Wang HY *et al* / Acta Pharmacol Sin 2003 Jan; 24 (1): 74-84

Quantitative determination of omapatrilat and its metabolites in human plasma by HPLC coupled with tandem mass spectrometry [1]

WANG Hong-Yun [2], HU Yu-Fang, JIANG Ji, HU Pei

Clinical Pharmacology Research Center, Peking Union Medical College Hospital and Chinese Academy of Medical Sciences, Beijing 100730, China

KEY WORDS omapatrilat; BMS-186716; high pressure liquid chromatography; mass spectrometry

ABSTRACT

AIM: To develop a sensitive and specific analytical method for the quantitative determination of omapatrilat (BMS-186716) and its metabolites (BMS-196087, 225308, 198433, and 253653) in human plasma. **METHODS:** Methyl acrylate (MA) was selected to react with BMS-186716, 196087, and 253653 to protect the free sulfhydryl groups. High pressure liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was used to detect the analytes. **RESULTS:** The method was validated over the concentration range of 0.2-250 μg/L for BMS-186716, 0.5-250 μg/L for BMS-196087, 1-250 μg/L for BMS-225308, 2-250 μg/L for BMS-198433, and 10-2500 μg/L for BMS-253653. The limit of quantitation was in turn 0.2, 0.5, 1, 2, and 10 μg/L, respectively. The extraction recovery was on average 60.5 %, 88.6 %, 76.3 %, 71.2 %, and 26.6 %, respectively. Inter- and intra-day precision of quality control samples (QC) was all within ± 15 % and accuracy was within 85 %–115 %. The analytes in human plasma were found to be stable after three cycles of freeze-thaw and for at least 6 h at room temperature (25 ℃). No significant change was found in reconstituted reagent after 24 h at room temperature and results of long-term stability showed all the analytes in human plasma were stable for at least 3 months at -30 ℃ freezing condition. **CONCLUSION:** This method is rapid, sensitive and specific for the pharmacokinetics study of omapatrilat and its metabolites.

INTRODUCTION

Vasopeptidase inhibitors are a new class of cardiovascular compounds that represent a new therapeutic principle including both inhibition of angiotensin-converting enzyme (ACE) and neutral endopeptidase (NEP). Simultaneous inhibition of NEP and ACE enhances peptides with vasodilatory properties, such as atrial natriuretic peptide, brain natriuretic peptide, and C type natriuretic peptide, and this inhibits the production of the vasoconstrictor angiotensin II. Vasopeptidase inhibitors have opened a new era in cardiovascular therapy[1-4].

Omapatrilat (BMS-186716) is the most clinically advanced agent in the vasopeptidase inhibitors. It is being developed by Bristol-Myers Squibb Pharmaceutical Company for the treatment of hypertension and

[1] Project supported by Bristol-Myers Squibb Pharmaceutical Company.
[2] Correspondence to Dr WANG Hong-Yun.
Phn 86-10-6529-6574. Fax 86-10-6529-6573.
E-mail wanghongyunpumc@sohu.com
Received 2002-01-26 Accepted 2002-10-14

congestive heart failure[5]. A number of reports indicated that omapatrilat would play an important role in the treatment of cardiovascular diseases[6-9].

Omapatrilat undergoes substantial presystemic first-pass metabolism after oral administration to produce metabolites such as BMS-196087, BMS-225308, BMS-198433, and BMS-253653[10,11]. It needs to be pointed out that omapatrilat, BMS-196087 and BMS-253653 are unstable thiolic compounds, and their free sulfhydryl groups are needed to be covalently protected with methyl acrylate (MA) to form MA adducts, so as to prevent *in vitro* oxidation of the sulfhydryl groups. Although several methods have been reported to assay omapatrilat in rat and dog plasma[12,13], no information is available on the quantitation of its metabolites by far. The aim of this paper is to describe a reliable and sensitive high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantitative determination of both omapatrilat and its metabolites simultaneously. The method has been well validated and applied to measure plasma concentrations of biological samples obtained from phase I clinical trial of omapatrilat in Chinese healthy volunteers.

MATERIALS AND METHODS

Chemicals and regents Omapatrilat (BMS-186716), BMS-196087, BMS-225308, BMS-198433, BMS-253653, and their corresponding isotopic internal standards d_5-BMS-186716, d_5-BMS-196087, d_5-BMS-225308, d_5-BMS-198433, and d_5-BMS-253653 (Fig1) were provided by Bristol-Myers Squibb Pharmaceutical Research Institute (New Brunswick, Canada). Methyl-tert butyl ether (HPLC grade), acetonitrile (HPLC grade) were purchased from Fisher (Fair Lawn, NJ, USA). Methyl acrylate was from Aldrich (Milwaukee, USA). Hydrochloric acid (37 %, v/v), acetic acid (AR grade), potassium hydrogen phosphate (AR grade), and ammonium acetate (AR grade) were purchased from Peking Chemical Plant (Beijing, China). Laboratory-deionized water was produced with a Milli-Q water purifying system (Millipore, Bedford, USA).

Preparation of stock solution (500 mg/L) BMS-186716, BMS-196087, BMS-253653, and their deuterate internal standards 12.5 mg, were weighted respectively and transferred to six 25-mL volumetric flasks containing 12.5 mL of phosphate buffer 0.01 mol/L and 50 μL methyl acrylate, used as derivative reagent[12,13]. The flasks were alternately sonicated and vortexed until the compounds completely dissolved and reacted with MA to form corresponding MA adducts (Tab 1, Fig 2), the reaction time lasted about 10 min. The flasks were then made up to the mark with acetonitrile.

