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Determination of Methylphenidate and Its Main Metabolite in Plasma by Gas Chromatography–Chemical Ionization Mass Spectrometry[1)]

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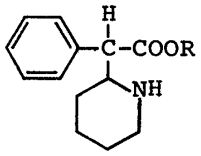
A method is described for the determination of methylphenidate and its main metabolite, ritalinic acid, in plasma from various species, based on gas chromatography-chemical ionization mass spectrometry using ethylphenidate as an internal standard. Methylphenidate in plasma is extracted with cyclohexane and converted to the pentafluoropropionyl (PFP) derivative. Ritalinic acid is extracted with 2-propanol from the salt-saturated remaining aqueous phase, converted to the parent drug by treatment with a mixture of methanol and sulfuric acid (2:1, v/v), and then converted to the PFP derivative. The protonated molecular ions at *m/z* 380 for the PFP derivative of methylphenidate and *m/z* 394 for that of the internal standard were used for selected ion monitoring analysis. The hydrolysis of methylphenidate in plasma before the addition of the internal standard could be prevented completely by holding the plasma sample at around 0 °C. The limit of quantitation was 0.5 ng for methylphenidate and 2.5 ng for ritalinic acid using 0.5 ml of plasma. The present method is highly sensitive and reliable, and should be satisfactory for application to pharmacokinetic studies of methylphenidate in laboratory animals and man.

Keywords——methylphenidate; ritalinic acid; GC-MS; selected ion monitoring; chemical ionization; plasma; pentafluoropropionyl derivative; stability

Methylphenidate (methyl α-phenyl-2-piperidineacetate; Fig. 1) is a sympathomimetic agent with a stimulant effect, and is used in the treatment of hyperkinesia in children and narcolepsy. In studies on the disposition of methylphenidate in animals and man,[2)] it was found that the plasma concentration of methylphenidate after oral administration was quite low because the drug is rapidly metabolized to ritalinic acid (α-phenyl-2-piperidineacetic acid; Fig. 1). Thus for studies on the pharmacokinetics of methylphenidate in laboratory animals and man, it is necessary to have a reliable microanalytical method to determine the concentration of methylphenidate and its main metabolite, ritalinic acid, in plasma.

Several analytical methods for determination of methylphenidate and/or ritalinic acid in plasma or serum have been developed. High-performance liquid chromatographic methods using an ultraviolet detector have been reported for the determination of methylphenidate,[3a)] or ritalinic acid[3b)] in human serum. However these methods have low sensitivity. Several gas chromatographic (GC) methods using flame ionization, electron capture and nitrogen phosphorus detectors have been reported for determination of methylphenidate alone,[4,5)] or



1: R=CH_3 2: R=H 3: R=C_2H_5

Fig. 1. Structures of Methylphenidate, Ritalinic Acid and Ethylphenidate

1, methylphenidate; **2**, ritalinic acid; **3**, ethylphenidate.

methylphenidate and ritalinic acid[6,7] in human or horse plasma. These GC methods required relatively large volumes of plasma (1—10 ml) and some[4,6] of them did not include an internal standard or a derivatization of methylphenidate to prevent thermal decomposition in the gas chromatograph. The method[7] based on the trifluoroacetylation of methylphenidate, and methylation of ritalinic acid with diazomethane followed by trifluoroacetylation is time-consuming and hazardous. Several gas chromatographic-mass spectrometric (GC-MS) methods for determination of methylphenidate alone[8,9] or methylphenidate and the metabolite[10,11] in human plasma and serum or rat plasma have also been reported. The GC-MS method in the electron impact (EI) mode reported by Milberg *et al.*[10] did not include an internal standard or a derivatization procedure. The methods in the EI mode reported by Gal *et al.*[8] and Chan *et al.*[9] based on selected ion monitoring (SIM) of the *m/z* 180 ion common to the mass spectra of the trifluoroacetyl (TFA) derivatives of the drug and internal standard were unsatisfactory because complete chromatographic separation of their compounds could not be confirmed. The method[11] in the chemical ionization (CI) mode using methane as the carrier gas is based on SIM of protonated molecular ions in the mass spectra of the TFA derivatives of the drug and internal standard. Although the lower limit of quantitation of methylphenidate was 1 ng in 2 ml of plasma, the analytical procedure for ritalinic acid involved a lyophilization step and derivatization with diazomethane.

