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GAS CHROMATOGRAPHIC ANALYSIS OF MONOALKYLHYDRAZINES

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SUMMARY

A quantitative electron-capture gas chromatographic assay procedure was developed for the analysis of monoalkylhydrazines in biological samples. Application to the analysis of phenelzine was demonstrated. Four monoalkylhydrazines were analyzed in whole blood by reaction with pentafluorobenzaldehyde to form stable hydrazone derivatives which were extracted and subsequently reacted with pentafluoropropionic anhydride to give products which were very sensitive to electron-capture detection when analyed by gas chromatography. Methylhydrazine, benzylhydrazine, phenelzine and pheniprazine each yielded single derivatives with this procedure suggesting that the analytical procedure has a broad application to the analysis of other monoalkylated hydrazines. The method was applied to monitor whole blood levels of phenelzine in rats treated intravenously with phenelzine sulphate.

INTRODUCTION

The analysis of monoalkylated hydrazines in biological samples is known to be complicated by poor extraction efficiencies possibly due to auto-oxidation and decomposition under alkaline conditions [1–3]. In addition, reactions of hydrazines with acylating reagents frequently give rise to multiple derivatives [4–6], and steps must be incorporated into assay procedures to compensate for these undesired reactions. Jindal et al. [1] have demonstrated that phenelzine (2-phenylethylhydrazine) reacts quantitatively in aqueous solution with pentafluorobenzaldehyde (PFBA) to form the corresponding hydrazone derivative, which

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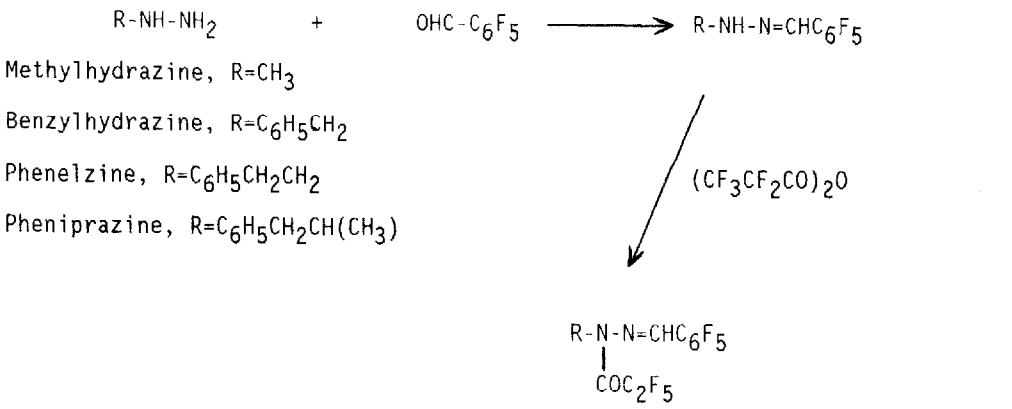

Fig. 1. Reaction sequence for generation of perfluoroacylated hydrazone derivatives of monoalkylhydrazines.

can be assayed by a mass spectrometric procedure. Furthermore, Dyck [4] has demonstrated that the acetone hydrazone of phenelzine readily reacts with dansyl chloride to form a monodansylated hydrazone. These two observations suggested to us that monoalkylhydrazines might be conveniently analyzed in biological systems by converting them to PFBA hydrazones prior to their reaction with a perfluoroacylating reagent to produce single derivatives sensitive to gas chromatography (GC) with electron-capture detection (ECD).

In this report we describe an assay procedure based upon dual derivatization with PFBA and pentafluoropropionic anhydride (PFPA), by which simple monoalkylated hydrazines can be determined in aqueous biological samples.

EXPERIMENTAL

Instrumentation

Analyses were performed using a Perkin-Elmer Model 3B gas chromatograph equipped with an electron-capture detector (^{63}Ni) and a 20 m×0.32 mm I.D. DB-5 fused-silica capillary column (J&W Scientific, Palo Alto, CA, U.S.A.). Oven temperature was programmed from 110 to 250°C at a rate of 4°C/min. Carrier gas was helium, and argon–methane (95:5) was added as make-up gas.

Mass spectra were obtained on a Hewlett-Packard Model 5985 mass spectrometer in series with a Hewlett-packard Model 5840 A gas chromatograph which contained a 30 m×0.32 mm I.D. HP-5 fused-silica capillary column (Hewlett-Packard, Palo Alto, CA, U.S.A.). Chromatographic conditions were: initial temperature 80°C, increased at 20°C/min to 300°C and held for 3 min.

