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INDUSTRIAL HYGIENE AIR MONITORING OF PHENYLHYDRAZINE*

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SUMMARY

Two gas chromatographic (GC) methods for the analysis of phenylhydrazine have been examined with phenyl- and *p*-chlorophenylhydrazine and a new method using thin-layer chromatography (TLC) was developed. The NIOSH GC method (2-furalphenylhydrazone derivatization), which is classified as "proposed", was found to yield two derivatization peaks, which could only be separated with capillary columns. The other GC method (acetone phenylhydrazone derivatization) gave a single peak, however, of limited stability. In the newly developed TLC method the fluorescamine derivative of phenylhydrazine is separated and detected either visually or with a TLC–fluorescence scanner after fluorescence enhancement with paraffin wax. With ordinary TLC techniques the quantification of phenylhydrazine was possible down to 200 pg per spot. The sensitivity of the TLC method is generally sufficient for the requirements of industrial hygiene air monitoring of phenylhydrazine.

INTRODUCTION

Phenylhydrazine is used in the chemical industry in various syntheses, *e.g.*, of dyestuffs and pharmaceuticals. In order to protect workers from its undesirable biological effects (mainly anaemia) the American Conference of Governmental Industrial Hygienists (ACGIH) established in 1956 a threshold limit value (TLV) of 5 ppm (22 mg/m^3) for industrial exposure. This value has been adopted and retained in most national TLV lists, although in 1978 the National Institute for Occupational Safety and Health (NIOSH) proposed to lower this value to 0.14 ppm (0.6 mg/m^3) because of the possible carcinogenicity of phenylhydrazine[1]. Based on the presently available data, it would be prudent to consider phenylhydrazine and its derivatives as potential weak carcinogens[2].

Only a few methods for the industrial hygiene air monitoring of phenylhydrazine can be found in the literature. NIOSH has published a sampling and analysis method, which is based on the collection of phenylhydrazine from air on sulphuric acid-coated silica gel tubes, desorption and derivatization with 2-furaldehyde, extrac-

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tion of the derivatives into ethyl acetate and gas chromatographic (GC) quantification[3]. The method is classified by NIOSH as "proposed" because of limited experience of its reliability and field applicability (proposed: a new unproven or suggested method not previously used by industrial hygiene analysts but which gives promise of being suitable for the determination of a given substance). In another, older method published by a Russian author, phenylhydrazine is sampled by impinger collection in aqueous solution, and quantified spectrophotometrically at 500 nm after colour development with $(NH_4)_2S_2O_8$ and pyramidone solutions[4]. The specificity and sensitivity of this method with respect to other contaminants present in industrial air is not known, but is expected to be lower than that of the NIOSH GC method. An alternative method for the detection of traces of phenylhydrazine has been pointed out by an industrial hygiene group at Los Alamos, who developed a spot test using fluorescamine for fluorescence detection of phenylhydrazine[5]. Colorimetric detection of phenylhydrazine has also been described using phosphomolybdic acid to form a molybdenum blue complex[6]. However, this method is not specific and other hydrazines are also indicated.

Other methods, which could possibly be applied to phenylhydrazine, have been published for hydrazine. In one method hydrazine is derivatized with acetone and the resulting azine determined by GC[7]. This method has already been successfully used for the control of phenylhydrazine production[8]. In another method 2,4-pentanedione has been used to form substituted pyrazole derivatives, which were analyzed by GC[9]. Electron capture detection of the derivative formed with pentafluorobenzaldehyde has been used for detection of hydrazine in cigarette-smoke[10]. However, no data are available on the performance of these methods with respect to industrial hygiene air monitoring of phenylhydrazine.

The purpose of the present paper is three-fold:

(a) to test the proposed NIOSH method[3],

(b) to test the acetone derivatization GC method[7,8],

(c) to develop further the fluorescence detection method[5] using thin-layer chromatography (TLC) as an additional analytical tool, using as test compound either phenylhydrazine or *p*-chlorophenylhydrazine.

