

## HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF HYDRAZINE IN WATER USING 2-HYDROXYNAPHTHALDEHYDE AS A DERIVATIZING REAGENT

M Y Khuhawar\*, A D Rajper and F M A Rind

Dr M A Kazi Institute of Chemistry, University of Sindh, Jamshoro, Sindh, Pakistan

(Received 12 March 2001; accepted 3 January 2002)

Hydrazine (HZ) derivative with 2-hydroxynaphthaldehyde (HN) eluted with methanol: acetonitrile: water (95:1:4 v/v/v) from reverse phase HPLC column Microsorb C-18, 5 $\mu$ m with UV detection at 330nm. Hydrazine, thiosemicarbazide (TSC) and phenylthiosemicarbazide (PTSC) derivatives of HN also separated when eluted with methanol: Chloroform: acetonitrile: water (65:4:2:29 V/V/V/V) with a flow rate 1 ml/minute, with detection limit in a range 0.54 to 16 ng/ injection (10 $\mu$ l). Linear calibration curve for HZ was obtained with 0.54-2.7 $\mu$ g/ml. The effect of variables on the determination of HZ was examined and method has been proposed for the determination of HZ from water.

**Key words:** Hydrazine derivation, 2-Hydroxynaphthaldehyde, Liquid chromatography.

### Introduction

Hydrazine (HZ) is a toxic agent that may induce mutagenesis, carcinogenesis and hepatic injury (Manes *et al* 1988). HZ is used in boiler feed water as reducing agent to control the presence of dissolved oxygen. The determination of HZ in below down water has an environmental importance. HZ could be determined by titrimetry (Budkuley 1992; Hernandez Mendez *et al* 1984), spectrophotometry (Amlathe and Gupta 1990; Yatsimirsky *et al* 1994; Molto and Font 1989; Alvarez de Laviada *et al* 1987; Collins and Rosepehorson 1993 and 1994), electro analytical techniques (Colonnese and Lannieuo 1989; Dias and Jaselskis 1983; Lovering *et al* 1985), gas (Matsui *et al* 1983; Wang and Xin 1988; Lovering *et al* 1985) and liquid chromatography (Zhou and Wang 1993; Kester and Danielson 1984; Shustina and Lesser 1989). The methods are based on spectrophotometry, spectrofluorimetry and chromatography involving derivatization with aromatic aldehydes including different substituted benzaldehydes, salicylaldehydes, *o*-phthalaldehyde, vanillin and 2-hydroxynaphthaldehyde (HN). Liquid chromatographic methods involved UV or electrochemical detection. Manes *et al* (1987 & 1988) have used HN for spectrophotometric determination of HZ in feed waters and as fluorimetric reagent for the analysis of HZ in isoniazid and its formulation. Manes *et al* (1989) have subsequently used HN for the determination of HZ in whole blood using either spectrophotometer or spectrofluorometer. The present work examine HN for HPLC determination of HZ in water.

\*Author for correspondence

### Experimental

Spectrophotometric studies were carried with Hitachi 220 spectrophotometer equipped with 1cm quartz cuvettes. HPLC studies were carried out with Hitachi 655 A liquid chromatograph connected with variable wavelength UV monitor, Rheodyne 7125 injector and Hitachi 2500 chromato-integrator. Column Microsorb C-18, 5 $\mu$ m (150x4.6mm, id) was used throughout the study, pH measurements were made with Orion 420A pH meter, connected with glass electrode and internal reference electrode. Hydrazine hydrated (Fluka), 4-phenylthiosemicarbazide (PTSC) (Merck), thiosemicarbazide (TSC) (Merck), 2-hydroxynaphthaldehyde (HN) (Fluka), chloroform (Fluka), methanol (Fluka) and acetonitrile (Fluka) were used. Buffer solutions in the pH range 1 to 10 were prepared from the following:

Hydrochloric acid (0.1M), potassium chloride (1M), acetic acid (1M), sodium acetate (1M), ammonium acetate (1M), ammonia (25%), sodium bicarbonate (1M) and sodium carbonate (saturated).

