Journal of Food and Drug Analysis

Volume 13 | Issue 4 Article 2

A novel liquid chromatography/mass/mass spectrometry system to determine procaterol levels in human plasma

Journal of Food and Drug Analysis, Vol. 13, No. 4, 2005, Pages 312-318 藥物食品分析 第十三卷 第四期

A Novel Liquid Chromatography/Mass/Mass Spectrometry System to Determine Procaterol Levels in Human Plasma

TU-WEN YU[1], RUSSEL RHEI-LONG CHEN[2] AND CHURN-SHIOUH GAU[1]*

[1.] School of Pharmacy, College of Medicine, National Taiwan University, 1 Jen-Ai Rd., Sec. 1, Taipei City 100, Taiwan, R.O.C.
[2.] School of Pharmacy, Ta-Jen Institute of Technology, Ping-Tung, Taiwan, R.O.C.

(Received: May 10, 2005; Accepted: July 27, 2005)

ABSTRACT

A highly sensitive and accurate liquid chromatography/mass/mass spectrometry (LC/MS/MS) system was developed to determine the plasma concentration of procaterol in human subjects. The chromatographic system used for this study included a Lichrospher® Silica column with a mobile phase of 10 mM ammonium acetate aqueous solution/acetonitrile (30/70, v/v) and 1.0 mL/min flow-rate. Procaterol and betaxolol, the latter used as the internal standard, were detected using mass spectrometry at ionization mode (ESI+), a source block temperature of 90°C and desolvation temperature of 500°C. The detection mass was 290.99 > 273.64 for procaterol and 308.33 > 116.31 for betaxolol. The calibration curve over a concentration range of 0.005~1.0 ng/mL showed good linearity, with a correlation coefficient of 0.9998. The results of within-run and between-run precision tests for the method have coefficient variation values of no more than 10.2%. The average recovery rates of procaterol in human plasma samples over a wide range of concentrations exceeded 86%. The concentration detection and quantitation limits of the proposed LC/MS/MS method were determined to be 0.001 and 0.005 ng/mL, respectively. The pharmacokinetic parameters from volunteers were similar to that previously reported in the literature in a study on patients administered with the same procaterol HCl dose. All results indicate that the LC/MS/MS method proposed in this study is a sensitive, selective, precise, and accurate method to determine concentrations of procaterol in human plasma and hence to evaluate the pharmacokinetic characteristics of human subjects.

Key words: procaterol, LC/MS/MS assay method, pharmacokinetics

INTRODUCTION

Procaterol hydrochloride (procaterol HCl: (±)-erythro-8-hydroxy-5-[1-hydroxy-2-(isopropylamino)butyl] carbostyril hydrochloride) is a potent sympathomimetic amine with selective beta-2 adrenergic receptor agonist activity used in the treatment of reversible bronchospastic diseases[1-3]. Therapeutic dose regimens for procaterol are smaller relative to all other beta-2 adrenergic agonists (e.g., salbutamol, fenoterol and terbutaline[2-4]). For example, procaterol HCl is administered in microgram doses to treat asthma, i.e., 10~25 μg daily for children and 50~100 μg daily for adults[5,6].

Because it is administered in such minute dosages, the concentration of procaterol in plasma and urine should, in almost all circumstances, fall below the minimum level of UV spectrophotometer sensitivity. Referenced methods currently used to determine the concentration of procaterol in human or animal subject samples include radiochemical (C^{14}-procaterol)[7], gas chromatography-mass spectrometry (GC/MS)[4,8], high performance liquid chromatography (HPLC)[9], and radioimmunoassay[10,11]. The detection limits from these methods vary widely (30 ng/mL[7], 5 ng/mL[4], 0.010 ng/mL[8], 4.3 ng/mL[9], and 0.015 ng/mL[10], respectively, for the above mentioned tests). Of these, only the gas chromatography-mass spectrometry method developed by Huang *et al.*[8] and the radioimmunoassay method developed by Eldon *et al.*[10] have proven able to detect procaterol in human plasma in a concentration range of 0.015~2 ng/mL after oral administration of 75 μg procaterol HCl. Other methods are unable to detect procaterol even at maximum plasma concentrations (C_{max}) of around 0.23 ng/mL, as reported in literature[10]. However, radioimmunoassay method experimental procedures in most conditions are quite complicated. Plasma samples must be incubated with fixed amounts of antibody and ^{125}I-labeled procaterol, after which antibody bound fractions must be precipitated with sheep anti-rabbit immunoglobulin and then counted in a gamma counter. Difficult disposal of isotope waste, complicated procedures, and sophisticated instrumentation and working environments limit the applicability of current testing methodologies in a general chemical laboratory. Therefore, in this study, a novel method using a sensitive and specific LC/MS/MS system for routine analysis of procaterol in human plasma was developed and validated. This LC/MS/MS system was also applied in a preliminary pharmacokinetics clinical trial to assess its feasibility in human studies.

