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Short Communication

Determination of hydrazine in human plasma by high-performance liquid chromatography

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ABSTRACT

A simple and sensitive liquid chromatographic method for the determination of hydrazine in human plasma and serum is described. Samples were prepared in a single-step reaction by protein denaturation with trichloroacetic acid and derivatization to a stable azine with 4-hydroxybenzaldehyde. Chromatographic separation was carried out on a reversed-phase (octadecylsilane) column with methanol–water (60:40) as mobile phase and ultraviolet detection at 340 nm. Linearity was found in the range 5–1000 ng/ml. The detection limit of spiked plasma was 1 ng/ml. The coefficient of variation ranged from 1.7 to 3.8%. No degradation of hydrazine was found in spiked plasma and serum, even after storage at room temperature for one week. An increased hydrazine level was found after *in vitro* degradation from isoniazid in human serum.

INTRODUCTION

Hydrazine is a hazardous chemical widely used in the laboratory and industry. It is employed as a corrosion inhibitor in boiler feedwater, in waste water treatment for the removal of halogens, as a reducing agent in nickel plating, as a chain extender in the polymerization of polyurethanes, as soldering fluxes, as a reactant in military fuel cells, and in rocket propellants. Several pharmaceuticals and agricultural chemicals are derivatives of hydrazine. It is a minor metabolite of the antituberculosis agent isoniazid [1] and appears as an impurity of the growth-retardant maleic hydrazide, which is used in tobacco cultivation and in potato and onion storage [2]. At low levels, hydrazine represents a frequent risk to humans. It has been detected in cigarette smoke, edible mushrooms, and processed foods [3].

The toxic effects of hydrazine are multiple. Effects on the nervous system, the liver and kidney have been described as have immuno- and genotoxic effects [3]. Experimental studies with rats and mice indicate that hydrazine can produce tumours in the lung and liver. Because of insufficient data in humans, hydrazine is considered to be a potential carcinogen in humans [4,5]. Exposure can occur by inhalation, ingestion or skin contact. Death from acute exposure results from convulsions, respiratory arrest and cardiovascular collapse [6]. Chronic toxicity by inhalation has been described in a case report of an occupational exposure. Early signs include lethargy, conjunctivitis and tremors, extending to vomit-

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ing, fever and diarrhea. Later, abdominal pain and incoherence develop [7].

Occupational medical monitoring is necessary for persons who are exposed to hydrazine by air or who handle hydrazine-containing fluids and solids. Spectrophotometric and fluorometric methods are widely used [8,9], but at the lower ng/ml level these methods are not specific enough to distinghuish hydrazine from other interfering components. The sensitivities of reported high-performance liquid chromatographic (HPLC) methods are at the μg/ml level, and therefore not sufficient for occupational medical monitoring [10,11]. Sensitive and specific gas chromatographic–mass spectrometric (GC–MS) methods require sophisticated technology and ^{15}N-labelled hydrazine as an internal standard [12]. In an HPLC method for isoniazid, 4-hydroxybenzaldehyde is used as a derivatization reagent to form a UV-detectable hydrazone [13].

This paper describes a simple HPLC determination of hydrazine as an azine derivative of 4-hydroxybenzaldehyde. The sensitivity and specifity of this method make it suitable for accurate occupational medical monitoring.

EXPERIMENTAL

Reagents and chemicals

4-Hydroxybenzaldehyde was obtained from Fluka (Neu-Ulm, Germany), hydrazine (sulphate salt) and isoniazid from Sigma (Deisenhofen, Germany), acetic acid hydrazide from Aldrich (Steinheim, Germany), and trichloroacetic acid and methanol from Merck (Darmstadt, Germany).

Apparatus and chromatographic conditions

The HPLC apparatus (Merck/Hitachi, Darmstadt, Germany) consisted of a Model L6000 HPLC pump, a Model AS2000A autosampler, and a Model L4000 UV spectrophotometric detector. A Model SE120 recorder (BBC-Goerz Metrawatt, Nürnberg, Germany) was connected to the detector. The chromatographic column was a LiChrospher 100 RP 18 (125 × 4 mm I.D., particle size 5 μm) purchased from Merck. The mobile phase was methanol–water (60:40, v/v); both components were HPLC-grade and degassed ultrasonically before analysis. The flow-rate was maintained at 1.0 ml/min. The detector wavelength was set at 340 nm and the sensitivity at 0.01 a.u.f.s.

