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## Note

# Thin-layer and high-performance liquid chromatography of biologically active agents

## II. Semicarbazones

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In this paper we describe a convenient thin-layer chromatographic (TLC) method for the separation and identification of semicarbazones, which is also suitable for testing thiosemicarbazones[1], and an high-performance liquid chromatographic (HPLC) separation of some semicarbazones.

Di Modica and Spriano[2] separated aliphatic semicarbazones by partition chromatography. Priel and Fisher[3] used semicarbazones as reagents in order to separate aromatic aldehydes. Camp and O'Brien[4] separated the semicarbazones of some common aldehydes by TLC. Tumlinson *et al.*[5] reported a gas–liquid/thin layer chromatographic technique for the identification of carbonyl compounds which involves formation of 2,4-dinitrophenylsemicarbazone derivatives.

The semicarbazones are of interest because of their use as herbicides and in the identification of aldehydes[6] and ketones. Pilgram[7] demonstrated that several semicarbazones obtained from aldehydes are effective as herbicides. Ogura *et al.*[8] reported UV data for semicarbazones and thiosemicarbazones with aliphatic aldehydes, which act as antibiotics.

## MATERIALS AND METHODS

The semicarbazones were synthesized according to the method of Shriner and Turner[6].

### *TLC*

Thin-layer chromatographic plates were prepared from silica gel G (SMI, octadecyl silane, 20 cm × 20 cm, thickness 0.25 mm) according to the manufacturer's instructions. The silica gel G was air-dried, activated at 110°C for 3 h and stored in a desiccator. The solvent systems comprised benzene–chloroform–methanol (9:3:2) and chloroform–methanol (3:1).

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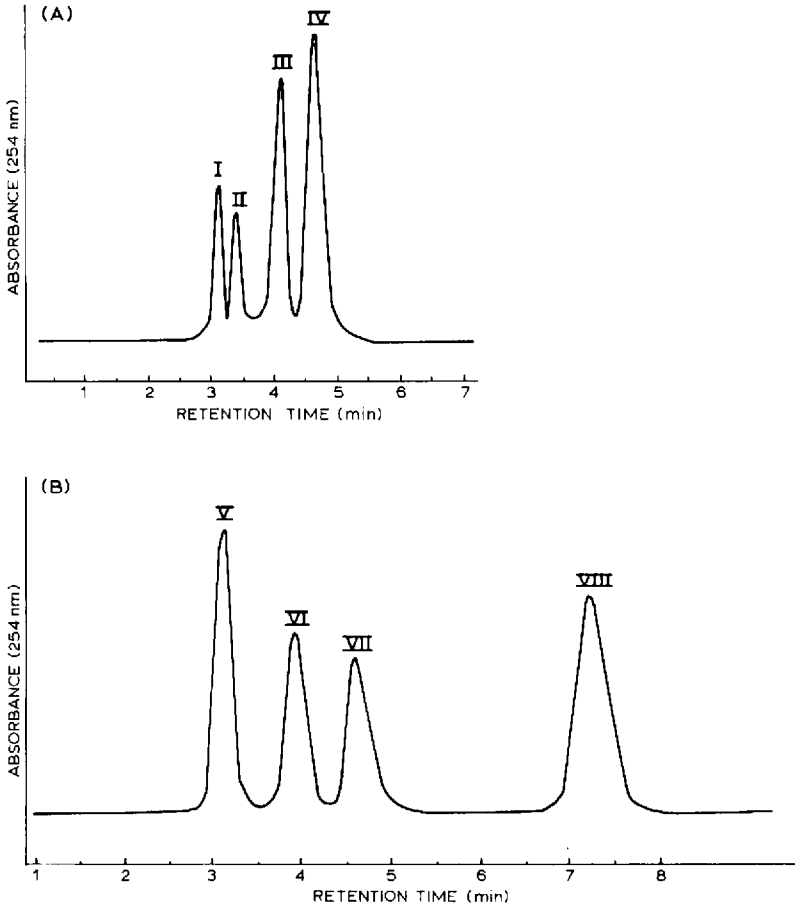


Fig. 1. Chromatograms of some semicarbazones. HPLC conditions as described in the Experimental section. Peaks: (A) I = Sc *p*-HO-Bz; II = Sc Fur; III = Sc Bz; IV = Sc Acetophenone; (B) V = Sc *m*-HO-Bz; VI = Sc 2-Butanone; VII = Sc Cyclohexanone; VIII = Sc *o*-HO-Bz.

A 1% methanolic solution of each compound was prepared and 1 $\mu$l (corresponding to 10 $\mu$g of each compound) was spotted 2.0 cm from the edge of the plate. The solvent was allowed to migrate 15 cm from the starting line. The chromatograms were developed at room temperature (20 ± 3°C) in a normal chromatographic chamber presaturated with the solvents for at least 30 min. Two different spray reagents were used. One was ferric chloride in butanol (1%, w/v); in this case, heating at 110°C was required. The other was a sensitive reagent, 2,4-dinitrophenylhydrazine dissolved in ethanol (3%, w/v).