Hydrazine hydrate was standardized by titration with standard sulfuric acid (0.1N) using methyl orange as indicator (Furniss *et al* 1989).

**Analytical procedure.** Solution (5ml) containing (7.2 to 28.7 $\mu$ g), of HZ PTSC (up to 268ng) and TSC (up to 180ng) were added sodium acetate acetic acid buffer pH 5 (2ml), derivatizing reagent HN (0.3% in ethanol) 1.5ml and the solution was heated on water bath for 20 min. The solution was allowed to cool and chloroform was added (5ml). The mixture was shaken for 3-4 min and layers were allowed to separate. The organic layer (10 $\mu$ l) was injected on the column

Microsorb C-18 (150x4.6mm id) and eluted with methanol: chloroform: acetonitrile: water (63:4:2:29 V/V/V/V) for 6 min, followed by methanol for 7 min. The detection UV was at 330nm.

**Analysis of HZ in water.** Water (15ml) was spiked with 1-3 $\mu$ g of HZ and the analytical procedure was followed. The chloroform extract (5ml) was transferred to sample vial and solvent was evaporated gently on water bath, the residue was dissolved in methanol (0.5ml). Solution 10 $\mu$ l was injected on the column and eluted as analytical procedure. The amount found was evaluated from calibration curve.

## Results and Discussion

Hydrazine reacts with HN in 1:2 molar ratio to form 2, 2-dihydroxyl-naphthaldazine with absorbance in visible region at 410nm and 430nm with molar absorptivities of  $2.7 \times 10^{-4}$  and  $2.2 \times 10^{-4}$  L mole<sup>-1</sup> cm<sup>-1</sup> respectively. (Fig 1) The derivative formed was stable for more than 24 hr. Similarly HN reacts with PTSC and TSC which absorbed maximally at 390nm and 375nm; and 376nm and 363nm with molar absorptivities of 9200 and 9375 L mole<sup>-1</sup> cm<sup>-1</sup> and 9576 and 11250 L mole<sup>-1</sup> cm<sup>-1</sup> respectively. The absorbance of the solution after derivatization and after 24hr was recorded and no change in absorbance was observed.

The effect of pH, heating time and concentration of HN on the spectrophotometric response of HZ were examined at 410nm. The buffer solution (2ml) of different pH within the range 1-10 was added and analytical procedure was followed. The pH which gave a maximum absorbance was considered as optimum. Maximum absorbance was observed within pH range 4-7 to pH 5 was considered optimal. Heating time at 95°C was varied between 5 to 25min at an interval of 5 min. It was observed that absorbance remained constant after 15 min and heating time of 20 min was selected. Reagent concentration (0.3% in ethanol) was varied between 0.5 to 2.5 ml at an interval of 0.5ml. The absorbance remained constant up to 1.5ml and was used.

The determination of HZ in water has an environmental importance and therefore HPLC method was examined. The HZ after derivatization was injected on the column Microsorb C-18 5 $\mu$ m, (150x4.6mm id) and eluted isoratically with

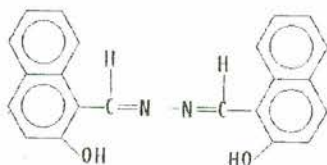


Fig 1. The structural diagram of 2,2-dihydroxyl-1-naphthaldazine derivative.

methanol, methanol-water or methanol-acetonitrile-water. Each time HN and HZ derivative eluted but the complete separation between derivatizing reagent (HN) and hydrazine derivative was obtained when eluted with methanol: acetonitrile: water (95:1:4 V/V/V) using flow rate of 1ml/min with uv detection at 330nm (Fig 2). A linear calibration curve was obtained with 0.54 to 2.7 $\mu$ g/ml HZ using 10 $\mu$ l injection with coefficient of correlation ( $r^2$ ) 0.9981. The detection limit measured as three times the background noise was observed 54ng/ml corresponds to 0.54ng/injection (10 $\mu$ l). The effect of amount of water on the derivatization, extraction and the determination of HZ was investigated. A constant amount of HZ 2.1 $\mu$ g was added to different volumes of water between 5 to 50ml and same analytical procedure was carried out. Average peak height (n=3) was used to examined the response. The similar response was observed with the volume of water used between 5 to 15ml, but somewhat decrease in peak height was observed by increasing the volume of water up to 50ml. Extraction of 2.1 $\mu$ g of HZ from 15ml was extracted and the amount extracted was evaluated from calibration curve. The amount extracted was in the range 90-96% with preconcentration factor of 3.

