Journal of Chromatography, 573 (1992) 227–234
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6135

Determination of hydrazine in biofluids by capillary gas chromatography with nitrogen-sensitive or mass spectrometric detection

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(First received July 1st, 1991; revised manuscript received September 2nd, 1991)

ABSTRACT

Plasma and liver levels of hydrazine were determined at 10, 30, 90 and 270 min in rats given 0.09, 0.27, 0.84 and 2.53 mmol of hydrazine per kg body weight orally by capillary gas chromatography–mass spectrometry of its pentafluorobenzaldehyde adduct (DFBA, *m/z* 388) using selected ion monitoring with $^{15}N_2$-labelled hydrazine as the internal standard (adduct, *m/z* 390). The mean half-life for hydrazine in the plasma was approximately 2 h but varied with dose. Urinary excretion (0–24 h) of hydrazine and its metabolite acetylhydrazine were determined employing nitrogen–phosphorus detection of the adducts utilising a novel internal standard, pentafluorophenylhydrazine, the adduct of which structurally resembles DFBA. The fraction of the original dose excreted as hydrazine (and acetylhydrazine) declined with increasing dose.

INTRODUCTION

Several pharmaceuticals including the anti-depressant phenelzine and the anti-hypertensive agent hydralazine are derivatives of hydrazine (Fig. 1). Indeed hydrazine and acetylhydrazine are toxic minor metabolites of isoniazid, the tuberculostatic drug [1]. The hydrazine function of procarbazine is required for its anti-neoplastic effect in the treatment of Hodgkin's disease. Hydrazine sulphate itself is undergoing evaluation as a treatment for cancer cachexia [2]. Human exposure to hydrazines also occurs in the industrial environment. Hydrazines are impurities of a number of agrochemicals, among them the plant growth retardant maleic hydrazide, which is

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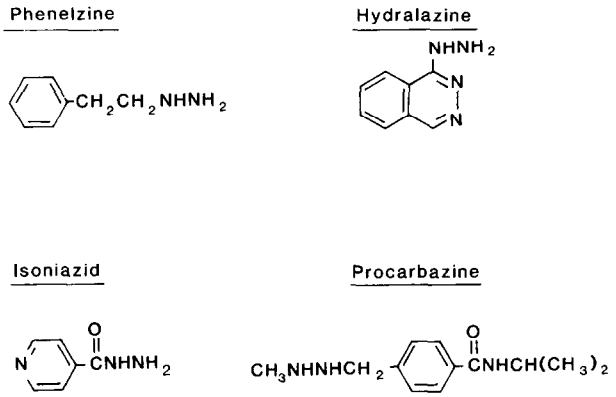

Fig. 1. Structures of some hydrazine-based drugs.

widely used in tobacco and potato cultivation [3], and diaminozide, which is used to delay apple and peanut ripening. Hydrazine compounds have numerous other applications, and exposure to them may occur during the preparation of boiler feed water, jet propellants and blowing agents such as azidodicarbonamide used in the manufacture of plastics. When given to animals hydrazine and its derivatives initiate seizures in the central nervous system [4] and cause fatty disposition in the liver [5]. Some reports imply that trace exposure to hydrazine can induce cancer [6–8]. Further, despite their ubiquity, the disposition, metabolism and toxicity of hydrazines are poorly understood.

A critical problem associated with the investigation of hydrazine is the lack of a reliable method for its quantification in biological samples in which it is rapidly autoxidised. Gas chromatographic (GC) methods for the determination of hydralazine [9] and isoniazid and its metabolite acetylhydrazine [10] take advantage of the nucleophilic character of the hydrazine function by quickly derivatising it with benzaldehyde. It is advantageous to complete derivatisation as rapidly as possible before autoxidation can occur. In this respect the activated electrophile pentafluorobenzaldehyde (PFB) is preferable because it reacts rapidly at room temperature with acidified aqueous solutions of hydrazine and substituted hydrazine compounds which possess a free N-NH_2 group, *e.g.* acetylhydrazine to form decafluorobenzalazine (DFBA, i, Fig. 2) and acetylpentafluorobenzaldehyde hydrazone(s) (PFAhz, ii), respectively. Amines present in biofluids possessing a free C-NH_2 group do not form Schiff bases or amides with PFB under these conditions so there is little interference from other nitrogen compounds. Chromatographic methods for hydrazine [3] and phenelzine [11,12] have used PFB to derivatise the hydrazine function.

