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Pharmacokinetics of Methylphenidate in the Rat Using Single-Ion Monitoring GLC–Mass Spectrometry

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Abstract □ A GLC–mass spectrometric assay for methylphenidate in biological fluids was developed using the ethyl ester homolog of the drug as the internal standard. The procedure has a lower level of sensitivity of 1.2 ng/ml and is based on GLC–mass spectrometic monitoring of the *m/e* 180 ion common to the mass spectra of the *N*-trifluoroacetyl derivatives of the drug and internal standard. The brain and plasma levels of methylphenidate in rats were determined after intravenous administration of 0.5 mg/kg of the drug. The two-compartment open pharmacokinetic model fit the data.

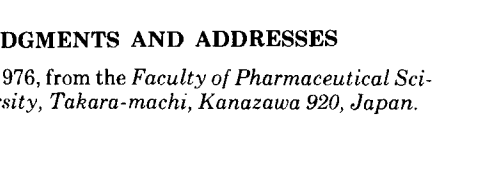
Keyphrases □ Methylphenidate—GLC–mass spectrometric analysis in biological fluids, pharmacokinetics in rats □ GLC–mass spectrometry—analysis, methylphenidate in biological fluids, pharmacokinetic study in rats □ Pharmacokinetics—methylphenidate, GLC–mass spectrometric study in rats □ Stimulants, central—methylphenidate, GLC–mass spectrometric analysis in biological fluids, pharmacokinetics in rats

Methylphenidate [(±)-*threo*-methyl α-phenyl-2-piperidineacetate, I] has pharmacological properties similar to those of dextroamphetamine (1). It is used to treat depressed states of various origins and hyperkinesis in children (2). In addition, I has become a drug of abuse in many countries (3–5).

The metabolic fate of I in humans and several other species was studied by Faraj *et al.* (6), who also reviewed previously published work. The main urinary metabolite in humans, accounting for 30% of the dose, is the hydrolysis product of I, ritalinic acid (II).

BACKGROUND

Studies on the disposition of radioactive I in the rat were reported (7), as was information on plasma I levels in humans (6, 8). While the drug appears to be essentially completely absorbed after oral administration, the concentration of I after a 20-mg oral dose of I hydrochloride was reported not to exceed 20 ng/ml of plasma (6). Plasma I levels in humans were 10–60 ng/ml 1–3 hr after a 0.4-mg/kg oral dose of I hydrochloride (8). Such low levels of I achieved after therapeutic doses may partially explain the paucity of I pharmacokinetic data.



I: $R_1 = CH_3$, $R_2 = H$
II: $R_1 = R_2 = H$
III: $R_1 = CH_2CH_3$, $R_2 = H$
IV: $R_1 = CH_3$, $R_2 = COCF_3$
V: $R_1 = CH_2CH_3$, $R_2 = COCF_3$

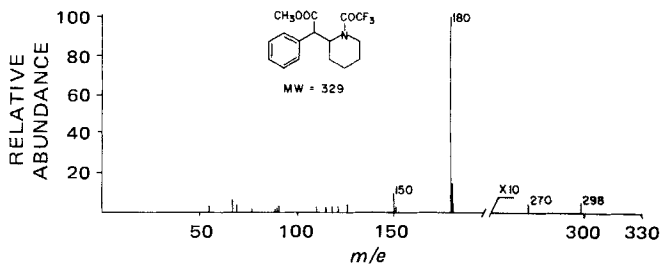


Figure 1—*Mass spectrum of IV obtained by GLC–mass spectrometry under the described conditions.*

Several analytical methods for I were reported (6–10), but none appears capable of measuring I reliably and routinely at the low levels required. *N*-Acylation of I with trichloroacetyl chloride followed by electron-capture GLC gave (9) a lower limit of detection of 9 ng/ml in plasma. Another procedure (10), also using electron-capture GLC, involved reduction of the methoxycarbonyl function of I to $-CH_2OH$ using lithium aluminum hydride, followed by derivatization with pentafluoropropionic anhydride. The sensitivity of the assay was 2 ng of I/ml of plasma. Recently, an analytical procedure for I based on GLC–mass spectrometry, with a sensitivity limit of 1.5 ng/ml in plasma, was described (8).

None of these methods (8–10) included an internal standard. A further difficulty with the published (8) GLC–mass spectrometric assay is that the procedure involved the GLC of underivatized I. Underivatized I may undergo extensive and variable decomposition in the injection port of the gas chromatograph, making quantitative determination difficult at best (11).

