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Simultaneous enantiomeric determination of a gastroprokinetic agent mosapride citrate and its metabolite in plasma using α_1-acid glycoprotein HPLC column[1]

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Abstract

Mosapride citrate, a novel benzamide-type gastroprokinetic agent, is clinically prescribed as a racemate and is metabolized to its des-4-fluorobenzyl structure (M-1). In order to analyze simultaneously the enantiomers of mosapride and M-1 in plasma, a simple and reproducible high-performance liquid chromatographic (HPLC) method has been developed. The enantiomeric separation and determination were successfully achieved using an α_1-acid glycoprotein column and gradient elution with a fluorimetric detection (excitation 314 nm/emission 352 nm). Both enantiomers of mosapride and M-1 were well separated between 20 and 22 min at pH 4.4 and between 4 and 7 min at pH 5.0, respectively. Accurate determinations are possible in the concentration ranges of 10–5000 ng ml^{-1} for mosapride enantiomers and 50–5000 ng ml^{-1} for M-1 enantiomers. The intra- and inter-day coefficients of variation are satisfactory for the pharmacokinetic study of mosapride. © 1997 Elsevier Science B.V.

Keywords: Mosapride citrate; Gastroprokinetic agent; Enantiomeric separation; Enantiomeric determination; Chiral α_1-acid glycoprotein column

1. Introduction

Mosapride citrate, a novel gastroprokinetic agent with a benzamide ring, behaves as a selective 5-HT_4 receptor agonist and enhances only upper gastroprokinetic motor activity [1,2]. Mosapride is a racemic mixture of (R)- and (S)-enantiomers, which exhibit similar activity, and is metabolized to its des-4-fluorobenzyl structure (M-1, Fig. 1) [3–5]. The pharmacokinetic profile of mosapride and M-1 enantiomers remains unknown. Thus, analytical determination of the enantiomers of M-1 as well as those of mosapride is important for insight into their pharmacokinetics and pharmacodynamics.

Recently, enantiomeric separations of racemic drugs [6–17] on chiral stationary phases by high performance liquid chromatography (HPLC) have been widely investigated since some racemic drugs

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were found to show different pharmacokinetics between the two enantiomers in animals and humans.

In this study, enantiomeric separation of racemic mixtures of mosapride and M-1 was examined by HPLC using α_1-acid glycoprotein (AGP) stationary phase column. An efficient analytical method for simultaneous enantiomeric determination of mosapride and M-1 has been developed and validated. Further, the method was applied to determine plasma concentrations of mosapride and M-1 enantiomers in rats after oral administration of mosapride citrate.

2. Experimental

2.1. Materials

Mosopride citrate ((±)-4-amino-5-chloro-2-ethoxy-*N*-[[4-(4-flurobenzyl)-2-morpholinyl]methyl]benzamide citrate), (R)-(+)- and (S)-(−)-enantiomers of mosapride, its des-4-fluolobenzyl metabolite (M-1) ((±)-4-amino-5-chloro-2-ethoxy-*N*-[(2-morpholinyl)methyl]benzamide hydrochloride), and (R)-(−)- and (S)-(+)-enantiomers of M-1 were synthesized in the laboratory. AD-9675 ((+)-4-amino-5-chloro-2-ethoxy-*N*-[[4-Propyl-2-morpholinyl]methyl]benzamide citrate) was used as an internal standard. All other chemicals were of HPLC or analytical-reagent grade.

The mobile phases were freshly prepared, filtered through 0.45-μm filters, and degassed under vacuum prior to use.

2.2. Chromatographic system

The HPLC system consists of an HP 1050 pump (Hewlett Packard, USA), a Waters 717 autosampler equipped with a sample loop injector of 250-μl capacity (Waters, USA) and a Jasco fluorescence detector (Nihon-Bunkou, Japan) with the excitation wavelength set at 314 nm and the emission wavelength set at 354 nm. The chromatograms were recorded and the peak areas were calculated using Waters Maxima 820 software.

For purification of the collected fraction containing mosapride, M-1 and I.S., the plasma sample was pre-treated by solid-phase extraction and was followed by reversed-phase HPLC using a 5-μm Shim-pack CLC-ODS column (150 × 4 mm i.d., Shimadzu, Japan), before which a μ Bondapak C_{18} guard column (20 × 9 mm i.d., Waters, USA) was used. The enantiomeric separation of mosapride and M-1 isomers was preformed on a 10-μm Chiral AGP column (100 × 4 mm i.d., 1 Chrom Tech AB, Sweden); a Chiral AGP guard column (10 × 3 mm i.d.) was used.