EXPERIMENTAL

Instrumental methods

For the GC determinations a Varian Vista 44 gas chromatograph was used, together with packed glass columns and on-column injection or Pyrex glass capillaries with a glass split injector. The capillaries were coated by the dynamic method after thermal deactivation at 500°C. The chromatographic peaks were detected either with a flame ionization detector (FID) or with a thermionic specific detector (TSD). In all cases nitrogen was used as a carrier gas. Quantification of peaks was by electronic peak area integration. If necessary, the raw data of the chromatograms were stored on diskettes.

For TLC analysis, commercial silica gel plates (thickness 0.25 mm) were used with and without fluorescence indicator (Merck). The samples were spotted manually using disposable 5-μl micropipettes (CAMAG). The plates were developed in a conventional tank according to standard techniques with no further precautions. Fluo-

rescence detection was carried out either visually with a UV lamp (Kontron) at 366 nm or instrumentally using a Zeiss Scanner and a strip chart recorder. Quantification was by peak height estimation.

Air sampling methods

For the sampling of phenylhydrazine from air the NIOSH sampling method using sulphuric acid-coated silica gel tubes is recommended[3]. For phenylhydrazine salts, *e.g.*, the hydrochloride, glass fibre filters (diameter 37 mm, Millipore) were used in conjunction with an ordinary membrane pump (Reciprotor) or an explosion-proof model (Charles Austen) and a gas volume counter. The samples were worked up as soon as possible due to stability problems of phenylhydrazine in air. If necessary the samples were stored frozen at −20°C in the dark together with corresponding standard samples in order to be able to estimate eventual decomposition losses.

Analysis methods

Chemicals used for analytical purposes were either of analytical grade or checked for purity with the appropriate method (GC or TLC). For the 2-furaldehyde method the derivatization procedure outlined by NIOSH was followed[3]. In the acetone derivatization method[7] the silica gel or filter was extracted with acetone–ethanol (2:8) and injected into the gas chromatograph after standing for *ca.* 30 min at room temperature.

For TLC analysis the samples were extracted with buffer solution pH 8 (Sörensen), derivatized with fluorescamine (Fluram®, Roche Diagnostica) in dioxane according to the Roche Diagnostica standard procedure[11] and spotted on prewashed TLC plates. The spots were developed with toluene–ethanol (85:15) in the dark (R_F = 0.15). After drying, the plates were dipped in a 10% paraffin wax solution for fluorescence enhancement, as described in the literature[12]. The instrumental settings for TLC fluorescence quantification were 364 nm for excitation and 479 nm (cut-off) for emission. In all operations the fluorescamine derivatives were protected from direct light.

RESULTS AND DISCUSSION

2-Furaldehyde derivatization GC method (NIOSH)[3]

The NIOSH analysis method was tested with respect to derivative formation with phenylhydrazine and *p*-chlorophenylhydrazine. As expected, in both cases the *syn*- and *anti*-phenylhydrazones were formed, which could be verified by gas chromatography–mass spectrometry (GC–MS) (Fig. 1, Table I). The *syn*- and *anti*-isomers have been assigned tentatively on the basis of thermodynamic arguments, peak intensities and ratios. On packed columns the isomer peaks could not be separated

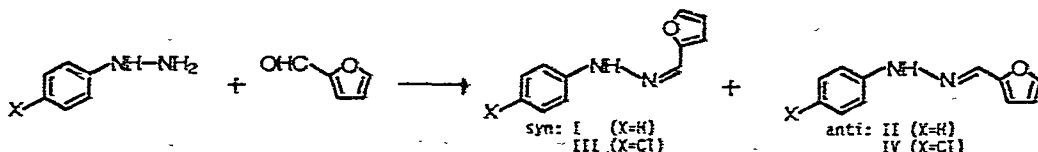

Fig. 1. Derivatization reaction involved in the NIOSH analysis method for phenylhydrazine[3].

TABLE I

TENTATIVE ASSIGNMENT OF *syn*- AND *anti*-ISOMERS AND MS DATA OF THE 2-FURAL-PHENYLHYDRAZONE DERIVATIVES (SEE FIG. 2)

The italicized *m/e* values represent base peaks.