Some of these methods capitalise on the high nitrogen content of hydrazine derivatives by employing nitrogen-sensitive detectors [9], whereas fluorinated derivatives have been detected by electron capture [3,12] and mass spectrometry (MS) [11]. Note, however, that poly-fluorination restricts the use of certain deuterated standards [10] required for selected ion monitoring (SIM). Recently we have reported a nuclear magnetic resonance (NMR) method to identify several metabolites of $^{15}N_2$-labelled hydrazine [13], many of which can not be derivatised. In the parallel studies reported here we have measured administered unlabelled hydrazine and its metabolite acetylhydrazine by more accurate chromatographic methods for comparison. A specific aim has been to estimate the half-life of hydrazine and so assess the likely rate of autoxidation (and of hepatic metabolism) occurring *in vivo*. To this end we have determined hydrazine and acetylhydrazine (following PFB derivatisation) in the urine of rats given various doses of hydrazine. Separation of PFB adducts has been achieved by capillary GC using a nitrogen-sensitive detector, utilising a novel internal standard, the PFB adduct of pentafluorophenylhydrazine (PFPhz, iii, Fig. 2), the structure and chromatographic properties of which are similar to those of DFBA. We

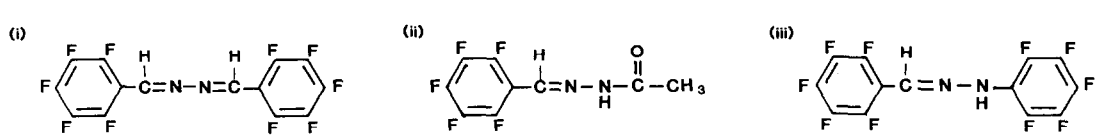

Fig. 2. Structures of (i) decafluorobenzalazine (DFBA), (ii) acetylpentafluorobenzaldehyde hydrazone (PFAhz) and (iii) pentafluorophenylpentafluorobenzaldehyde hydrazone (PFPhz).

have also measured hydrazine at lower concentrations (following PFB derivatisation) in the liver and plasma of similarly treated rats by GC–MS using $^{15}N_2$-labelled hydrazine as the internal standard and employing SIM. Finally we have investigated the applicability of the methodology to a variety of hydrazine compounds in greater detail than previously reported and the suitability of the various detection methods available.

EXPERIMENTAL

Apparatus

Two identical fused-silica columns, 12 m × 0.22 mm I.D. with an OV-1 bonded phase (0.25 μm) were purchased from Waters Chromatography (Northwich, UK). Analyses were performed using: (1) a Perkin Elmer 8410 gas chromatograph fitted with a nitrogen–phosphorus detector (NPD) with a minimum detectivity (determined) of ~100 ng of nitrogen (atom) s^{-1} or an electron-capture detector (ECD) with a minimum detectivity (determined) of ~1 ng of fluorine (atom) s^{-1}; or (2) a Hewlett Packard 5890A gas chromatograph linked to a VG 12-250 quadrupole mass spectrometer with undetermined but superior detectivity.

Materials

All reagents were of analytical grade. $^{15}N_2$-Labelled hydrazine sulphate was obtained from MSD Isotopes (Merck Frost, Montreal, Canada). All other reagents were purchased from Sigma (Poole, UK).

Syntheses

Methyl, *tert.*-butyl, *tert.*-butylcarbazyl, phenyl, isonicotinoyl, pentafluorophenyl, acetyl and formidyl PFB-hydrazones and decafluorobenzaldehyde azine were synthesised by reacting PFB with an equimolar quantity of methylhydrazine, *tert.*-butylhydrazine, *tert.*-butylcarbazate, phenylhydrazine, isoniazid, pentafluorophenylhydrazine or acetylhydrazine or half-molar quantities of semicarbazide or hydrazine in methanol–water (50:50) for 2 h at 60°C, respectively. Confirmation of the adducts' structures were obtained by electron-impact (EI) MS and NMR spectroscopy (Table I).

