

SEPARATION OF 2,4-DINITROPHENYLHYDRAZONES OF CARBONYL COMPOUNDS BY THIN-LAYER CHROMATOGRAPHY

E. DENTI AND M. P. LUBOZ

SORIN, *Centro Ricerche Nucleari, Saluggia (Italy)*

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INTRODUCTION

When carrying out a study on the radiolysis of cyclohexanone, the need for separating and identifying some carbonyl compounds was recognized.

Various methods for separating and identifying aldehydes and ketones by paper, column or thin-layer chromatography are already known from the literature. The last-mentioned technique seems to be the most interesting, being simple and rapid.

Some authors¹⁻⁸ have already described separations of 2,4-dinitrophenylhydrazones (2,4-DNPH) of carbonyl compounds by the technique of STAHL⁹; however, many data on the compounds studied by us, and on the separation of different 2,4-DNPH mixtures obtained in our above-mentioned study, are still lacking.

The problem has been solved by thin-layer chromatography using silica gel and alumina (in some cases with AgNO₃) according to the technique described by BARRET, DALLAS AND PADLEY¹⁰.

METHODS

Preparation of the 2,4-DNPH

The 2,4-DNPH were prepared by the method described by BRADY¹¹ and recrystallized twice from methanol.

Adsorbents

Layers of silica gel G* (I), alumina G* (II), silica gel G* + 25% AgNO₃ (III) and alumina G* + 25% AgNO₃ (IV) were employed.

Layer preparation

Glass plates, 20 × 20 cm, were used. The different layers, 300 μ thick, were prepared with the "Stratomat" apparatus (Chemetron, Milan).

The plates were subsequently treated as follows:

(a) *Layers of I and III.* After drying in air for 5 min, the plates were dried for 30 min in an oven at 110° and stored in a dry-box over CaCl₂ before use. The plates with layers containing AgNO₃ (III) have to be kept in the dark.

(b) *Layers of II and IV.* After drying for 5 min in air the plates were dried for 30 min in an oven at 110° and subsequently deactivated for 12 h by exposing them

* E. Merck A.G., Darmstadt.

to air at room temperature ($\sim 60\%$ relative humidity). The plates carrying a layer containing AgNO_3 (IV) were kept in the dark.

Solvents

All the solvents used were pure compounds, chromatography grade.

For the separations mentioned in this paper the following mixtures proved to be the most useful:

- (I) benzene-petroleum ether (b.p. $40-70^\circ$) (60:40),
- (II) chloroform-petroleum ether (b.p. $40-70^\circ$) (75:25),
- (III) benzene-*n*-hexane (50:50),
- (IV) cyclohexane-nitrobenzene-petroleum ether (b.p. $40-70^\circ$) (30:15:10).

Development

The plates were placed in a Shandon chamber (type 2842) and developed up to about 14 cm from the starting point. The temperature during development was $18-22^\circ$.

RESULTS AND DISCUSSION

Since the R_F values are dependent on the adsorbent and its activity, as well as the eluant, special care was taken to standardize the techniques for layer deposition and activation.

Nevertheless the R_F values obtained show some variations, and the reproducibility of the results is better when the data are defined as a function of the R_F for a 2,4-DNPH of a compound present in the mixtures which is taken as a standard.

The results reported here are relative values, with the R_F of formaldehyde-2,4-DNPH taken as unity.

In Tables I-IV, instead of the R_F values, the R_{for} values are tabulated, where

$$R_{for} = \frac{R_F \text{ of the 2,4-DNPH of the compound listed}}{R_F \text{ of formaldehyde-2,4-DNPH}}$$

Good separations are possible for all the compounds considered by choosing the best combinations of solvents and substrates.

Figs. 1-3 show some separations of 2,4-DNPH mixtures from various aldehydes and ketones.

In the preparation of the 2,4-DNPH from carbonyl compounds in mixtures of varying composition, recrystallization was avoided, in order to avoid losing some of the more soluble 2,4-DNPH. In these mixtures some free 2,4-dinitrophenylhydrazine is present, as can be seen in Figs. 2 and 3.

Figs. 2 and 3 are chromatograms on adsorbents containing AgNO_3 . Sharp black spots may be obtained in this case if the plates are exposed to diffuse (not direct) daylight for about 12 h. Spots containing about 0.2 γ of 2,4-DNPH are easily detectable.

By spraying the plates with a 2% solution of NaOH in 90% ethanol, the spots are seen more easily. In our experiments the spots changed from yellow to a more or less dark brown.