MATERIALS AND METHODS

I. *Chemicals and Reagents*

Procaterol HCl hemihydrate (lot no. 8611, purity

* Author for correspondence. Tel: +886-2-23123456 ext. 8395; Fax: +886-2-23416347; E-mail: csg@ntu.edu.tw

Journal of Food and Drug Analysis, Vol. 13, No. 4, 2005

100.4%) obtained from Edmond Pharma s.r.l. (Milano, Italy) was used as a standard. Betaxolol HCl (lot no. 214, purity 100.3%) purchased from Sigma, Inc. (Steinheim, Germany) was used as the internal standard. Procaterol HCl pellet capsules (lot no. 212) 25 μg/cap were manufactured by U-Chu Pharmaceutical Co., Ltd.; and Meptin®-mini tablets 25 μg/tab (lot no. 1K84) made by Otsuka Pharmaceutical Co., Ltd. were purchased from the market. Acetonitrile of HPLC-grade was purchased from Merck KGaA (Darmstadt, Germany). Ammonium acetate, formic acid, di-potassium hydrogen phosphate, 2-propanol and hydrochloric acid were the products of Sigma, Inc. (Steinheim, Germany).

II. *Liquid Chromatography/Mass/Mass Spectrometry System*

The LC/MS/MS system used in this study comprised a Liquid Chromatograph system (Waters® 1795 Separations Module, Waters, USA), a Mass Selective Detector (Quattro Ultima, Micromass, USA), and a Lichrospher® Silica column (125 mm × 4.6 mm I.D., 5 μm, E. Merck Darmstadt, Germany) thermostated at 40°C by a column heater. The mobile phase was composed of 10 mM ammonium acetate aqueous solution and acetonitrile (30:70, v/v) eluted with a flow-rate of 1.0 mL/min. The injection volume was 15 μL, and post column split 1/10 to Mass Selective Detector and 9/10 to waste. The Mass Selective Detector was set as follows: ionization mode (ESI+), ESI voltage 3.0 kV, source block temperature 90°C, desolvation temperature 500°C, and detection mass for procaterol (MW 290.36 g/mol) parent ion at *m/z* 290.99 > fragment ion at *m/z* 273.64 and for betaxolol (MW 307.43 g/mol) parent ion at *m/z* 308.33 > fragment ion at *m/z* 116.31.

(I) *Stock and Working Solutions*

Stock solutions of both procaterol HCl and betaxolol HCl (internal standard) of 1000 μg/mL in 50% acetonitrile solution were prepared and stored at 4°C. The working solutions of procaterol HCl and betaxolol HCl in blank plasma, obtained from healthy adult volunteers, were freshly prepared with procaterol HCl concentrations of 0.001, 0.01, 0.1, 1.0, and 10.0 μg/mL, respectively, and a betaxolol HCl concentration of 0.02 μg/mL.

(II) *The Stability Studies of Procaterol HCl in Human Plasma*

Studies of freeze-thaw stability as well as short-term and long-term stability were conducted to confirm the stability of procaterol in plasma. Samples of procaterol HCl in drug-free human plasma in two concentrations (0.015 ng/mL and 0.8 ng/mL) were prepared and used for a freeze-thaw stability study that included three cycles and six replicates.

Samples of procaterol HCl in drug-free human plasma in four concentration levels (0.005, 0.015, 0.08 and 0.8 ng/mL) were used to assess both short-term and long-term stability after one freeze-thaw cycle. The short-term study was carried out at 0, 2, and 4 hr after thawing the sample with no further preparation. For the long-term study, samples were examined at 0 and 24 hr following thawing and further preparation (in accordance with preparation procedures for pharmacokinetic study samples).