Chemicals and reagents

Phenelzine sulphate and benzylhydrazine dihydrochloride were obtained from Sigma (St. Louis, MO, U.S.A.). Methylhydrazine, PFBA and PFPA were obtained from Aldrich (Milwaukee, WI, U.S.A.). (±)-Pheniprazine hydrochloride was a gift from Merrell Pharmaceuticals (Concord, Canada).

All solvents were ACS grade (Fisher Scientific, Fair Lawn, NJ, U.S.A.) and glass-distilled prior to use. Water was distilled and deionized using a Milli-Q reagent water system (Millipore, Bedford, MA, U.S.A.).

Analysis of methylhydrazine, phenelzine, benzylhydrazine and pheniprazine in blood

To 0.6 ml of whole blood were added methylhydrazine, benzylhydrazine, phenelzine and pheniprazine (methylhydrazine 20 ng; others 100 ng). The solution was mixed for 30 s in an IKA-Vibrax-VXR multi-tube vortex mixer (Terochem, Edmonton, Canada), and 1 ml of 0.4 *M* perchloric acid was added to precipitate protein. After centrifugation for 10 min (Fisher microcentrifuge Model 235B at 10 000 *g*), the supernatant was retained and the pH was adjusted to between 6.8 and 7.0 with solid sodium bicarbonate. After addition of 0.04 ml of PFBA (4%, v/v in dimethylformamide), the samples were shaken at room temperature for 30 min after which time excess solid sodium bicarbonate was added and the solution was extracted with 5 ml of ethyl acetate. After shaking for 30 min, the organic layer was retained and 5 μl of *n*-dodecane were added (cf. ref. 1). The volume was reduced under a gentle stream of nitrogen to 0.5 ml, and the organic phase remaining was transferred to a 1.5-ml polypropylene microcentrifuge tube and evaporated to dryness. PFPA (65 μl) and dry ethyl acetate (25 μl) were then added and acylation was allowed to proceed for 30 min at 70°C. The reaction mixture was evaporated to dryness and the residue was dissolved in 0.6 ml of toluene. After washing with 0.5 ml of 0.1 *M* ammonium hydroxide, a 1-μl portion of the toluene solution was injected into the gas chromatograph.

Standard curves

To prepare standard curves, benzylhydrazine (internal standard, 100 ng) and phenelzine (5, 50, 100, 500, 1000 and 2000 ng) were added to six whole blood samples (1.0 ml) which were extracted as described above. A calibration curve was constructed by plotting phenelzine/benzylhydrazine peak-area ratios against phenelzine concentration, using the regression equation.

Analysis of phenelzine in blood

Jugular canullae were inserted in rats, and the animals were allowed to recover before being administered phenelzine (15 mg/kg free base). Blood samples (0.6 ml) were withdrawn 30, 60, 90 and 120 min after drug administration. Benzylhydrazine (100 ng) was added to each sample and derivatization and extraction were carried out as described above.

RESULTS AND DISCUSSION

Earlier reports on the analysis of phenelzine have described steps necessary to minimize or compensate for poor extraction efficiencies and formation of multiple derivatives [1,4–6]. The procedure described by us is superior to previously reported procedures in that a single, stable derivative of phenelzine, sensitive to GC–ECD, is obtained. Similar derivatives of three other monoalkyl hydrazines were also obtained; it is possible that this procedure may have broad application.