On the other hand, hydrolysis of methylphenidate in pooled human plasma[2] and buffer solution[13] has been reported. However, most[3-12] of the analytical methods reported previously have neglected the possible hydrolysis of the drug in the plasma after blood sampling. In addition, the possible hydrolysis of the drug in plasma from other species remains unclear.

In this paper, we describe a highly sensitive and reliable GC-MS method in the CI mode for the determination of methylphenidate and ritalinic acid in plasma from various species. The method should be suitable for pharmacokinetic studies of methylphenidate in animals and man.

Experimental

Chemicals——Methylphenidate hydrochloride and ritalinic acid were obtained as generous gifts from CIBA-GEIGY (Japan) Ltd. (Takarazuka). Commercially available tablets (Ritalin®) containing 10 mg of methylphenidate hydrochloride were purchased from CIBA-GEIGY (Japan) Ltd. Ethylphenidate hydrochloride used as an internal standard (I.S.; Fig. 1) was synthesized in our laboratory by esterification of ritalinic acid according to the method of Gal *et al.*[8]; white crystals were obtained with a decomposition point of 171—172 °C (lit. value 171—173 °C[8]). Pentafluoropropionic anhydride (PFPA) and trifluoroacetic anhydride were purchased from Pierce Chemical Co. (Rockford, Illinois). Ethylenediaminetetraacetic acid, disodium salt (EDTA) was from Wako Pure Chemical Industries (Tokyo). Plasma of beagle dogs was kindly supplied by Chugai Pharmaceutical Co. (Tokyo) and that of Arab horses was kindly provided by the Chemical Research Laboratory of Racing Chemistry (Tokyo). All other chemicals and reagents used were of reagent grade.

Apparatus and Chromatographic Conditions——The GC-MS system used in this study was composed of a JGC-20K gas chromatograph, a JMS D-300 mass spectrometer equipped with a multiple ion detector, and a JMA-2000 data processing system including a JEC-980B computer (JEOL, Tokyo). The chromatographic column was silanized and coiled glass tubing (1 m × 2 mm i.d.) packed with 2% OV-17 on Chromosorb W AWDMCS, 80—100 mesh (Wako Pure Chemical Industries). The gas chromatograph was operated at column and injection-port temperatures of 220 and 230 °C, respectively, with a helium flow-rate of 35 ml/min (inlet pressure of 0.8 kg/cm^2). The mass spectrometer was operated in the CI mode. Isobutane was used as the reactant gas at a CI chamber pressure of approximately 1 Torr. The ion-source temperature was maintained at 200 °C. The ionization current was 300 μA with an electron energy of 200 eV.

Extraction and Derivatization Procedures——Methylphenidate: Extraction of methylphenidate from plasma was performed by a minor modification of the method of Shults *et al.*[4a] To 0.5 ml of plasma in a 10 ml glass-stoppered centrifuge tube were added 100 μl of ethanol containing 250 ng per ml of the I.S., 1 ml of saturated aqueous solution of sodium tetraborate and 4 ml of cyclohexane. These procedures were carried out on ice. The tube was then capped, shaken mechanically for 5 min and centrifuged at 1670 *g* for 5 min. The upper organic phase was transferred to a clean

tube. The remaining aqueous phase was saved for the determination of ritalinic acid as described below. The organic phase was evaporated to dryness on a rotary vacuum evaporator, then 0.5 ml of *n*-hexane, 40 μl of 5% pyridine in benzene and 40 μl of PFPA were added to the residue. The mixture was allowed to react for 30 min at ambient temperature. Then 4 ml of 0.1 N sodium hydroxide to decompose the excess PFPA and 2 ml of *n*-hexane were added. The whole was agitated vigorously for 30 s. After centrifugation, the organic phase was transferred to a clean tube and evaporated gently to dryness under nitrogen gas at ambient temperature. The residue was redissolved in 40 μl of *n*-hexane and 1 to 3 μl of this solution was injected into the column.

