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Short communication

Enantioselective determination of sibutramine and its active metabolites in human plasma

Wonku Kang*, Kyoungjin Bae, Keumhan Noh

College of Pharmacy, Catholic University of Daegu, Kyoungbuk 712-702, South Korea

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ABSTRACT

Although racemic sibutramine has been widely used for the treatment of obesity, its enantioselective detection method has not been elucidated in human plasma. In this report we introduce a validated analytical method for the determination of sibutramine and its two active metabolites, desmethylsibutramines using LC–MS/MS. *R*- and *S*-isomers of those compounds in human plasma were extracted using diethyl ether–hexane (4:1, v/v) followed by an addition of NaOH solution. After removing the organic layer, the residue was reconstituted in the mobile phase 10 mM ammonium acetate solution adjusted to pH 4.0 with acetic acid–acetonitrile (94:6, v/v). Both isomers in the extract were separated on a Chiralcel AGP chiral stationary-phase column and were quantified in a tandem mass spectrometry. The assay method was in accordance with FDA regulations for the validation of bioanalytical methods. This method was successfully used to profile the plasma concentrations of the stereoisomers of sibutramine and its two active metabolites with time in healthy volunteers.

1. Introduction

Racemic sibutramine is widely used for the treatment of obesity due to the inhibition of serotonin and noradrenaline reuptake in synapse. Its mode of action results in enhancing satiety and energy expenditure [1]. It has been well known that sibutramine is mainly metabolized *in vivo* to mono- and di-desmethyl active metabolites [2,3], and a chiral chromatography method was recently introduced to separately determine each stereoisomer in rat plasma, and the time courses of plasma concentrations of both isomers of those three compounds were clearly elucidated for the first time in rat [4]. Interestingly, the systemic exposure of the *S*-isomers of desmethylsibutramines was much greater than that of the *R*-isomers representing much more potent compared to the *S*-enantiomers [4,5]. On the basis of the previous experience, we tried to develop an analytical method to determine both isomers of sibutramine, monodesmethylsibutramine (MDS) and didesmethylsibutramine (DDS) in human plasma. The present method was successfully applied to characterize the time courses of plasma concentrations of the stereoisomers of sibutramine as well as its two active metabolites in human, following an oral administration of racemic sibutramine commercially available.

2. Experimental

2.1. Reagents and materials

Sibutramine, MDS, and DDS were kindly donated by Yuhan Pharmaceutical Co. (Seoul, Korea) and the purity of three compounds is more than 99.5%. Domperidone (internal standard, IS) was purchased from Sigma (Seoul, Korea), and all other chemicals and solvents were of the highest analytical grade available. *R*- and *S*-isomers were separated at a Medicinal Chemistry Laboratory in College of Pharmacy, Catholic University of Daegu [6].

2.2. Preparation of standards and quality controls

Sibutramine, MDS, DDS, and the IS were dissolved in methanol to obtain a concentration of 1.0 mg/ml. These solutions were diluted serially with the mobile phase (10 mM ammonium acetate buffer adjusted to pH 4.03 with acetic acid–acetonitrile, 94:6, v/v), and 50 μl of each solution was added to 850 μl of drug-free plasma, to obtain final concentrations at 0.1, 0.25, 0.5, 1, 2.5, and 5 ng/ml for sibutramine and MDS; and at 0.25, 0.5, 1, 2.5, 10, and 25 ng/ml for DDS. Using linear regression, six calibration graphs were derived from the ratio between the area under the peak of each compound and the IS. Quality control samples were prepared in 850 μl of blank human plasma by adding 50 μl of serially diluted solutions of each of the three racemates, to obtain low, intermediate, and high concentrations in control samples. These samples were used to evaluate the between days and within day precision and accuracy of the assay.

* Corresponding author. Tel.: +82 53 850 3619; fax: +82 53 850 3602.
E-mail address: wonkuk@cu.ac.kr (W. Kang).

