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Note

Determination of fenoctimine in plasma by capillary gas chromatography with nitrogen—phosphorus detection

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Fenoctimine sulfate {4-(diphenylmethyl)-1-[(octylimino)methyl] piperidine sulfate (1:1) hemihydrate} is a new gastric anti-secretory agent [1–3]. The drug has been tested clinically in the 100–150 mg range. Maximum plasma concentrations of fenoctimine under these conditions are $\leqslant 10$ ng/ml, which is below detection limits of packed-column methods for this compound.

This paper reports the development of a sensitive and reproducible capillary gas chromatographic (GC) assay using nitrogen–phosphorus detection (NPD) which uses both hot-needle and splitless injection modes. The assay has a detection limit of 0.5 ng/ml and has been employed successfully in analyzing biological samples from clinical and non-clinical studies.

EXPERIMENTAL

Apparatus

Gas chromatography. A Hewlett-Packard 5880A capillary gas chromatograph equipped with a splitless injector and a nitrogen–phosphorus ionization detector was used. An SE-54 fused-silica capillary column (15 m × 0.32 mm I.D.; 0.12 μm film thickness, J & W Scientific, Rancho Cordova, CA, U.S.A.) was used with helium as carrier gas at a flow-rate of 4 ml/min at 210°C. The injector and detector temperatures were 300°C and oven temperature programming was employed from 210 to 250°C at 30°C/min. Splitless injection with a purge at 0.5 min was used in conjunction with a hot-needle technique. Air (as a 2-μl plug) was drawn into a 20-μl Hamilton syringe (Reno, NV, U.S.A.) immediately before and after the sample (5 μl) so that the syringe needle barrel contained no solvent before and after injection. The syringe needle was introduced into the injector 10 s before injection to acquire injector temperature

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and stayed for an additional 10 s after injection. Oven temperature programming was started 45 s following injection.

Data acquisition. A Hewlett-Packard 3354 laboratory automation system with software developed at McNeil Pharmaceutical (Spring House, PA, U.S.A.) was used for data acquisition and processing.

Reagents and supplies

Solvents. Nanograde methanol and diethyl ether were obtained from Mallinckrodt (Paris, KY, U.S.A.) and used without further purification. Triply purified, distilled water was obtained from Ephrata Mountain Water (Manheim, PA, U.S.A.).

Reagents. Ammonium carbonate and ammonium hydroxide (58%), analytical-reagent grade, were purchased from Mallinckrodt.

Other supplies. C_{18} Bond-Elut cartridges, 500 mg capacity, and the Vac-Elut manifold were purchased from Analytichem International (Harbor City, CA, U.S.A.).

A nonylimino analogue of fenoctimine was used as the internal standard. Fenoctimine sulfate and the internal standard were obtained in-house (McNeil Pharmaceutical). Structures of these compounds are given in Fig. 1.

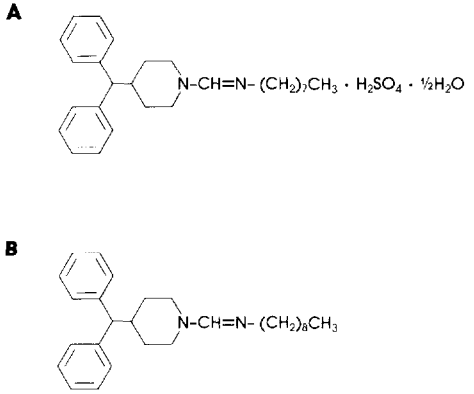

Fig. 1. Structural formulae for fenoctimine sulfate (A) and the internal standard (B).

