

## Separation of the semicarbazones of some common aldehydes by thin-layer chromatography

Numerous articles have appeared in the literature on the separation and identification of carbonyl compounds by means of paper, and thin-layer chromatography<sup>1-3</sup>. The usual procedure has been to chromatograph the 2,4-dinitrophenylhydrazones of the carbonyl compounds<sup>4-6</sup>. With some of the aromatic aldehydes, the DNP-hydrazones are difficult to crystallize and purify.

Generally, carbonyl compounds ( $> 5C$ ) react readily with semicarbazide to yield crystalline derivatives that are easily separated and purified from the reaction mixture. PRILL AND FISHER demonstrated the potential use of paper chromatography in separating aromatic aldehydes as their semicarbazones<sup>7</sup>. The lack of a suitable chromogenic reagent for semicarbazones in micro-quantities has limited the use of this carbonyl derivative for paper and thin-layer chromatography. The purpose of this communication is to describe a procedure for the thin-layer chromatography of some common aldehydes as the semicarbazone.

Glass plates (200 × 200 mm) were prepared by mixing 60 g of Silica Gel G and 62 ml of distilled water. The mixture was shaken for 90 seconds and applied to the plates with a Desaga-Brinkman adjustable applicator to a thickness of 0.25 mm. When NaOH plates were prepared, 0.5 N NaOH was substituted for distilled water in preparing the Silica Gel slurry<sup>8</sup>. The coated plates were dried in an oven at 105° for 2 hours. The base line was fixed at a distance of 20 mm from the edge of the plates, and the compounds were applied in a methanol solution by means of a lambda pipette. The distance between samples on the same plate was approximately 10 mm. The chromatograms were developed in one dimension in a glass tank lined with filter paper at room temperature. A line was drawn at an arbitrary distance of 160 mm above the base line, and the plates were left in the solvent system for 115 min. After the plates were removed from the developing chamber, they were air-dried at 105° for 1 h. The chromogenic agent used was 10 % phosphomolybdic acid in 50 % ethanol<sup>9</sup>. After being sprayed lightly, the plates were heated at 105° to develop color.

When solvent system  $S_9$  was employed, interfering impurities in the adsorbent necessitated pretreatment of the plates with ethanol-chloroform-water (10:10:1). After the solvent was allowed to migrate to within 8-10 mm of the top edge, the plate was dried at 105° for 30 min, then scribed and spotted as described above.

Best results were obtained by spotting minimum detectable quantities and restricting the spray to a light coat of very fine mist. Colors usually develop within 30 min upon heating to 105°, and they vary as shown in Table II. Allowing the plates to stand at room temperature at least 24 h after color development concentrates some of the spots and improves color variation.

Impurities in the adsorbent caused no interference with solvent systems  $S_1$  through  $S_8$ . In order to develop the maximum color of the semicarbazones on the chromatoplates that were developed in solvent systems containing N,N-dimethylformamide, it was usually necessary to heat the chromatoplates for several hours after spraying with phosphomolybdic acid.

The  $R_F$  values presented in Table I in conjunction with the color development given in Table II demonstrate the potential use of thin-layer chromatography as a means of separating and identifying aldehydes as the semicarbazone.

