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Determination of procaterol in human plasma by gas chromatography/electron impact ionization mass spectrometry[1]

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KEY WORDS gas chromatography/mass spectrometry; procaterol; pharmacokinetics

ABSTRACT

AIM: To improve a gas chromatography/electron impact ionization mass spectrometry (GC/MS) method for determining the concentration of procaterol in human plasma. **METHODS**: GC/MS was developed with capillary column. Samples were extracted by liquid phase before derivated. Imipramine was used as an internal standard. The injector and GC/MS interface temperatures were set at 280 ℃ and 250 ℃, respectively. The carrier gas (helium) was 0.8 mL·min^{-1}, and injections were made in the pulse-splitless mode. The MS source and MS Quad temperature were 230 ℃ and 150 ℃, respectively. **RESULTS**: The detection limit of plasma procaterol was 5 ng·L^{-1}. The assay was linear over the range of 10-10000 ng·L^{-1} with correlation coefficient of 0.9987. The coefficients of variation were less than 10 % for procaterol detection at high, medium and low concentration levels ($n = 5$). The average recovery of the assay was 99.1 % ± 1.3 %. **CONCLUSION**: This assay was sensitive, precise, and accurate for evaluating the clinical pharmacokinetics of procaterol.

INTRODUCTION

Procaterol is a new β_2-adrenergic bronchodilator. Because of its potency and selectivity, procaterol is used worldwide for treatment of asthma[1]. To study the pharmacokinetics of procaterol, we developed a simple and sensitive gas chromatography/electron impact ionization mass spectrometry (GC/MS) technique for determination of procaterol in human plasma. This paper describes the development and validation of this method.

MATERIALS AND METHODS

Chemicals and drugs Dry powder of procaterol, to be used as a standard, and procaterol hydrochloride capsules and tablets were obtained from Da Kang Pharmaceutical Research Institute (Changsha, China). Imipramine was purchased from Sigma Chemical Co (St louis, MO). N-O-Bis (trimethylsilyl)-fluoroacetamide (BSTFA) was purchased from Macherey-Nagel (Germany). All other chemicals were of analytical-reagent grade unless specified otherwise.

Standard solutions Stock solutions of procaterol and imipramine (200 mg/L) were prepared in water and kept at -20 ℃. Working solutions of procaterol were freshly prepared in blank plasma, except for imipramine which was prepared in water.

Sample preparation Plasma 0.5 mL was mixed with imipramine (an internal standard, IS, 20 mg/L) 100 μL, double distilled water 0.5 mL, and K_2CO_3 1.0 g. The sample was extracted with 5 mL acetone and vortexed for 3 min. After centrifuged at 1500 × g for 10 min, the organic phase 4 mL was transferred into a conical tube, and was evaporated to dryness under a stream of nitrogen at 37 ℃. The residues were dissolved in 50 μL of BSTFA, and left overnight at room temperature for trimethylsilylation (TMB). Solution 5 μL was injected for analysis.

GC/MSD The GC/MSD system consisted of an HP 6890 Plus Gas Chromatograph, an HP 7673 Automatic Liquid Sampler, an HP 5973 Mass Selective Detector, a capillary column (HP-5, 30 m × 0.25 mm ID). The data system was a HP MSD ChemStation controller with a HP LaserJet printer. The apparatus were purchased from Hewlett-Packard (Palo Alto CA, USA). The injector and GC/MS interface temperatures were set at 280 ℃ and 250 ℃, respectively. The carrier gas (helium) was 0.8 mL·min^{-1} and injections were made in the pulse-splitless

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mode. The MS Source and MS Quad temperature were 230 ℃ and 150 ℃, respectively. The oven temperature was held at 180 ℃ for 2 min, then increased to 210 ℃ at 15 ℃ per min, and held at 210 ℃ for 2 min, then increased to 240 ℃ at 20 ℃ per minute.

