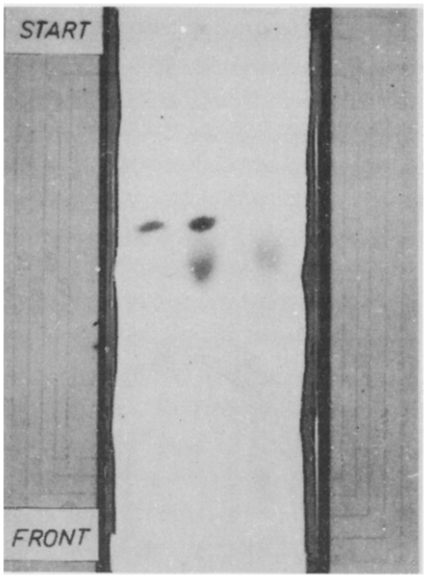

Fig. 1. Chromatogram of a simple mixture of 2,4-dinitrophenylhydrazones. From left to right: formaldehyde, formaldehyde + methyl ethyl ketone (mixture); methyl ethyl ketone.

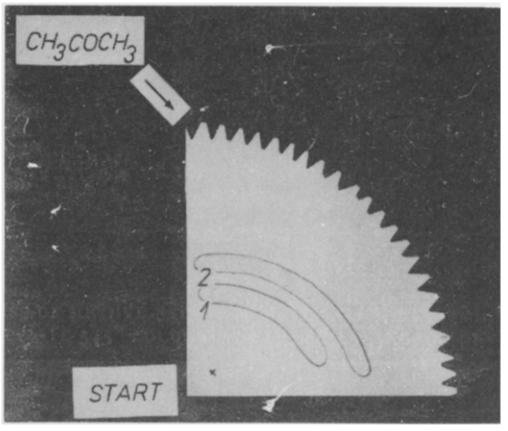

Fig. 2. Separation of a mixture of the 2,4-dinitrophenylhydrazones of formaldehyde (1) and acetaldehyde (2) (overflowing technique).

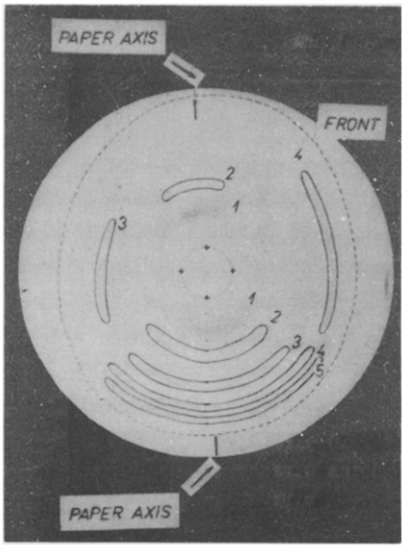

Fig. 3. Separation of a mixture of the 2,4-dinitrophenylhydrazones of some carbonyl compounds, using centrifugal chromatography. (1,2) furfural, *cis* + *trans*; (3) acetone; (4) methyl ethyl ketone; (5) cyclohexanone.

from this figure that the quality of separation is much better than that obtained with modifications of centrifugal chromatography where the separation is based on adsorption.

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BOOK REVIEWS

Chromatographic and Electrophoretic Techniques, edited by IVOR SMITH, William Heinemann Medical Books Ltd., London, 1960; Vol. 1: *Chromatography*, xv + 617 pages, price 65 s; Vol. 2: *Zone Electrophoresis*, viii + 215 pages, price 30 s.

The editor of these two volumes has tried to prepare a practical handbook for chromatographic and electrophoretic methods that contains sufficient details for work without consultation of the literature to be possible. He was aided in this task by 22 authors for chromatography and 8 authors for electrophoresis.

We agree entirely with the editor that it would be highly desirable to have a volume that presents chromatographic methods in the form of cook-book recipes, so that a technician or collaborator may be told to look up a certain method and apply it directly to, for example, amino acids in body fluids. What is questionable at the moment is whether work on chromatography is sufficiently advanced as yet to permit the suggestion, for example of one single apparatus or whether detailed methods can be given for fields where new problems keep coming up every day.

It may be said, however, that the two books succeed to the extent of about 90% in providing good and detailed techniques. It is regrettable however that there are several techniques and whole chapters that look rather perplexing to the reviewer. We shall pick out a few: on page 18 (Vol. 1) it is suggested that samples should be placed on the paper with a platinum loop, and the platinum wire flamed for cleaning. No limitations of this technique are mentioned and the reviewer hates to think