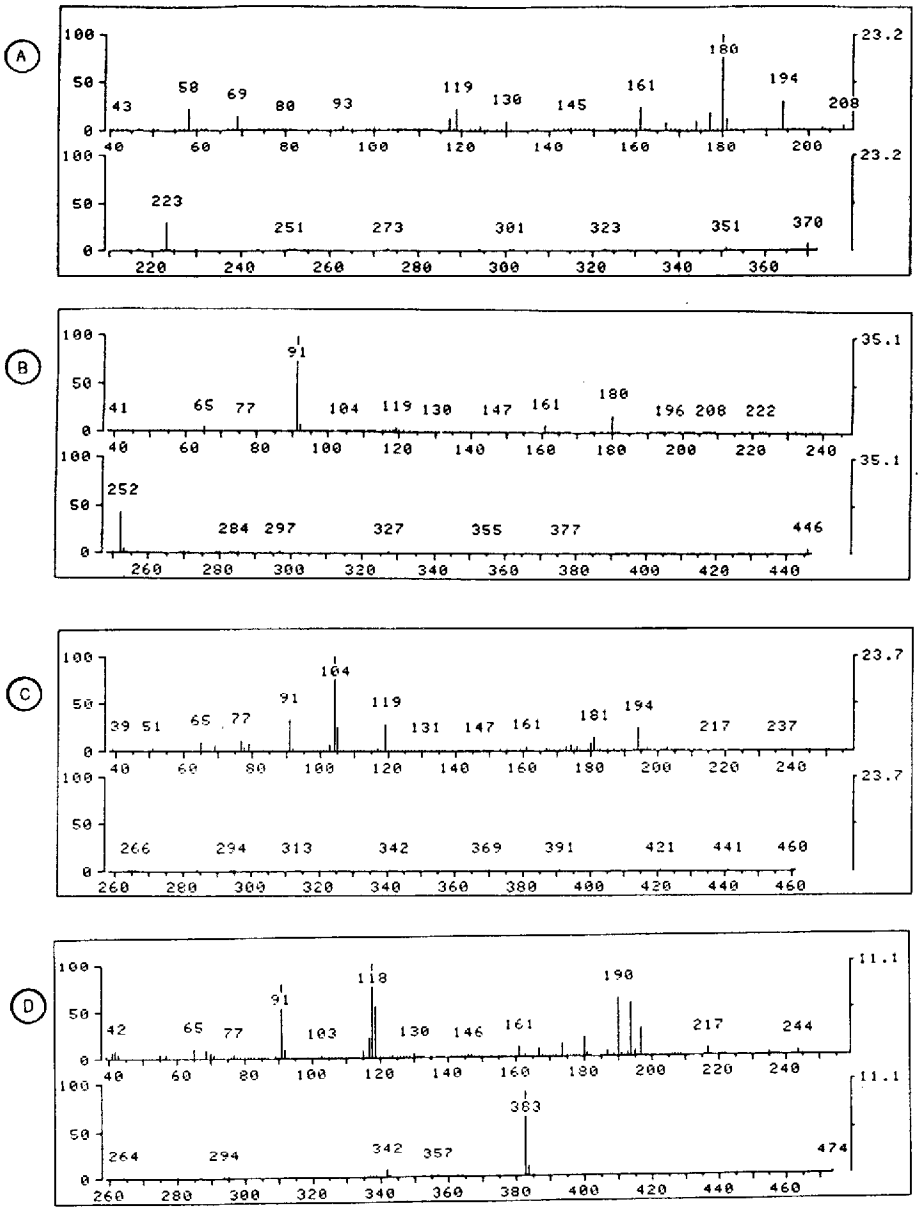

Fig. 2. Electron-impact mass spectra of (A) methylhydrazine, (B) benzylhydrazine, (C) phenelzine and (D) pheniprazine after derivatization with PFBA and PFPA. Molecular ions of the derivatives are of *m/z* 370, 446, 460 and 474, respectively.

The reaction sequence involved in our procedure is depicted in Fig. 1. By reacting the hydrazines first with PFBA as described by Jindal et al. [1], each was converted to an easily extracted stable hydrazone. In our hands, these hydrazones were not sufficiently ECD-sensitive, and to increase this characteristic, further derivatization with PFPA was necessary. Whereas others [5,6] have observed the formation of multiple perfluoroacylated derivatives of monoalkylated hydra-