Phenylhydrazone isomer (tentative)	*GC retention time (min)*		*Mass spectra (GC–MS), m/e*
	Capillary	*Packed column*	
I (*syn*)	7.28	9.37	*186* (M^+), 157, 92, 77, 65
II (*anti*)	6.16	10.50	*186* (M^+), 157, 92, 77, 65
III (*syn*)	11.69	14.65	*220* (M^+), 191, 126, 111, 99
IV (*anti*)	13.19	16.20	*220* (M^+), 191, 126, 111, 99

entirely. For a baseline peak separation the use of a capillary column was necessary (Fig. 2).

Double peak formation upon derivatization appears to be the only drawback of this analytical method. This can be overcome by either special integration methods or application of capillary column techniques.

Acetone derivatization GC method[7,8]

Phenylhydrazine gives only a single derivatization product with acetone in contrast to the NIOSH method. In the case of *p*-chlorophenylhydrazine this derivatization reaction has been confirmed by GC–MS (Fig. 3). As a limiting factor it was

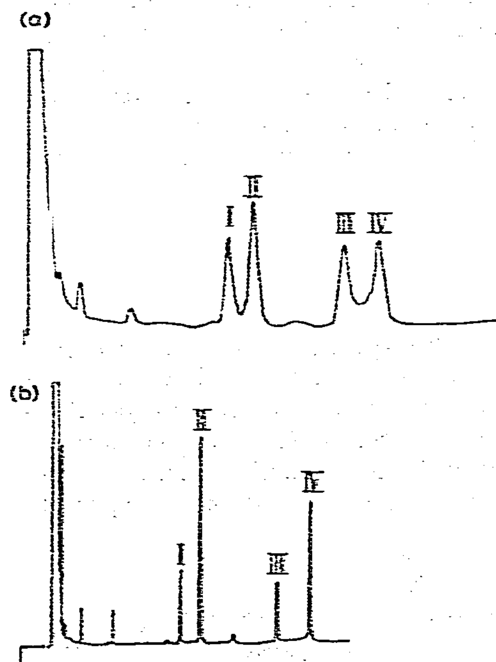

Fig. 2. GC separation of the 2-furalphenylhydrazone derivatives formed in the NIOSH analysis method (SE-30, FID, column temperature raised from 150°C to 250°C at 3°/min). (a) Packed column (2 m × 2 mm I.D.) containing 5% SE-30 on Chromosorb G AW DMCS (70–80 mesh), nitrogen flow-rate 30 ml/min. (b) Capillary column (SE-30) (22 m × 0.3 mm I.D.), splitting ratio 1:80, nitrogen pressure 12 p.s.i.

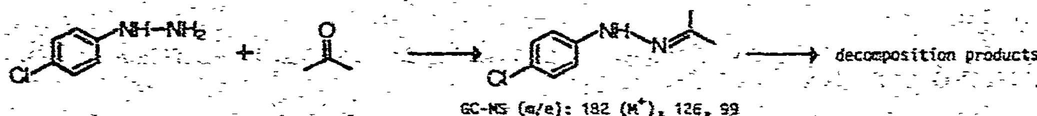

Fig. 3. Reaction of *p*-chlorophenylhydrazine with acetone.

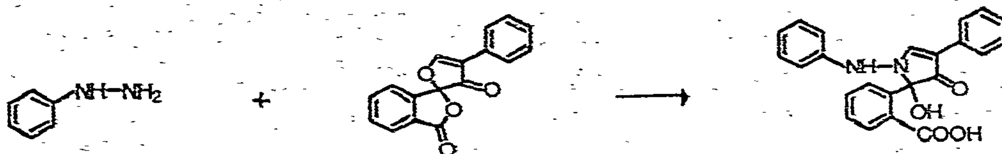

Fig. 4. Postulated derivatization reaction of phenylhydrazine with fluorescamine.

found that the acetone *p*-chlorophenylhydrazone derivative is not stable in solution and decomposes gradually. This behaviour is expected, since, *e.g.*, acetone phenylhydrazone is known to rearrange to 2-methylindole (Fischer indole synthesis)[13] as well as to form self-condensation products[14], depending on the reaction conditions. For quantitations it is therefore recommended that the GC injections be always made at the same time after derivatization and not later than after 1 h. With respect to sensitivity, the acetone method appeared to be comparable to the NIOSH method[3].