Stock solutions of BMS-198433 and BMS-225308 and their internal standards, were prepared by dissolving 12.5 mg of the corresponding compounds with acetonitrile in 25-mL volumetric flasks, respectively.

Preparation of MA adducts in unknown human blood Seven mL of blood was drawn from healthy

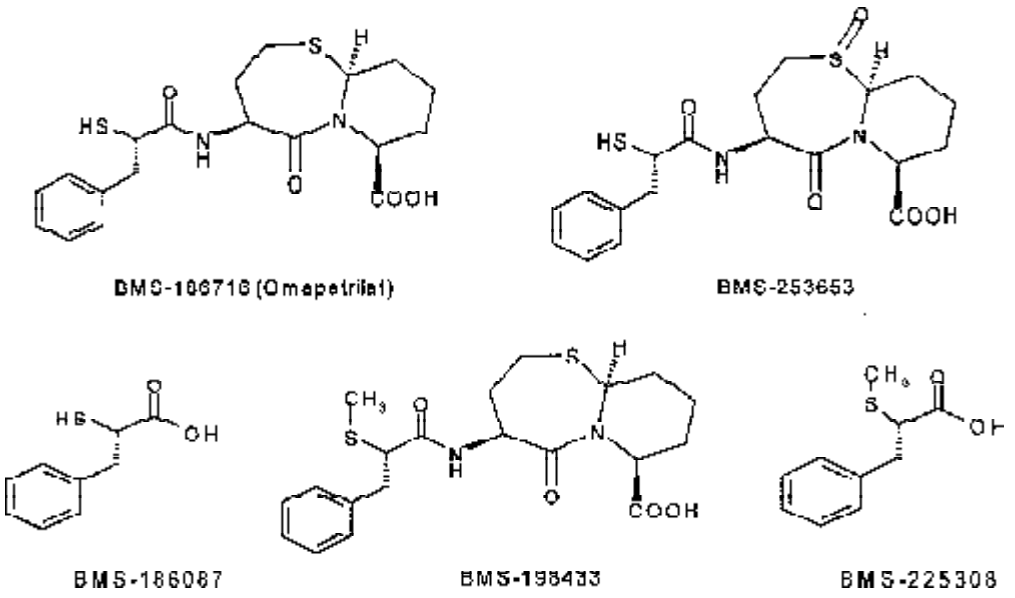

Fig 1. Chemical structures of BMS-186716 and its metabolites, and their corresponding deuterated internal standards were labeled at phenyl.

Tab 1. Molecular weights and corresponding MA adducts of the analytes.

Compound	M_r	MA adducts
BMS-186716	408	494
BMS-198433	422	Not applicable
BMS-196087	182	268
BMS-225308	196	Not applicable
BMS-253653	424	510
d_5-BMS-186716	413	499
d_5-BMS-198433	427	Not applicable
d_5-BMS-196087	187	273
d_5-BMS-225308	201	Not applicable
d_5-BMS-253653	429	515

volunteers at every blood drawing point on the day of clinical trial, and transferred immediately to the vacutainers containing 70 μL methyl acrylate, Tri-potassium ethylene diamine tetraacetate (K_3EDTA) was used as an anticoagulant. After spiking, the blood samples were kept at room temperature for 10 min, and then centrifuged at 2500×*g* for 10 min. Plasma was taken out and stored at -30 °C until use.

Biological matrix Drug-free human plasma and blood used in the research was purchased from Peking Union Medical College Hospital blood bank. Fresh human plasma from different donors was pooled and used to prepare calibration standard and quality control plasma samples. No significant interference was found in the determination.

Preparation of calibration curves and quality control samples in human plasma Calibration curve and quality control samples were prepared by adding corresponding stock solutions to drug-free human plasma (Tab 2).

Extraction procedure of plasma samples To each 0.5-mL volume of plasma sample, 50 μL of the internal standard working solution (500 μg/L of d_5-BMS-186716, d_5-BMS-196087, d_5-BMS-225308, and d_5-BMS-198433, 5000 μg/L of d_5-BMS-253653) was added and mixed. Then 0.5 mL of hydrochloric acid solution 0.1 mol/L was added using an Eppendorf repeater pipette, followed by the addition of 3 mL methyl-tert butyl ether to each tube. The tubes were then capped and shaken for 10 min on a shaker. The aqueous and the organic layers were separated by centrifugation at 2500×*g* for 10 min. The organic layer was transferred to a clean test tube and evaporated to dryness under gentle stream of nitrogen at 40 °C. The residue was dissolved with 150 μL of mobile phase and vortexed, then centrifuged by a micro-spin filter tube (0.2 μm nylon, Alltech) at 8000×*g* for 3 min. The filtrate was collected and stored at -30 °C until analysis.