Extraction procedure

To a 2-ml plasma sample in a 14.5-ml disposable screw-top bottle, 20 μl of methanol containing 20 ng of internal standard and 10 ml of diethyl ether are added. The capped bottle is shaken for 15 min on a table top shaker (Eberbach) at 240 oscillations/min and centrifuged at 700 *g* for 20 min. An 8-ml aliquot of the supernatant ether layer is pipetted into a 12-ml centrifuge tube and evaporated to dryness under a stream of nitrogen at room temperature. The residue is reconstituted in 0.5 ml of 1% (w/v) ammonium carbonate—methanol (2:3, v/v). This is mixed on a Vortex Genie (Scientific Instruments, Springfield, MA, U.S.A.) at a speed setting of 6 for 10 s. All of the resulting solution is decanted into the reservoir of a conditioned C_{18} Bond-Elut cartridge. Cartridges are conditioned by washing twice with 3 ml of methanol and once with 3 ml of 1% (w/v) ammonium carbonate—methanol (2:3, v/v). Following the application of the sample, the cartridge is rinsed twice with 3 ml of 1% (w/v) ammonium

carbonate—methanol (2:3, v/v) and once with 3 ml of 0.5% (v/v) ammonium hydroxide in methanol.

The sample is eluted using 5 ml of 0.5% (v/v) ammonium hydroxide in methanol. The eluent is transferred to a centrifuge tube and evaporated to dryness using a gentle nitrogen stream. The sides of the tube are washed with 1 ml of methanol and the solution is again evaporated to dryness.

The dried residue is reconstituted with 30 μl of methanol. A 5-μl aliquot is injected into the capillary gas chromatograph for analysis.

Standard curves

To establish a calibration curve, a series of fenoctimine standard solutions (0.1—10.0 ng/μl) were prepared in methanol. Appropriate aliquots (10—60 μl) of these solutions were added to 2 ml of plasma and the samples were extracted according to the procedure above. Duplicate standard curves were run on each analysis day. The peak-height ratios of fenoctimine and the internal standard were weighed by 1/variance and plotted against the fenoctimine concentrations. Linear regression analysis gave a calibration line which was used to calculate fenoctimine concentrations in unknown samples and frozen seeded controls.

As an additional control, seeded plasma pools were prepared at three concentrations (3, 7 and 13 ng/ml fenoctimine), separated into 2-ml aliquots and frozen. Two samples from each pool were analyzed with each calibration curve to assess the precision of the assay procedure.

RESULTS AND DISCUSSION

Gas chromatography

Chromatograms of plasma with and without fenoctimine and the internal standard are shown in Fig. 2. The retention times of fenoctimine and the internal standard are 8.66 and 10.56 min, respectively. No significant interfering peaks appear in the chromatogram of blank plasma and the two compounds are well separated. Additionally, owing to the selectivity of the cartridge procedure and the resolution of the capillary column, none of the following drugs interfere with the determination of fenoctimine in plasma: phenobarbital, morphine, aspirin, acetaminophen, ibuprofen, chlorothiazide and propranolol.

Standard curves

Duplicate calibration curves run on three consecutive days were linear over the concentration range (0.5—16.0 ng/ml) studied here (Table I). The linear regression line which represents the best fit of the fenoctimine data had an equation of $y = 0.184x + 0.062$ (y = drug/internal standard peak-height ratio and x = fenoctimine concentration). The correlation coefficient for the three-day composite curve was 0.995. The relative standard deviations at each concentration were less than 10.4% except at the detection limit of 0.5 ng/ml (20.3%). The average back-calculated concentration was within 10% of the seeded value at each concentration. The average measured concentrations of the frozen seeded control plasma samples (n=6) were within 11% of their theoretical concentrations with relative standard deviations of less than 12% (data not shown).

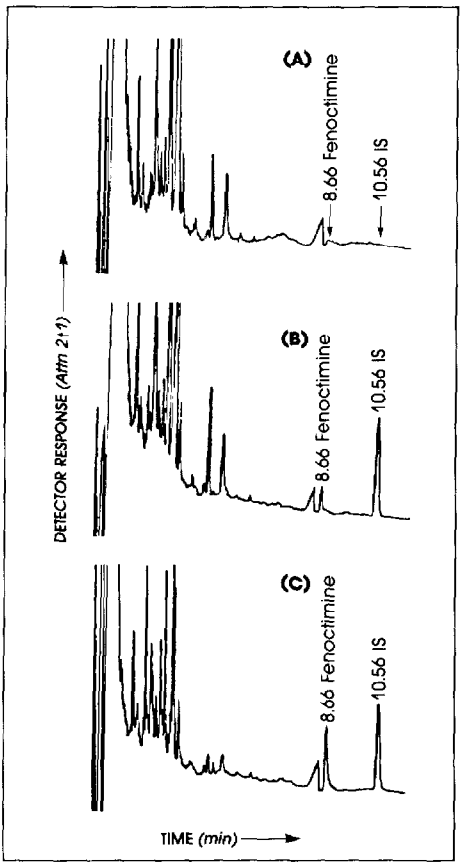

Fig. 2. Chromatograms of blank human plasma (A), human plasma spiked with 2.0 ng/ml fenoctimine and 10 ng/ml internal standard (IS) (B) and a clinical sample containing 5 ng/ml fenoctimine (C).

