THIN-LAYER CHROMATOGRAPHY OF SUBSTITUTED BENZALDEHYDE 2,4-DINITROPHENYLHYDRAZONES

K. THOMAS FINLEY AND ROBERT E. GILMAN
Department of Chemistry, Rochester Institute of Technology, Rochester, N.Y. (U.S.A.)
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INTRODUCTION

Since its introduction 35 years ago, 2,4-dinitrophenylhydrazone (2,4-DNPH) has proven to be generally useful as a derivative for carbonyl compounds[1,2]. Numerous studies of the chromatographic separation of these compounds have been reported in the literature. These include: column[3], paper[4], and gas[5] applications as well as the more recent thin-layer technique[6,7].

Recent interest in the thin-layer chromatography of aromatic aldehyde 2,4-DNPH's[8] prompts us to report our experiments with an extentive series of mono-substituted benzaldehyde 2,4-DNPH's. A further purpose of this work was to examine and evaluate the Eastman Chromagram precoated sheet and sandwich-type developing chamber.

EXPERIMENTAL

Materials

The 2,4-DNPH derivatives of the benzaldehydes included in this study were all prepared by the procedure of SHRINER, FUSON AND CURTIN[9] and were recrystallized until the melting points agreed with the literature values. In most cases methanol was the solvent used, but with the nitro- and amino-substituted compounds tetrahydrofuran was preferable.

Solvents used for spotting, developing, and recrystallizing were Eastman "White Label" and were used without further purification.

The adsorbent employed was silica gel in all cases. The major portion was supplied by Eastman on Chromagram sheet at a thickness of 100 μ. For comparison, a 250 μ layer of Merck Silica Gel G spread on glass plates using STAHL's method was adopted.

All chromatograms were developed with benzene.

Procedure

Since an evaluation of apparatus and conditions was desired in addition to the separations themselves, careful standardization of procedure was rigorously observed[10]. This was especially important in view of the fact that a large number of inexperienced workers were obtaining the data. The following standard procedures were used:

(1) Spots of 3–5 λ (6–10 λ for mixtures) were applied from 0.1 mg per ml solutions in chloroform or tetrahydrofuran so that their diameter did not exceed 3 mm.

(2) Spots were air dried for 15 min.

(3) Desaga tanks were lined with Whatman No. 1 filter paper and the ground glass covers were sealed with silicone grease.

(4) Sandwiches were carefully dried before each development.

(5) Chromagram sheets and glass plates were activated for 30 and 60 min, respectively. Chromagram sheets were stored in a desiccated chamber and glass plates were used immediately.

(6) All development was over a distance of 100 mm.

(7) Spots were located 20 mm from the bottom of the sheet or glass plate. The distance from the edge and between adjacent spots was also 20 mm.

(8) Three spots of a given binary mixture (benzaldehyde and a substituted benzaldehyde 2,4-DNPH) and three spots of each component of the mixture were chromatographed on a 20 × 20 cm sheet or glass plate as illustrated in Fig. 1.

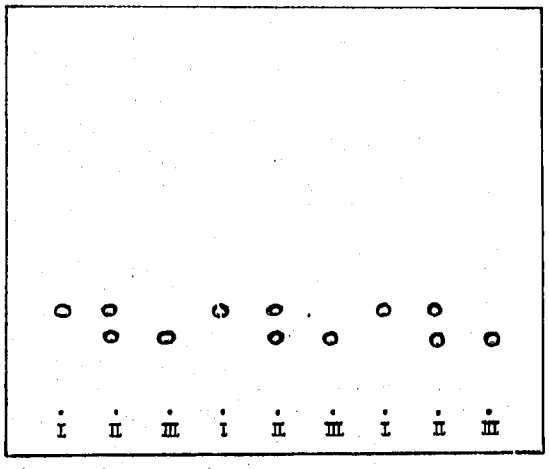

Fig. 1. Separation of 2,4-DNPH mixtures. Adsorbent: silica gel. Solvent: benzene. I = Benzaldehyde 2,4-DNPH; II = mixture of benzaldehyde and 3-methoxybenzaldehyde 2,4-DNPH's; III = 3-methoxybenzaldehyde 2,4-DNPH.

RESULTS

It is generally agreed that a much more useful comparison of chromatographic values is found when they are reported relative to a standard compound[7,8]. For our purposes, the unsubstituted benzaldehyde is most convenient; thus, we define:

$$R_{\text{benzaldehyde}} = \frac{R_F \text{ substituted benzaldehyde 2,4-DNPH}}{R_F \text{ benzaldehyde 2,4-DNPH}}$$

The relative values obtained are presented in Table I. A comparison of the precision of the various apparatus and conditions studied is presented in Table II.