Ritalinic Acid: The aqueous phase containing ritalinic acid saved as mentioned above was washed once with 4 ml of cyclohexane and then with 4 ml of dichloromethane to remove trace amounts of methylphenidate and the I.S. One ml of the upper aqueous phase was transferred to a clean tube, then 5 ml of 2-propanol and 1 g of anhydrous potassium carbonate were added. The mixture was agitated vigorously for 30 s and further shaken for 5 min, then centrifuged for 5 min at 1670 *g*. Four ml of the upper organic phase was transferred to a clean tube and evaporated to dryness on a rotary vacuum evaporator. Then 0.2 ml of a mixture of methanol and sulfuric acid (2 : 1, v/v) was added to the residue. This mixture was allowed to react for 15 min at 85 °C in a water bath, then cooled on ice, and 3 ml of 1 M carbonate buffer solution (pH 10), 100 μl of ethanol containing 500 ng per ml of the I.S. and 4 ml of cyclohexane were added. The resulting mixture was processed according to the procedure for methylphenidate.

Preliminary Examination of Derivatization——A sample of 100 ng of methylphenidate or ethylphenidate (used as the I.S.) was taken up in 1 ml of saturated aqueous solution of sodium tetraborate and 4 ml of cyclohexane. After shaking and centrifugation of the mixture in the same as described above, 3 ml of the organic phase was transferred to a clean tube and evaporated to dryness. Methylphenidate or the I.S. in the residue was then converted to the PFP derivative by the method described above and to the TFA derivative by the method of Gal *et al.*[8] In the present method, the excess PFPA in the mixture after the reaction was decomposed in the same way as described above, then 2 ml of *n*-hexane phase was transferred to a clean tube, and evaporated to dryness. In the method of Gal *et al.*, methylphenidate or the I.S. in the residue was derivatized by heating at 70 °C for 15 min with 50 μl of acetonitrile and 50 μl of trifluoroacetic anhydride, and the mixture after reaction was evaporated gently to dryness under nitrogen gas at ambient temperature.

Each residue was redissolved in 200 μl of *n*-hexane and 3 μl of the solution was injected into the column. Peak heights of *m/z* 380 for the PFP derivative of methylphenidate and *m/z* 394 for that of the I.S., or *m/z* 330 for the TFA derivative of methylphenidate and *m/z* 344 for that of the I.S. were measured and the coefficient of variation was calculated.

Stability Test——One ml of the solution of methylphenidate containing 100 ng per ml, prepared by dissolving methylphenidate hydrochloride in methanol, was placed in a glass-stoppered 10 ml centrifuge tube and the solvent was evaporated off. To the residue was added 0.5 ml of human (obtained from 3 healthy volunteers), horse, dog, rabbit or rat plasma. The plasma sample was incubated for appropriate time intervals at 0, 0—4 (on ice), 25 or 37 °C, and analyzed by the methods described for extraction and derivatization. Similar experiments with 0.5 ml of 1/15 M phosphate buffer solution (pH 7.4) containing 100 ng of methylphenidate, and 0.5 ml of human or rabbit plasma containing 100 ng of the drug and 2.5×10^{-6} mol of EDTA were carried out at 37 °C. These samples were incubated for various time intervals up to 48 h.

Calibration Curves——A series of solutions containing various concentrations of methylphenidate and ritalinic acid was prepared by dissolving each compound in methanol (ng per ml of methylphenidate/ng per ml of ritalinic acid: 0.5/2.5, 1/5, 2/10, 5/25, 10/50, 25/100, 50/200, 100/300 and 200/400). One ml of each solution was placed in a centrifuge tube and the solvent was evaporated off. To each residue was added 0.5 ml of ice-cold human plasma. These samples were then assayed by the methods described for extraction and derivatization. The peak height ratios of *m/z* 380 for the PFP derivative of methylphenidate *versus* *m/z* 394 for that of the I.S. were calculated and plotted against the known amounts of methylphenidate and ritalinic acid.