The reversed-phase (RP) TLC solvent systems comprised water–acetonitrile, water–methanol and water–tetrahydrofuran (10:90, 25:75, 50:50).

*HPLC*

HPLC was carried out with a MCHS Varian liquid chromatograph equipped

TABLE I

COLOUR REACTIONS AND ULTRAVIOLET SPECTRA OF SEMICARBAZONES

Sc = Semicarbazone; Bz = benzaldehyde; Thph = thiophenecarbaldehyde; Fur = furaldehyde; AcNaph = acetylnaphthalene. Solvents: A = benzene–chloroform–methanol (9:3:2); B = chloroform–methanol (3:1). $D_1$ = 2,4-dinitrophenylhydrazine; $D_2$ = ferric chloride. Colours: Y = yellow; Br = brown; R = red; P = pink; Or = orange; L = light. Each $R_F \cdot 100$ value represents the mean of five determinations. Absorbent: silica gel G.

| *Semicarbazone* | $R_F \cdot 100$ *in solvent* | | *Colour reaction* | | *Ultraviolet spectra* | |
|---|---|---|---|---|---|---|
| | *A* | *B* | $D_1$ | $D_2$ | $\lambda_{max.}$ | *log ε* |
| Sc Bz | 54 | – | Y | Y | 282 | 4.29 |
| Sc *o*-OH-Bz | 40 | – | Y | Y | 310/286/278 | 4.12/4.26/4.32 |
| Sc *m*-OH-Bz | 35 | – | Y | Y | 280 | 3.96 |
| Sc *p*-OH-Bz | 31 | – | Br-R | Br-R | 320 | 4.45 |
| Sc 2,3-diOH-Bz | – | 68 | Or | Or | 314/288 | 4.38/4.12 |
| Sc 3,4-diOH-Bz | – | 82 | Y | Or | 286 | 4.36 |
| Sc 2,4-diOH-Bz | – | 88 | Y | Or | 312/284/236 | 4.24/4.22/4.08 |
| Sc 3,4-diOH-5-$OCH_3$-Bz | 20 | – | Or | Or | 306/238 | 4.24/4.13 |
| Sc Fur | 37 | – | R | R | 292 | 4.39 |
| Sc 2-Thph | 51 | – | Or | Or | 304 | 4.21 |
| Sc Acetophenone | 79 | – | Or | LY | 268 | 4.12 |
| Sc 5-$NO_2$-2-Fur | 37 | – | Y | Y | 360/259 | 4.01/3.98 |
| Sc Cyclohexanone | 78 | – | Y | LBr | 240 | 3.59 |
| Sc 9-Anthraldehyde | 75 | – | Br-R | Br-R | 383/364/348/254 | 3.86/3.84/3.65/4.72 |
| Sc AcNaph | 62 | – | Y-R | LY | 304/290/260/238 | 4.35/4.37/4.41/4.34 |
| Sc Acetone | 71 | – | Y | LBr | 236 | 3.39 |
| Sc 2-Pentanone | 64 | – | Y-R | LP | 238 | 3.45 |
| Sc Benzophenone | 87 | – | Y | Y | 278 | 4.06 |
| Sc Acetaldehyde | 52 | – | P | P | 244 | 2.54 |
| Sc 2-Butanone | 62 | – | Y | LBr | 236 | 3.42 |
| Sc Methyl isopropyl ketone | 68 | – | Y-R | LP | 238 | 3.40 |

with a variable-wavelength UV detector. An octadecyl silane reversed-phase column with 5-$\mu$m particles was used (30 cm × 0.4 cm). The mobile phase was water–acetonitrile (45:55) at a flow-rate of 1 ml/min (3000 p.s.i.). All operations were carried out at 35°C. The working solutions contained 1–5 mg of each substance in 2 ml of acetonitrile. Samples of 50 $\mu$l were injected and the peaks were detected at 254 nm.

## RESULTS AND DISCUSSION

The chromatographic results on silica gel G are shown in Table I. Each $R_F \cdot 100$ value represents the mean of five determinations. Each series of determinations showed only slight variations within the limits of experimental error.

The detection limit was found to be *ca.* 1 $\mu$g for each compound. The spray, 2,4-dinitrophenylhydrazine, located the semicarbazone spots unequivocally and did not require heating.

The convenience of polyamide as an adsorbent was examined but incomplete separations were obtained. Sharp spots free from tailing were found only on silica gel G.

The $R_F$ values obtained in TLC on silica gel G were adequate for the separation and identification of these compounds.

RP-TLC using octadecyl silica as stationary phase was not an adequate method because the $R_F$ values were very similar.

Fig. 1 shows a typical example of the HPLC elution pattern for some semicarbazones.

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