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Note

Quantitation of selective dopaminergic drugs in plasma by gas chromatography–mass spectrometry following solid-phase extraction

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The substituted benzazepines represent a diverse series of compounds with varied pharmacologic properties. Of particular interest are five benzazepines with high selectivity for central nervous system dopamine-1 receptors (D-1) and peripheral nervous system dopamine-1 receptors (DA-1): (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine, a potent DA-1 receptor antagonist (I), (*R*)-(+)-7-iodo-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine, a potent DA-1 receptor antagonist (II), (*R*)-(+)-6-chloro-7,8-dihydroxy-1-(3-hydroxyphenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine, a selective DA-1 receptor agonist (III), (*S*)-(−)-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine, an inactive isomer of I (IV) and 7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine, a DA-1 receptor agonist (V) The actions of these drugs are summarized in Table I and their structures shown in Fig 1.

Selective dopaminergic drugs have been developed with higher affinities than dopamine for the D-1 and DA-1 receptors and many of the pharmacologic actions have been investigated in humans and dogs. However, these pharmacologic properties are often studied without quantitating their concentrations in biological fluids This is in part due to the fact that current analytical methods for these drugs either lack sensitivity or require extensive sample preparation prior to analysis Published reports include a high-performance liquid chro-

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TABLE I

ACTIONS OF THE SUBSTITUTED BENZAZEPINES

Drug	Receptor activity	Structure[a]	Reference
SCH-23390	D-1 antagonist	I	1
SKF-103108A	D-1 antagonist	II	2
SKF-82526	D-1 agonist	III	3
SCH-23388	D-1 agonist	IV	4
SKF-38393	Inactive isomer of I	V	1

[a]See Fig 1

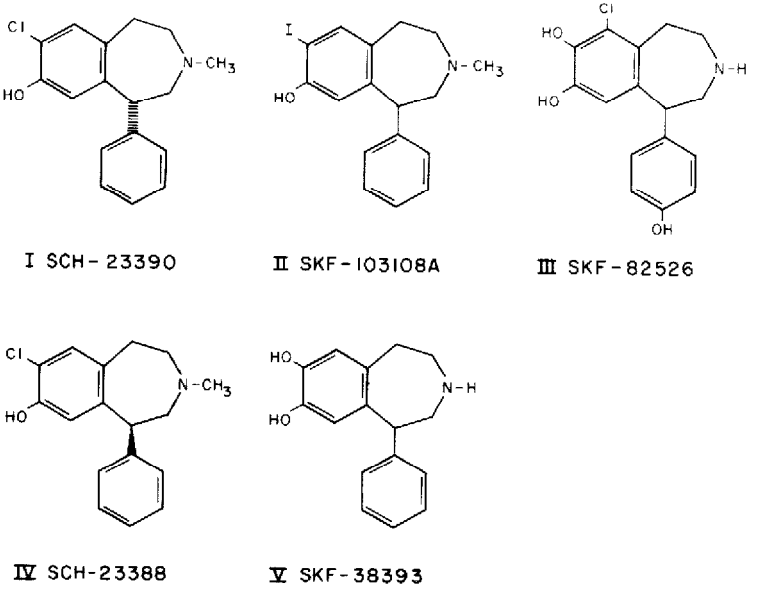

Fig 1 Structures of the five benzazepines investigated (see also Table I)

matographic (HPLC) method for I with a detection limit in the ng/ml range [5] and a gas chromatography method for a structural analogue of I, *d*-7,8-dimethoxy-3-methylphenyl-2,3,4,5-tetrahydro-1*H*-benzazepine acid maleate, capable of detecting 500 pg/ml [6]. We report a general gas chromatographic–mass spectrometric (GC–MS) analysis scheme for the quantification of the members of this class of compounds in plasma using one of the series as internal standard. This analysis scheme is exemplified by the quantitation of I using II as internal standard. This method allows quantitation at levels down into the range of 10 pg/ml of plasma utilizing a simplified solid-phase extraction scheme to isolate these compounds from the plasma matrix. The GC–MS properties of the additional compounds are also presented and discussed

EXPERIMENTAL

Materials

All solvents used were HPLC-grade solvents purchased from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Cyanopropyl solid-phase extraction columns, Baker 10SPE CN disposable columns, were from J T Baker (Phillipsburg, NJ, U.S A.). Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane was purchased from Pierce (Rockford, IL, U S.A.). II, III and V were generous gifts from Smith, Kline and French and I and IV were purchased from Research Biochemicals (RBI, Natick, MI, U S.A.).

