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Short communication

Quantitation of iptakalim in human plasma by high-performance liquid chromatography–tandem mass spectrometry

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Abstract

This paper describes a rapid and sensitive analytical method for the quantitation of iptakalim, a novel antihypertensive drug, in human plasma. The method is based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) using sildenafil as internal standard. Sample preparation involved liquid–liquid extraction with dichloromethane–diethyl ether (2:3, v/v) in a basic environment. Chromatography was carried out on an amino column with a mobile phase consisting of acetonitrile–water (55:45, v/v, water containing 0.5% formic acid). Detection employed electrospray ionization (ESI) tandem mass spectrometry in the multiple-reaction-monitoring (MRM) mode. The assay was linear in the concentration range of 0.5–100 ng/ml with a lower limit of quantitation (LLOQ) of 0.5 ng/ml. Intra- and inter-day precision (R.S.D.) were <4.5% and <12.0%, respectively and the accuracy (R.E.) was in the range ±5%. The method was successfully applied to a single oral dose pharmacokinetic study in human volunteers.

Keywords: Iptakalim; LC–MS/MS; Human plasma

1. Introduction

Iptakalim (*N*-(1-methylethyl)-1,1,2-trimethylpropylamine, IPT, Fig. 1), a novel cardiovascular ATP-sensitive potassium channel opener [1], shows antihypertensive and neuroprotective effects in a variety of in vivo and in vitro preparations [2,3]. It is a promising antihypertensive drug with the ability to protect against endothelial dysfunction through activating ATP-sensitive potassium channels in endothelial cells [4]. The compound is now undergoing extensive clinical trials in China.

IPT is a small molecular weight compound without a significant chromophore. This limits its detection by UV or fluorescence spectroscopy such that pharmacokinetic studies in rat have been based on assay of GC–MS [5]. However, the GC–MS method requires a derivatization step, arduous sample preparation, and long chromatographic run times. In the last decade, LC–MS/MS has become popular for the determination of drugs in biological matrices because of its excellent specificity, speed, and sensitivity [6,7]. This paper presents a sensitive LC–MS/MS method for the determination of IPT in plasma involving sample preparation by liquid–liquid extraction and sildenafil as internal standard. The method has been successfully applied to a clinical pharmacokinetic study involving a single 5 mg oral dose.

2. Experimental

2.1. Chemicals and reagents

IPT hydrochloride (99.0%) and internal standard (I.S.) sildenafil (99.1%) (Fig. 1) were kindly supplied by the Department of Clinical Pharmacology, Chinese PLA General Hospital, Beijing, China. Methanol and acetonitrile were HPLC grade purchased from Fisher Scientific (Fair Lawn, NJ, USA), and other reagents were analytical grade used without further purification.

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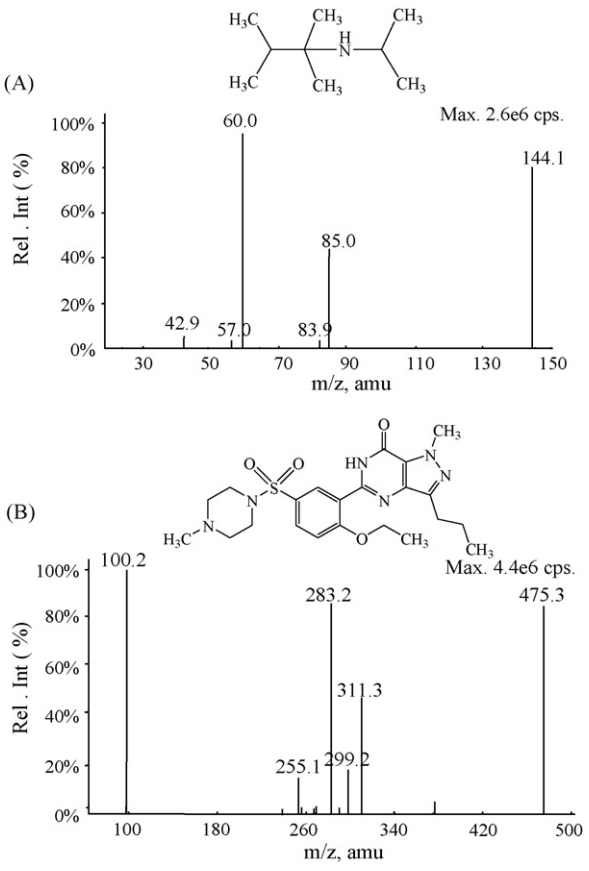