Fluorescamine derivatization TLC method

Fluorescamine reacts efficiently with primary amino groups to form fluorescent pyrrolinone chromophores and has been widely applied to the detection of primary amines[15,16], amino acids and peptides[17]. In the case of aliphatic amines the pyrrolinone structure has been firmly established[18]. For convenience, fluorescamine is usually applied in TLC as a spray reagent[19–22]. However, other derivatization techniques such as prelabelling[17], dipping[23], predipping[24] and overspotting[25] have also been successfully used. The detection limit of these methods has been found to be considerably lower than that of the spraying technique, probably due to a higher derivatization efficiency, *e.g.*, in the dipping procedure an approximately ten-fold increase in sensitivity was achieved[23].

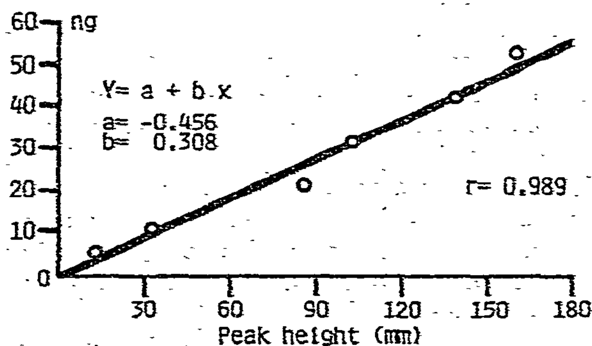

Fig. 5. Typical calibration curve for TLC determination of phenylhydrazine using fluorescamine.

TABLE II

APPROXIMATE DETECTION LIMITS OF THE FLUORESCAMINE TLC- AND THE NIOSH GC-SAMPLING AND ANALYSIS METHODS

Sampling method	*Typical air volume sampled*	*Detection limit in air ($\mu g/m^3$)*	
		Fluorescamine TLC method	*NIOSH GC method*[3]
Silica gel tube	96*	0.57**	5.0***
Glass fibre filter	4800‡	0.03‡‡	0.16‡‡

* Portable sampling pump, 200 ml/min, 8 h.
** Extraction volume 1 ml.
*** Extraction volume 2 ml.
‡ Stationary membrane pump, 10 l/min, 8 h.
‡‡ Extraction volume 3 ml.

As expected from the literature, phenylhydrazine formed a strongly fluorescent derivative in good yield. Based on the reaction sequence of fluorescamine with primary amines, the corresponding pyrrolinone structure can be postulated for the phenylhydrazine derivative (Fig. 4). Infrared and mass spectra of the isolated, crystalline product showed the presence of the main structural elements, but did not allow a final structure identification [IR in KBr: 1712, 1694 cm^{-1}, MS/EI: 368 (M − 18), 352, *323*, 77, MS/CI: 369 (M + H − 18), 353, *325*, 94]. For a full structure determination more analytical research is necessary.

Quantification down to 200 pg (Fig. 5) was possible on ordinary TLC plates without special precautions, which is usually sufficient for industrial hygiene air monitoring. The visual detection limit was found to be comparable to the fluorescence spot test[5], at about 5 ng.

The paraffin wax treatment of the plates was found to produce a fluorescence enhancement of about 2. If necessary, *e.g.*, for environmental air or water monitoring, it will certainly be possible to lower further the detection limit by using modern high-performance TLC techniques and more sophisticated equipment[26,27]. If other hydrazines or amines are present, interferences are possible, when the pyrrolinone derivatives have identical R_F values. For example, the phenylhydrazone and *p*-chlorophenylhydrazone derivatives, which were found to have comparable fluorescence intensities, could not be separated with the TLC solvents used in this study. Hydrazine, on the other hand, did not interfere with phenylhydrazine in the given analytical procedure (fluorescence at $R_F = 0$). In addition, preliminary experiments using toluene–methanol–acetic acid (8:1:1) for TLC separation revealed that hydrazine can form more than one product upon derivatization with fluorescamine (three fluorescent spots detected). Thus, it appears that the fluorescamine TLC method would not be optimal for hydrazine detection, although the detection limits for phenylhydrazine and hydrazine are similar in the fluorescamine spot test[5].

In conclusion, the results obtained suggest that the fluorescamine TLC method may be a useful and simple analytical tool for industrial hygiene air monitoring of

phenylhydrazine, as well as for other applications requiring low detection limits (Table II).

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