2.3. Standard solutions

Stock solutions of mosapride, M-1 and I.S. were prepared by dissolving each compound in distilled water at a concentration of 100 μg ml^{-1} and stored at 4°C. Working standard solutions were obtained each day by further dilution of the stock solutions with distilled water.

2.4. Influence of mobile phase pH on enantiomeric separation of mosapride and M-1 isomers

For mosapride and M-1, separation of their enantiomeric isomers was examined on an AGP

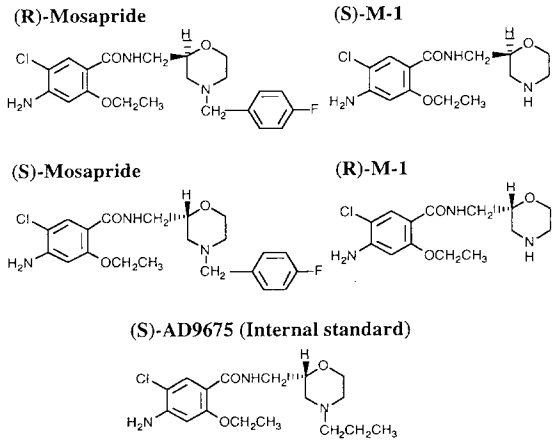

Fig. 1. Chemical structures of enantiomers of mosapride, M-1, and I.S.

Table 1
Influence of mobile phase pH on retention times and resolutions of mosapride and M-1 enantiomers

Mobile phase NaH_2PO_4:Citric acid:MeOH[1]	pH[2]	Mosapride enantiomers		
		Retention time (min)		Resolution
		(R)-isomer	(S)-isomer	
80:0:20	9.1	17.5	8.0	5.8
70:10:20	7.4	15.0	9.4	3.3
60:20:20	6.5	12.8	12.4	—
55:25:20	5.8	8.9	11.8	1.8
50:30:20	4.9	3.8	6.4	2.9
45:35:20	4.4	2.9	4.3	2.4
40:40:20	4.0	1.8	2.4	1.2
30:50:20	3.6	1.3	1.5	0.6
Mobile phase NaH_2PO_4:Citric acid[1]	pH	M-1 enantiomers		
		Retention time (min)		Resolution
		(R)-isomer	(S)-isomer	
80:20	6.5	16.6	27.8	2.9
75:25	6.1	10.7	17.9	3.0
70:30	5.6	6.2	9.4	2.3
65:35	5.0	3.7	4.8	1.3
60:40	4.5	2.6	3.2	1.0
55:45	4.1	2.2	2.4	0.3
50:50	3.8	1.8	1.9	—
40:60	3.4	1.4	1.5	—

[1] The concentrations of Na_2HPO_4 and citric acid; 0.02 M.
[2] Apparent pH.

column using various mobile phase pH values. The compositions of the mobile phases for separating mosapride and M-1 enantiomers are indicated in Table 1. The standard solutions were injected into the chiral AGP column at a flow-rate of 1.0 ml min^{-1}.

2.5. Sample preparation

2.5.1. Solid phase extraction

To 1 ml of plasma sample, 50 μl of I.S. (100 μg ml^{-1}) and 2 ml of pH 7.0 sodium phosphate buffer were added. The mixture was applied to a solid-phase extraction column (Sep-Pak C_{18}, Waters, USA), which was washed with distilled water (3 ml twice) followed by elution with 2 ml of methanol. The extraction column was pre-conditioned with methanol (3 ml twice) and distilled water (3 ml twice) and then equilibrated with 0.02 M phosphate buffer (pH 7.0) (2 ml twice).

2.5.2. Purification by reversed-phase HPLC

The elute evaporated to dryness under reduced pressure at 50°C was dissolved in 400 μl of the mobile phase composed of 0.05% (w/v) acetic acid-methanol (55/45, v/v) and then centrifuged for 10 min at 5000 rpm. A 180-μl aliquot was injected into the reversed-phase HPLC using a Shim-pack CLC-ODS column at a flow-rate of 1.3 ml min^{-1}. The mobile phase was followed by programmed gradient elution (Table 2). A fraction containing mosapride, M-1 and I.S. (t_R: 9.0–14.0 min) was collected for purification.