Validation study A series of procaterol of 10, 25, 50, 100, 200, 500, 1000, and 10 000 ng·L^{-1} were prepared with blank plasma. The compound was quantified by the peak area ratio using a calibration curve. Three standard solutions containing, respectively, 25, 200, and 1000 ng·L^{-1} were prepared to test the accuracy and precision of the assay. The lower limit of detection 5 ng·L^{-1} was defined as a signal-to-noise ratio of 3∶1. The potential interference from endogenous sources was examined with blank plasma.

RESULTS

Chromatography No endogenous interfering peaks were found according to the representative chromatograms, which were obtained from the blank plasma of volunteers (Fig 1A), the plasma with procaterol and imipramine (Fig 1B), and an unknown plasma from a volunteer after oral administration of 100 μg procaterol hydrochloride (Fig 1C). The procaterol, and IS were rapidly eluted with complete resolution and sharp symmetrical peaks. The retention time for procaterol and IS was within about 10.5 min and 9.2 min, respectively, which was much shorter than that reported previously[2].

GC/MSD The mass spectra of TMS derivative internal standard revealed had base peak at m/z 234 (Fig 2A). The mass spectra of TMS derivative procaterol revealed had base peak at m/z 407 accounting for 30 % of the total ions (Fig 2B).

Calibration curve The calibration curves for procaterol are linear over the range of 10 – 10000 ng·L^{-1}. Results from a typical regression analysis were: Response (peak area) = 12.7 × Amt (procaterol concentration ng·L^{-1}) – 127; $r = 0.9987$.

Validation test The average recovery of procaterol ranged from 98 % to 101 % (Tab 1). Coefficients of both intra- and inter-day variations (CV) were less than 10 % (Tab 1). The detection limit was 5 ng·L^{-1}.

Application of the method This method was applied to study the relative bioavailability of procaterol capsule in one volunteer taking procaterol hydrochloride tablet as reference. The curves of concentration-time and main pharmacokinetic parameters of them were shown in Fig 3 and Tab 2, respectively.

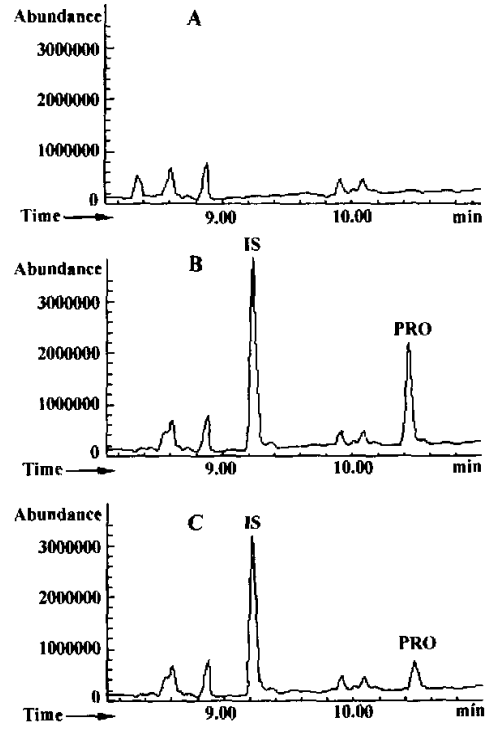

Fig 1. Chromatograms of blank plasma (A), plasma (B), and unknown plasma from a subject who took procaterol (C).

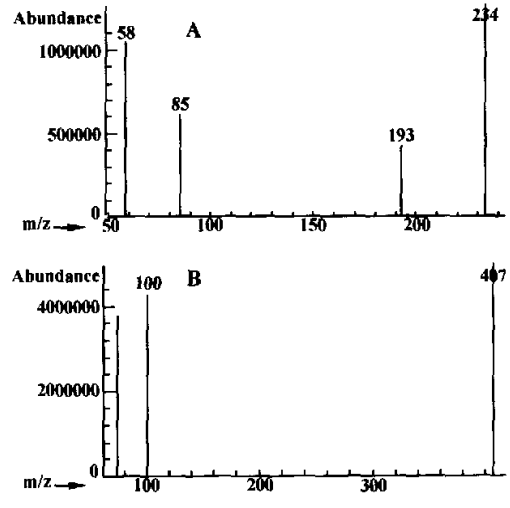

Fig 2. Mass spectrometry of imipramine (A) and procaterol (B).