Preparation of standards

Concentrated stock solutions of I and II were prepared in methanol and diluted as needed prior to use A series of dog plasma standards were prepared by addition of the appropriate amount of I to yield samples with final concentrations of 10 p*M*, 100 p*M*, 10 n*M* and 100 n*M* for each analysis set. These standards were treated in an identical manner as the specimens submitted for analysis and were used to determine response ratios for quantitation.

Preparation of samples

A 1.0-ml volume of plasma was transferred to a glass tube and 0.01 nmol of II added to yield a final internal standard concentration of 10 n*M*. The plasma was then deproteinized through the addition of 200 μl of 10% (w/v) trichloroacetic acid (Aldrich, Milwaukee, WI, U.S.A.) to each sample [7]. The samples were subsequently centrifuged for 15 min at 4500 *g* and the pH of the supernatant was adjusted to 9 0 by gradual addition of 1 *M* sodium hydroxide. The cyanopropyl solid-phase extraction columns were washed with 5 ml of methanol and 10 ml of distilled water and the pH-adjusted supernatant was applied to the column The column was washed with three 0 5-ml aliquots of water and the analytes were eluted in 1.5 ml of methanol

GC–MS analysis

Derivatization The methanol was evaporated at 60°C under a stream of argon and the residue transferred to a 1-ml reaction vial with three 200-μl aliquots of fresh methanol. The samples were again evaporated to dryness under argon and the residue was silylated in a mixture of 100 μl of acetonitrile and 100 μl of BSTFA at 60°C for 30 min. The reagent mixture was then evaporated and the residue reconstituted in 20 μl of BSTFA for analysis. At times an appreciable amount of insoluble material was present following the derivatization step. In those cases, the samples were centrifuged again and the supernatant (consisting of the silylating reagent mixture) transferred to a clean reaction vial prior to evaporation and reconstitution in 20 μl of BSTFA. This insoluble material was generally encountered in samples which precipitated

larger amounts of protein and was a particular problem for samples of dog plasma

GC–MS A Finnigan MAT 8230 GC–MS system with a Varian gas chromatograph equipped with a 30 m × 0.32 mm DB-1 dimethyl polysiloxane capillary column with a 25 μm film thickness (J&W Scientific, Folsom, CA, U.S.A.) was used. Samples (1–2 μl) were injected on-column at 70°C and the oven temperature was increased to 300°C at 15°C/min with a linear velocity of 50 to 60 cm/s. The column was interfaced directly into the ion source through a transfer line heated to approximately 280°C; the ion source temperature was maintained at approximately 250°C. Data were acquired in the selected-ion monitoring mode at a mass resolution of 750 Ions at *m/z* 451, 359, 268 and 267 were monitored at a rate of 1.5 cycles/s. Electron ionization with 70-eV electrons was used

RESULTS AND DISCUSSION

The mass spectra of the trimethylsilyl derivatives of I and II are shown in Fig. 2. Molecular ions of high relative intensity were observed for both compounds with high-relative-intensity fragment ions at *m/z* 267 and 268 also being observed for both compounds. These ions were, therefore, chosen to be monitored although the fragmentation processes which lead to the ions at *m/z* 267 and 268 are not known. Quantitation was always based on the ratios of the integrated peak areas in the mass chromatograms of the molecular ions with the mass chromatograms of *m/z* 267 and 268 providing additional confirmation of the qualitative identification of the analyte peaks.