TABLE I

 $R_F$  VALUES ( $\times 100$ ) OF THE SEMICARBAZONES OF SOME COMMON ALDEHYDES

	$R_F \times 100$										
	$S_1^b$	$S_2^b$	$S_3^b$	$S_4^n$	$S_5^n$	$S_6^b$	$S_6^n$	$S_6^b$	$S_7^b$	$S_8^n$	$S_9^n$
Anisaldehyde	35	45	47	56	52	52	15	10	50	78	58
Benzaldehyde	41	46	49	56	58	58	19	13	55	78	65
<i>o</i> -Chlorobenzaldehyde	53	52	54	60	69	68	22	—	66	76	69
<i>p</i> -Chlorobenzaldehyde	41	43	48	55	61	59	20	13	54	78	67
<i>p</i> -Nitrobenzaldehyde	36	39	45	51	55	52	14	8	51	76 <sup>t</sup>	63
Cinnamaldehyde	43	44	49	56	63	59	20	13	57	77	65
Furfural	38	36	41	—	56	52	16	9	52	72	60
Phenylacetaldehyde	45	46	48	58	66	63	23	16	57	74	64
Crotonaldehyde	43	40	42	52	65	61	24	15	56	72	65
Salicylaldehyde	12	28 <sup>t</sup>	27	29	45 <sup>t</sup>	34 <sup>t</sup>	9	3	32 <sup>t</sup>	70 <sup>t</sup>	48
Semicarbazide·HCl	12 <sup>t</sup>	6 <sup>t</sup>	3 <sup>t</sup>	9 <sup>t</sup>	10 <sup>t</sup>	9 <sup>t</sup>	1 <sup>t</sup>	1 <sup>t</sup>	7 <sup>t</sup>	10 <sup>t</sup>	7 <sup>t</sup>

<sup>b</sup> Basic Silica Gel G.<sup>n</sup> Neutral Silica Gel G.<sup>t</sup> Tendency to streak. $S_1$  Benzene-*N,N*-dimethylformamide (3:1). $S_2$  Benzene-ethanol-chloroform (2:1:1). $S_3$  Benzene-ethanol-ethyl acetate (2:1:1). $S_4$  Benzene-ethanol-chloroform (1:1:1). $S_5$  Cyclohexane-*N,N*-dimethylformamide-chloroform (1:1:1). $S_6$  Cyclohexane-*N,N*-dimethylformamide-chloroform (4:1:1). $S_7$  Benzene-*N,N*-dimethylformamide-ethyl acetate (1:1:1). $S_8$  Cyclohexane-*N,N*-dimethylformamide-ethyl acetate (1:1:1). $S_9$  *n*-Butanol-acetic acid-water (4:1:1).

TABLE II

LIMITS OF IDENTIFICATION OF SEMICARBAZONES

Compound	Limit of identification ( $\mu$ g)	Color
Anisaldehyde	6	medium blue
Benzaldehyde	6	medium blue
<i>o</i> -Chlorobenzaldehyde	20	medium blue
<i>p</i> -Chlorobenzaldehyde	15	medium blue
<i>p</i> -Nitrobenzaldehyde	12	light blue
Cinnamaldehyde	20	brown
Furfural	6	brown
Phenylacetaldehyde	6	dark blue
Crotonaldehyde	15	blue-gray
Salicylaldehyde	12	blue-gray
Semicarbazide·HCl	30	dark blue

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## **A simple apparatus for holding paper chromatograms during equilibration and development**

The variation in  $R_F$  values encountered when reversed-phase paper chromatograms are developed before coming to equilibrium<sup>1</sup> led us to construct an apparatus for holding the undeveloped chromatograms in a saturated atmosphere for some hours, enabling us to lower the chromatograms into the solvent without taking off the lid of the tank. The device is suitable for ascending chromatograms only. It consists of a frame that fits into the standard (22 × 22 × 10 cm), rectangular, glass chromatography tanks<sup>2</sup> and will allow the simultaneous equilibration of two papers. Although it is possible to develop more sheets simultaneously on conventional frames in larger tanks<sup>3</sup> the atmosphere in these may not be so evenly saturated because the end chromatograms are adjacent to the paper lining the walls of the tank while the middle papers are between other, unequilibrated sheets.

The apparatus (Fig. 1) consists of a stainless steel or aluminium frame (depending on the solvents used) with adjustable feet that can compensate for any irregularity in the floor of the glass tank. Three lateral bars hold the structure rigid and prevent the paper lining the tank from touching the chromatograms should it become detached from the walls. The centre holes in the end sections hold a glass rod carrying a sheet of paper saturated with solvent.

The chromatograms are suspended by small wire loops from a narrow glass tube (0.5 cm diam.), a strong cylindrical magnet (0.3 cm diam.) being fitted into each end with like poles towards the centre. The glass tubes should be as long as possible without the magnets touching the walls of the tank. Soda glass tubes are preferable

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