2.5.3. Enantiomeric separation by chiral AGP column

The collected fraction was evaporated to dryness under reduced pressure at 50°C and then redissolved in 200 μl of the mobile phase consisting of 0.02 M sodium hydrogen phosphate and 0.02 M citric acid (65/35, v/v). A 100-μl aliquot was injected into the chiral AGP column at a flow rate of 1.0 ml min^{-1}. Mosapride and M-1 enantiomers were separated using pH-controlled gradient elution. The composition of the mobile phase in accordance with the time programmed gradient elution is indicated in Table 3.

Table 2
Timetable[a] for reversed-phase HPLC

Time (min)	Mobile phase	
	A(%)	B(%)
	0.05% AcOH	MeOH
0	80	20
7	61.3	38.7
9	10	90
13	10	90
13.5	80	20
18	80	20

[a] Total run time: 18 min.

Table 3
Timetable[a] for chiral-AGP HPLC

Time (mn)	Mobile phase		
	A(%)	B(%)	C(%)
	Na_2HPO_4	Citric acid	MeOH
0	65	35	0
4	65	35	0
10	60	35	5
13	60	35	5
14	48	37	15
25	40	35	25
26	65	35	0
30	65	35	0

[a] Total run time: 30 min.

2.6. Animal experiments

Male S.D. rats, purchased from Nippon Clea (Japan), were fasted overnight before experiments with water *ad libitum*. Mosapride citrate suspended in a 0.5% solution of tragacanth gum was orally administered at a dose of 30 mg kg^{-1}. Blood samples were obtained by cardiac puncture at 0.25, 0.5, 1, 2, 3 and 4 h after oral administration and then centrifuged. Plasma samples were stored at −20°C until analysis.

3. Results and discussion

3.1. Influence of mobile phase pH on enantiomeric separation of mosapride and M-1 isomers

Table 1 and Fig. 2 show the influence of the mobile phase pH (isocratic elution) on the enantiomeric separation of mosapride and M-1 isomers. For both compounds, the mobile phase pH has a strong effect on the retention and resolution.

Retention times of mosapride and M-1 enantiomers, except for mosapride at pH above 6.5, are made longer with increasing pH. It is well known that AGP is a very acidic protein and has net negative charges at pH above 2.7 (isoelectric point) [18,19]. The electrostatic interaction between the positive charge(s) of basic drug and negative charges in or near its binding site is considered to be important [20]. Therefore, pH increase within the examined range may cause their favourable electrostatic interaction, resulting in elongation of the retention. For mosapride at pH above 6.5, pH increase causes elongation of retention times of (R)-isomer of mosapride, whereas retention times of its (S)-isomer are made shorter with increasing pH. The intermolecular interaction mode between the basic drug and AGP stationary phase at pH above 6.5 may be considered to be different from that at pH below 6.5.

Resolution between (S)- and (R)-enantiomers of M-1 was improved with increasing pH. This may be ascribed to the fact that at higher pH the