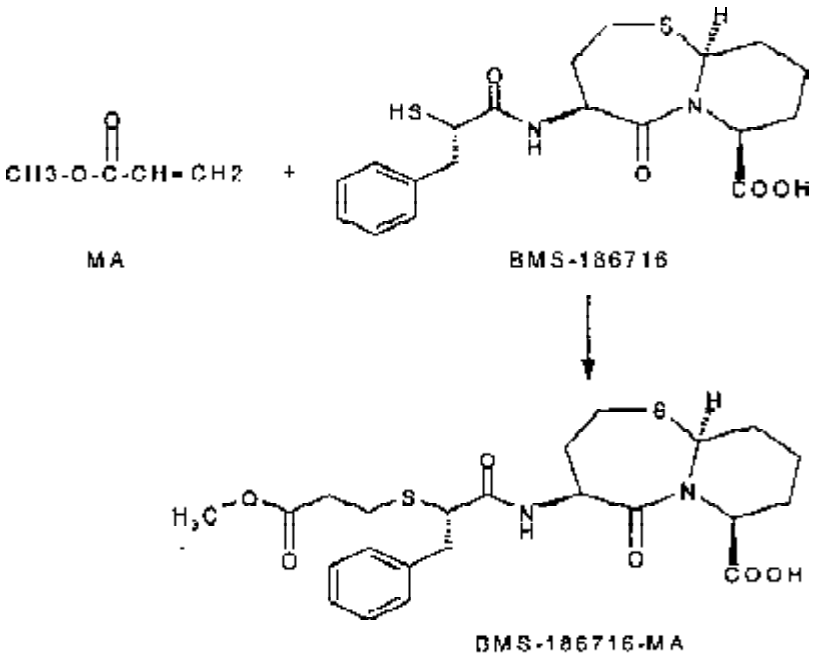

Fig 2. Typical reaction of BMS-186716 with MA (the reaction was also applied to treat with BMS-196087, BMS-253653, and their corresponding isotopic internal standards).

Tab 2. Summary of concentration range for calibration curves and quality control sample in human plasma.

Compound	Calibration curve/μg·L^{-1}	Quality control/μg·L^{-1}	Internal standard/ μg·L^{-1}
BMS-186716	0.2, 0.5, 1, 2, 5, 12.5, 25, 50, 125, 250	2, 100, 200	50
BMS-196087	0.5, 1, 2, 5, 12.5, 25, 50, 125, 250	2, 100, 200	50
BMS-225308	1, 2, 5, 12.5, 25, 50, 125, 250	4, 100, 200	50
BMS-198433	2, 5, 12.5, 25, 50, 125, 250	8, 100, 200	50
BMS-253653	10, 20, 50, 125, 250, 500, 1250, 2500	40, 1000, 2000	500

Chromatographic conditions HPLC system consisted of Waters 510 HPLC pump, Waters automated gradient controller (Waters, USA) and a PE series 200 autosampler from Perkin-Elmer (Foster city, CA, USA). The analytical column was BDS Hypercil C_8 (3 μm, 50 mm×2 mm ID, Keystone Scientific, INC) proceeded by Hypercil C_8 guard column. The mobile phase consisted of 62.5 % water and 37.5 % acetonitrile, with ammonium acetate 1 mmol/L at pH 5.5. The flow rate was 0.2 mL/min and the injection volume was 25 μL.

Mass spectrometer conditions The HPLC system was coupled to Perkin-Elmer SCIEX API-3000 triple-quadruple mass spectrometer (Foster city, CA, USA) equipped with ESI source. Probe temperature was set at 350 °C with ultrahigh-purity nitrogen as curtain gas (10 L/min) and nebulizer gas (10 L/min). Auxiliary gas (nitrogen) was optimized daily. The sprayer voltage (IS), deflector voltage (DF), and multiplier voltage (CEM) were set at -3600 V, 300 V, and 2200 V, respectively. Mass dependent parameters such as orifice voltage (OR), ring voltage (RNG), Q2 rod offset voltage (RO_2), RF-stubbies voltage (ST_3) , and Q2 rod offset voltage (RO_3) were optimized specially for each analyte (Tab 3). Multiple reactions monitoring (MRM) was employed using nitrogen as collision gas (6 L/min) with a dwell time of 200 ms for each transition. The ionization was performed in negative mode and the analytes were detected by monitoring the following ion transitions: *m/z* 493.0→407.1 for BMS-186716-MA and *m/z* 498.0 → 412.2 for d_5-BMS-186716; *m/z* 181.1 → 147.2 for BMS-196087-MA and *m/z* 186.1→ 152.1 for d_5-BMS-196087; *m/z* 195.1 → 147.1 for BMS-225308 and *m/z* 200.1→152.0 for d_5-BMS-225308; *m/z* 421.0 → 246.1 for BMS-198433 and *m/z* 426.0→ 251.1 for d_5-BMS-198433; *m/z* 509.1→423.2 for BMS-253653-MA and *m/z* 514.0→ 428.2 for d_5-BMS-253653-MA.

Comparison between a calibration curve of BMS-186716-MA in human plasma and a calibration curve of BMS-186716 reacted with MA in human blood Because the reaction between MA and unknown biological samples occurred directly in human blood on the day of blood drawing, and this was different from the processing of standard solutions, comparison was designed to evaluate the applicability of the method. A calibration curve of BMS-186716-MA in human plasma and a calibration curve of BMS-186716 reacted with MA in human blood, were respectively prepared by spiking an amount of BMS-186716-MA or BMS-186716 into human plasma or human blood that contained MA (10 μL per mL of blood). The final nominal concentrations of the two calibration curves were identical since the same amount of BMS-186716 was weighed and used. After spiking, the blood samples were kept for 10 min before they were centrifuged at 2500×*g* to form plasma. The plasma samples of both

Tab 3. Summary of mass-dependent parameters for the analytes.

Compound	OR/V	RNG/V	RO_2/V	ST_3/V	RO_3/V
BMS-186716-MA	-45	-115	28	48	30
BMS-196087-MA	-32	-80	24	44	26
BMS-225308	-30	-78	20	40	22
BMS-198433	-48	-116	33	53	35
BMS-253653-MA	-52	-125	32	52	34
d_5-BMS-186716-MA	-50	-115	27	45	29
d_5-BMS-196087-MA	-38	-92	25	45	27
d_5-BMS-225308	-22	-60	21	38	23
d_5-BMS-198433	-46	-108	36	56	38
d_5-BMS-253653-MA	-52	-125	32	52	34

OR: orifice voltage; RNG: ring voltage; RO_2: Q2 rod offset voltage; ST_3: RF-stubbies voltage; RO_3: Q2 rod offset voltage.

calibration curves were extracted and analyzed followed by addition of internal standard, comparison was done by assessing the deviations between the two curves.