(III) *Validation Studies*

For validation studies, a series of standard solutions of procaterol HCl in 0.5 mL of drug-free human plasma in concentrations of 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5 and 1.0 ng/mL were freshly prepared. The plasma sample was mixed with a 5-μL internal standard working solution (0.02 μg/mL betaxolol HCl) and 25 μL 1 M K_2PO_4 solution, and then extracted with 5.0 mL of 2-propanol and vortexed for 2 min. After centrifugation at 3000 rpm for 10 min, the supernatant layer was transfer into a conical tube. A solution of 100 μL of 3% formic acid was added to the conical tube and mixed by vortexing for 30 sec. The sample was centrifuged again at 3000 rpm for 10 min, and the organic layer was removed. The aqueous solution was evaporated to dryness under a stream of nitrogen gas for 5 min at ambient condition. The residues were dissolved in 200 μL of acetonitrile and vortex-mixed for 1 min. We then transferred 150 μL of the solution to a sample vial. A volume of 15 μL was then injected into the LC/MS/MS system. A calibration curve was established by plotting the peak area ratio, i.e., peak area of procaterol over peak area of betaxolol, as a function of procaterol HCl concentration. Recovery, within-run, and between-run tests were conducted to ensure the reliability of our proposed method. The sample concentrations were then calculated by interpolating the peak area ratio for each sample into the calibration curve. All experiments were repeated six times. All data were presented as mean ± S.D. The lowest limit of detection was defined as a detection concentration at a signal-to-noise ratio of 3:1.

III. *A Preliminary Clinical Trial of Two Procaterol HCl Preparations*

Four healthy male Taiwanese subjects, each having provided written informed consent, were enrolled in this preliminary clinical trial. The trial was designed as a single dose, non-blinded, randomized, balanced, two-sequence, and two-period crossover study. Before the trial, the physical and biochemical condition of each volunteer was examined. Subjects were aged between 20 and 25 years (22.8 ± 2.2 years), weighed between 57.4 and 75.6 kg (65.0 ± 7.8 kg), and ranged from 167.4 to 177.3 cm in height (171.0 ± 4.4 cm). Each healthy subject received either three procaterol HCl pellet capsules (25 μg/capsule) or three Meptin®-mini tablets (25 μg/tablet), administered orally with 200 mL of water. A washout period of 7 days was maintained between the two administrations. Prior to

administering each dose, a 20 mL sample of blood from each subject was taken as blank, and a 10 mL sample of blood was drawn at 0.33, 0.67, 1, 1.67, 2, 2.5, 3, 4, 6, 8, 10, 12, 14 and 16 hr after dose administration. Blood samples were collected from the antecubital vein using coded vacutainers and were centrifuged immediately at 3000 rpm, 4°C for 10 min using a Kubota® KN-70 (Double Enterprise Co., Ltd., Japan). The plasma layer was then transferred to a labeled test tube and stored at -20°C until analysis. The plasma concentrations of procaterol were then assayed in accordance with our proposed LC/MS/MS method.