Fig. 1. Full-scan product ion spectra of $[M+H]^+$ for (A) iptakalim and (B) sildenafil.

Drug-free plasma was obtained from the Jilin Blood Donor Service. Distilled water, prepared from demineralized water, was used throughout the study.

2.2. Preparation of standards and quality control samples

All concentrations of IPT refer to the free base form. Two aqueous stock solutions of IPT (1 mg/ml) were prepared independently. One was used for assay validation and calibration and the other for preparation of quality control (QC) samples. Standard solutions containing 0.5, 1, 3, 10, 30, 60 and 100 ng/ml were prepared in water. An I.S. working solution containing 500 ng/ml was prepared by dilution with 30% methanol. All solutions were stored at 4 °C until use. Spiked human plasma standards were freshly prepared on each assay day by mixing 0.1 ml standard solution with 0.1 ml blank human plasma. High, medium and low QC samples were prepared in the same way as standards at concentrations of 1, 30 and 80 ng/ml, respectively.

2.3. Sample preparation

Plasma samples were thawed at room temperature and an aliquot (0.1 ml) mixed with 0.1 ml water, 0.1 ml I.S. and 50 μl 1 M sodium hydroxide solution in a 10 ml capped test-tube. Analytes were extracted by vortexing with 3 ml of dichloromethane–diethyl ether (2:3, v/v) for 1 min followed by shaking on a mechanical shaker for 10 min and centrifuging for 5 min at 3000 × *g*. The organic layer was transferred to another 10 ml test-tube followed by 0.5 ml 1% formic acid (v/v). Volatile solvent was evaporated under a gentle stream of nitrogen at 20 °C after which the remaining aqueous phase was vortexed for 1 min, transferred to an autosampler vial, and injected (20 μl) into the LC–MS system.

2.4. Instrumentation

The HPLC system (Agilent Technologies, Palo Alto, CA, USA) consisted of a binary pump, an autosampler, a column oven at 30 °C and an Ultra Amino analytical HPLC column (4.6 mm × 150 mm, 5 μm; Restek, USA). An Applied Biosystems Sciex API 4000 mass spectrometer (Applied Biosystems Sciex, Ontario, Canada) equipped with an ESI source was used as detector. Chromatography was performed using acetonitrile–water (55:45, v/v, water containing 0.5% formic acid) as mobile phase in the isocratic mode at a flow-rate of 0.8 ml/min. The mobile phase was degassed by sonication before use.

2.5. LC–MS/MS conditions

ESI was performed in the positive ion mode with nitrogen as nebulizer, heater and curtain gas. Gas flow parameters were optimized by flow injection of mobile phase into the ionization source at 0.2 ml/min. Optimum values for nebulizer, heater and curtain gas flow rates were 55, 50 and 30 units, respectively. The instrument response was optimized for IPT and I.S. by syringe pump infusion of a solution containing 100 ng/ml of each into the mobile phase using a post-column "T" connection at a flow-rate of 10 μl/min. Optimal response was obtained with declustering potential and collision energy settings of 30 V, 100 eV for IPT and 16 V, 54 eV for I.S. The TurboIonSpray temperature was set at 500 °C. MRM at unit resolution was employed for quantitation based on the transitions of the protonated molecular ions of IPT at *m/z* 144.1 → 60.0 and I.S. at *m/z* 475.3 → 283.2.