Validation of method Calibration curve samples were prepared in duplicate every day for 5 d. The linear regression between the plasma concentration of each analyte and the peak area ratio to the internal standard was weighted by $1/x^2$. The limit of quantitation (LOQ) of BMS-186716 and its metabolites were set at the concentration of the lowest non-zero calibration standard. Intra- and inter-day precision and accuracy (relative recovery) were determined by calculation of QC samples at low, medium, and high concentrations. The extraction recoveries of the analytes were determined by comparing extracted QC samples at low medium and high concentrations to unextracted calibration standard solutions at the same concentration. The stability of the analytes in human plasma at room temperature (25 °C) was investigated by assessing QC samples after 0, 3, 6, and 24 h. Freeze-thaw cycle's stability was done after three times of cycles and the stability in reconstituted reagent was tested after 24 h at room temperature. Long-term stability in plasma was assessed after QC samples were stored at -30 °C freezing condition for 3 months.

RESULTS

Representative MRM chromatograms of blank, plasma spiked with drugs, and unknown plasma sample from a volunteer were obtained under the selected LC-MS/MS conditions (Fig 3-5). It was demonstrated that no significant interfering substances were found in the retention time of the analytes, and metabolite BMS-253653 was not detected in the unknown human plasma samples.

Calibration curves were prepared in duplicate every day for 5 d. The linear regression between the plasma concentration of each analyte and the peak area ratio to the internal standard was weighted by $1/x^2$. Calibration curves were validated over the concentration range of 0.2-250 μg/L for BMS-186716, 0.5-250 μg/L for BMS-196087, 1-250 μg/L for BMS-225308, 2-250 μg/L for BMS-198433 and 10-2500 μg/L for BMS-253653, respectively. The deviations of the back-calculated concentrations of calibration standards from their nominal values were within ±15 %. Typical equations of calibration curves were as followes:

BMS-186716: Y=0.00494+0.01909X (r=0.9996)

BMS-196087: Y=0.00316+0.02254X (r=0.9963)

BMS-225308: Y=0.002662+0.02182X (r=0.9972)

BMS-198433: Y=0.003836+0.03139X (r=0.9983)

BMS-253653: Y=0.04312+0.02182X (r=0.9967)

Here, Y represented peak area ratio, and X described plasma concentration.

The limit of quantitation (LOQ) under described conditions was 0.2 μg/L for BMS-186716, 0.5 μg/L for BMS-196087, 1 μg/L for BMS-225308, 2 μg/L for BMS-198433, and 10 μg/L for BMS-253653, respectively.

Because BMS-186716 and its metabolites BMS-196087 and BMS-253653 were unstable thiolic compounds, good stability became the precondition of accurate quantitation. The analytes in human plasma were found to be stable after three cycles of freeze-thaw and for at least 6 h at room temperature (25 °C). No significant change was found in reconstituted reagent after 24 h at room temperature and results of long-term stability showed all the analytes were still stable for at least 3 months at -30 °C freezing condition.

Intra-assay and inter-day precision (RSD %) were within ±15 % for BMS-186716, BMS-196087, BMS-225308, BMS-198433, and BMS-253653 (Tab 4). Accuracy (relative recovery) ranged from 97.4 % to 108.1 %, 94.4 % to 108.0 %, 100.3 % to 101.7 %, 97.9 % to 103.3 % and 99.3 % to 101.2 % for the five analytes in the same order as above. The extraction recovery was on average 60.5 % for BMS-186716, 88.6 % for BMS-196087, 76.3 % for BMS-225308, 71.2 % for BMS-198433, and 26.6 % for BMS-253653, respectively (Tab 5).

Comparison between a calibration curve of BMS-186716-MA in human plasma and a calibration curve of BMS-186716 in human blood with MA added, where the reaction between BMS-186716 and MA occurred directly in blood, showed acceptable deviation, and this explained the applicability of this method at different conditions (Tab 6).

This method was used to the pharmacokinetics study of omapatrilat (BMS-186716) and its metabolites (BMS-196087, BMS-225308, BMS-198433, and BMS-253653) in Chinese healthy volunteers. But metabolite BMS-253653 was not found in all the unknown plasma samples, and this showed that the present method was not sensitive enough to determine metabolite BMS-253653, and method needed for further improvement (Fig 5). So only mean plasma concentration-time curves for BMS-186716, BMS-196087, BMS-225308, and BMS-198433 were acquired (Fig 6).