TABLE I

THIN-LAYER CHROMATOGRAPHY ON SILICA GEL G OF SOME 2,4-DNPH DERIVATIVES

2,4-DNPH from	R_{for} (average from 5 determinations)			
	I*	II	III	IV
Formaldehyde	(I)	(I)	(I)	(I)
Propionaldehyde	1.38	1.36	1.37	1.72
Butyraldehyde	1.68	1.41	1.55	2.10
Isobutyraldehyde	1.90	1.63	2.10	2.24
Methyl ethyl ketone	1.50	1.46	1.62	1.95
<i>n</i> -Valeraldehyde	1.92	1.60	2.05	2.41
3-Pentanone	2.00	1.51	2.14	2.34
2-Pentanone	1.76	1.41	1.81	2.25
Cyclopentanone	0.88	1.03	0.98	1.53
<i>n</i> -Caproaldehyde	2.36	1.74	2.22	2.58
α -Methyl- <i>n</i> -valeraldehyde	0.84	0.98	0.90	1.43
4-Methyl-2-pentanone	1.95	1.57	2.05	2.61
2-Hexenal	2.18	1.49	2.08	2.28
5-Hexen-2-one	1.72	1.42	1.67	2.12
Cyclohexanone	1.14	1.44	1.16	2.35
Oenanthaldehyde	2.68	1.79	2.38	2.86
Benzaldehyde	1.85	1.39	1.90	1.65

* The numbers refer to the solvents used.

TABLE II

THIN-LAYER CHROMATOGRAPHY ON ALUMINA G OF SOME 2,4-DNPH DERIVATIVES

2,4-DNPH from	R_{for} (average from 5 determinations)			
	I*	II	III	IV
Formaldehyde	(I)	solvent front	(I)	(I)
Propionaldehyde	1.23	solvent front	1.33	1.17
Butyraldehyde	1.36	solvent front	1.47	1.21
Isobutyraldehyde	1.46	solvent front	1.71	solvent front
Methyl ethyl ketone	1.46	solvent front	1.64	solvent front
<i>n</i> -Valeraldehyde	1.71	solvent front	1.99	solvent front
3-Pentanone	1.67	solvent front	1.95	solvent front
2-Pentanone	1.55	solvent front	1.70	solvent front
Cyclopentanone	1.20	solvent front	1.18	1.39
<i>n</i> -Caproaldehyde	1.54	solvent front	1.68	solvent front
α -Methyl- <i>n</i> -valeraldehyde	1.16	solvent front	1.26	1.21
4-Methyl-2-pentanone	1.68	solvent front	1.91	solvent front
2-Hexenal	1.40	solvent front	1.47	solvent front
5-Hexen-2-one	1.46	solvent front	1.54	solvent front
Cyclohexanone	1.34	solvent front	1.25	solvent front
Oenanthaldehyde	1.57	solvent front	1.79	solvent front
Benzaldehyde	0.96	solvent front	0.93	solvent front

* The numbers refer to the solvents used.

TABLE III

THIN-LAYER CHROMATOGRAPHY ON SILICA GEL G + 25% AgNO₃ OF SOME 2,4-DNPH DERIVATIVES

2,4-DNPH from	<i>R_f</i> (average from 5 determinations)			
	I*	II	III	IV
Formaldehyde	(I)	(I)	(I)	(I)
Propionaldehyde	1.46	1.23	1.73	1.49
Butyraldehyde	1.81	1.22	2.11	1.92
Isobutyraldehyde	1.96	1.60	2.48	2.09
Methyl ethyl ketone	1.43	1.01	1.77	1.51
<i>n</i> -Valeraldehyde	1.90	1.45	2.21	2.17
3-Pentanone	1.83	1.20	2.40	1.92
2-Pentanone	1.73	1.04	1.95	1.84
Cyclopentanone	0.90	0.47	1.03	1.11
<i>n</i> -Caproaldehyde	2.13	1.57	2.49	solvent front
α -Methyl- <i>n</i> -valeraldehyde	0.78	0.64	0.99	0.86
4-Methyl-2-pentanone	2.12	1.07	2.17	2.09
2-Hexenal	1.90	1.16	2.25	1.98
5-Hexen-2-one	0.18	0.20	0.26	0.14
Cyclohexanone	0.98	0.35	1.24	1.15
Oenanthaldehyde	2.46	1.74	2.47	solvent front
Benzaldehyde	1.75	1.10	1.74	1.46

* The numbers refer to the solvents used.