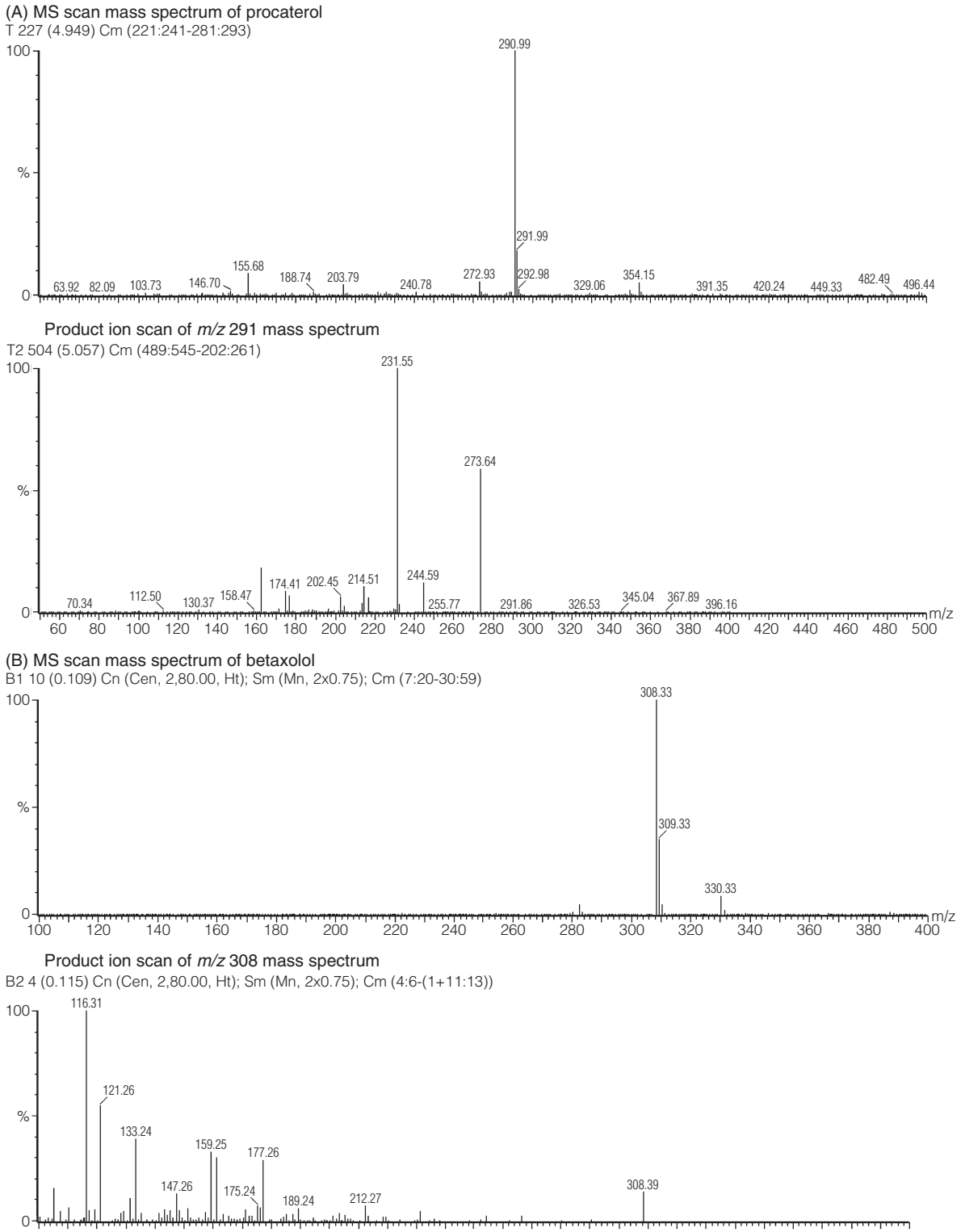

Figure 1. Mass spectra of standards of (A) procaterol and (B) betaxolol.

Journal of Food and Drug Analysis, Vol. 13, No. 4, 2005

(I) *Sample Preparation*

A plasma sample of 0.5 mL was pipetted accurately and mixed with 5 μL of internal standard working solution (betaxolol, 0.02 μg/mL). The remaining preparation procedures were similar to those described in the section on validation studies. Following preparation, 15 μL of sample solution was injected into the LC/MS/MS system.

(II) *Pharmacokinetic Analysis*

Area under the curve (AUC), maximum plasma concentration (C_{max}), time at maximum concentration (T_{max}), mean residence time (MRT), and elimination half life ($t_{1/2}$) were determined from procaterol plasma concentration-time profiles using methodologies recommended by Gibaldi[12].

(III) *Statistical Treatments*

The log-transformed data (geometric means) of $AUC_{0\rightarrow16}$, $AUC_{0\rightarrow\infty}$, and C_{max} and the MRT data with sequence, period, and treatment effects were analyzed using an analysis of variance (ANOVA) model by the Statistical Analysis System (SAS) GLM Procedure, version 8.1. Differences in data set values of $p < 0.05$ were considered statistically significant.