Recovery——The recovery of methylphenidate from human plasma was examined at 3 different amounts (10, 50 and 100 ng). The analytical procedure described above was modified slightly; the I.S. (20 ng) was added to cyclohexane extracts (3 ml) after completion of the extraction, and 2 ml of the *n*-hexane phase after derivatization with PFPA was evaporated to dryness. The recovery of ritalinic acid was examined at 3 different amounts (20, 100 and 200 ng). The analytical procedure was carried out as described above. The peak height ratios (*m/z* 380/394) of the PFP derivative of methylphenidate to that of the I.S. obtained from the plasma samples were calculated, and compared with the peak height ratios obtained by direct assay after PFP derivatization of standards equivalent to 100% extraction yield in each extraction step. The recovery of ethylphenidate (the I.S.) from human plasma was determined by using methylphenidate as an internal standard.

Drug Administration——Rabbit: Methylphenidate dissolved in normal saline was administered intravenously to a male white rabbit through an ear vein at 0.5 mg/kg. About 1 ml of blood was collected from the opposite ear vein with a heparinized disposable syringe at appropriate time intervals up to 8 h after administration and each sample was immediately transferred to a centrifuge tube cooled on ice. Plasma was separated by centrifugation (automatic refrigerated centrifuge, model 20PR-5, Hitachi Koki Co., Tokyo) at 1620 *g* for 10 min at 0 °C.

Human: A healthy adult male volunteer, aged 31 years and weighing 69 kg, participated in this study. After an

overnight fast, two Ritalin® tablets were administered orally to the volunteer. No food was permitted for 4 h after administration. About 1 ml of blood was withdrawn through an indwelling cannula using a heparinized disposable syringe at appropriate time intervals up to 24 h after administration. Plasma was immediately separated in the same way as described above.

Each plasma sample was immediately frozen in ethanol cooled with dry ice and stored at −20 °C until assay on the next day.

Results and Discussion

Preliminary Examination of Derivatization

Methylphenidate could not be chromatographed directly without volatile derivatization, since the drug undergoes partial thermal decomposition in the injection port of a gas chromatograph.[14] In a preliminary examination of derivatization, the reproducibilities of fluoroacylation of methylphenidate and ethylphenidate (used as the I.S.) with PFPA by the present method and with trifluoroacetic anhydride by the method of Gal *et al.*[8] were compared. The coefficients of variation of 12 determinations each for methylphenidate and the I.S. in the present method were 4.5 and 3.9%, respectively, whereas those in the method of

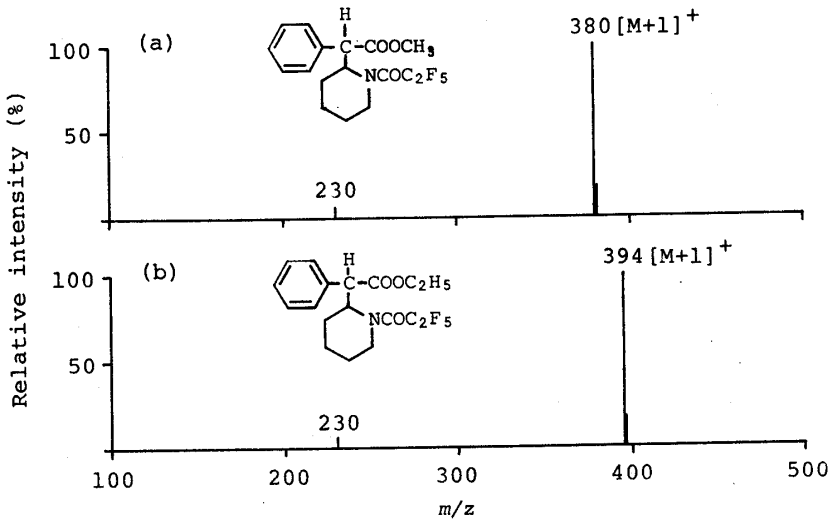


Fig. 2. Chemical Ionization Mass Spectra of Pentafluoropropionyl Derivatives of (a) Methylphenidate and (b) Ethylphenidate (Used as an Internal Standard)