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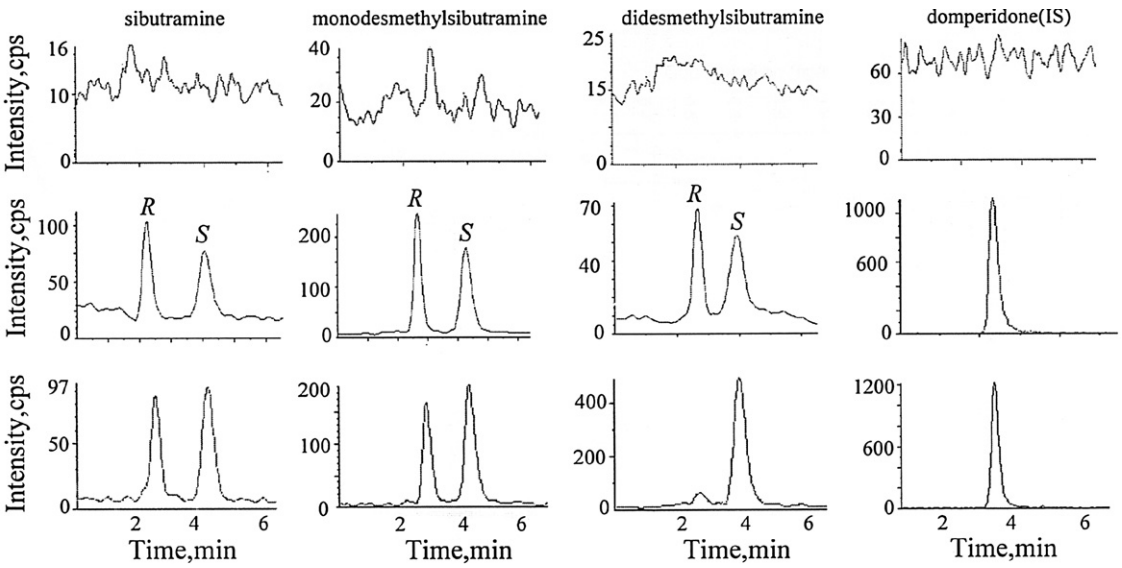

Fig. 1. Chromatograms of sibutramine, monodesmethylsibutramine, and didesmethylsibutramine enantiomers and domperidone. Top, double-blank plasma; middle, plasma spiked with 5 ng/ml sibutramine, monodesmethylsibutramine, and didesmethylsibutramine, and 100 ng/ml domperidone (IS); bottom, plasma sample of *R*- and *S*-isomers equivalent to 0.8 and 1.8 ng/ml for sibutramine, 0.6 and 3.2 ng/ml for monodesmethylsibutramine, and 0.0 and 4.8 ng/ml for didesmethylsibutramine, respectively, in a sample obtained from a volunteer 1 h after oral administration of 8.73 mg racemic sibutramine.

2.3. Analytical system

Plasma concentrations of sibutramine, MDS, and DDS were quantified using an API 4000 LC–MS/MS system (Applied Biosystems, Foster City, CA, USA) equipped with an electrospray ionization interface that was used in the positive ion mode ($[M+H]^+$).

The compounds were separated on a chiral stationary-phase column (Chiralcel AGP, 100 mm × 2.0 mm inner diameter, 5-μm

Table 1
Precision and accuracy of the assay of sibutramine, MDS and DDS enantiomers ($n = 5$).