PTSC and TSC also formed derivatives with HN at the conditions optimized for the HZ and was therefore examined for the simultaneous elution and separation from the HZ. PTSC and TSC derivatives also eluted with the solvent system used for HZ derivative, but the optimal separation between TSC, PTSC and HN was obtained when eluted with methanol:chloroform:acetonitrile:water (60:2:4:34 V/V/V/V) using flow rate of 1ml/min. However using eluent the HZ derivative was long retained. It was therefore after elution of TSC, PTSC and HN (6min), HZ derivative was eluted with methanol (Fig 3). The resolution factor ( $R_s$ ) between TSC and PTSC and HN was calculated 2.25 and 2.9 respectively. The detec-

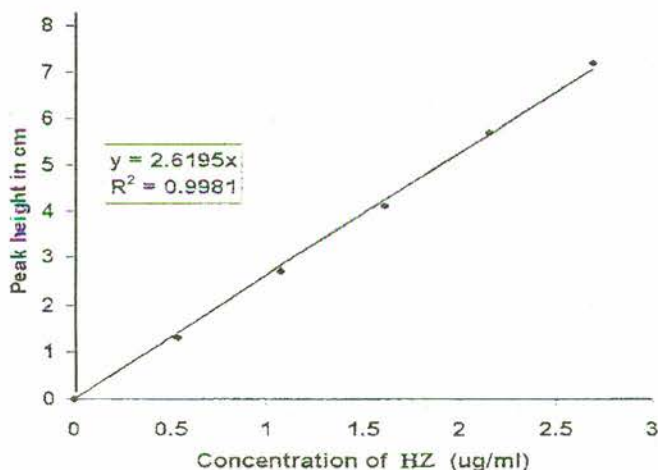
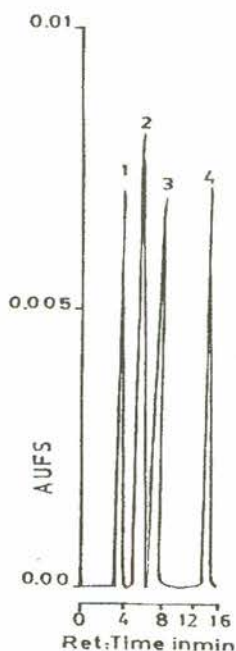


Fig 2. The calibration curve of hydrazine.



**Fig 3.** HPLC separation of (1) TSC (2) PTSC (3) HN (4) HZ-HN, Colum Microsorb C-18 (150x4.6mm), elution with methanol: chloroform: acetonitrile: water (63:4:2:29 V/V/V/V) for six min, followed by 100% methanol up to 13 min, with the flow rate 1ml/min, UV detection at 330nm.

tion limits for PTSC and TSC were found 1.6 $\mu$ g/ml corresponding to 10.6ng/injection (10 $\mu$ l).

Replicate analysis of HZ (2.1 $\mu$ g) (n=5) was carried out and the relative standard deviation was observed 3.5%. Test solutions of water were analyzed for the contents of HZ and amounts were evaluated from the calibration curve. The relative % error was observed within  $\pm$ 3%.

The work reports a simple liquid chromatographic method for the determination of HZ in water at mg/ml level with %RSD within 3.5%. The work also reports the possible separation and determination of HZ, PTSC and TSC simultaneously.

Water samples spiked with hydrazine were analyzed with preconcentration factor of 3, with % extraction with 90-96%.