Chromatography

Typical conditions used for GC–NPD were isothermal (140°C) (injection temperature, 300°C; detection temperature, 350°C) using helium (1 ml/min) as the carrier gas (10:1 split ratio; 5-μl volume). Retention times were: PFAhz, 2.4 min; DFBA, 4.7 min; PFPhz, 7.3 min. Identical retention times were obtained for these adducts using the ECD.

GC–MS–SIM was performed using an identical column with a temperature programme (2-μl volume, splitless injection to maximise sensitivity). The column was maintained at 35°C for 3 min, then ramped to 180°C at 20°C min^{-1}. Ionisation was effected by EI and the ion current was detected by SIM under computer control. DFBA (retention time, 10.8 min) was monitored at m/z 388 and the labelled internal standard at m/z 390.

Animal treatment

Rats had free access to drinking water and were fed *ad libitum* until 24 h prior to dosing. Hydrazine in distilled water was administered by stomach tube to 60 male Sprague–Dawley rats (200 g) at doses of 0.09, 0.28, 0.84 and 2.53 mmol hydrazine per kg body weight while control rats received saline. Fifteen rats were used at each dose, three rats per time point. Forty-eight animals were diethyl ether-anaesthetised at 10, 30, 90 and 270 min after dosing. Blood (3–5 ml) was taken from the vena cava into heparinised syringes, then into heparinised tubes, centrifuged at 800 *g* for 15 min, and the plasma removed and stored at –80°C until analysis. In addition the liver was removed from the animal, weighed and 5 g were homogenised in 20 ml of methanol–water (50:50, v/v). The homogenate was centrifuged and the supernatant removed and kept at −80°C until analysis.

For the GC–MS–SIM assay, 0.1 ml of the internal standard (0.02–0.5 m*M* [$^{15}N_2$]hydrazine sulphate, dependent on dose) was added to 0.9 ml of plasma sample or 4.9 ml of liver supernatant, respectively, before freezing. The remaining twelve rats (and three controls which received saline) were kept in purpose-built metabolic chambers to collect urine for 24 h. For the GC–NPD assay, 1 ml of the internal standard (0.01–0.1 *M* pentafluorophenylhydrazine, dependent on dose)

TABLE I

MS AND NMR DATA FOR PENTAFLUOROBENZALDEHYDE DERIVATIVES WITH HYDRAZINE AND HYDRAZINE COMPOUNDS

Parent hydrazine compound	Electron impact mass spectrometry[a]	^{1}H NMR[b] (ppm)	^{19}F NMR[b] (ppm)
Decafluorobenzalazine (DFBA)	388, $M^{+\cdot}$; 369, M − 19, F; 194, M − 194, C_6F_5CHN; 180, C_6F_5CH; 167, C_6F_5	8.64 (s) C_6F_5CH	*o* -139.1 *m* -149.2 *p* -161.6
Methyl	224, $M^{+\cdot}$; 194, M − 30, CH_3NH; 180, M − 44, CH_3N_2H	7.12 (s) C_6F_5CH 2.86 (d) CH_3 8.36 (d) NH	*o* -145.3 *m* -159.5 *p* -163.6
tert.-Butyl	266, $M^{+\cdot}$; 251, M − 15, CH_3; 210, M − 56, $CH_2=C(CH_3)_2$; 209, M − 57, $C(CH_3)_3$	8.02 (s) C_6F_5CH 1.22 (s) $C(CH_3)_3$ 8.90 (s) NH	*o* -143.6 *m* -156.0 *p* -162.8
tert.-Butyl carbazyl	310, $M^{+\cdot}$; 210, M − 100, $CO_2CH_2=C(CH_3)_2$; 253, M − 57, $C(CH_3)_3$	8.65 (s) C_6F_5CH 1.47 (s) $C(CH_3)_3$ 8.08 (s) NH	*o* -142.5 *m* -153.9 *p* -162.4
Phenyl	286, $M^{+\cdot}$; 209, M − 77, C_6H_5	7.74 (s) C_6F_5CH 7.05 *o* (d) 6.83 *m* (t) 7.24 *p* (m) 10.88 (s) NH	*o* -144.2 *m* -157.2 *p* -163.1
Isonicotinoyl	315, $M^{+\cdot}$; 237, M − 78 and 78, C_5NH_4; 209, M − 106 and 106, C_5NH_4CO	8.59 (s) C_6F_5CH 7.83 *o* (d) 8.81 *m* (d)	*o* -141.4 *m* -152.2 *p* -162.0
Pentafluorophenyl (PFPhz)	376, $M^{+\cdot}$; 194, M − 182 and 182, C_6F_5NH; 167, C_6F_5	8.14 (s) C_6F_5CH 10.71 (s) C_6F_5NH	-143.1 -155.2 -162.8 -163.8 -168.2
Acetyl (PFAhz)	252, $M^{+\cdot}$; 210, M − 42, $CH_2=CO$; 190, M − 62, 43 CH_3CO	8.15 (s) C_6F_5CH 2.15 (s) CH_3CO 8.27 NH	*o* -142.0 -142.7 *m* -153.2 -153.6 *p* -162.9
Formidyl (PFFhz)	253, $^{+\cdot}$; 210, M − 43; 190, M − 63; 44, NH_2CO	7.89 (s) C_6F_5CH 10.7 (s) NH 6.33 (s) NH_2	*o* -142.8 *m* -154.4 *p* -162.5