Validation procedure

The assay linearity, accuracy, precision and detection limit were validated by adding various amounts of hydrazine to pooled human plasma. For testing the linearity and recovery, standard solutions and spiked plasma samples at 0, 5, 10, 20, 40, 70, 100, 200, 500 and 1000 ng/ml were assayed in triplicate. The accuracy and precision were tested at three different concentrations of hydrazine added to plasma (20, 80 and 200 ng/ml; $n = 15$). The detection limit was checked by a discriminating test of pooled plasma and spiked plasma with 1 and 2 ng/ml ($n = 9$).

Sample preparation

To 200 μl of plasma, 200 μl of 2% methanolic 4-hydroxybenzaldehyde (recrystallized) and 100 μl of 10% trichloroacetic acid in water were added, vortex-mixed, and incubated on a shaking apparatus for 10 min at room temperature. The tubes were then centrifuged at 12 000 *g* for 2 min. A 50-μl volume of the supernatant was injected into the HPLC system.

Stability study

Plasma (heparin or EDTA) and serum, spiked with 50 ng/ml hydrazine, were stored at room temperature (20°C), at 4°C, and at −18°C, and hydrazine was assayed in duplicate 0, 1, 3, 5 and 7 days after adding. In addition, serum spiked with 50 ng/ml was stored at 20°C, 4°C, and −18°C and assayed 3, 4, and 5 weeks after adding. A serum sample from a patient treated with isoniazid (serum concentration 4.2 μg/ml) was stored at 4°C, and assayed 4, 8, 10, and 14 days after the first determination.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatograms of a reagent

blank (A), a standard solution of 40 ng/ml (B), a plasma (C) and a plasma spiked with 40 ng/ml (D). The reagent blank shows the 4-hydroxybenzaldehyde peak at a retention time of 1.6 min (absorbance maximum at 280 nm) and small impurities at 3.0 and 5.1 min. The peak at 5.1 min appears much greater without recrystallization of the reagent. The retention time of the azine derivative of hydrazine is 3.5 min (absorbance maximum at 340 nm). No significant degeneration of the chromatographic conditions was found after at least 500 injections into the column. The mobile phase is a simple mixture of methanol and water. Isoniazid appears in front of the reagent peak and does not interfere with hydrazine. Even acetic acid hydrazide is eluted in front of the reagent and cannot be determined under the present chromatographic conditions.