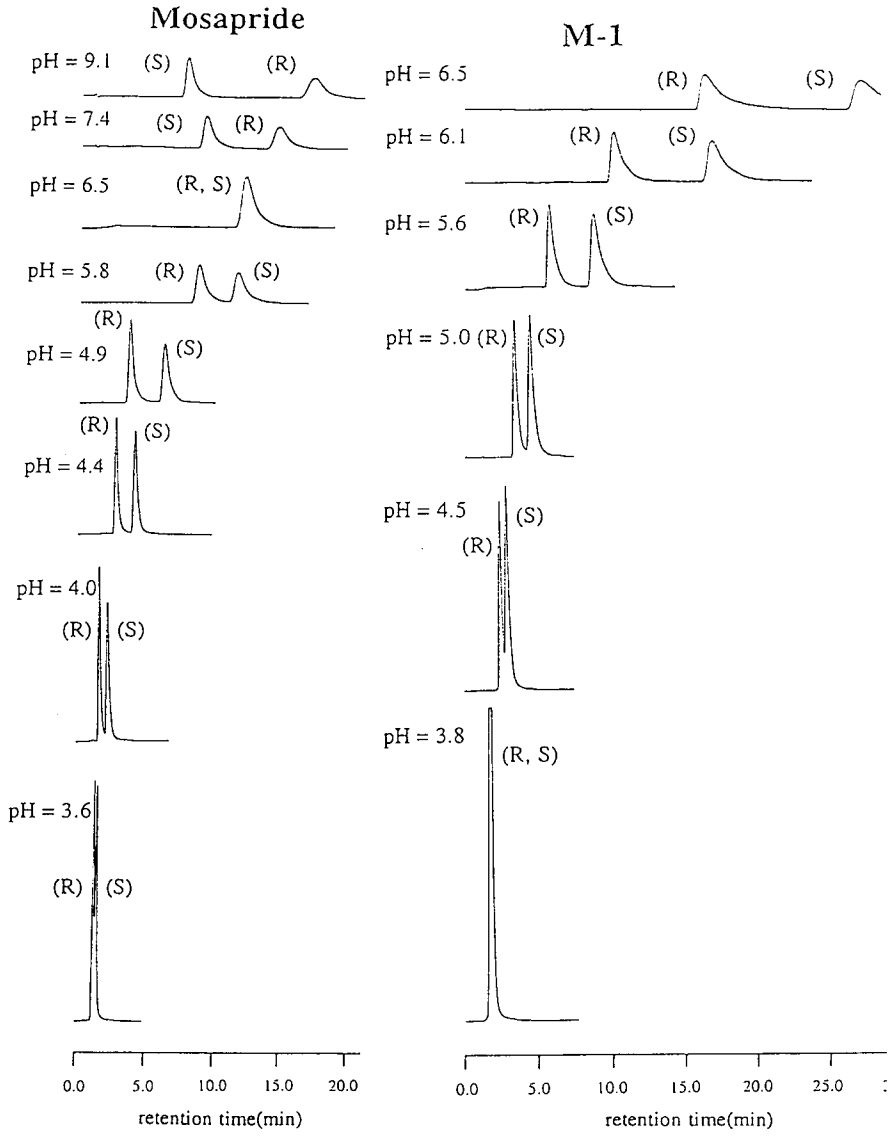

Fig. 2. Influence of mobile phase pH on the enantiomeric separation of mosapride and M-1. The composition of the mobile phase and its pH for mosapride and M-1 are shown in Table 1.

electrostatic interaction between the (S)-isomer of M-1 and AGP is much stronger than that between its (R)-isomer and AGP. On the other hand, the (R)-isomer of mosapride eluted before its (S)-isomer at pH below 6, whereas (S)-isomer eluted first at pH above 6. The conversion of the retention order of mosapride isomers may be due to different binding modes of the solute with AGP stationary phase.

3.2. Reversed phase HPLC

An illustrated reversed-phase chromatogram obtained by injection of a plasma sample contain-