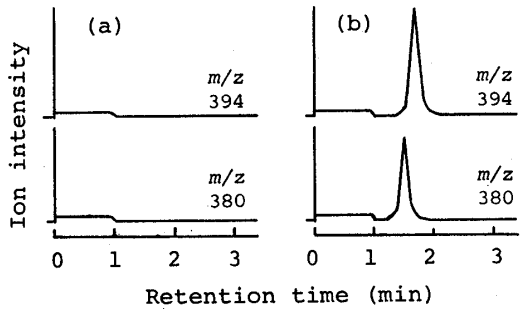


Fig. 3. Selected Ion Monitoring Chromatograms of Human Plasma

(a) blank human plasma; (b) human plasma containing 12.5 ng of methylphenidate and 25 ng of the internal standard.

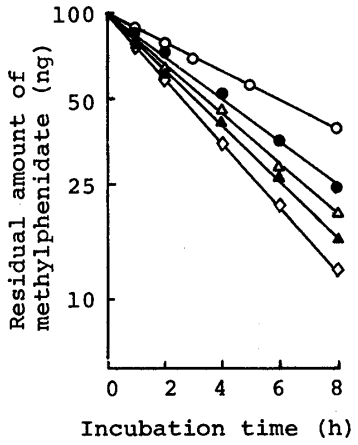


Fig. 4. Stability of Methylphenidate in Plasma from Various Species at 37 °C

The data represent the mean of 3 determinations. ○, man; ●, dog; △, horse; ▲, rabbit; ◇, rat.

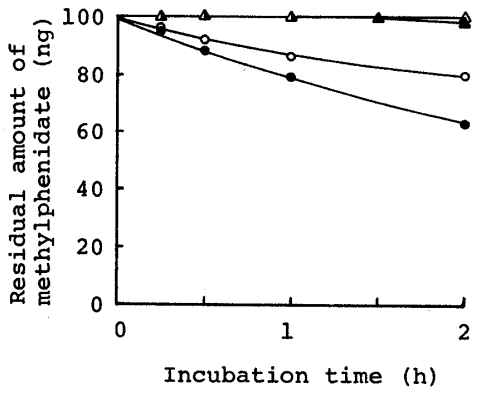


Fig. 5. Effect of Temperature on the Stability of Methylphenidate in Rabbit Plasma

The data represent the mean of 3 determinations. ●, 37 °C; ○, 25 °C; ▲, 0—4 °C (on ice); △, 0 °C.

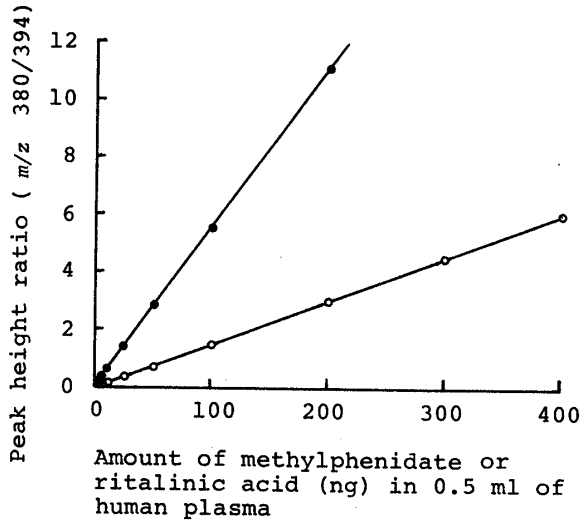


Fig. 6. Calibration Curves for Determination of Methylphenidate and Ritalinic Acid in Human Plasma

The amount of the internal standard added was 25 ng for the determination of methylphenidate and 50 ng for that of ritalinic acid. ●, methylphenidate; ○, ritalinic acid.