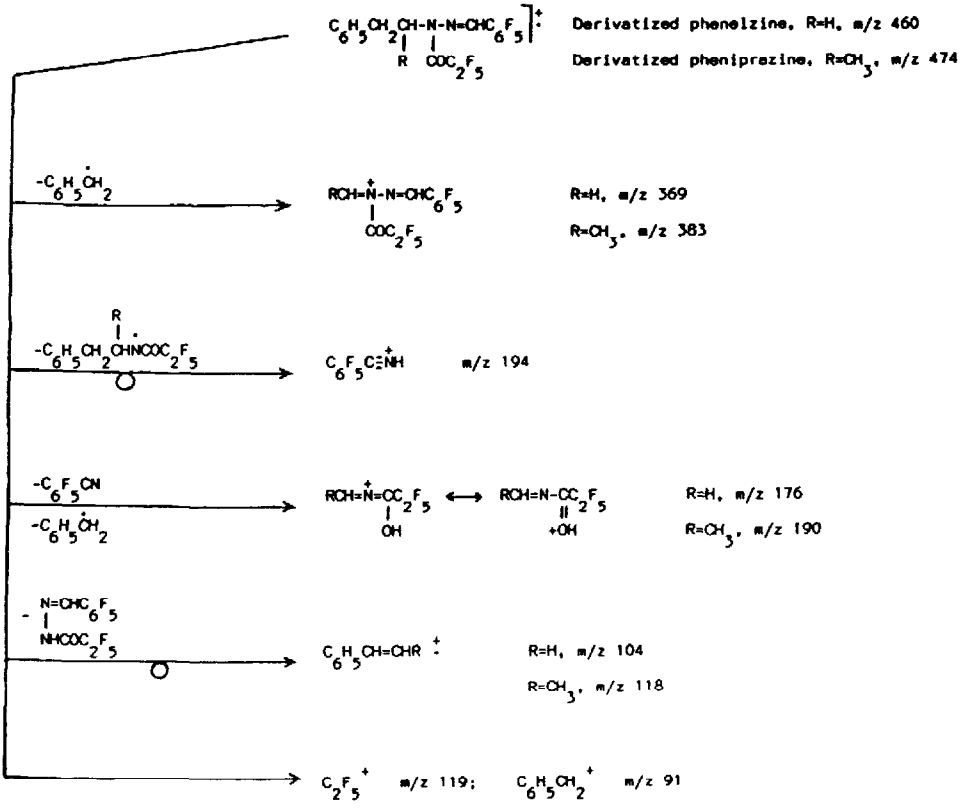

Fig. 3. Proposed fragmentation pathways in the electron-impact mass spectra of derivatized phenelzine and pheniprazine.

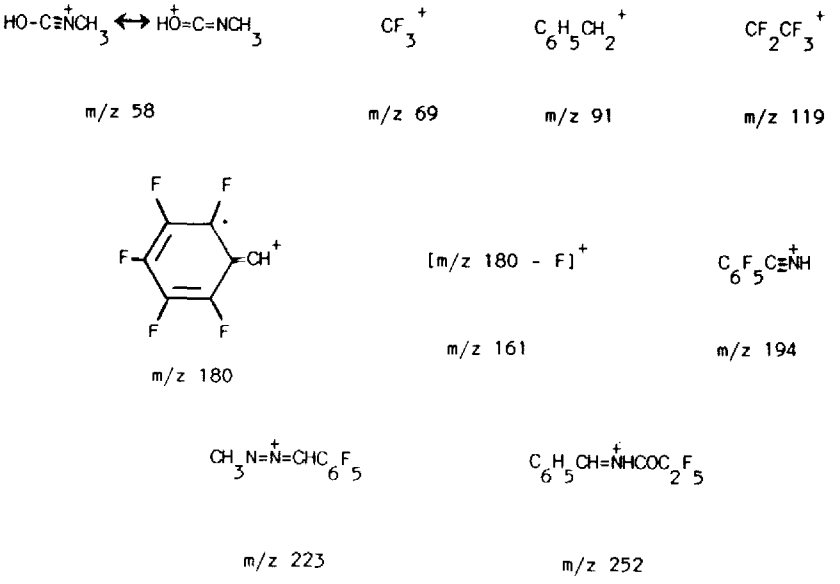

Fig. 4. Diagnostic fragment ions in the electron-impact mass spectra of derivatized methylhydrazine and benzylhydrazine.