## References

- Alvarez de Laviada T, Romero F J, Anton V, Anton V Jun, Roma J, Vina J, Saez G T 1987 Simple micro-assay for the determination of hydrazine in biological samples. Effect of hydrazine and isoniazid on liver and brain glutathione. *J Anal Toxicol* **11** 260-262.
- Amlathe S, Gupta V K 1990 Some observation on a field procedure for sampling and determination of low concentration of hydrazine in air. *Microchem J* **42** 331-335.
- Budkuley J S 1992 Determination of hydrazine and sulfid in the presence of one another, *Mikrochim. Acta* **108**(1-2) 103-105.
- Collins G E, Rose-Pehrsson S L 1994 Fluorescent detection of hydrazine, monomethylhydrazine and 1, 1-dimethylhydrazine by derivatization with aromatic dicarboaldehydes. *Analyst* **119** 1907-1913.
- Collins G E, Rose-Pehrsson S L 1993 Sensitive, fluorescent detection of hydrazine via derivatization with 2, 3-naphthalene dicarboxaldehyde, *Anal Chem Acta* **284** 207-215.
- Colonnese R, Lannieu R M 1989 Determination of hydrazine in poly (vinylpyrrolidinone) by derivatization and square wave voltammetry, *Mikrochim. Acta* **11** 7-14.
- Dias F X, Jaselskis B 1983 Voltammetric determination of hydrazine and hydroxylamine. *Analyst* **1282** 76-80.
- Furniss B, Hannafords A, Smith W G, Tatchel A R H 1989 *Vogels Text Book of Practical Organic Chemistry*. Fifth ed. ELBS 436.
- Hernandez Mendez J, Gonzalez Perez C, Caldern Diez M D 1984 Volumetric determination of organic compounds with iodine-poly (vinyl pyrrolidinone II Indirect determination. *Stud Chem Univ Salamanca* **9** 65-75.
- Kester P E, Danielson N D 1984 Determination of hydrazine and 1, 1-dimethylhydrazine as salicylaldehyde derivative by liquid chromatography with electrochemical detection. *Chromatographia* **18** 125-128.
- Lovering E G, Matsui F, Robertson D, Curran N M 1985 Determination of hydrazine in pharmaceuticals IV. Hydrazine in isocarboxazid. *J Pharm Sci* **74** 105-107.
- Manes I, Campillos P, Font G 1987 Extraction-spectrophotometric Determination of Hydrazine with 2-hydroxyl-1-naphthaldehyde. *Analyst* **112** 1183-1184.
- Manes J, Gimeno M J, Molto J C, Font G 1988 Fluorimetric determination of hydrazine in isoniazid formulation with 2-hydroxyl-1-naphthaldehyde. *J Pharma Biomed Anal* **61** 1023-1027.
- Matsui F F, Robertson D L, Lovering E G 1983 Determination of hydrazine in Pharmaceuticals III, Hydrazine and isoniazid by GLC. *J Pharm Sci* **72** 948-951.
- Molto J C, Font G 1989 Spectrophotometric and spectrofluorimetric determination of hydrazine in whole blood. *Quim Anal* **8** 69-73.
- Shustina R, Lesser J H 1989 Liquid chromatographic determination of hydrazine carbohydrazide and thiocarbohydride in aqueous solutions. *J Chromatogr* **464** 208-212.
- Wang Y, Xin Y 1988 Determination of free hydrazine in isoniazid by gas chromatography. *Yaowen Fenxi Zazhi* **8** 181-183.
- Yatsimirsky A K, Yatsimirskaya N T, Kashina S B 1994

Micellar catalysis and product stabilization in hydrazone formation reactions and micellar modified determination of hydrazine and phenyl hydrazine. *Anal Chem* **66** 2232-2239.

Zhou J X J, Wang E K 1993 Electro catalysis and determination of hydrazine compounds in liquids chromatography at a mixed-valent cobalt oxide/cynocobaltate film electrode. *Talanta* **40** 943-948.