[a] Figures represent principal fragments in the mass spectrum and are followed by their source; $M^{+\cdot}$ = the molecular ion. EI-MS was performed on a VG 12-250 quadrapole mass spectrometer.

[b] Figures represent chemical shifts w.r.t. TMS or CCl_3F and are followed by their multiplicities [(s) singlet, (d) doublet, (t) triplet, (m) multiplet], and their likely source (*o*, *ortho*-, *p*, *para*-, *m*, *meta*-position). ^{1}H NMR was performed on 500-MHz JEOL and ^{19}F NMR at 235 MHz on a Bruker magnetic resonance spectrometer on dry d_6-DMSO solutions of the adducts.

was added to the samples before freezing. These animals were killed four days after dosing at which time liver tissue was taken for histological examination.

Sample preparation

A 0.2-ml volume of 1 *M* hydrochloric acid and 2.3 ml of ammonium sulphate (5 *M*) solution (to precipitate residual protein) was added to urine,

plasma or liver supernatant, respectively, in a screw-top tube, vortex-mixed thoroughly and centrifuged at 1200 *g* for 20 min. The supernatant was then removed and an equal volume of citrate buffer (pH 5) was added to maintain the hydrazine and acetylhydrazine in aqueous solution by protonation. To this 20 ml of dichloromethane were added, and the mixture was vortex-mixed to extract interfering lipids. The layers were separated and the derivatising agent PFB was added in excess (20 μl) to the aqueous layer containing the hydrazines. This was left to react for 30 min before final extraction of the adducts with three 5-ml volumes of chloroform for GC–MS or three 5-ml volumes of ethyl acetate for GC–NPD (chloroform and other chlorinated solvents can react and inactivate the rubidium bead within the NPD). This was followed by rotary evaporation to concentrate the sample for injection if needed.

RESULTS AND DISCUSSION

Derivatisation and chromatography

All the hydrazine compounds investigated in the study (see *Syntheses* and Table I) were found to rapidly react with PFB in aqueous solution to form the expected adducts. The structures of these adducts [PFB-hydrazones and in the case of hydrazine itself DFBA (Fig. 1, i)] were confirmed by EI-MS and ^{1}H and ^{19}F NMR spectroscopy. Simple amino compounds such as ammonia, methylamine and urea, also present in urine, were unreactive under these derivatisation conditions. This decreased reactivity of PFB with C-NH_2 groups compared with N-NH_2 groups was exemplified in the same compound, semicarbazide, which only formed PFB-formidylhydrazone (PFFhz), and not decafluorobenzaldehyde semicarbazine, the product of reaction at both -NH_2 termini.