DISCUSSION

Separation of binary mixtures

Ten of the nineteen substituted benzaldehyde 2,4-DNPH's that were chromato-

TABLE I

$R_{benzaldehyde}$ VALUES OF SUBSTITUTED BENZALDEHYDE 2,4-DNPH's

Substituent	*1**	*2*	*3*
4-OH	0.07	0.07	0.09
3-OH	0.09	0.10	0.11
4-CN	0.36	0.37	0.30
4-NO_2	0.51	0.53	0.54
3-NO_2	0.64	0.63	0.63
3-OCH_3	0.73	0.82	0.76
4-OCH_3	0.73	0.78	0.72
4-$N(CH_3)_2$	0.73	0.75	0.72
2-OH	0.78	0.82	0.70
4-$N(C_2H_5)_2$	0.93	0.91	0.87
4-F	0.96	0.98	0.93
4-Cl	0.98	0.97	0.96
4-Br	0.98	1.04	1.11
4-$CH(CH_3)_2$	0.98	1.04	1.06
3-Cl	1.00	1.01	1.00
3-Br	1.00	0.99	1.06
3-F	1.02	1.01	0.96
3-CH_3	1.07	1.00	1.04
4-CH_3	1.07	1.03	1.00

* Apparatus and conditions: (1) tank, benzene, Chromagram sheet, silica gel; (2) Chromagram sandwich, benzene, Chromagram sheet, silica gel; (3) tank, benzene, glass plate, silica gel G.

graphed as components of binary mixtures with benzaldehyde 2,4-DNPH were found to be separable. This may in all cases be attributed to the presence of a polar substituent. It was further found that the methoxy-, hydroxy-, nitro-, cyano-, and amino-compounds all migrate more slowly than benzaldehyde 2,4-DNPH.

The extremely small R_F values for 3- and 4-hydroxybenzaldehyde 2,4-DNPH's are due to the strong hydrogen bonding between the polar group and the silica gel. This effect, as well as the large R_F value for 2-hydroxybenzaldehyde 2,4-DNPH, has been reported in an earlier publication[8]. The influence of the 2-hydroxy group can be easily understood as a consequence of intramolecular hydrogen bonding.

TABLE II

PRECISION OF BENZALDEHYDE 2,4-DNPH R_F VALUES

*Apparatus and conditions**	R_F	±	σ
1	0.45	±	0.022
2	0.74	±	0.033
3	0.46	±	0.028

* For footnote, see Table I.

The conditions used fail to separate all isomeric compounds with the exception of the 2-hydroxy just discussed and the strongly polar nitrosubstituent.

An interesting case is found in the observed results with 4-dimethylamino- and 4-diethylaminobenzaldehyde 2,4-DNPH's. Under the conditions studied, the former is clearly separable while the latter shows an $R_{\text{benzaldehyde}}$ value much closer to 1.00 and in several cases was only partially separated. This may be a result of the greater hydrocarbon character of the diethyl molecule.

The remaining nine compounds, those containing either a halogen or alkyl substituent, all showed R_F values close to that of benzaldehyde 2,4-DNPH and when chromatographed as mixtures were totally inseparable. In early attempts to improve the separations, a substantial number of additional solvent systems were tried. Ethyl acetate, chloroform and tetrahydrofuran, as well as the mixtures benzene–chloroform, benzene–ligroine, and benzene–ethyl acetate were investigated. All of these attempts failed to produce the desired results and in most cases were less satisfactory.

Since at the time of this study Eastman Chromagram sheet was available only with silica gel coating, it was not possible to vary the nature of the adsorbent.

Comparison of apparatus and conditions

The precision of R_F values was determined as the standard deviation of 100 values submitted by 44 different workers. The maximum value of $\pm$ 0.033 obtained in this manner is well within the limits of $\pm$ 0.05 that is generally considered satisfactory. This is true of tanks and sandwiches, Chromagram sheet, and glass plates. Typical results are shown in Table II.

Agreement among a smaller number of determinations (12 to 24) for the various substituted compounds was generally better than $\pm$ 0.02. A given individual was able to reproduce R_F values with still greater precision. In neither instance did more than an occasional average deviation exceed $\pm$ 0.02.

A striking observation is the large difference in R_F values for a given compound in tanks and sandwiches. The large R_F values in the sandwiches result from the inherent difference in the two techniques. The tanks are presaturated with solvent whereas the atmosphere inside the sandwich is saturated by the moving solvent front as it advances. Since more solvent passes through a given spot of compound in the sandwich, the R_F value is larger[11].

The use of the relative value, $R_{\text{benzaldehyde}}$, produced the agreement shown in Table I and discussed above. It should be noted that this is true not only for the comparison of Chromagram sheets in tanks and sandwiches, but includes the values obtained on glass plates as well. This clearly illustrates the greater utility of R_{standard} values as compared to simple R_F values.

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SUMMARY

Benzaldehyde 2,4-DNPH and 19 mono-substituted benzaldehyde 2,4-DNPH's have been chromatographed by thin-layer techniques. Conditions are reported for the separation of ten of the substituted compounds from binary mixtures with benzaldehyde 2,4-DNPH.

Comparisons are reported of Eastman Chromagram precoated sheets with glass plates and of Desaga tanks with Chromagram sandwiches.

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