Data obtained in recovery studies with the ^{125}I analogue of II show 91% recovery in the methanol fraction with the remaining 9% lost during the water washes. These data indicate that this compound is recovered in high yield using our sample isolation scheme. For this analysis, II was chosen as the internal standard because of the structural similarity between it and the analyte I. This structural similarity would indicate that the extraction efficiency for both compounds should be similar provided a simple extraction procedure is used. An equally high extraction yield was, therefore, expected for the analyte I in this very simple solid-phase extraction protocol Extracted standards were, however, always used to determine response ratios for quantitation. A calibration curve for this analysis was found to be linear over the range studied, correlation coefficient = 0.998 with a slope of $1\,23 \cdot 10^{-4} \pm 3 \cdot 10^{-6}$ where the amount of analyte (pg) is plotted on the *x*-axis and the ratio of the analyte peak area to the standard peak are is plotted on the *y*-axis.

Typical chromatograms obtained for the analysis of fortified dog plasma samples are shown in Fig. 3. A number of extraneous peaks were observed in both the analyte and internal standard traces but these peaks did not interfere with the integration of the areas for either of the peaks of interest. It was

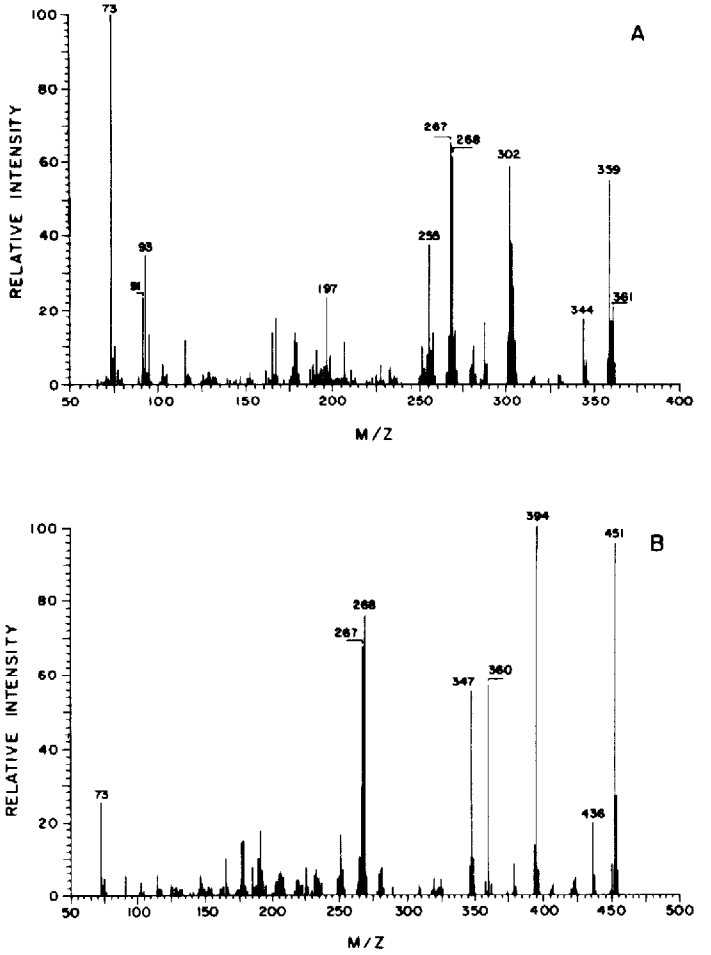

Fig 2 Mass spectra of the trimethylsilyl ether derivatives of (A) I and (B) II

interesting to note that the proteins present in the sample appeared to represent the most significant source of these extraneous peaks and sample handling difficulty Samples which precipitated large amounts of protein routinely showed many more extraneous chromatographic peaks while samples which precipitated relatively smaller amounts of protein were chromatographically cleaner Samples with high protein also often required additional handling in the derivatization step to remove insoluble residue (as noted in the Experimental section) It is not expected that this material would affect recovery or quantitation but again extracted standards are recommended to control for any effects. The combination of these problems made dog plasma more difficult to analyze than human plasma.