Gal *et al.*[8] were 14.3 and 13.7%, respectively. Thus, PFPA was chosen as a suitable fluoroacylation reagent for these compounds.

Mass Spectra and Selected Ion Monitoring

CI mass spectra of the PFP derivatives of methylphenidate and the I.S. using isobutane as a reactant gas are shown in Fig. 2. A protonated molecular ion peak was observed in both spectra as the base peak. Thus these ions at m/z 380 for the derivative of methylphenidate and m/z 394 for that of the I.S. were used for SIM analysis. Ethylphenidate was used as an internal standard for the determination of methylphenidate and ritalinic acid in plasma, because its structure is similar to that of methylphenidate. Typical chromatograms obtained from blank human plasma and human plasma supplemented with methylphenidate and the I.S. are shown in Fig. 3. No endogenous peak interfering with the determination of these compounds was observed on the chromatograms of blank plasma. The retention times of the PFP derivatives of methylphenidate and the I.S. were approximately 1.5 and 1.7 min, respectively.

Stability of Methylphenidate in Plasma

The results on the stability of methylphenidate in plasma samples from 5 species at 37 °C are shown in Fig. 4. Methylphenidate was degraded according to a pseudo-first-order rate process in all species, and the half-lives in rat, rabbit, horse, dog, and human plasma were 2.68 ± 0.20, 3.30 ± 0.41, 3.52 ± 0.95, 4.22 ± 0.94 and 6.43 ± 0.72 h (mean $\pm$ standard deviation, $n = 3$), respectively. These results indicate that the drug is unstable in plasma, but the extent of its instability is species-dependent. The findings in human plasma described above were in good agreement with those reported in the previous paper.[2] On ther other hand, ritalinic acid was found in every plasma sample, and its amount increased gradually with the decrease of that of methylphenidate. The sum of the amounts of methylphenidate and ritalinic acid was approximately 100 ng as methylphenidate at each time (data not shown). These results indicate that the decomposition product was mainly ritalinic acid produced by deesterification of the drug. Hungund *et al.*[13] reported in the experiments of protein binding of methylphenidate that the addition of 1.25×10^{-6} mol of EDTA to 1.25 ml of human plasma prevented

TABLE I. Recovery of Methylphenidate, Ritalinic Acid and Ethylphenidate (Used as the Internal Standard) from Human Plasma

Added amount (ng)		Recovery Mean ± S.D.	C.V. (%)
Methylphenidate			
100		89.4 ± 1.4	1.6
50		89.0 ± 1.7	1.9
10		89.2 ± 1.8	2.0
	Mean	89.2	
Ritalinic acid			
200		88.8 ± 1.8	2.0
100		88.2 ± 1.3	1.5
20		88.3 ± 4.5	5.1
	Mean	88.4	
Ethylphenidate			
25 ng		88.0 ± 1.8	2.0

Each recovery is the mean ± standard deviation (S.D.) and each coefficient of variation (C.V.) is the mean of 5 determinations.

the hydrolysis of the drug for at least 24 h at 37 °C. Thus, we examined the effect of EDTA on the degradation of methylphenidate in human and rabbit plasma at 37 °C. The apparent half-lives of the drug in human and rabbit plasma containing 2.5×10^{-6} mol of EDTA were 9.5 and 3.7 h (mean, $n=2$), respectively. In spite of the larger added amount of EDTA, the results in this study were not in agreement with the previous report.[13] The half-life of the drug in 1/15 M phosphate buffer solution (pH 7.4) without addition of plasma or EDTA was 12.4 h (mean, $n=2$). This degradation was due to specific base catalysis.[14] From these findings, it is suggested that the degradation of methylphenidate in plasma may involve both enzymatic and nonenzymatic processes. We then examined the effect of temperature on the degradation of the drug in plasma. The results are shown in Fig. 5. It was found that the degradation of methylphenidate could be prevented significantly by cooling the plasma sample immediately on ice. Quantitative recovery was obtained when blood collected was immediately transferred to a clean tube cooled on ice, plasma was separated by centrifugation at 0 °C, and the sample was immediately analyzed or immediately frozen and stored at −20 °C until analysis on the next day.