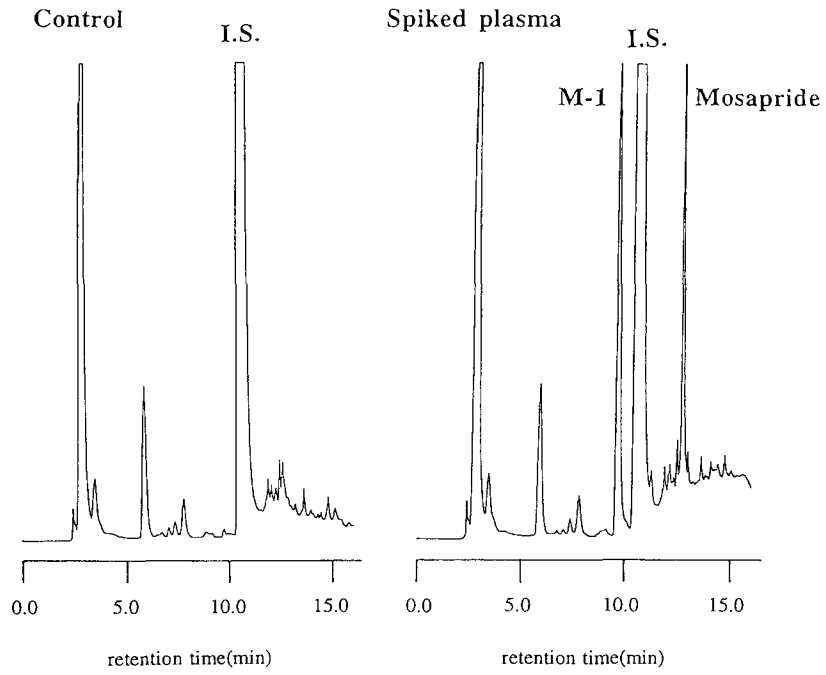

Fig. 3. Chromatograms of mosapride, M-1 and I.S. in plasma on a reversed-phase HPLC column: (left) control and (right) spiked plasma.

ing mosapride and M-1 at a concentration of 500 ng ml^{-1} each is shown in Fig. 3. The retention times of mosapride, M-1, and I.S. were 9.5, 11.0 and 12.5 min, respectively. Because of medium hydrophobicity between mosapride and M-1, I.S. was eluted between them. A fraction eluted from 9.0 to 14.0 min was collected so that interfering constituents in plasma could be eliminated.

3.3. HPLC using a chiral AGP column

In terms of resolution and sensitivity, the optimal pH values for appropriate enantiomeric separation of mosapride and M-1 are considered to be 4.4 and 5.0, respectively (Table 1 and Fig. 2). In order to analyze simultaneously the enantiomers of mosapride and M-1 in a single chromatogram, programmed gradient elution capable of achieving the pH variation was found to be necessary. The timetable for chiral-AGP HPLC is indicated in Table 3. Inclusion of methanol into the mobile phase (up to 25% (v/v)) was due to rapid elution of mosapride with more hydrophobic property than M-1. A typical chromatogram obtained by injection of plasma sample containing racemic mosapride and M-1 each at a concentration of 5000 ng ml^{-1} is shown in Fig. 4, as compared with that of control plasma. (R)- and (S)-enantiomers of mosapride were eluted between 20 and 22 min at pH 5.0 and (R)- and (S)-enantiomers of M-1 eluted between 4 and 6 min at pH 4.4.

3.4. Calibration and reproducibility

Calibration ranges of the enantiomers of mosapride and M-1 are 10–5000 and 50–5000 ng ml^{-1}, respectively. The correlation coefficients of the calibration curves ($n = 3$) were greater than 0.997. The intra- and inter-day coefficients of variation of mosapride enantiomers at concentrations of 50, 500, and 5000 ng ml^{-1} were less than 5.4 and 2.6% respectively, and those of M-1 enantiomers were less than 2.7% and 8.9% respectively (Table 4). Accuracies of mosapride and M-1

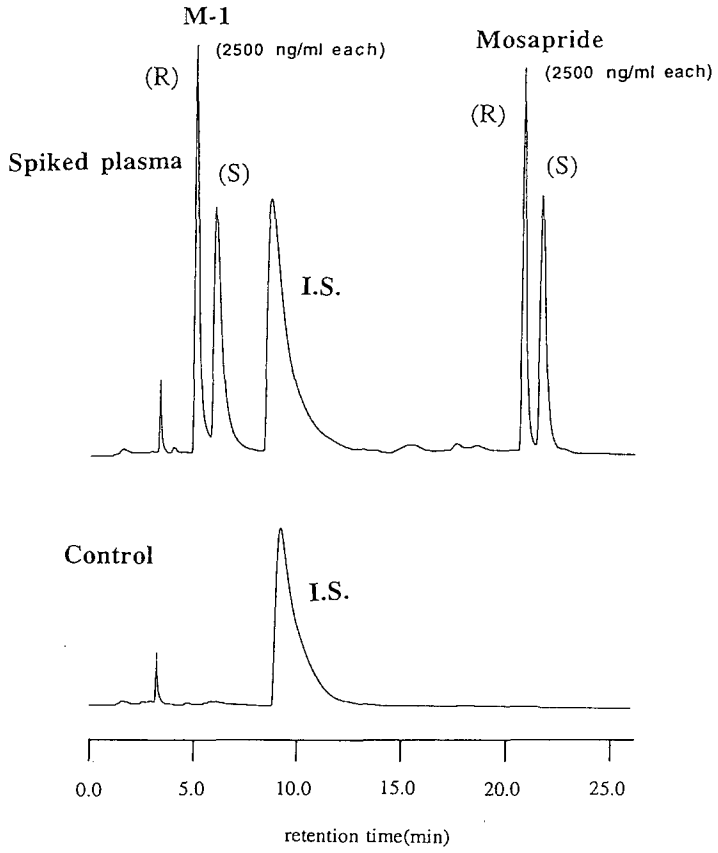

Fig. 4. Chromatograms of enantiomers of mosapride and M-1 in plasma on a Chiral-AGP HPLC column: (upper) spiked plasma and (lower) control. Configuration assignments are indicated in the chromatogram.