A new GLC–mass spectrometric assay for I in biological fluids and tissues is now described. The procedure involves the addition of the ethyl ester homolog of I, Compound III (12), to the biological medium, extraction of the drug and internal standard under mildly basic conditions, concentration of the extract, and derivatization with trifluoroacetic anhydride. GLC–mass spectrometric analysis was then performed with the mass spectrometer focused on an ion common to the spectra of both I and the internal standard. The lower limit of sensitivity of the assay is 1.2 ng of I/ml of plasma. The procedure was applied to the study of the pharmacokinetics of I in the rat, and the data obtained were examined in terms of classical pharmacokinetic models.

EXPERIMENTAL

Materials—Internal standard III was prepared *via* two different methods. A solution of II[1] (0.5 g) in 55 ml of absolute ethanol containing

[1] Ciba-Geigy Corp.

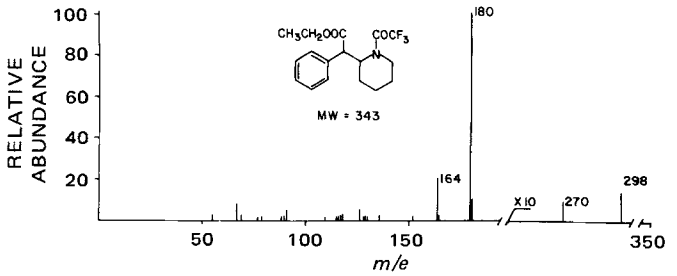


Figure 2—*Mass spectrum of V obtained by GLC–mass spectrometry under the described conditions.*

dry 10% (w/w) HCl was refluxed overnight. The solvent was then evaporated, and the residue was recrystallized from ethanol–ether to give III hydrochloride, mp 171–173° [lit. (12) mp 172–173°]. In the second method, I hydrochloride[1] (0.2 g) was treated in the same way, but the reflux time was 3 weeks. Stock solutions of I hydrochloride in methanol and III hydrochloride in ethanol were used in the described analytical procedures.

Animal Procedures—Male Sprague–Dawley rats, 200–250 g, were given an intravenous injection of I hydrochloride in normal saline equivalent to 0.5 mg of free base/kg; 0.5 ml of solution/100 g of animal weight was used. At the appropriate times, the animals were lightly anesthetized with ether, and blood was collected by cardiac puncture with a heparinized syringe. The brains were removed and immediately frozen. The whole blood was centrifuged (3000×*g*), and aliquots of plasma were taken for analysis.

Extraction and Sample Preparation—The brain was homogenized in twice the volume of saturated ammonium carbonate solution containing 500 pmoles of internal standard, and the homogenate was extracted as described for the plasma samples. To a 2-ml aliquot of plasma were added 500 pmoles of the internal standard and 0.6 g of ammonium carbonate, and the mixture was extracted with 6 ml of hexane by shaking[2] for 15 min. The mixture was then centrifuged for 10 min, 4.5 ml of the organic layer was transferred to a 5-ml microcentrifuge tube[3], and the solvent was evaporated in a nitrogen stream.

The residue in each tube was treated with 50 μl of acetonitrile and 50 μl of trifluoroacetic anhydride, and the solution was swirled on a mixer and then heated at 70° for 15 min. The solvent and excess derivatizing agent were evaporated in a nitrogen stream. The residue was treated with 50 μl of acetonitrile and swirled[4]. Analysis by GLC–mass spectrometry was then performed.

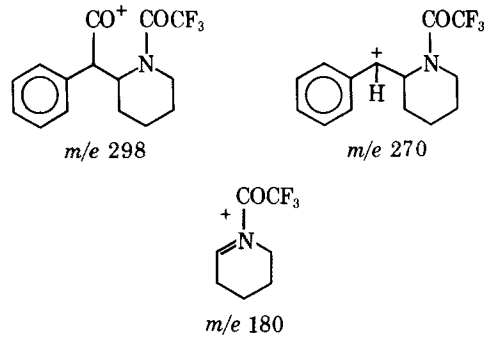
A standard curve was routinely obtained by adding 0–2000 pmoles of I to a series of extraction tubes containing 2 ml of a solution of plasma proteins[5] and 500 pmoles of the internal standard. These tubes were then carried through the assay procedure.