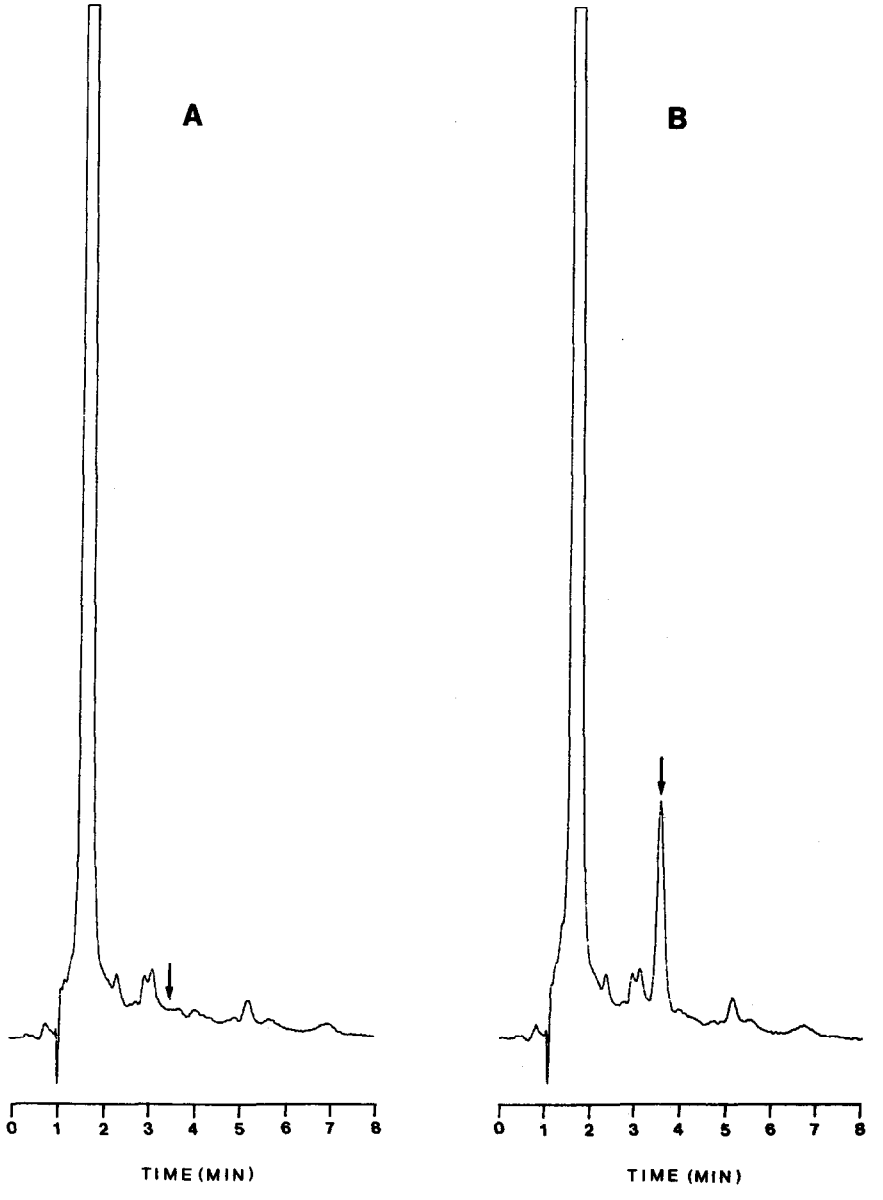

Fig. 1.

(Continued on p. 160)

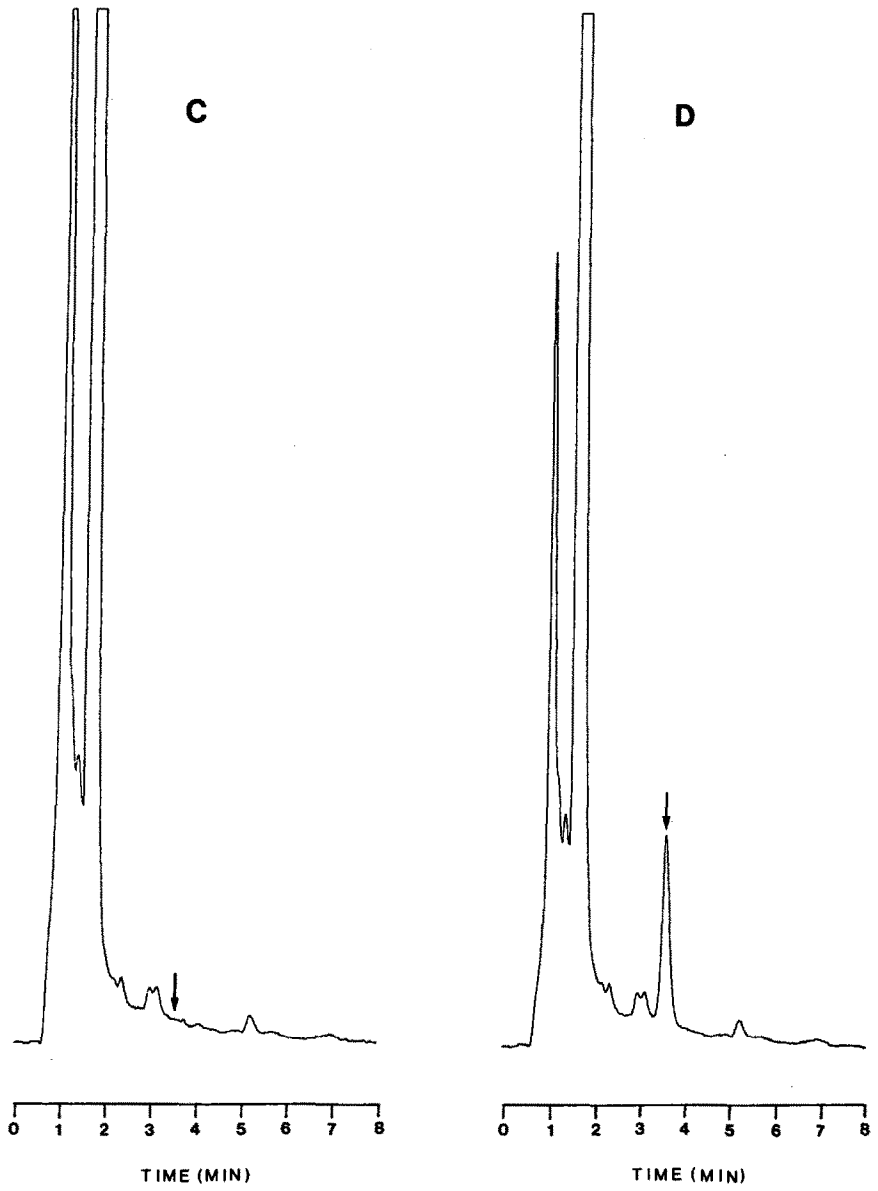

Fig. 1. Representative chromatograms from (A) a reagent blank, (B) a hydrazine standard solution (40 ng/ml), (C) a plasma blank, and (D) plasma spiked with 40 ng/ml. The arrows on the chromatograms refer to the elution of hydrazine derivative with 4-hydroxybenzaldehyde. Chromatographic conditions are described in Experimental.