The chromatograms shown in Fig. 3 are for the analysis of samples contain-

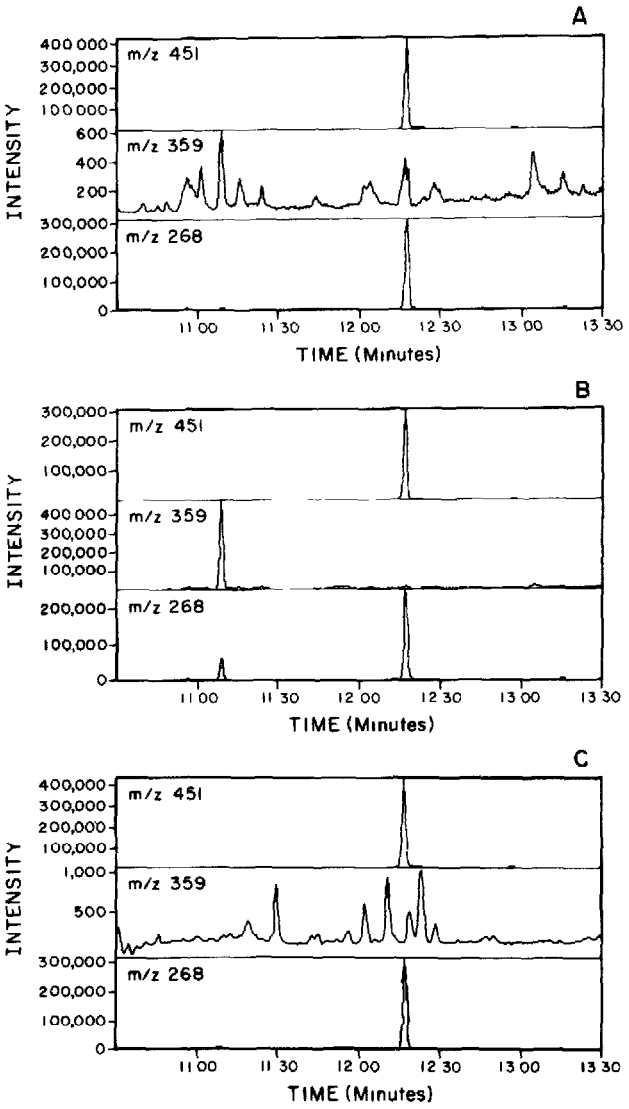

Fig 3 Reconstructed mass chromatograms for the analysis of I in dog plasma samples Sample concentrations are (A) 10 p*M*, (B) 10 n*M* and (C) blank

ing 3 pg (10 p*M*) and 3 ng (10 n*M*) of the analyte, respectively As can be seen, a good signal-to-noise ratio was still obtained for the pg sample indicating a limit of detection as low as 1 pg in 1-ml specimen.

Fig. 4 contains the mass spectra of the trimethylsilyl derivatives of three other benzazepines, III, IV and V. The mass spectra of these compounds also show molecular ions of high intensity. Compound III is an exception in that it