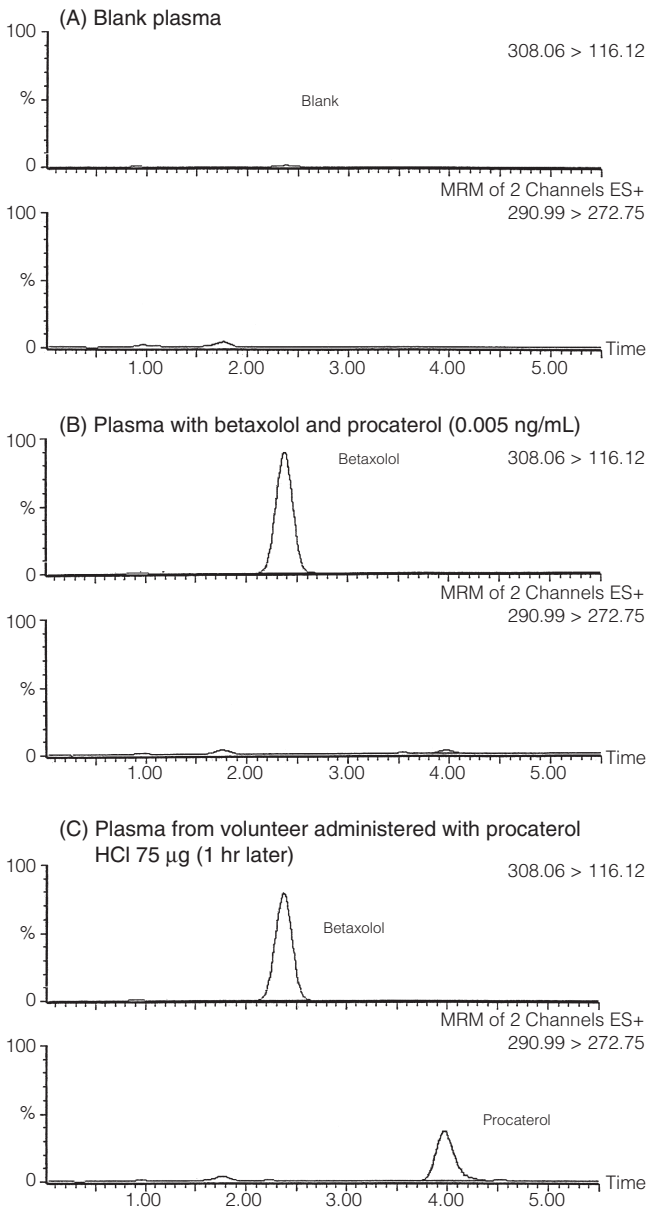

Figure 2. Chromatograms after mass selective ion detector for (A) blank plasma, (B) plasma with betaxolol and procaterol (0.005 ng/mL), (C) plasma from volunteer who took procaterol 75 μg HCl (1 hr later). The base peaks of procaterol and betaxolol were revealed at *m/z* 273.64 and 116.31, respectively.

RESULTS AND DISCUSSION

I. *Chromatographic Analysis*

Figure 1A depicts the procaterol HCl standard mass spectra (MW 290.36 g/mol), with a base peak at *m/z* 273.64 from the parent fragment at *m/z* 290.99. In Figure 1B, the mass spectra of internal standard betaxolol (MW 307.43 g/mol) shows a base peak at *m/z* 116.31 from the parent fragment at *m/z* 308.33. In order to check for possible distortions due to the methodology used to assay material extracted from plasma samples we employed an LC/MS/MS system to analyze the samples of blank plasma from volunteers; plasma mixed with betaxolol and procaterol at the lowest concentration (0.005 ng/mL), i.e., used for the standard solution series, and volunteer plasma samples that had been treated with 75 μg procaterol hydrochloride mixed with betaxolol. The resultant chromatograms are presented in Figures 2A, 2B, and 2C, respectively. While no significant peak appears in Figure 2A, the procaterol and betaxolol peaks in Figures 2B and 2C chromatograms are sharp and symmetrical, with high resolution and with the retention times of approximately 2.3 min and 4.0 min, respectively. The procaterol retention time for the proposed system is much shorter than that previously reported data (e.g., 5.0 min by Ishigami *et al.*[4], 15.1 min by Hunag *et al.*[8] and 16.5 min by Wright *et al.*[9]). The fact that no endogenous interfering peak appeared in our chromatograms suggests that the developed LC/MS/MS assay system is valid.

II. *Validation of the Assay Method*

Table 1 shows the results of stability studies on procaterol HCl in plasma. Differences in the determined concentration of procaterol HCl between samples at time 0 and samples after the first, second and third freeze-thaw cycles, respectively, were all within 3%. Determined concentration differences of procaterol HCl between time 0 and at the times monitored for short-term and long-term stability were all within 5%. Results indicated that