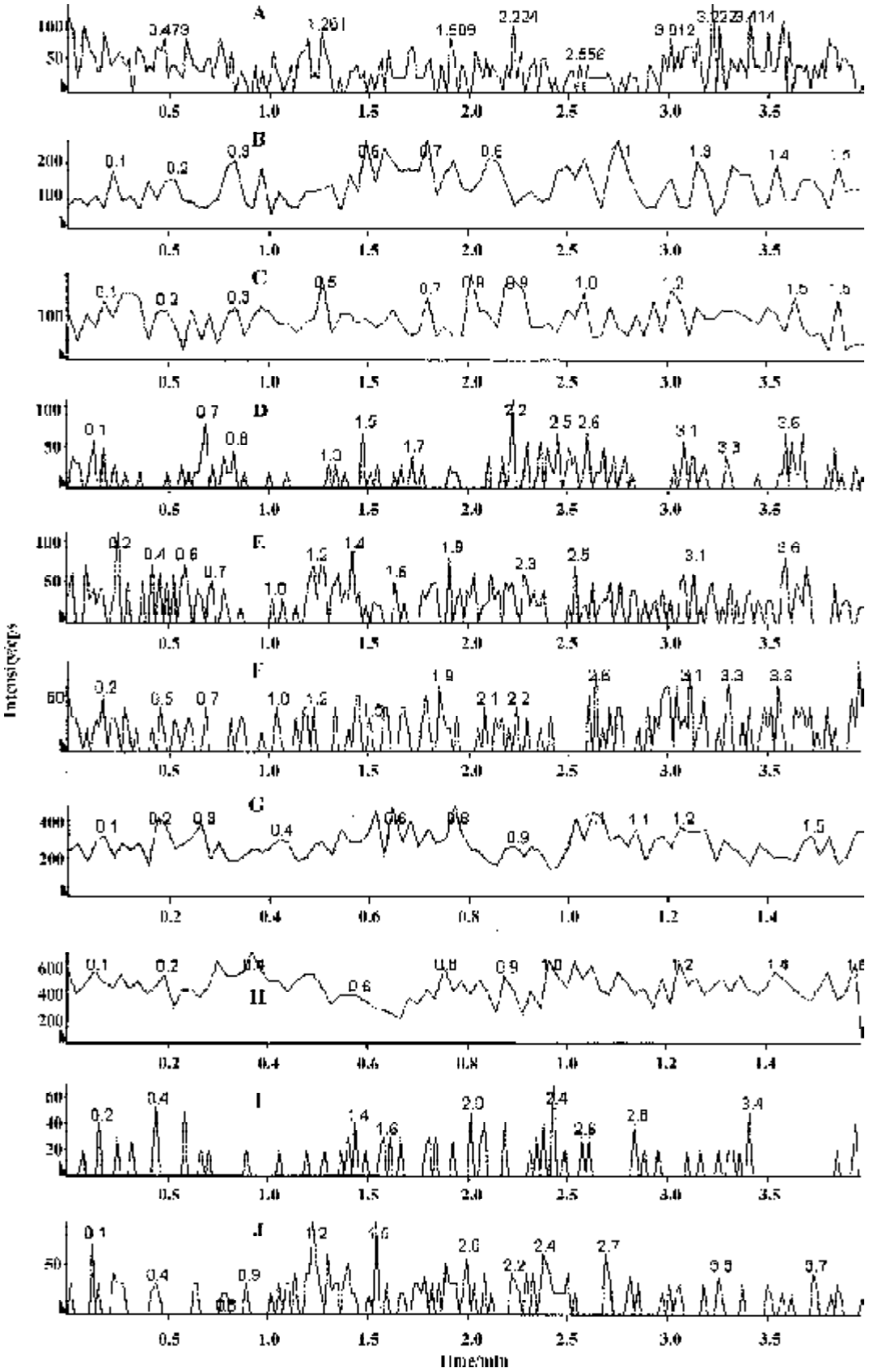

Fig 3. Representative MRM chromatograms of drug-free human plasma without any drugs added: (A) BMS-186716-MA, (B) BMS-196087-MA, (C) BMS-225308, (D) BMS-198433, and (E) BMS-253653-MA. Representative MRM chromatograms of drug-free human plasma without internal standards added: (F) d_5-BMS-186716-MA, (G) d_5-BMS-196087-MA, (H) d_5-BMS-225308, (I) d_5-BMS-198433, and (J) d_5-BMS-253653-MA.

