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Paper chromatography of 2,4-dinitrophenylhydrazones. Extension of the Huelin method

During the course of certain studies on the carbonyl components of cigar tobacco smoke, it became necessary to separate these compounds as their 2,4-dinitrophenylhydrazones (DNPH's). For this purpose the paper chromatographic method of HUELIN[1] was selected and used with only slight modifications.

Admittedly, there have been newer methods for 2,4-dinitrophenylhydrazones published since the work of HUELIN and, as MACEK states[2], this method may no longer be in great use. Initially, several of these newer techniques were tried, but they failed to give satisfactory results in our hands. For example, one method[3] failed to give sufficient movement or discrete spots, while another[4] was not reproducible due to lack of information from the authors. The precoated-paper systems[5–7] seemed too troublesome to control. In contrast, the method of HUELIN was found to be very simple, highly reproducible and provided good separation and spot formation.

To establish R_F values under our own conditions, some 25 known DNPH's were investigated. Since HUELIN's paper dealt with only a limited number of DNPH's, it seemed advisable to submit our data as an extension of the original work. These are presented in Table I. Each R_F value listed is the average of several runs on different days. The reproducibility was generally good, varying only a few hundredths, except in the cases where a range is given. The only compound presenting any difficulty was propanal-DNPH, which exhibited extremely poor reproducibility.

The variation between these data and those of HUELIN may be attributed to differences in operating conditions. The temperature was probably the most significant factor in this; HUELIN failed to mention any operating temperature.

In the present work, chromatograms were run in a cylindrical glass jar 22 cm in diameter and 46 cm high. The solvent container at the bottom was a crystallizing dish 15×7.5 cm in size. Whatman No. 1 paper was used in sheets 42×30 cm. Samples were placed along a line 2 cm from the bottom and 3 cm apart. The solvent system

TABLE I

R_F VALUES OF AUTHENTIC 2,4-DINITROPHENYLHYDRAZONES

Ketone-DNPH	R_F	*Aldehyde-DNPH*	R_F
Acetone	0.41	Formaldehyde	0.17
Methyl ethyl	0.55–0.59	Acetaldehyde	0.27
Methyl isopropyl	0.69	Propanal	0.37–0.48
Methyl isobutyl	0.76	Butanal	0.49
Methyl *n*-amyl	0.83–0.87	Heptanal	0.84
Diisopropyl	0.85–0.91		
Di-*n*-propyl	0.94		
Diisobutyl	0.94		
Unsaturated-DNPH	R_F	*Dicarbonyl-DNPH*	R_F
Crotonal	0.41	Acetylacetone (mono)	0.40
2-Hexenal	0.65	Dilevulinic acid (mono)	0.45
2-Heptenal	0.77	Glyoxal (bis)	0.00
2-Nonenal	0.92	Methyl glyoxal (bis)	0.00
Phorone	0.85	Dimethyl glyoxal (bis)	0.00
		Acetonylacetone (bis)	0.00
		3-Hexene-2,5-dione (bis)	0.00

consisted of heptane–methanol (2:1, v/v). (HUELIN used a petroleum fraction boiling between 95° and 105° in place of heptane.). This mixture was shaken and allowed to separate into two phases. At 25°, the upper, heptane-rich phase was 57 %, while the lower, methanol-rich phase was 43 % of the total volume. The upper phase was used for development and the lower phase for equilibration, as prescribed by HUELIN. The remainder of the procedure followed the original work except that the temperature was maintained at 25 ± 1°.

Instead of the usual alkali spray for detection of spots on the completed chromatograms, observation under ultraviolet light was employed. This proved to be a more sensitive technique.

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