Table 1. The data of the stability studies of procaterol HCl in human plasma

Spike concentration (ng/mL)		0.005	0.015	0.08	0.8
Freeze-thaw stability					
Measured	Initial	—	0.0155 ± 0.0007	—	0.7871 ± 0.0650
concentration[a] (ng/mL)	1st cycle	—	0.0151 ± 0.0008 (-2.58)	—	0.7702 ± 0.0481 (-2.15)
(% difference to initial)	2nd cycle	—	0.0154 ± 0.0010 (-0.65)	—	0.7911 ± 0.0501 (0.50)
	3rd cycle	—	0.0155 ± 0.0007 (0.0)	—	0.7823 ± 0.0404 (-0.61)
Short-term stability					
Measured	0 hr	0.048 ± 0.0003	0.0152 ± 0.0002	0.0794 ± 0.0006	0.8137 ± 0.0070
concentration[a] (ng/mL)	2 hr	0.0050 ± 0.0005 (4.57)	0.0150 ± 0.0002 (-1.10)	0.0795 ± 0.0015 (0.21)	0.8110 ± 0.0046 (-0.33)
(% difference to initial)	4 hr	0.0051 ± 0.0006 (4.98)	0.0149 ± 0.0003 (-1.76)	0.0789 ± 0.0021 (-0.58)	0.8107 ± 0.0085 (-0.37)
Long-term stability					
Measured	0 hr				
concentration[a] (ng/mL)	24 hr	0.0057 ± 0.0001	0.00151 ± 0.0040	0.0815 ± 0.0013	0.8142 ± 0.0060
(% difference to initial)		0.0055 ± 0.0002 (-3.51)	0.0150 ± 0.0003 (-0.66)	0.0811 ± 0.0009 (-0.49)	0.8143 ± 0.0049 (0.01)

[a]All the data are mean ± S.D. (n = 6).

Table 2. Recovery rate of procaterol and betaxolol in plasma of healthy human subjects[a]

Concentration (ng/mL)	Peak area of procaterol	Peak area of betaxolol	Peak area ratio	Recovery (%)
0.005	66.51 ± 3.85	3134.59 ± 110.81	0.021 ± 0.001	86.15 ± 4.86
0.01	147.51 ± 4.32	4018.57 ± 128.33	0.037 ± 0.002	109.34 ± 8.51
0.02	170.83 ± 6.85	3031.89 ± 123.73	0.056 ± 0.005	94.02 ± 9.98
0.05	436.39 ± 13.85	3721.51 ± 101.71	0.117 ± 0.007	88.14 ± 6.03
0.1	870.57 ± 24.92	3207.55 ± 103.08	0.271 ± 0.015	107.85 ± 6.29
0.2	1407.29 ± 35.48	2789.05 ± 120.42	0.505 ± 0.035	102.38 ± 7.19
0.5	3143.74 ± 124.67	2625.79 ± 123.04	1.197 ± 0.103	98.28 ± 8.49
1.0	7279.02 ± 208.92	2992.92 ± 136.36	2.432 ± 0.101	100.29 ± 4.18

[a]Mean ± S.D. of six repeated experiments.

Table 3. Results of within-run and between-run tests

Spiked concentration (ng/mL)	Within-run test			Between-run test		
	Measured conc.[a] (ng/mL)	S.D.	CV (%)	Measured conc.[a] (ng/mL)	S.D.	CV (%)
0.005	0.0051	0.0002	4.7	0.0054	0.0004	8.0
0.01	0.0099	0.0009	8.6	0.0105	0.0007	6.7
0.02	0.0197	0.0020	10.2	0.0195	0.0017	8.7
0.05	0.0506	0.0030	5.9	0.0459	0.0023	5.0
0.1	0.0992	0.0063	6.4	0.102	0.008	7.8
0.2	0.205	0.014	6.8	0.194	0.011	5.7
0.5	0.483	0.042	8.7	0.484	0.025	5.2
1.0	1.01	0.04	4.0	1.03	0.02	1.9

[a]Mean of six repeated experiments in six days.

procaterol in human plasma exhibits good stability at the experimental conditions employed for this study.

The calibration curve for the ratio of peak area response (procaterol/betaxolol) vs. that for the concentration of procaterol HCl standard solutions analyzed by our proposed LC/MS/MS system demonstrated a linear relationship over a concentration range of 0.005~1.0 ng/mL, with a regression line of $y = 2.414x + 0.0106$ and a correlation coefficient of $r^2 = 0.9998$. Table 2 lists the results of the recovery test for procaterol in plasma solutions over the same concentration range. The average recovery rate (n = 6) for each solution ranged from 86.15% to 109.34%, which was considered acceptable. Table 3 summarizes the results of within-run and between-run tests for eight procaterol in plasma standard solutions, presented in standard deviation (S.D.) and coefficient of variance (C.V.) formats. All data are derived from the results of six repeated experiments performed on each concentration over three separate days. C.V. values ranged from 4.0% to 10.2% for the within-run test and from 1.9 % to 8.7% for the between-run test. The above results indicate that the proposed assay method is quite reliable.