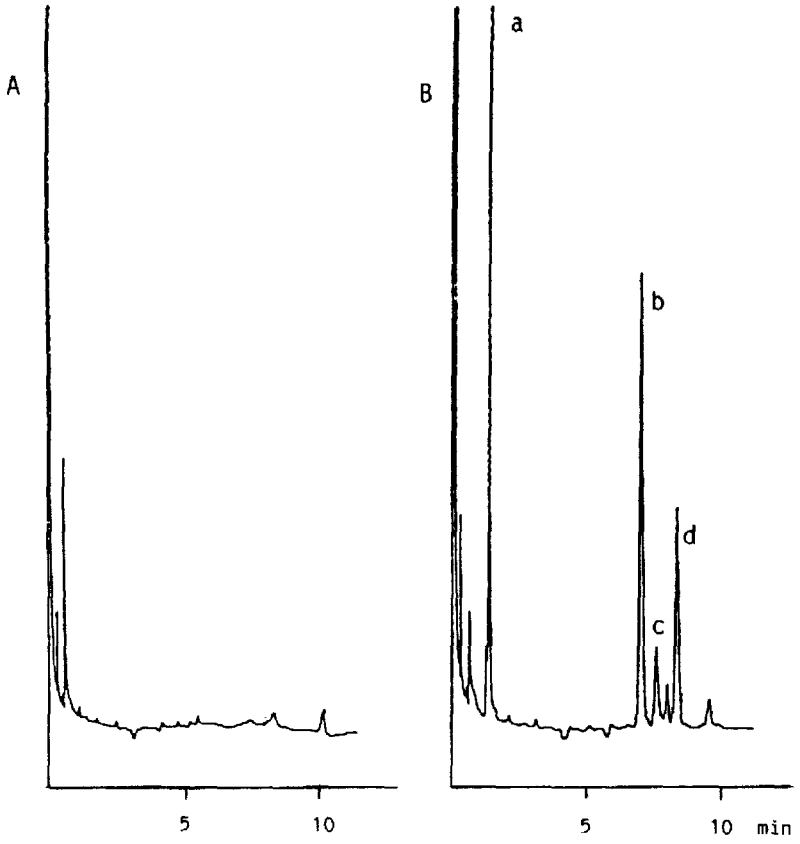

Fig. 5. Gas chromatogram (ECD) of (A) blank blood extract and (B) extract of derivatized blood containing (a) 20 ng/ml methlhydrazine, (b) 100 ng/ml benzylhydrazine, (c) 100 ng/ml pheniprazine and (d) 100 ng/ml phenelzine.

zines, single derivatives of methylhydrazine, phenelzine, benzylhydrazine and pheniprazine were obtained using our reaction conditions. The initial formation of the hydrazone and the subsequent perfluoroacylating reaction are apparently quantitative. The identity of each derivatized hydrazine was confirmed by mass spectrometry. The electron-impact mass spectra of the four derivatized hydrazines are provided in Fig. 2. All spectra contained molecular ions of low abundance. The derivatives of phenelzine and pheniprazine gave spectra that resulted from similar fragmentation pathways which are depicted in Fig. 3. The spectra of derivatized methylhydrazine and benzylhydrazine also contained diagnostic ions which are identified in Fig. 4.

After derivatization, all four model hydrazones employed in our experiments were chromatographically well separated, both from each other and from interfering substances (Fig. 5). GC of mixtures of methylhydrazine, benzylhydrazine, phenelzine and pheniprazine, after derivatization, indicated greatest detector sensitivity towards methylhydrazine and lowest sensitivity toward pheniprazine.

The reasons for these differences in detector sensitivity are not known. The high sensitivity towards methylhydrazine may be of value in the analysis of the low levels of this hydrazine that are found in mushrooms [7] or in biological samples from patients who have ingested methylhydrazine-containing mushrooms.

Standard curves for the analysis of phenelzine in whole blood were linear over the range 5–2000 ng/ml when benzylhydrazine was used as internal standard. Averages of triplicate measurements were plotted; the coefficient of variation was less than 4% at all concentrations except at the 5-ng level for which the coefficient of variation was 10.8%. The line through the data points is described by $y=0.00506x-0.0778$ ($r^2=0.998$). The limit of detection was 2 ng/ml and sufficiently sensitive to study the kinetics of this antidepressant in human patients [1]. When applied to the analysis of phenelzine in rat blood, levels detected 30, 60, 90 and 120 min after intravenous injection of phenelzine (15 mg/kg) were 10.47 ± 1.54, 4.35 ± 0.87, 0.80 ± 0.17 and 0.42 ± 0.12 μg/g ($n=4$), respectively. These levels are appreciably higher than those reported by Rao et al. [6] in rat brain after a similar dose was administered intraperitoneally. These differences may be due to changes in route of administration, in sampling times or in tissues examined. Our results agree more closely with those of Dyck [4] who reported a phenelzine concentration of 500 ng/g in rat striatum 2 h after intraperitoneal administration of a higher drug dose.

In summary, we have demonstrated that the analysis of simple hydrazine substances in biological samples can be accomplished after conversion of each hydrazine to a hydrazone by treatment with PFBA, followed by perfluoroacylation with PFPA to give a diderivatized product. This procedure generates single derivatives which are sensitive to GC–ECD and easily quantified. Using this method, phenelzine can be readily analyzed quantitatively in rat blood.

ACKNOWLEDGEMENTS

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