GLC–Mass Spectrometry—A quadrupole GLC–mass spectrometry system[6] was used under the following conditions: helium carrier gas, 35 ml/min; injector temperature, 250°; and column temperature, 170°. The 0.9-m × 2-mm i.d. glass column was packed with 3% phenyl methyl silicone[7] coated on acid-washed dimethylchlorosilane-treated diatomite[8] support. The temperature of the membrane separator was 170°, and that of the transfer line was 250°. The ionizing energy was 70 ev.

A digital selected-ion monitor (13) was used to monitor the ion current at *m/e* 180. The retention times of the *N*-trifluoroacetyl derivatives IV and V were 2.8 and 3.4 min, respectively. No interfering peaks were observed when brain and plasma samples from animals not given the drug were analyzed without adding the internal standard.

RESULTS AND DISCUSSION

Since I may decompose when injected directly into a gas chromatograph (11), the assay developed involved the derivatization of I with trifluoroacetic anhydride before GLC. The mass spectrum of the *N*-trifluoroacetyl derivative IV of I is shown in Fig. 1. The molecular ion is not discernible. Weak ions are seen at *m/e* 298 and 270 with relative intensities of 0.5 and 0.4%, respectively.



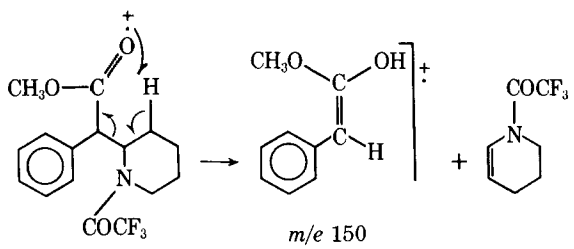
The *m/e* 298 ion likely results from the loss of the methoxyl radical from the molecular ion, while the *m/e* 270 ion arises from the loss of the entire methoxycarbonyl group. The ion of *m/e* 150 (8%) appears to be formed *via* the McLafferty rearrangement, frequently occurring during the fragmentation of carbonyl-containing compounds (Scheme I). The base peak in the spectrum is the *m/e* 180 ion, arising *via* β-cleavage to nitrogen. All other ions in the spectrum are weak, <5%.

The mass spectrum of the *N*-trifluoroacetyl derivative V of the ethyl ester homolog III is shown in Fig. 2. The spectrum is similar to that of IV (Fig. 1). The molecular ion is not seen, the base peak is at *m/e* 180, and the ions of *m/e* 270 and 298 are present. The ion arising *via* the McLafferty rearrangement appears, as expected, at *m/e* 164.

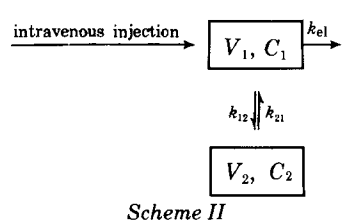
The ethyl ester III was selected to serve as the internal standard in the assay for I. This homolog is readily obtained by esterification of amino acid II or by transesterification of I in ethanolic hydrochloric acid. Homologs, rather than stable-isotope-labeled analogs, were used as internal standards in some selected-ion monitoring GLC–mass spectrometric assays (14, 15), and the two approaches were critically compared (16). In the present case, III proved highly suitable as an internal standard due to its chemical similarity to I and the similarity of the mass spectrum of its *N*-trifluoroacetyl derivative (V) (Fig. 2) to that of IV (Fig. 1). The ion of *m/e* 180 is the base peak in the spectra of both compounds and was chosen for selected-ion monitoring. The mass spectrometer could thus be used in the *single*-ion monitoring mode, serving as a sensitive and selective GLC detector. The two trifluoroacetyl derivatives are sufficiently resolved under the GLC conditions used (Fig. 3).

Compounds I and III were extracted with hexane from plasma or rat brain homogenate after saturation with ammonium carbonate (pH 8.8–9.0) (17) since these esters rapidly hydrolyze (18) under the strongly basic conditions often used to extract basic drugs (19). The overall recovery of I from plasma was 96% at 117 ng/ml and 101% at 23 ng/ml. The relative standard deviation of the slopes of three calibration curves was 4.2%. The coefficient of variation in the determination of five 2-ml plasma samples containing 1.2 ng of I/ml was 14%. This concentration is considered to be the lower limit of sensitivity of the assay; attempted determination of three samples of 0.6 ng of I/ml gave an average value of 0.3 ng/ml with a coefficient of variation of 100%.