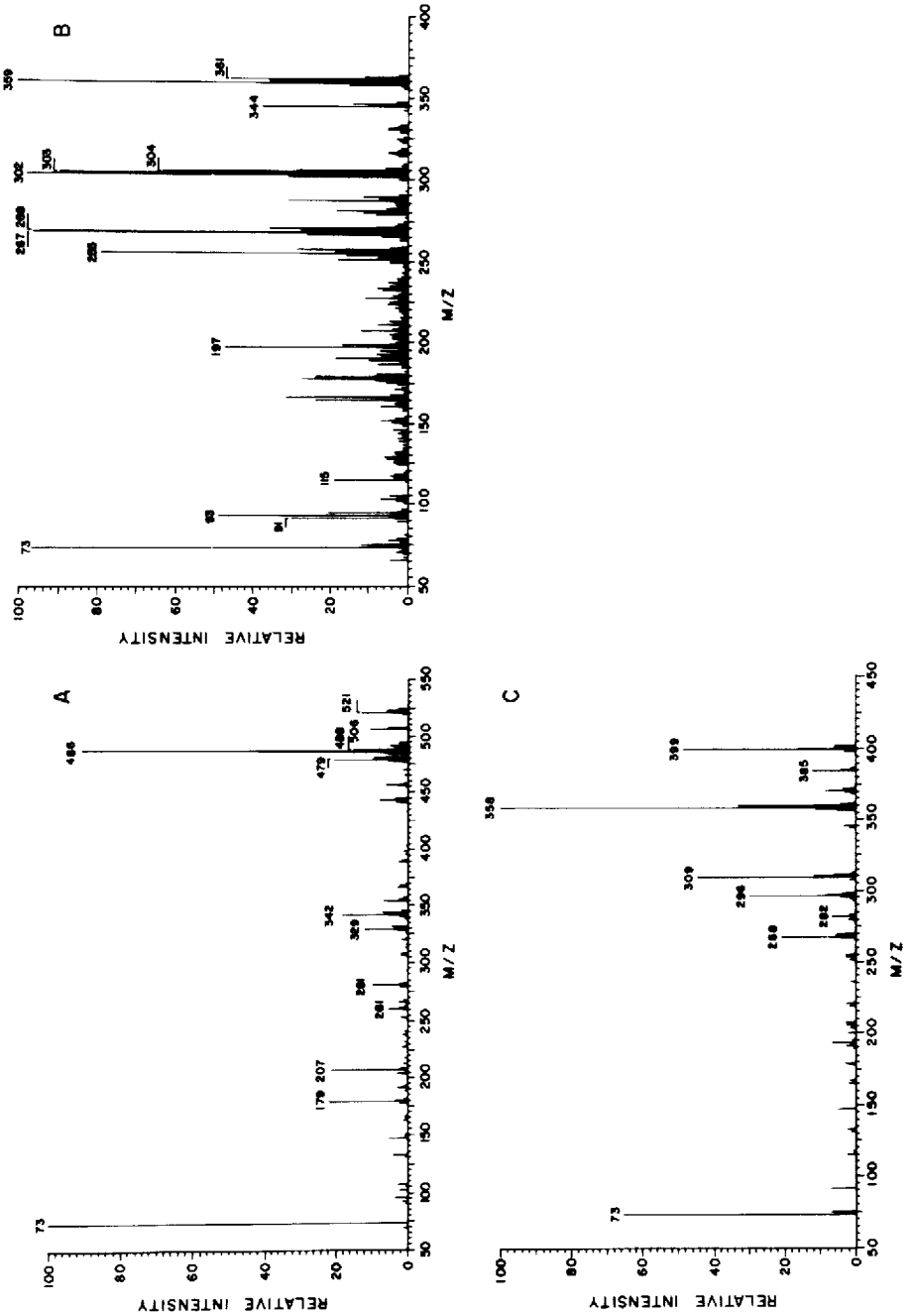

Fig 4 Mass spectra of the trimethylsilyl ether derivatives of (A) III, (B) IV and (C) V

show a less intense molecular ion, but the spectrum does show a large fraction of the ion current carried by the ion resulting from the loss of $Cl^{\bullet}$ making this ion ideal for quantitative analysis by GC–MS. Since these compounds all continue to be structurally similar to I and II, similar extraction and derivatization protocols would be recommended. Any of these compounds would function well as the internal standard for the analysis of the others provided extracted standards are used to determine response ratios The only exception would be that I and IV could not serve as internal standards for each other since they are not chromatographically separated and have the same molecular mass. The result is a flexible analysis scheme capable of incorporating a number of different compounds with different pharmacological properties This scheme should, therefore, aid in the study of the properties of these compounds and dopamine receptors through practical, sensitive quantitation.

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