Detection limit and quantitation limit concentrations for the proposed LC/MS/MS method were identified as 0.001 and 0.005 ng/mL, respectively. The detection limit concentration in this method is below the lowest detectable values of 0.010 ng/mL using the GC/MS method[8] and 0.015 ng/mL using the radioimmuno-assay method[10].

Journal of Food and Drug Analysis, Vol. 13, No. 4, 2005

Table 4. The pharmacokinetic parameters of procaterol HCl pellets capsules and Meptin®-mini tablets after oral administration to healthy male adults (Mean ± S.D., n = 4)

Parameters	Procaterol HCl pellets capsule	Meptin®-mini tablet	ANOVA *p*-value[a]
$AUC_{0\rightarrow16}$ (hr • ng/mL)	0.906 ± 0.334	0.870 ± 0.216	0.269
$AUC_{0\rightarrow\infty}$ (hr • ng/mL)	1.03 ± 0.37	0.932 ± 0.214	0.583
$AUC_{0\rightarrow16}$/ $AUC_{0\rightarrow\infty}$ (%)	87.6 ± 3.1	93.0 ± 2.4	
C_{max} (ng/mL)	0.211 ± 0.078	0.219 ± 0.091	0.950
MRT (hr)	7.36 ± 0.98	5.76 ± 1.12	0.128
T_{max} (hr)	1.00 ± 0.27	0.835 ± 0.191	0.176
$T_{1/2}$(hr)	5.90 ± 0.72	4.63 ± 1.05	0.122

[a] $p < 0.05$ as the breaking point for statistical significant difference.

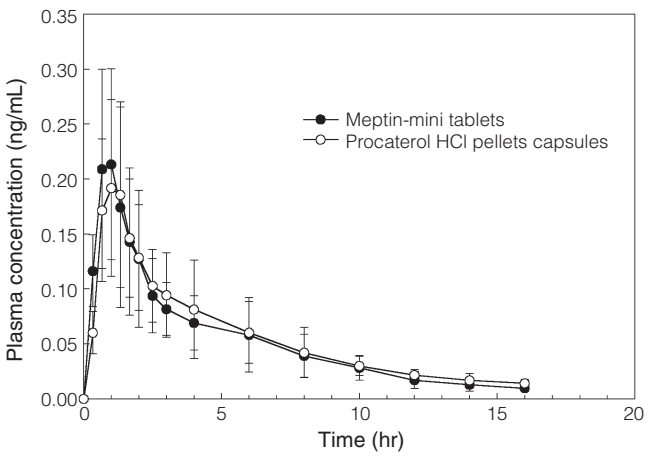

Figure 3. Mean plasma procaterol concentration vs. time for healthy adult subjects (n = 4) after single dose oral administration of procaterol HCl pellets capsules (–○–) and Meptin®-mini tablets (–●–).

In other words, the sensitivity achieved by the proposed LC/MS/MS method is greater than that of other reported methods. Other advantages of the proposed LC/MS/MS method include the absence of isotope waste handling issues, easy operations and relative simple experimental procedures, and reduced processing times.

III. *Application to a Preliminary Human Study*

We applied the proposed LC/MS/MS system on samples from healthy adult human subjects to determine procaterol plasma concentrations. The resultant kinetics curves representing mean drug plasma concentration *versus* time after oral administration of two procaterol HCl preparations are depicted in Figure 3. The two curves are almost superimposed on each other. The proposed LC/MS/MS method was able to detect procaterol in a plasma concentration range of 0.005~1.0 ng/mL. The calculated pharmacokinetic parameters for each group are summarized in Table 4 as mean ± S.D. Table 4 lists the *p*-values of each parameter for both formulations. There was no significant difference ($p > 0.05$) between the two treatment groups in terms of pharmacokinetic parameters (e.g., $AUC_{0\rightarrow16}$, $AUC_{0\rightarrow\infty}$, C_{max}, MRT, T_{max} and $T_{1/2}$). They are similar to results reported in the literature on an experiment with patients treated with the same procaterol HCl dose[10], where $AUC_{0\rightarrow\infty}$ was 1.380 ± 0.286 hr • ng/mL, C_{max} was 0.233 ± 0.299 ng/mL, T_{max} was 0.835 ± 0.191 hr, and $T_{1/2}$ was 2.0 ± 0.55 hr. These results demonstrate that the LC/MS/MS method developed here is suitable for use in pharmacokinetic studies to detect procaterol at very low plasma concentrations.