TABLE IV

THIN-LAYER CHROMATOGRAPHY ON ALUMINA G + 25% AgNO₃ OF SOME 2,4-DNPH DERIVATIVES

2,4-DNPH from	<i>R_f</i> (average from 5 determinations)			
	I*	II	III	IV
Formaldehyde	(I)	(I)	(I)	(I)
Propionaldehyde	1.10	1.25	1.20	1.08
Butyraldehyde	1.14	1.10	1.26	1.12
Isobutyraldehyde	1.19	1.40	1.26	1.19
Methyl ethyl ketone	1.12	0.65	1.17	1.08
<i>n</i> -Valeraldehyde	1.18	1.05	1.47	1.17
3-Pentanone	1.22	0.74	1.53	1.10
2-Pentanone	1.19	0.56	1.41	1.06
Cyclopentanone	0.86	0.30	0.75	0.75
<i>n</i> -Caproaldehyde	1.19	1.08	1.37	1.20
α -Methyl- <i>n</i> -valeraldehyde	0.85	0.35	0.87	0.77
4-Methyl-2-pentanone	1.20	0.53	1.35	1.13
2-Hexenal	1.11	0.53	1.25	1.10
5-Hexen-2-one	0.20	0.03	0.13	0.03
Cyclohexanone	0.74	0.15	0.70	0.69
Oenanthaldehyde	1.19	1.20	1.33	1.23
Benzaldehyde	0.95	0.47	0.90	0.99

* The numbers refer to the solvents used.

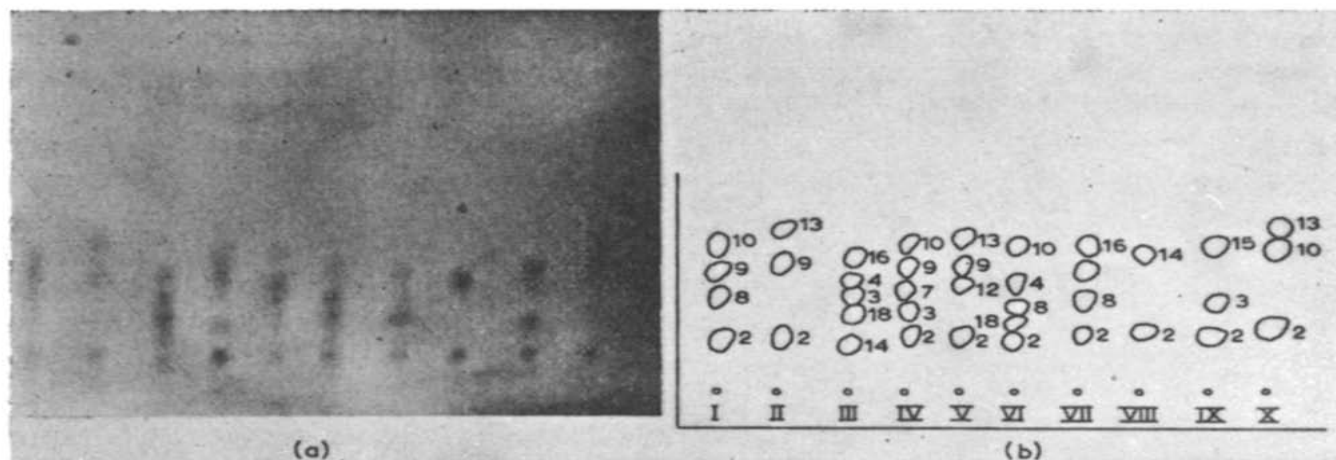


Fig. 1. Separation of 2,4-DNPH mixtures. Adsorbent: silica gel G. Solvent: benzene-petroleum ether (b.p. 40–70°) (60:40). (b). (I) Mixture of 2,4-DNPH from: formaldehyde (2), methyl ethyl ketone (8), 3-pentanone (9), *n*-caproaldehyde (10). (II) Mixture of 2,4-DNPH from: formaldehyde (2), 3-pentanone (9), oenanthaldehyde (13). (III) Mixture of 2,4-DNPH from: cyclopentanone (15), cyclohexanone (18), propionaldehyde (3), butyraldehyde (4). (IV) Mixture of 2,4-DNPH from: formaldehyde (2), propionaldehyde (3), 2-pentanone (7), 3-pentanone (9), *n*-caproaldehyde (10). (V) Mixture of 2,4-DNPH from: formaldehyde (2), isobutyraldehyde (12), 3-pentanone (9), oenanthaldehyde (13). (VI) Mixture of 2,4-DNPH from: formaldehyde (2), cyclohexanone (18), methyl ethyl ketone (8), butyraldehyde (4), *n*-caproaldehyde (10). (VII) Mixture of 2,4-DNPH from: formaldehyde (2), methyl ethyl ketone (8), 5-hexen-2-one (17), 2-hexenal (16). (VIII) Mixture of 2,4-DNPH from: formaldehyde (2), *n*-valeraldehyde (14). (IX) Mixture of 2,4-DNPH from: formaldehyde (2), propionaldehyde (3), 2-hexenal (16). (X) Mixture of 2,4-DNPH from: formaldehyde (2), *n*-caproaldehyde (10), oenanthaldehyde (13).