Quality control	Sibutramine				MDS				DDS			
	R-isomer		*S*-isomer		*R*-isomer		*S*-isomer		*R*-isomer		*S*-isomer	
	Accuracy[a]	RSD[b]	Accuracy	RSD	Accuracy	RSD	Accuracy	RSD	Accuracy	RSD	Accuracy	RSD
Within day assay												
LOQ[c]	102.2 ± 7.6	7.3	104.6 ± 3.4	2.8	97.3 ± 11.4	11.7	101.0 ± 4.4	4.4	105.2 ± 8.6	8.1	101.1 ± 16.7	16.5
MOQ_1[d]	89.5 ± 4.5	5.0	93.6 ± 7.6	8.2	97.5 ± 6.4	6.6	98.0 ± 8.0	8.1	91.8 ± 4.6	5.3	95.1 ± 6.9	7.2
MOQ_2[e]	108.6 ± 5.1	4.7	106.5 ± 1.3	1.2	99.6 ± 2.2	2.2	101.3 ± 2.0	2.0	89.1 ± 4.8	5.4	87.9 ± 2.8	3.2
HOQ[f]	102.8 ± 7.0	6.8	102.4 ± 3.0	2.9	101.8 ± 1.1	1.1	104.1 ± 3.1	3.0	112.7 ± 1.5	1.4	110.9 ± 2.7	2.4
Between days assay												
LOQ[c]	101.9 ± 5.9	5.8	101.7 ± 3.3	3.3	102.5 ± 4.8	4.7	101.4 ± 5.5	5.5	105.6 ± 6.9	6.6	101.5 ± 2.6	2.5
MOQ_1[d]	92.8 ± 6.0	6.4	94.2 ± 3.7	3.9	94.3 ± 2.8	2.9	99.1 ± 2.8	2.8	90.6 ± 1.4	1.6	91.0 ± 4.0	4.4
MOQ_2[e]	104.0 ± 6.9	6.7	103.0 ± 3.0	2.9	95.5 ± 3.7	3.8	95.7 ± 5.1	5.3	90.2 ± 1.0	1.1	87.2 ± 0.9	1.0
HOQ[f]	101.4 ± 2.4	2.4	100.6 ± 3.4	3.4	104.1 ± 3.2	3.0	104.2 ± 1.1	1.1	111.1 ± 4.1	3.7	112.6 ± 1.5	1.3

[a] Mean % ± S.D.
[b] RSD (relative standard deviation, %) = S.D. × 100/mean.
[c] 0.1 ng/ml for sibutramine and MDS, 0.25 ng/ml for DDS.
[d] 0.5 ng/ml for sibutramine and MDS, 1.0 ng/ml for DDS.
[e] 1.0 ng/ml for sibutramine and MDS, 2.5 ng/ml for DDS.
[f] 5.0 ng/ml for sibutramine and MDS, 25.0 ng/ml for DDS.

Table 2
Stability of sibutramine, MDS and DDS enantiomers.

Stability condition	Sibutramine				MDS				DDS			
	R-isomer		*S*-isomer		*R*-isomer		*S*-isomer		*R*-isomer		*S*-isomer	
	0.5 ng/ml	5 ng/ml	0.5 ng/ml	5 ng/ml	0.5 ng/ml	5 ng/ml	0.5 ng/ml	5 ng/ml	0.5 ng/ml	5 ng/ml	0.5 ng/ml	5 ng/ml
Room temp. (12 h)	87.1 ± 2.5[a]	97.5 ± 7.0	88.3 ± 3.3	93.7 ± 5.2	97.0 ± 3.3	99.8 ± 6.6	99.5 ± 4.8	99.2 ± 5.2	88.9 ± 3.2	89.3 ± 3.1	90.5 ± 2.0	89.6 ± 2.4
Post-extraction (12 h)	90.5 ± 3.9	96.6 ± 6.4	89.3 ± 2.5	97.3 ± 2.5	85.7 ± 1.5	93.1 ± 3.7	88.2 ± 1.2	91.4 ± 4.9	89.3 ± 4.2	91.1 ± 4.0	89.6 ± 4.2	90.4±3.2
Freeze–thaw	90.7 ± 3.1	104.3 ± 1.6	88.3 ± 2.5	98.2 ± 2.3	90.4 ± 3.2	97.5 ± 0.7	85.4 ± 0.2	100.2 ± 2.0	106.0 ± 3.5	99.0 ± 3.3	89.2 ± 2.1	96.3 ± 5.1
Long-term (4-week)	90.6 ± 2.1	88.6 ± 3.6	87.2 ± 1.5	94.8 ± 3.1	92.6 ± 1.5	92.0 ± 3.5	91.2 ± 3.4	90.8 ± 2.0	88.0 ± 2.5	85.9 ± 0.8	94.7 ± 2.7	90.8 ± 1.6

[a] Mean % ± S.D. ($n = 3$).