CONCLUSIONS

A sensitive LC/MS/MS system was developed to determine the presence of plasma procaterol at extremely low concentration levels for use in studies on human subjects. The data in this paper supports that the proposed LC/MS/MS method has good selectivity, accuracy and precision, with detection sensitivity reaching concentrations of 0.001 ng/mL. The LC/MS/MS system is a valid method for use in human pharmacokinetic studies.

ACKNOWLEDGEMENTS

We would like to thank the U-Chu Pharmaceutical Corporation of Taiwan for their kind support of this project. Our sincere gratitude also goes to the staffs of the Analysis Division of the Protech Pharmaservices Corporation of Taiwan for their excellent technical assistance.

REFERENCES

1. Yamashita, S., Takai, M. and Yabuuchi, Y. 1978. Actions of procaterol (OPC-2009), a new β_2-adrenoceptor stimulant, on pulmonary resistance, contractions of the soleus muscle, and cardiovascular system of the anaesthetized cat. J. Pharm. Pharmacol. 30: 273-279.
2. Saitoh, Y., Hosokawa, T., Igawa, T. and Irie, Y. 1979. Effect of a selective β_2-adrenoceptor agonist, procaterol, on tissue cyclic AMP level. Biochem. Pharmacol. 28: 1319-1322.
3. Folco, G. C., Passoni, E., Vigano, T., Daffonchio, L., Rossoni, G., Brunelli, G. and Berti, F. 1983. New phar-

macological aspects of the bronchodilating activity of procaterol. Pharmacol. Res. Commun. 15: 909-921.

4. Ishigami, M., Saburomaru, K., Niino, K., Kido, M., Morita, S., Miyamoto, G. and Kohri, H. 1979. Pharmacokinetics of procaterol in the rat, rabbit and beagle dog. Arzneim. Forsh. Drug Res. 29: 266-270.
5. Bonder, A. L., Zanotto, C. E., Piacentini, G., Miglioranzi, P., Richelli, C. and Sette, L. 1990. Efficacy and duration of action of oral procaterol in asthmatic children after single administration of different dosages. J. Asthma 27: 21-30.
6. Kemp, J. P., Welch, M. J., Meltzer, E. O. and Orgel, H. A. 1985. Therapeutic efficacy and equivalence of tablet and syrup formulations of procaterol hydrochloride in childhood asthma. Ann. Allergy 55: 588-592.
7. Yasuda, Y., Fujisawa, N., Morita, S. and Kohri, H. 1979. Metabolic fate of a new β_2–adrenergic stimulant procaterol (OPC-2009). Arzneim. Forsh. Drug Res. 29: 261-265.
8. Huang, S. L., Wang W., Ou-Yang, D. S. and Zhou, H. H. 2000. Determination of procaterol in human plasma by gas chromatography/electron impact ionization mass spectrometry. Acta Pharmacol. Sin. 21: 564-566.
9. Wright, D. S., Pachla, L. A., Gibson, D. M. and Jordan, R. A. 1987. Sensitive high-performance liquid chromatographic method for procaterol in human urine. J. Chromatogr. 417: 223-228.
10. Eldon, M. A., Blake, D. S., Coon, M. J., Nordblom, G. D., Sedman, A. J. and Colburn, W. A. 1992. Clinical pharmacokinetics of procaterol: Dose proportionality after administration of single oral doses. Biopharm. Drug Dispos. 13: 663-669.
11. Eldon, M. A., Battle, M. M., Coon, M. J., Nordblom, G. D., Sedman, A. J. and Colburn, W. A. 1993. Clinical pharmacokinetics and relative bioavailability of oral procaterol. Pharm. Res. 10: 603-605.
12. Gibaldi, M. 1984. Biopharmaceutics and Clinical Pharmacokinetics. 3rd ed. pp.17-28. Lea and Febiger. Philadelphia, U. S. A.