Calibration Curves

The calibration curves for plasma assay constructed by adding known amounts of methylphenidate and ritalinic acid to drug-free human plasma are shown in Fig. 6. The calibration curves were linear in the ranges of 0.5—200 ng for methylphenidate and 2.5—400 ng for ritalinic acid, and on extrapolation, the lines passed close to the origin.

Recovery and Reproducibility

A recovery test was carried out by adding known amounts of methylphenidate, ritalinic acid or the I.S. to drug-free human plasma. The mean recoveries for methylphenidate, ritalinic acid and the I.S. were 89, 88 and 88%, respectively, and the reproducibilities were good (Table I). Similar results were obtained for rabbit plasma supplemented with methylphenidate and ritalinic acid. As shown in Table I, coefficients of variation in the recovery of ritalinic acid were similar to those of methylphenidate. This result indicates that variations in each extraction step and in derivatization of ritalinic acid before the addition of the I.S. were very small.

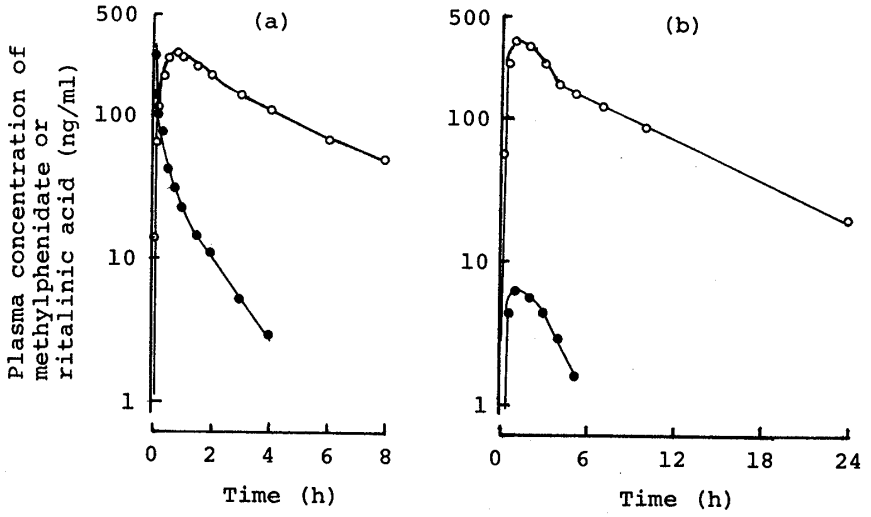


Fig. 7. Plasma Concentration–Time Curves of Methylphenidate and Ritalinic Acid after (a) Intravenous Administration of 0.5 mg/kg of Methylphenidate to a Rabbit and after (b) Oral Administration of 0.25 mg/kg of Methylphenidate to a Human Subject

●, methylphenidate; ○, ritalinic acid.

Application of the Method

The present method was applied to the determination of the concentrations of methylphenidate and its metabolite, ritalinic acid, in rabbit and human plasma.

Rabbit: A single dose of 0.5 mg/kg of methylphenidate was administered intravenously to a rabbit. Time courses of methylphenidate and ritalinic acid concentrations in plasma after administration are shown in Fig. 7(a). Methylphenidate was eliminated rapidly and the concentration was 2.9 ng/ml at 4 h later. The maximal plasma concentration of ritalinic acid was reached within 1 h after administration, and was 265 ng/ml. Thereafter the concentration decreased gradually and was 49 ng/ml at 8 h later.