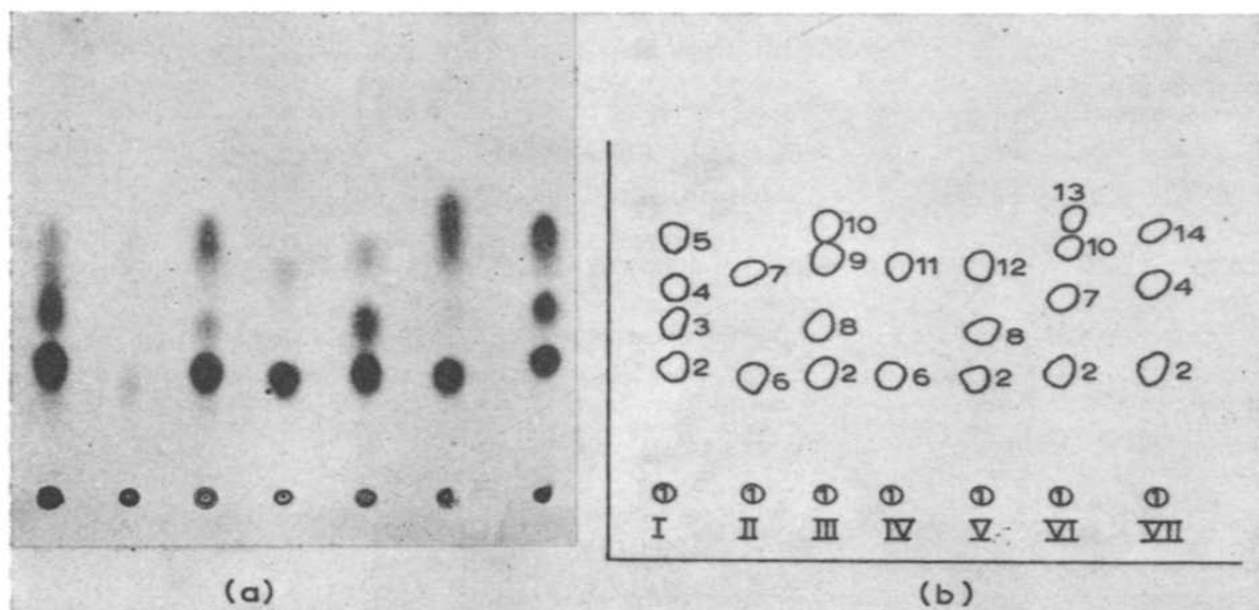


Fig. 2. Separation of 2,4-DNPH mixtures. Adsorbent: silica gel G + 25% AgNO₃. Solvent: benzene-petroleum ether (b.p. 40–70°) (60:40). (b). (I) Mixture of 2,4-DNPH (1), 2,4-DNPH from: formaldehyde (2), propionaldehyde (3), butyraldehyde (4), 4-methyl-2-pentanone (5). (II) Mixture of 2,4-DNPH (1), 2,4-DNPH from: formaldehyde (2), methyl ethyl ketone (8), 3-pentanone (9), 2-hexenal (10). (IV) Mixture of 2,4-DNPH (1), 2,4-DNPH from: α -methyl-*n*-valeraldehyde (6), benzaldehyde (11). (V) Mixture of 2,4-DNPH (1), 2,4-DNPH from: formaldehyde (2), methyl ethyl ketone (8), isobutyraldehyde (12). (VI) Mixture of 2,4-DNPH (1), 2,4-DNPH from: formaldehyde (2), 2-pentanone (7), *n*-caproaldehyde (10), oenanthaldehyde (13). (VII) Mixture of 2,4-DNPH (1), 2,4-DNPH from: formaldehyde (2), butyraldehyde (4), *n*-valeraldehyde (14).