TABLE I

SUMMARY OF STANDARD CURVE DATA FOR THE ANALYSIS OF FENOCTIMINE IN PLASMA

Duplicate standard curves were analyzed on three separate days ($n=6$).

Concentration seeded (ng/ml)	Mean concentration found (ng/ml)	Relative standard deviation (%)	Deviation from seeded value (%)
0.5	0.51	20.3	+1.3
1.0	0.99	7.2	−1.2
3.0	3.1	4.5	+2.3
5.0	4.5	10.4	−10.0
7.0	7.1	6.7	+0.9
10.0	9.7	8.4	−2.7
13.0	13.2	4.6	+0.6
16.0	15.8	7.7	−1.0

Recovery and stability

The extraction efficiency for fenoctimine was determined using ^{14}C-labeled compounds. At fenoctimine concentrations of 15 ng/ml, the extraction efficiency was 87 ± 4% (n=8). The extraction efficiency for the internal standard was 78 ± 2% at 10 ng/ml (n=16). Fenoctimine was found to be stable in frozen human plasma or urine at −5°C for at least three months.

Application of the procedure to plasma samples

This procedure has been used successfully in analyzing plasma samples from mouse, rat, dog and man. Fig. 3 shows a typical plasma concentration versus time profile from a subject following oral administration of a single 150-mg solution dose of fenoctimine sulfate. The plasma concentration of fenoctimine at the 12-h time point is below the detection limit of the assay (0.5 ng/ml).

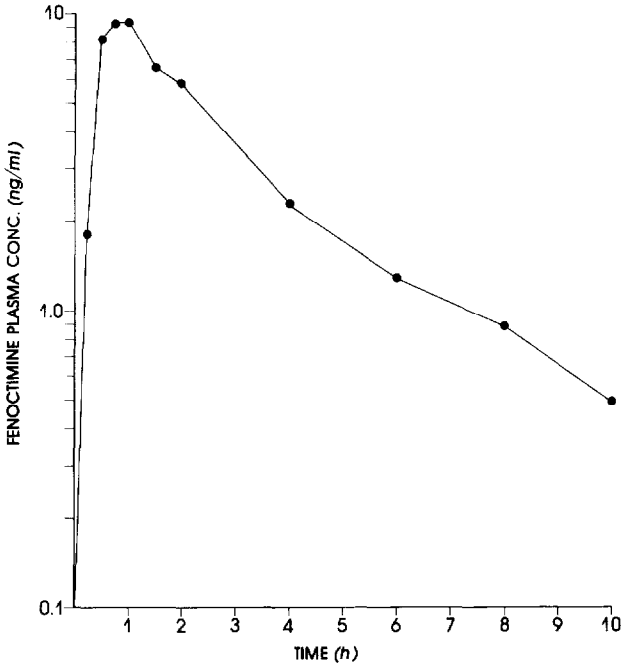

Fig. 3. Plasma concentration—time profile from a subject following oral administration of a 150-mg solution dose of fenoctimine sulfate.

In trace analysis, splitless injection can offer lower detection limits and shorter analysis times when compared to on-column injection. These advantages are achieved by using larger injection volumes (5 μl vs. 0.5 μl) and shorter temperature programmes. The larger variation of signal response with splitless injection (cf. on-column) can be overcome using a hot-needle injection technique and a close structural analogue of the compound of interest as an internal standard. The present assay illustrates that splitless and hot-needle injection modes can offer good assay sensitivity, speed and accuracy with acceptable precision.

In summary, this assay procedure provides a reliable and sensitive method for determining fenoctimine concentrations as low as 0.5 ng/ml in plasma.

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