The PFB adducts nearly all proved to have excellent chromatographic characteristics. Solutions of purified standards (Fig. 2) kept at room temperature for several months were considered completely inert as judged by their chromatographic purity and the unchanged spectra seen in repeated NMR analyses. These poly-fluorinated compounds are unreactive because of the inherent stability of the C-F bond. Consequently they produced no detectable non-physical interactions with the column. Poly-fluorinated derivatives show little intermolecular attraction for similar reasons, which was demonstrated by their high volatility. Only those adducts which possessed unmasked functionalities such as PFFhz were eluted from the column over periods longer than 10 s. The highly fluorinated adducts DFBA and PFAhz produced in the animal study and PFPhz (Fig. 2) gave sharp peaks which completely eluted within 3 s. Determination of the PFB adducts with an NPD rather than an ECD (which was fluorine-sensitive; results not shown) was preferable in this particular study. This was because the derivatising agent PFB overloaded the ECD because it was present in excess to ensure instant and complete derivatisation, but PFB itself was not detected by the NPD. The limit of detection (see *Apparatus*) with an NPD compared to an ECD was poorer, but insufficient detectivity was not a problem in determining hydrazines in the urine samples.

Hydrazine present in the plasma and liver samples (Fig. 3a and b, respectively) was determined by GC–MS with SIM for the PFB molecular ion [3,11,12]. The ratio of [^{14}N]- to [^{15}N] azine (388/390) was determined and used to calculate the concentration of [^{14}N]hydrazine. It was important to use approximately similar concentrations of internal standard as those expected in the sample (1–20 μmol) to overcome errors due to the presence of $^{13}C_2$-labelled and $^{13}C/^{15}N$-labelled isotopomers of DFBA which occur with an abundance of approximately 0.1% and contribute to the 390 molecular ion. Calibration curves prepared from hydrazine-spiked, derivatised biofluid samples were found to be approximately linear ($r \geq 0.94$) between 5 μg and 50 ng of DFBA by NPD and linear ($r \geq 0.97$) between 50 ng and 5 pg by MS–SIM injected on to the column.

GC–MS–SIM was the best method for ensuring equivalence of the internal standard. This was because any *post-mortem* autoxidation of plasma hydrazine, which occurred before derivatisation, was matched by an equal loss of the internal standard, $^{15}N_2$-labelled hydrazine (assuming kinetic isotope effects on the rate of reaction were inconsequential). Therefore the amount of hydrazine present in the plasma at the

time of removal was not underestimated. Pentafluorophenylhydrazine was chosen as the internal standard for the NPD for similar reasons; however, the rate of its autoxidation differed from that of free hydrazine. The recovered yields of spiked hydrazine samples (1–10 mmol) determined by GC–NPD in untreated rats' urine were 79 ± 14% (n = 20), whereas the recovered yields of spiked hydrazine samples (16–100 μmol) determined by GC–MS–SIM in untreated rats' plasma were 103 ± 9% (n = 20).

Liver and plasma levels of hydrazine

The levels of hydrazine in plasma and liver measured after each dose are shown in Fig. 3a and b respectively. Hydrazine levels in both plasma and liver showed an increase with dose as expected. Liver and plasma levels could not be measured accurately until at least 10 min after dosing because several minutes were required to induce anaesthesia and open the abdomen for removal of liver tissue and blood. After 30 min there was a time-dependent decrease in the levels of hydrazine in both plasma and liver following dosing. The mean half-life for hydrazine in plasma was approximately 2 h but this varied with dose. The non-ideal dose response in the liver (Fig. 3b) indicates errors may have occurred during the determination of hepatic hydrazine levels, possibly because the internal standard ($^{15}N_2$-labelled hydrazine) could not be added to the sample until after homogenisation. Prior addition would have required, assuming the rate of autoxidation of intracellular hydrazine during homogenisation was correctly matched by the internal standard on the surface of the tissue.

Urinary excretion of hydrazines

Rats receiving 2.53 mmol hydrazine per kg bodyweight were diuretic for 24 h post-dosing. They failed to gain weight and had fatty liver when they were killed at four days compared to the rats receiving lower doses of hydrazine or saline-dosed controls. Hydrazine-treated rats excreted greater quantities of hydrazine and acetylhydrazine with increasing dose over 24 h (Table II). However, the fraction of the original dose excreted as hydrazine (and acetylhydrazine) declined with increasing dose level.