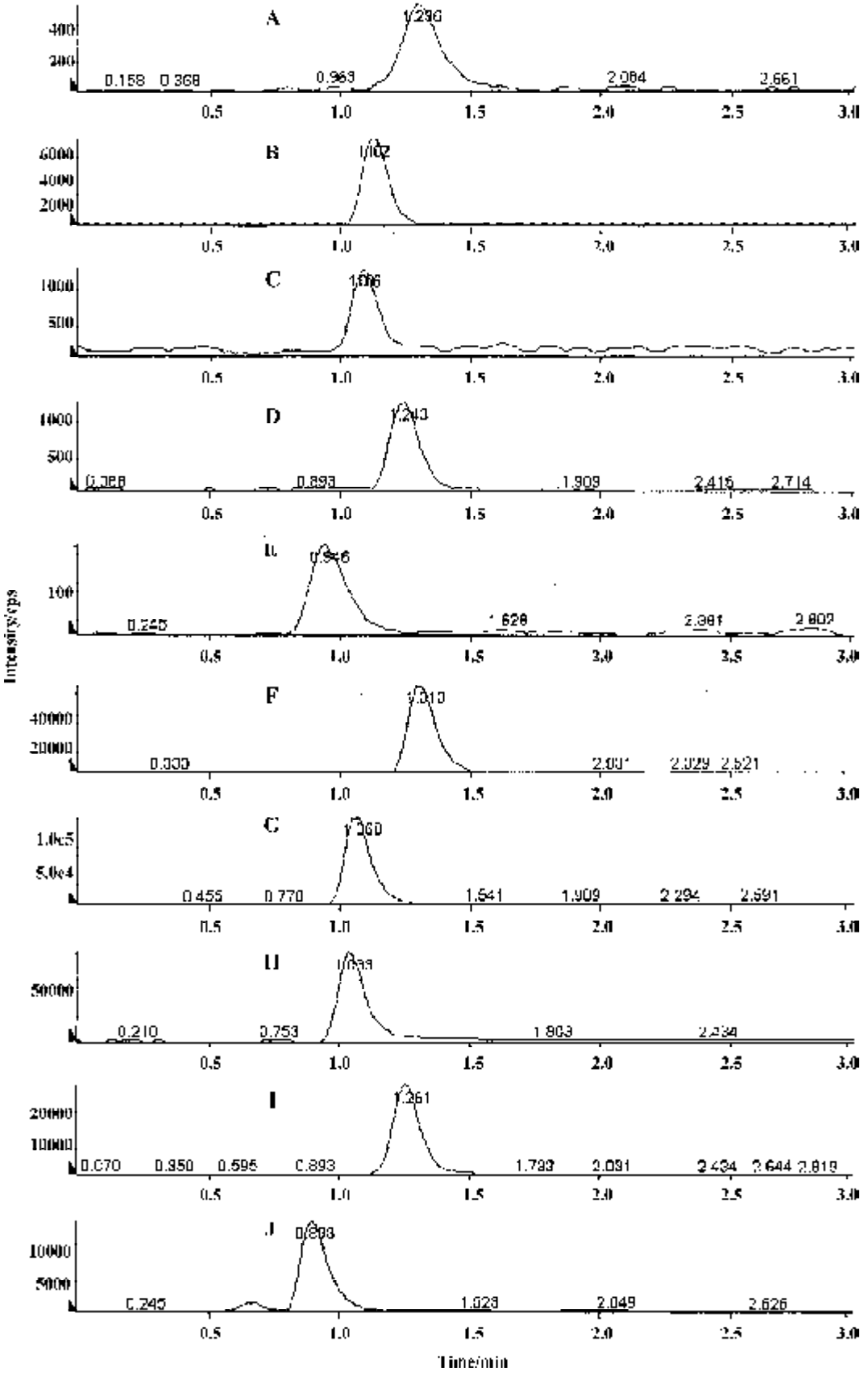

Fig 4. Representative MRM chromatograms of plasma sample spiked with drugs at LOQ concentration: (A) BMS-186716-MA, (B) BMS-196087-MA, (C) BMS-225308, (D) BMS-198433, and (E) BMS-253653-MA. Representative MRM chromatograms of plasma sample spiked with internal standards: (F) d_5-BMS-186716-MA, (G) d_5-BMS-196087-MA, (H) d_5-BMS-225308, (I) d_5-BMS-198433, and (J) d_5-BMS-253653-MA.

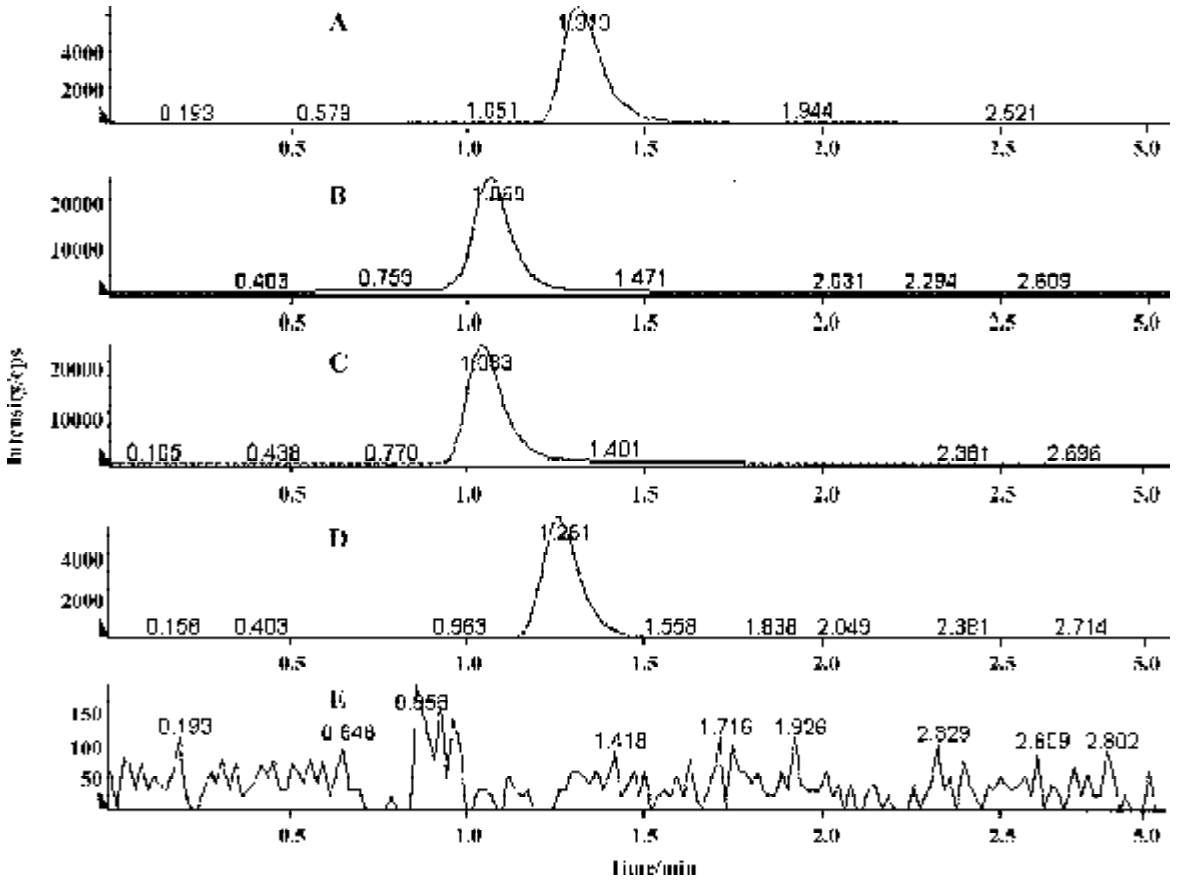

Fig 5. Representative MRM chromatograms of unknown human plasma sample from a volunteer, at 3 h after an oral dose of 100 mg: (A) BMS-186716-MA, (B) BMS-196087-MA, (C) BMS-225308, (D) BMS-198433, and (E) BMS-253653-MA.