Concentrations of I in rat plasma and brain are plotted in Fig. 4. The concentration in plasma (C_p) declined biexponentially, and the two-compartment open model (20) (Scheme II) fit the data. In Scheme II, V_1



Scheme I



Scheme II

[2] International bottle shaker, International Equipment Co., Boston, Mass.
[3] Microflex tube, Kontes Glass Co., Vineland, N.J.
[4] Vortex Genie, Scientific Industries, Springfield, Mass.
[5] Plasmanate, Cutter Laboratories, Berkeley, Calif.
[6] Model 5981A, Hewlett-Packard, Palo Alto, Calif.
[7] OV-17, Applied Science Laboratories, State College, Pa.
[8] Gas Chrom Q, Applied Science Laboratories, State College, Pa.

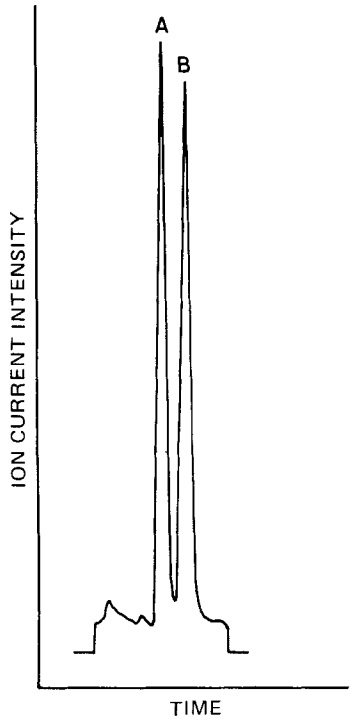


Figure 3—*Tracing of the ion current at* m/e *180 monitored by the GLC–mass spectrometer. Key: A, IV; and B, V.*

and V_2 are the volumes of the central and second compartments, respectively, and the k's are the rate constants for the movement of the drug as indicated by the arrows. A desk calculator[9] using a nonlinear regression program was employed to determine the four parameters A, B, α, and β of the equation $C_p = Ae^{-\alpha t} + Be^{-\beta t}$ that best fits the experimentally determined plasma I concentrations.

Analysis of the plasma data in terms of this model gave the results shown in Table I. The initial decline in plasma concentration is rapid and the β-phase is reached within a few minutes. Thus, transfer of I from plasma to tissues is rapid and distribution is extensive, as indicated by the ratio $C_p{}^0/B$, which was as high as 3.5. The volume of the central compartment (1.18 liters/kg) is about twice the volume of body water.

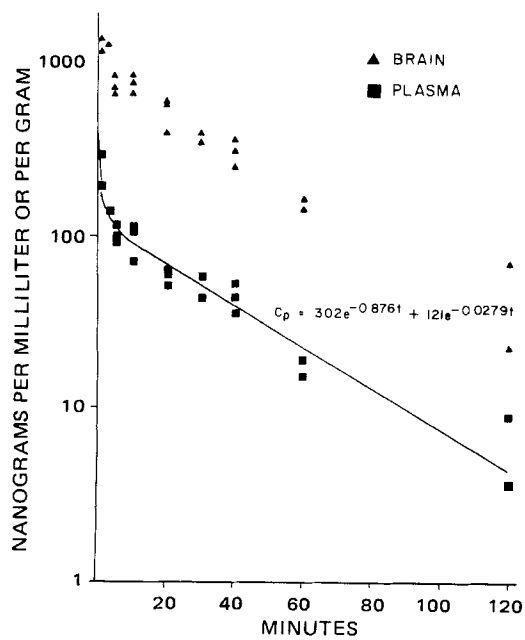


Figure 4—*Plasma and brain I levels. The line superimposed on the plasma data points was obtained by nonlinear regression analysis as described. The correlation coefficient for the plasma data on the calculated curve was 0.959.*

[9] Wang 600, Wang Laboratories, Tewksbury, Mass.