enantiomers were in the range of 0.2–6.3% (Table 5). These validation parameters shown in Tables 4 and 5 are considered to be satisfactory for the pharmacokinetic study of mosapride and M-1.

3.5. Animal experiment

Fig. 5 shows the plasma levels of enantiomers of mosapride and M-1 in rats after single oral administration of mosapride citrate (30 mg kg^{-1}). Plasma concentrations of (R)-isomer of mosapride were slightly but significantly higher than those of its (S)-isomer. Peak plasma concentrations of (R)- and (S)-isomers of mosapride were observed 0.5 h after oral administration with levels of 625 and 559 ng ml^{-1}, respectively. For both enantiomers, the half-lives were 0.8–0.9 h. Plasma levels of the (R)-isomer of M-1 tended to be slightly higher than those of the (S)-isomer of M-1, but the difference was not statistically significant.

4. Conclusions

A simple and reproducible high-performance liquid chromatographic method has been developed to determine simultaneously the enantiomers of mosapride and M-1 using a chiral AGP stationary phase and a time programmed gradient elution. The mobile phase pH has a strong effect on retention times and resolutions of mosapride

Table 4
Intra- and inter-day coefficients of variation for each isomer of mosapride and M-1 ($n = 3$)

Concentration (ng/ml)	C.V. (%)	Mosapride		M-1	
		(R)-isomer	(S)-isomer	(R)-isomer	(S)-isomer
50	Intra-day	0.65	4.45	2.64	1.54
	Inter-day	1.71	0.74	8.87	1.19
500	Intra-day	3.23	2.71	0.97	0.58
	Inter-day	2.51	1.03	2.18	0.53
5000	Intra-day	5.38	6.15	1.64	0.14
	Inter-day	1.12	0.39	1.09	1.05

Table 5
Accuracy for each isomer of mosapride and M-1 ($n = 3$)

Concentration (ng/ml)	Mosapride		M-1	
	(R)-isomer	(S)-isomer	(R)-isomer	(S)-isomer
Accuracy (%)				
50	5.45	3.09	0.91	1.54
500	4.26	0.36	3.75	1.69
5000	6.29	1.80	2.95	0.22

and M-1 isomers. It is interesting that the (R)-isomer of mosapride was eluted before its (S)-isomer at a pH below 6, whereas the (S)-isomer was eluted first at a pH above 6. The quantitative ranges of mosapride and M-1 enantiomers are 10–5000 and 50–5000 ng ml^{-1}, respectively. The intra- and inter-day coefficients of variation are satisfactory for the pharmacokinetic study of mosapride. The developed method was applicable to the simultaneous determination of plasma concentrations of the four enantiomers in rats after oral administration of mosapride citrate.

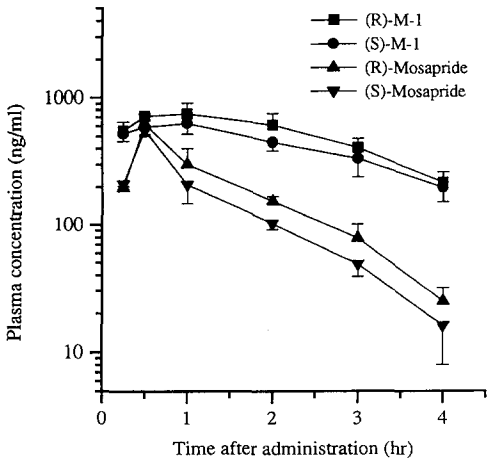

Fig. 5. Plasma concentrations of enantiomers of mosapride and M-1 in rats after a single oral administration of racemic mosapride at a dose of 30 mg kg^{-1}. Results are expressed as the mean ± S.E. of 3 rats.

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