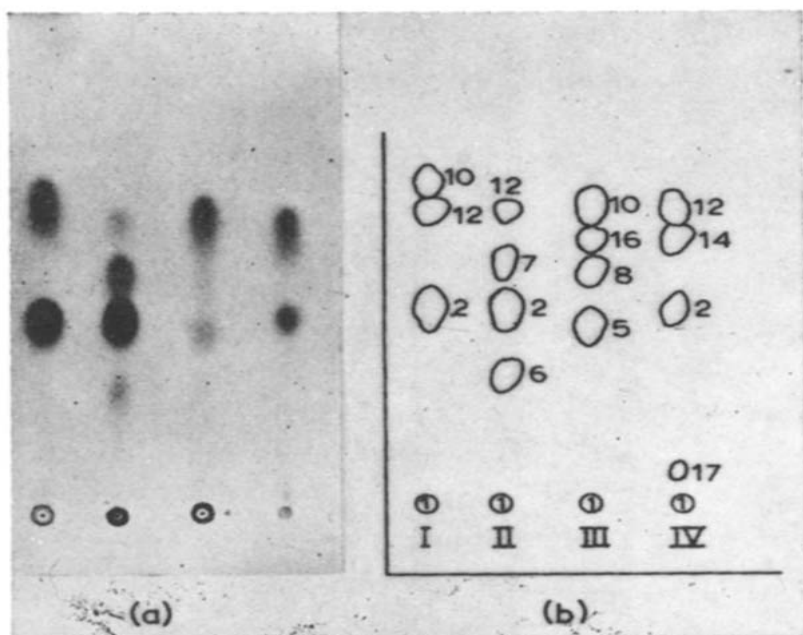


Fig. 3. Separation of 2,4-DNPH mixtures. Adsorbent: silica gel G + 25% AgNO_3 . Solvent: benzene-petroleum ether (b.p. 40-70°) (60:40). (b). (I) Mixture of 2,4-DNPH (1), 2,4-DNPH from: formaldehyde (2), isobutyraldehyde (12), *n*-caproaldehyde (10). (II) Mixture of 2,4-DNPH (1), 2,4-DNPH from: formaldehyde (2), α -methyl-*n*-valeraldehyde (6), 2-pentanone (7), isobutyraldehyde (12). (III) Mixture of 2,4-DNPH (1), 2,4-DNPH from: cyclopentanone (15), methyl ethyl ketone (8), 2-hexenal (16), *n*-caproaldehyde (10). (IV) Mixture of 2,4-DNPH (1), 2,4-DNPH from: formaldehyde (2), *n*-valeraldehyde (14), isobutyraldehyde (12).

The development times of the chromatograms are usually quite short. Good separations of the compounds mentioned here were carried out in 50-150 min, the time being different for the various solvents.

SUMMARY

A method is described for the separation of 2,4-dinitrophenylhydrazones (2,4-DNPH) of aldehydes and ketones by thin-layer chromatography.

The R_F values of various 2,4-DNPH compared with the R_F value of a given compound taken as a standard are tabulated for each adsorbent-solvent system studied.

REFERENCES

- 1 J. H. DHONT AND C. DE ROOY, *Analyst*, 86 (1961) 74.
- 2 K. OKAMI, *Nihon Kagaku Zasshi*, 73 (1952) 337.
- 3 J. ROSMUS AND Z. DEYL, *J. Chromatog.*, 6 (1961) 187.
- 4 C. BORDET AND G. MICHEL, *Compt. Rend.*, 256 (1963) 3482.
- 5 G. M. NANO AND P. SANCIN, *Experientia*, 19 (1963) 323.
- 6 G. M. NANO AND R. SANCIN, *Ann. Chim. (Rome)*, 53 (1963) 677.
- 7 E. F. L. J. ANET, *J. Chromatog.*, 9 (1962) 291.
- 8 G. URBACH, *J. Chromatog.*, 12 (1963) 196.
- 9 E. STAHL (Editor), *Dünnschicht-Chromatographie*, Springer-Verlag, Berlin, Göttingen, Heidelberg, 1962.
- 10 C. B. BARRET, N. S. J. DALLAS AND F. B. PADLEY, *Chem. Ind. (London)*, (1962) 1050.
- 11 O. L. BRADY, *J. Chem. Soc.*, (1931) 756.