Tab 4. Intra-day and inter-day precision (RSD %) of the analytes at low, medium, and high concentrations.

	BMS-186716	BMS-196087	BMS-225308	BMS-198433	BMS-253653
Concentration/μg·L^{-1}	2.0	2.0	4.0	8.0	40.0
Intra-day precision (RSD %, *n*=5)	5.9	13.3	6.5	7.5	7.2
Inter-day precision (RSD %, *n*=6)	7.2	11.7	11.6	8.3	10.9
Concentration/μg·L^{-1}	100.0	100.0	100.0	100.0	1000.0
Intra-day precision (RSD %, *n*=5)	5.5	5.3	4.7	9.8	8.1
Inter-day precision (RSD %, *n*=6)	3.1	3.5	6.3	7.7	6.7
Concentration/μg·L^{-1}	200.0	200.0	200.0	200.0	2000.0
Intra-day precision (RSD %, *n*=5)	5.2	6.8	5.3	7.4	12.7
Inter-day precision (RSD %, *n*=6)	3.9	4.6	6.3	12.8	11.9

DISCUSSION

Omapatrilat (BMS-186716) undergoes *in vivo* metabolism to produce several metabolites[11]. Because of instability of the thiolic analytes[12,13], to accurately study pharmacokinetics and metabolism of this drug in human being, it needs us to establish a highly sensitive and specific method to determine primary drug and metabolites simultaneously.

The impurity in plasma samples usually interferes with analysis, so MRM scan type was selected to insure the high specificity of this method. The molecular ions of the analytes were selected as parent ions in first-stage mass spectrometer (Q1), then collided by nitrogen in the second spectrometer (Q2), and finally the most intense fragment ions were selected as daughter ions in the third mass spectrometer (Q3), pairs of specific parent-daughter ions were formed in this way. As a result noise was reduced and good signal to noise ratio was acquired (Fig 3). Moreover, high specificity did not require all the analytes to be completely separated, so totally 3.0 min for each run was enough for accu-

Tab 5. Average extraction recoveries and accuracy (relative recovery) of the analytes at low, medium, and high concentrations. *n*=6. Data were presented as RSD %.

	BMS-186716	BMS-196087	BMS-225308	BMS-198433	BMS-253653
Concentration/μg·L^{-1}	2.0	2.0	4.0	8.0	40.0
Relative recovery	97.4	108.0	100.5	103.3	101.2
Extraction recovery	57.3	91.0	81.5	70.9	25.6
Concentration/μg·L^{-1}	100.0	100.0	100.0	100.0	1000.0
Relative recovery	106.0	94.4	101.7	98.7	99.3
Extraction recovery	64.8	88.7	77.5	80.6	28.0
Concentration/μg·L^{-1}	200.0	200.0	200.0	200.0	2000.0
Relative recovery	108.1	98.1	100.3	97.9	101.0
Extraction recovery	59.5	86.1	70.0	62.1	26.1

Tab 6. Comparison between a calibration curve of BMS-186716-MA in human plasma (Curve A) and a calibration curve of BMS-186716 reacted with MA in human blood (Curve B).

Nominal concentration/ μg·L^{-1}	[1)]Calculated concentration of Curve B/μg·L^{-1}	Deviation from nominal concentration/%
0.2	0.16	-21.7
0.5	0.40	-20.4
1	0.86	-13.8
2	2.36	17.8
5	5.63	12.6
12.5	14.36	14.9
25	28.30	13.2
50	61.20	22.4
125	146.50	17.2
250	279.60	11.8

1) Calculated concentrations of Curve B were calculated against Curve A.

rate quantitation of the analytes under the present chromatographic condition.

In the acquisition of MRM with negative mode, the first-stage mass spectra of tandem mass spectrometry should indicate the deproronated molecular ions [MA-H] of these MA adduct. However, the most intense ions selected in Q1 for BMS-196087-MA and d_5-BMS-196087-MA, were *m/z* 181 and *m/z* 186 [M-H], not *m/z* 267 and *m/z* 272 [MA-H]. The former *m/z* were molecular ions for BMS-196087 and d_5-BMS-196087, and the latter were for BMS-196087-MA and d_5-BMS-196087-MA. Considering the chemical structures of the two compounds, compared with other MA adducts such as BMS-186716-MA and BMS-253653-MA, the