Human: Two Ritalin® tablets were administered orally to a healthy volunteer. Time courses of methylphenidate and ritalinic acid concentrations in plasma after administration are shown in Fig. 7(b). Maximal plasma concentrations of methylphenidate and ritalinic acid were reached within 1 h after administration, and were 6.4 and 350 ng/ml, respectively. Thereafter the concentration of methylphenidate decreased rapidly, while that of ritalinic acid decreased slowly and was 21 ng/ml at 24 h later.

In conclusion, we found that the stability of methylphenidate in plasma was species-dependent. The degradation of the drug in plasma after sampling could be prevented completely by immediate treatment a temperature around 0 °C. As a result of the prevention of degradation in this way, highly sensitive and reliable analytical procedure for the determination of methylphenidate and its main metabolite, ritalinic acid, in plasma using the GC-CI-MS method with isobutane as a reactant gas was established. In addition, the present analytical procedure for ritalinic acid is simpler than the GC[6,7] and GC-MS[10,11] methods described previously, because lyophilization of the aqueous phase containing ritalinic acid followed by methylation with hazardous diazomethane were unnecessary in the present method. Consequently, the GC-CI-MS method described here should be useful for pharmacokinetic studies of methylphenidate in animals and man.

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References and Notes

1) A part of this work was presented at the 105th Annual Meeting of the Pharmaceutical Society of Japan, Kanazawa, April 1984.
2) W. Wargin, K. Patrick, C. Kilts, C. T. Gaultieri, K. Ellington, R. A. Mueller, G. Kraemer and G. R. Breese, *J. Pharmacol. Exp. Ther.*, **226**, 382 (1983).
3) *a*) S. J. Soldin, Y. M. Chan, B. M. Hill and J. M. Swanson, *Clin. Chem.*, **25**, 401 (1979); *b*) S. J. Soldin, B. M. Hill, Y. M. Chan, J. M. Swanson and J. G. Hill, *ibid.*, **25**, 51 (1979).
4) *a*) T. Shults, A. A. Kownacki, W. E. Woods, R. Valentine, J. Dougherty and T. Tobin, *Am. J. Vet. Res.*, **42**, 722 (1981); *b*) R. S. Ray, J. S. Noonan, P. W. Murdick and V. L. Tharp, *ibid.*, **33**, 27 (1972); *c*) R. Huffman, J. W. Blake, R. Ray, J. Noonan and P. W. Murdick, *J. Chromatogr. Sci.*, **12**, 382 (1974).
5) P. Jatlow, "Methodology for Analytical Toxicology," Vol. II, ed. by I. Sunshine and P. Jatlow, CRC Press, Florida, 1982, p. 109; B. D. Potts, C. A. Martin and M. Vore, *Clin. Chem.*, **30**, 1374 (1984).
6) B. Schubert, *Acta Chem. Scand.*, **24**, 433 (1970).
7) B. L. Hungund, M. Hanna and B. G. Winsberg, *Commun. Psychopharmacol.*, **2**, 203 (1978).
8) J. Gal, B. J. Hodshon, C. Pintauro, B. L. Flamm and A. K. Cho, *J. Pharm. Sci.*, **66**, 866 (1977).
9) Y. M. Chan, S. J. Soldin, J. M. Swanson, C. M. Deber, J. J. Thiessen and S. Macleod, *Clin. Biochem.*, **13**, 266 (1980).
10) R. M. Milberg, K. L. Rinehart, Jr., R. L. Sprague and E. K. Sleator, *Biomed. Mass Spectrom.*, **2**, 2 (1975).
11) C. R. Iden and B. L. Hungund, *Biomed. Mass Spectrom.*, **6**, 422 (1979).
12) S. Siegel, L. Lachman and L. Malspeis, *J. Am. Pharm. Assoc.*, **48**, 431 (1959).
13) B. L. Hungund, J. M. Perel, M. J. Hurwic, J. Sverd and B. G. Winsberg, *Br. J. Clin. Pharmacol.*, **8**, 571 (1979).
14) B. L. Flamm and J. Gal, *Biomed. Mass Spectrom.*, **2**, 281 (1975).