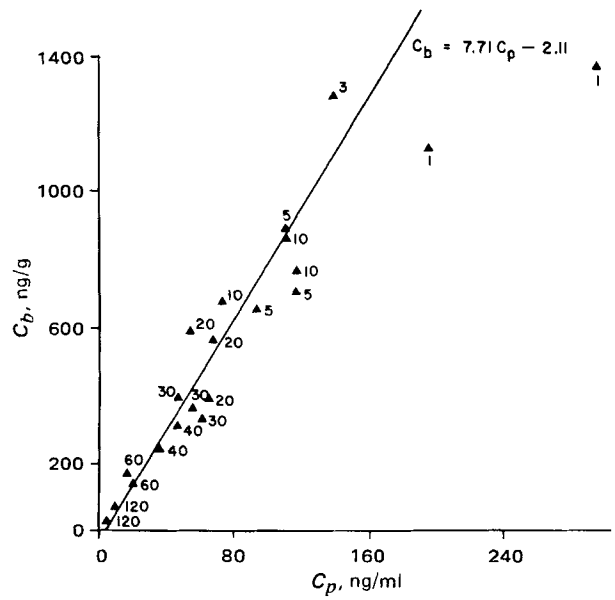


Figure 5—*Relationship of brain and plasma I concentrations* (C_b *and* C_p, *respectively). The number next to each data point is the time of sampling in minutes. The equation of the line was determined by least-squares linear regression analysis without the 1-min time points. (See text for explanation.) The correlation coefficient is 0.947.*

Compound I is metabolized extensively in the rat; less than 1% unchanged I was found in the 48-hr urine (6). The major route of metabolism in this species appears to be initial *para*-hydroxylation, and the resulting metabolite may undergo further transformations. In humans, the predominant route is hydrolysis of the ester portion of the molecule without *para*-hydroxylation (6). This difference between humans and rats in the metabolism of I is similar to the differences in the metabolism of amphetamine by the two species. The major route of *in vivo* biotransformation of amphetamine in the rat is *para*-hydroxylation; this pathway is minor in humans, however, with deamination and side-chain degradation being the predominant transformations (22). It was suggested (6) that metabolism of I is very rapid, in agreement with the rapid clearance of the drug from rat plasma (Table I). Compound I has been found to be poorly bound to human plasma proteins (6).

The concentration of I in rat brain was higher than the plasma concentration and declined parallel with the plasma concentration after the 1-min sampling point (Fig. 5), with a brain/plasma ratio of 7.7. The rapid entry of I into the brain is in agreement with its rapid and extensive tissue distribution and with its organic–aqueous partitioning characteristics (6). The brain appears to be part of the central compartment. The deviation in the 1-min sampling points (Fig. 5) may be the result of the delay caused by the mixing time of the bolus with the blood or of inaccurate measurement at the early time point when the change in concentration is very rapid.

Segal *et al.* (7) recently published a study on the disposition of ^{14}C-I in the rat, with emphasis on the time course of entry and distribution of the drug in the brain. In agreement with the present report, the concentration of I in rat plasma declined biexponentially. However, a detailed pharmacokinetic analysis in terms of an appropriate model was not described. The biological half-life (β-phase) was given (7) as 105 min, a value considerably larger than the finding (24.8 min) of the present report. The origin of this discrepancy is not clear. The assay of ^{14}C-labeled I used (7) was based on extraction of the biological medium with methylene chloride, and no further purification was performed. A metabolite could interfere in such determinations, since at least one lipid-soluble metabolite of I has been found in the rat (6). The rapid entry of I into rat brain after intravenous administration of the drug reported by Segal *et al.* (7) is in qualitative agreement with the findings of the present report, although significant quantitative differences in the half-life of I in the brain between the two studies exist.

The half-life of I in the rat is 24.8 min (Table I), while that of dextroamphetamine is 86.7 min (23), reflecting the faster metabolism of I.

Table I—Pharmacokinetic Analysis of Plasma Methylphenidate Concentrations[a]

Parameter	Value
C_p^0, ng/ml	424
V_1, liters/kg	1.18
V_2, liters/kg	2.35
$V_{d_{ss}}$, liters/kg	3.53
k_{12}, min^{-1}	0.542
k_{21}, min^{-1}	0.272
k_{el}, min^{-1}	0.090
Total body clearance, ml/min/kg	107.2
$t_{1/2}$, min	24.8

[a] The plasma levels were analyzed by nonlinear regression to the two-exponential function describing the two-compartment model (20). The volume of distribution at steady state, $V_{d_{ss}}$, is that defined by Riegelman *et al.* (21).

The volume of distribution of dextroamphetamine is 6.04 liters/kg (23), considerably larger than the value obtained for I, 3.53 liters/kg (Table I). The pKa values of I and dextroamphetamine are 8.8 (24) and 9.9 (25), respectively. The apparent heptane–water partition coefficient of I at pH 7.4 was determined (6) to be 0.63, while that of dextroamphetamine may be computed from published data (25) as 0.00593. Thus, I is ionized at physiological pH to a lesser extent than dextroamphetamine and is considerably more lipid soluble. The larger volume of distribution of amphetamine may be a reflection of the greater concentration of this compound, a stronger base than I, in the intracellular water of tissues, the pH of which is lower than that of extracellular water (26).

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