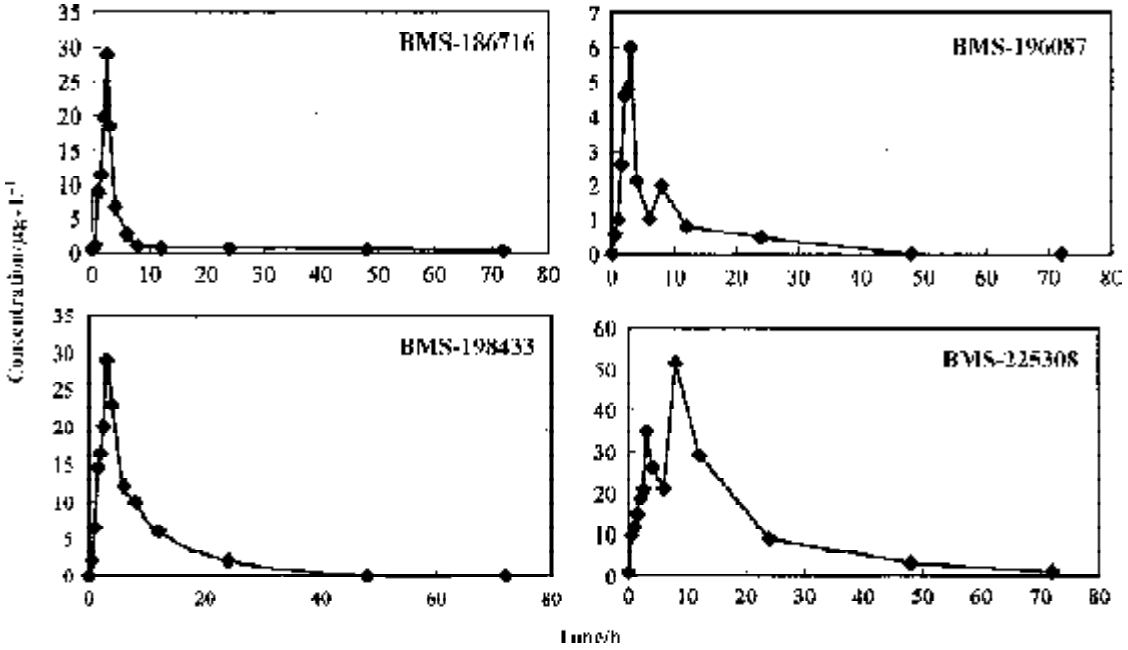

Fig 6. Mean plasma concentration–time curves of BMS-186716 and metabolites BMS-196087, BMS-2253080, and BMS-198433 after oral administration of 100 mg BMS-186716.

structures of BMS-196087-MA and d_5-BMS-196087-MA were simpler without big groups coupled with side chains. As a result, the protection from space resistance was much smaller, and the cleavage of the two compounds happened in ion source easily resulted in the loss of MA side chain. Finally, [M-H] ions at *m/z* 181 and *m/z* 185 were selected in Q1 as parent ions. Fortunately, final results of the research indicated that the cleavage happened in ion-source did not interfere with study.

Accurate analysis of unstable drugs and metabolites in complex biological matrix is always the bottleneck of clinical pharmaceutical research. A sensitive and specific method using advanced LC-MS/MS technique was established to determine omapatrilat and its metabolites in human plasma in this paper. This method has been applied to measure thousands of plasma samples related to the pharmacokinetics trials of omapatrilat in Chinese healthy volunteers.

REFERENCES

1 Cao Z, Burrell LM, Tikkanen I, Bonnet F, Cooper ME, Gilbert RE. Vasopeptidase inhibition attenuates the progression of renal injury in subtotal nephrectomized rats. Kidney Int 2001; 60 : 715-21.

2 Waeber B. Vasopeptidase inhibition: a new approach to the management of cardiovascular disease. J Clin Hypertens 2000; 2: 87-93.

3 Quaschning T, d'Uscio LV, Shaw S, Viswambharan H, Ruschitzka FT, Luscher TF. Chronic vasopeptidase inhibition restores endothelin-converting enzyme activity and normalizes endothelin levels in salt-induced hypertension. Nephrol Dialysis Transplant 2001; 16: 1176-82.

4 d'Uscio LV, Quaschning T, Burnett JC Jr, Luscher TF. Vasopeptidase inhibition prevents endothelial dysfunction of resistance arteries. Hypertension 2001; 37: 28-33.

5 Troughton RW, Rademaker MT, Powell JD, Yandle TG, Espiner EA, Frampton CM, *et al*. Beneficial renal and hemodynamic effects of omapatrilat in mild and severe heart failure. Hypertension 2000; 36: 523-30.

6 Sica DA, Liao W, Gehr TW, Khan S, Jemal M, Delaney CL, *et al*. Disposition and safety of omapatrilat in subjects with renal impairment. Clin Pharmacol Ther 2000 ; 68: 261-9.

7 Azizi M, Massien C, Michaud A, Corvol P. *In vitro* and *in vivo* inhibition of the 2 active sites of ACE by omapatrilat, a vasopeptidase inhibitor. Hypertension 2000; 35: 1226-31.

8 Weber M. Emerging treatments for hypertension: potential role for vasopeptidase Inhibition. Am J Hypertension 1999; 12: 139-47.

9 McClean DR, Ikram H, Garlick AH, Crozier IG. Effects of omapatrilat on systemic arterial function in patients with chronic heart failure. Am J Cardiol 2001; 87: 565-9.

10 Iyer RA, Mitroka J, Malhotra B, Bonacorsi S Jr, Waller SC, Rinehart JK, *et al*. Metabolism of [^{14}C] omapatrilat, a sulfhydryl-containing vasopeptidase inhibitor in humans. Drug Metab Dispos 2001; 29: 60-9.

11 Iyer RA, Mitroka J. Isolation and identification of human urinary metabolites of BMS-186716 from subjects dosed orally with [^{14}C] BMS-186716. Final report. New Brunswick: Bristol-Myers Squibb Pharmaceutical Research Institute (ca); 1999 Feb. Report No: 910057828.

12 Jemal M, Hawthorne DJ. Quantitative determination of BMS186716, a thiol compound, in dog plasma by high-performance liquid chromatography-positive ion electrospray mass spectrometry after formation of the methyl acrylate adduct. J Chromatogr B Biomed Sci Appl 1997; 693: 109-16.

13 Jemal M, Hawthorne DJ. Quantitative determination of BMS-186716, a thiol compound, in rat plasma by high-performance liquid chromatography-positive ion electrospray mass spectrometry after hydrolysis of the methyl acrylate adduct by the native esterases. J Chromatogr B Biomed Sci Appl 1997; 698: 123-32.