Dambrauskas and Cornish [14] also found hydrazine was rapidly metabolised and excreted into urine when measured 2 h after it was administered to the rat (2.0 mmol/kg). Liver and blood levels of hydrazine were equivalent as seen in this

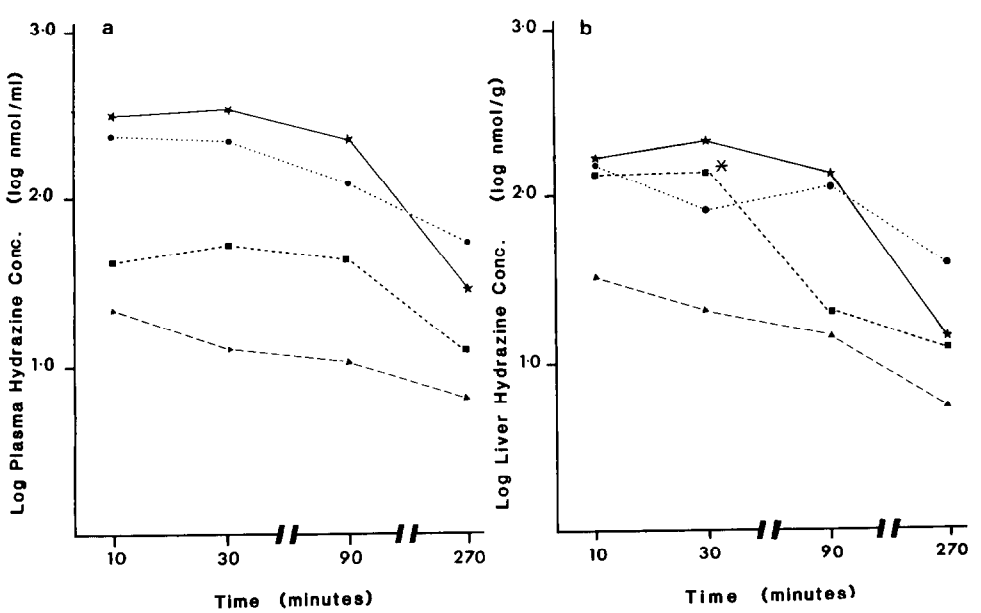

Fig. 3. Hydrazine was given to rats at doses of 0.09 (▲), 0.28 (■), 0.84 (●) and 2.53 (★) mmol/kg body weight orally. (a) Plasma; (b) liver. Points represent the mean of three rats (S.E.M. were less than 10% of the mean except at the asterisk).

TABLE II

EXCRETION OF HYDRAZINE AND ACETYLHYDRAZINE IN MALE RAT URINE (0–24h) AFTER ORAL ADMINISTRATION OF HYDRAZINE

Values represent the mean ± S.E.M. for three rats. Figures in parentheses represent the fraction of the original dose.

Hydrazine dose (mmol/kg)	Ammount excreted (μmol)		Acetylhydrazine/hydrazine ratio (%)
	Hydrazine	Acetylhydrazine	
0.09	8 ± 5 (35%)	1 ± 1 (6%)	17
0.27	26 ± 9 (42%)	3 ± 1 (4%)	10
0.84	39 ± 10 (21%)	4 ± 1 (2%)	9
2.53	99 ± 21 (18%)	6 ± 2 (1%)	6

study. Hydrazine levels in rat blood serum were also found to decline rapidly with time when measured at 1, 2 and 4 h after dosing [15]. There was a dose-related response over 0.09–2.0 mmol/kg. These groups used a spectrophotometric method involving the determination of the azine of *p*-dimethylaminobenzaldehyde. This method is moderately sensitive and the chromophore can be determined at even lower concentration by fluorescence [16] in acidic chloroform solution. However, the spectrophotometric method does not distinguish hydrazine from its monoacetylated derivative [17]. The studies reported here indicate that acetylhydrazine is a minor metabolite of hydrazine judging on the amount excreted into the urine (Table II). The other hydrazine metabolites [13], diacetylhydrazine and the cyclised 2-oxoglutarate adduct, THOPC, did not react with PFB and were therefore not detected by this assay (results not shown). McKennis *et al.* [18] found that some mono- and diacetylhydrazines were excreted by animals dosed with hydrazine, the second acetylation representing a detoxification step. Springer *et al.* [19] studied another detoxification step, the rapid conversion of $^{15}N_2$-labelled hydrazine to nitrogen gas by the rat using MS of the exhaled metabolite, which proved to be an important metabolic pathway.