Sample preparation was optimized with respect to the concentration of reagent, the reaction time and the analytical recovery. Excess reagent is needed for maximum azine formation. A concentration of greater than 1.6% gives constant signals. The reaction is complete after 6 min at room temperature (20°C). For good analytical recovery and minimum sample dilution, protein denaturation and derivatization were carried out in a single step. The azine derivative in the reaction mixture is stable for at least 24 h at room temperature.

The method was tested with hydrazine standard solutions and spiked human plasma sam-

TABLE I

REGRESSION STATISTICS FOR THE CALIBRATION CURVES

Data are the mean of three values.

Sample	Range (ng/ml)	n	Calibration graph	Correlation coefficient
Standard	5–200	7	$y = 0.812x - 0.06$	0.9999
Standard	100–1000	4	$y = 0.859x - 2.20$	0.9997
Spiked plasma	5–200	7	$y = 0.752x - 0.13$	0.9998
Spiked plasma	100–1000	4	$y = 0.715x + 7.07$	0.9994

TABLE II

ACCURACY AND PRECISION (INTRA-DAY)

Concentration added (ng/ml)	n	Concentration found (mean ± S.D.) (ng/ml)	C.V. (%)	Analytical recovery (%)
20	15	19.4 ± 0.64	3.3	97.0
80	15	75.2 ± 2.88	3.8	94.0
200	15	180.1 ± 3.03	1.7	90.1

ples and found to be in the range 5–1000 ng/ml, with good correlation coefficients between 0.9994 and 0.9999 (Table I).

The intra-day accuracy and precision at three different concentrations spiked to plasma in the range 20–200 ng/ml show an analytical recovery of 90.1–97.0% (Table II). The coefficient of variation (C.V.) was in the range 1.7–3.8%. The detection limit, checked by a discriminating test of pooled plasma and spiked plasma, was found to be 1 ng/ml.

A stability study of spiked samples with 50 ng of hydrazine per ml shows no degradation within 7 days, regardless of whether samples were stored at room temperature, refrigerated at 4°C or frozen at −18°C (Table III). No difference was ob-

TABLE III

STABILITY STUDY AND PRECISION DATA (INTER-DAY)

Samples were spiked with hydrazine (50 ng/ml) and assayed 0, 1, 3, 5 and 7 days after adding (mean of two).

Sample	Storage temperature (°C)	Slope (ng/ml per day)	Concentration (mean ± S.D.) (ng/ml)	C.V. (%)
Serum	+20	0.979	52.9 ± 3.63	6.9
	+ 4	0.409	50.2 ± 1.44	2.9
	−18	0.002	49.5 ± 2.22	4.5
Heparin plasma	+20	0.267	50.6 ± 2.70	5.3
	+ 4	0.149	49.5 ± 1.87	3.8
	−18	0.313	48.9 ± 1.88	3.8
EDTA plasma	+20	0.407	52.2 ± 1.72	3.3
	+ 4	−0.018	52.2 ± 1.83	3.5
	−20	0.358	52.1 ± 1.47	2.8

served between serum, heparin plasma and EDTA plasma. The inter-day precision shows a C.V. in the range 2.8–6.9%. The small increase of hydrazine in serum stored at room temperature with a slope of 0.979 ng/ml per day may result from an unknown drug-related degradation of the serum pooled from several patients. Prolonged storage over a period of 5 weeks results in a slow decrease to 68, 86 and 94%, respectively, if spiked plasma is stored at room temperature, refrigerated or frozen. There was a significant increase of hydrazine in a patient's serum sample treated with isoniazid and stored at 4°C for 14 days. Starting with an isoniazid concentration of 4.2 μg/ml and 21.7 ng/ml for hydrazine, the hydrazine level rises after 4, 8, 10, and 14 days to 27.6, 35.9, 36.5, and 38.1 ng/ml, respectively. The isoniazid concentration after 14 days at 4°C decreases to 3.8 μg/ml. Ten plasma samples from smokers and non-smokers were analyzed, but in all cases the hydrazine levels were below the detection limit of 1 ng/ml.

The method described here is simple, rapid, selective, reproducible and very sensitive in comparison with other available methods, and allows an accurate measurement of hydrazine in serum or plasma for occupational medical monitoring. Because of its advantages, the method is also suitable for large numbers of samples in metabolic studies of hydrazine or its derivatives. The method is not sensitive enough for monitoring environmental exposure to hydrazine in humans.

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