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Tab 1. Coefficients of variation (CV, %), and recovery ($\bar{x} \pm s$, %) of procaterol in plasma ($n = 5$).

Procaterol		CV %		Recovery
Added/ng·L^{-1}	Recovered/ng·L^{-1}	Intraday	Interday	%
25	24.4±2.3	8.1	9.1	98±9.4
200	201.4±10.3	5.1	6.3	101±5.1
1000	1004.2±31.6	3.1	5.5	100±3.1

Tab 2. Pharmacokinetic parameters of procaterol hydrochloride in capsule and in tablet after oral administration of 100 μg in one volunteer.

Parameters	Capsule	Tablet
T_{max}(h)	3.0	2.0
C_{max}(ng·L^{-1})	354	383
$T_{1/2}$(h)	4.9	5.4
Vd/F (L·kg^{-1})	709	0.11
CL/F (L·h^{-1})	42.5	46.0
AUC_{0-24}(ng·h·L^{-1})	2250	2023
F (%)	111	

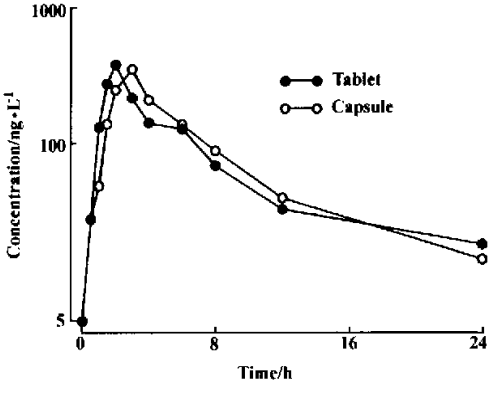

Fig 3. Plasma drug concentration-time curves in one subject who took procaterol hydrochloride tablet and capsule.

DISCUSSION

Procaterol is a new β_2-adrenergic receptor agonist used for treatment of asthma. The plasma procaterol is very low because of the low treatment dosage. It is necessary to develope a simple and sensitive method for detection of procaterol in human plasma.

The detection limit in this assay was 5 ng·L^{-1}. It was lower than that published by Scott Wright D *et al*[2] and the same as obtained by Ishigami M *et al*[3]. The sample preparation is simpler in this method compared with that described previously[3]. This method has been applied to study the relative bioavailability of procaterol capsule in one volunteer. The main pharmacokinetic parameters were similar with those reported[3]. In summary, we developed a simple and sensitive method for pharmacokinetic studies of procaterol.

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电子轰击离子化气相质谱法测定人类血浆中的丙卡特罗

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关键词 气相质谱法；丙卡特罗；药物动力学

目的：建立一种简便灵敏的气相质谱法用于测定丙卡特罗的血药浓度. **方法**：采用电子轰击离子化气相质谱法，以丙咪嗪做内标，样品采用液-液萃取、衍生化处理，分离柱为毛细管柱，进样器和接口温度分别为280 ℃和250 ℃，载气(氦气)流速为0.8 mL·min^{-1}，进样口选择脉冲不分流模式，离子源和四极杆的温度分别为230 ℃和150 ℃. **结果**：测定方法的检测限为5 ng·L^{-1}；线性范围为10-10 000 ng·L^{-1}；日内($n = 5$)和日间($n = 5$)变异系数均小于10%，平均回收率为99.1 %±1.3 %. **结论**：本方法灵敏、简便，可用于丙卡特罗的药代动力学研究.

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