In conclusion, capillary GC using NPD and MS with PFB derivatisation are reliable methods for determining free hydrazine in biofluids once it is derivatised if prior autoxidation can be minimised or matched by the internal standard. Similar methods for determining hydrazine-based drugs and their metabolites have been previously reported [3,9–12]. (Note that also methylated hydrazines which are known to be potent carcinogens can be determined by liquid chromatography [20].) Acethylhydrazine is thought to be metabolised to a reactive electrophile by liver mixed function oxidases [1] while hydrazine is known to inhibit pyridoxal phosphate-dependent enzymes [4] and gluconeogenesis [21] *in vivo*. The changes hydrazine ellicits in hepatic lipid metabolism resulting in fatty liver [5] are unknown. This study has shown the applicability of PFB derivatisation and subsequent capillary GC with NPD or MS for assaying hydrazine and its derivatives. Further, the study indicates that the rats' ability to rapidly excrete hydrazine may be overwhelmed with increasing dose which may lead to increased metabolic flux through other pathways. It should be possible to use the GC–MS–SIM method reported here (in reverse) to measure [$^{15}N_2$]hydrazine and its derivatives, in animals dosed for ^{15}N NMR studies [13], using non-labelled hydrazine as the internal standard.

ACKNOWLEDGEMENTS

This work was supported by the Wellcome Trust and Bayer AG.

REFERENCES

1 I. A. Blair, R. Mansilla Tinoco, M. J. Brodie, R. A. Clare, C. T. Dollery, J. A. Timbrell and I. A. Beever, *Human Exp. Toxicol.*, 4 (1985) 195.
2 J. Gold, *Nutr. Cancer*, 9 (1987) 59.

3 Y. Y. Liu, I. Schmeltz and D. Hoffmann, *Anal. Chem.*, 46 (1974) 885.
4 R. W. Horton, A. G. Chapman and B. S. Meldrum, *J. Neurochem.*, 33 (1979) 745.
5 R. G. Lamb and W. L. Banks, *Biochim. Biophys. Acta*, 574 (1979) 440.
6 D. Steinhoff and U. Mohr, *Exp. Pathol.*, 33 (1988) 133.
7 C. Biancifiori, *J. Natl. Cancer Inst.*, 44 (1970) 943.
8 W. S. Bosan, R. C. Shank, J. G. MacEwen, C. L. Gaworski and P. M. Newberne, *Carcinogenesis*, 8 (1987) 439.
9 O. Gyllenhaal, L. Grönberg and J. Vessman, *J. Chromatogr.*, 511 (1990) 303.
10 G. Karlaganis, E. Peretti and B. H. Lauterburg, *J. Chromatogr.*, 420 (1987) 171.
11 S. P. Jindal, T. Lutz and T. B. Cooper, *J. Chromatogr.*, 221 (1980) 301.
12 T. S. Rao, G. B. Baker, R. T. Coutts, J. M. Yeung, G. J. A. McIntosh and G. A. Torok-Both *J. Pharmacol. Methods*, 17 (1987) 297.
13 N. E. Preece, J. K. Nicholson and J. A. Timbrell, *Biochem. Pharmacol.*, 41 (1991) 1319.
14 T. Dambrauskas and H. H. Cornish, *Toxicol. Appl. Pharmacol.*, 6 (1964) 653.
15 B. A. Reynolds and A. A. Thomas, *Am. Ind. Hyg. Assoc. J.*, 26 (1965) 527.
16 S. Vickers and E. K. Stuart, *Anal. Chem.*, 46 (1974) 138.
17 T. Alvarez de Laviada, F. J. Romero, V. Anton, Jr., J. Roma and J. Vina, *J. Anal. Toxicol.*, 11 (1987) 260.
18 H. McKennis, Jr., A. S. Yard, J. H. Weatherby and J. A. Haggy, *J. Pharmacol. Exp. Ther.*, 114 (1985) 385.
19 D. L. Springer, B. M. Krivak, D. J. Broderick, D. J. Reed and F. N. Dost, *J. Toxicol. Environ. Health*, 8 (1981) 21.
20 E. S. Fiala and J. H. Weisburger, *J. Chromatogr.*, 117 (1976) 181.
21 P. D. Ray, R. L. Hanson and H. A. Lardy, *J. Biol. Chem.*, 245 (1970) 690.