2.6. Method validation

Quantitation was based on determination of peak areas. Specificity was checked by analyzing spiked plasma samples prepared using plasma from six different individuals. Matrix effects were evaluated by comparing peak areas of analyte and I.S. in QC samples spiked after extraction with the corresponding areas obtained by direct injection of standard solutions at the same concentrations. Linearity was assessed by weighted ($1/x^2$) least squares analysis of three different calibration curves. Precision was calculated using one-way ANOVA. Intra- and inter-day precision (as relative standard deviation (R.S.D.)) and accuracy (as relative error (R.E.)) were determined by analysis of high, medium and low QC samples ($n=6$) on 3 different days. The limit of detection (LOD) was determined as the concentration with signal-to-noise ratio of 3. LLOQ was the concentration

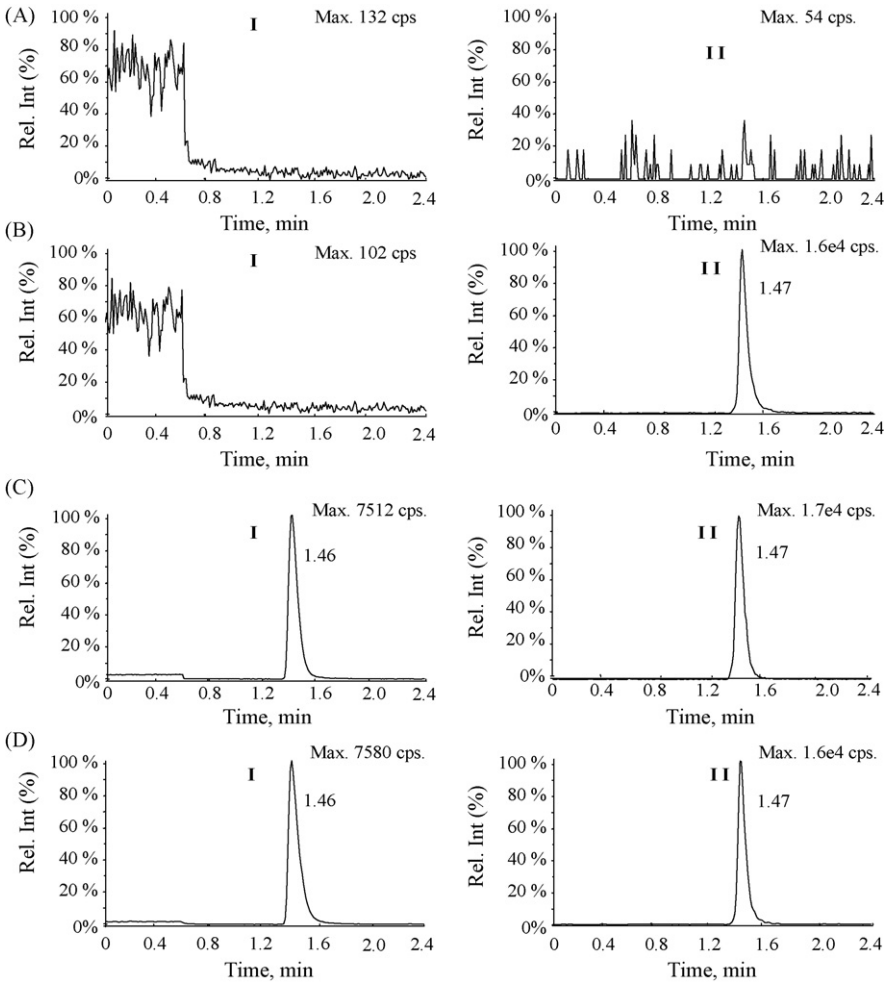

Fig. 2. Representative MRM chromatograms of (A) blank plasma, (B) blank plasma spiked with sildenafil (I.S.), (C) plasma spiked with iptakalim at the lower limit of quantitation (0.5 ng/ml) and (D) a plasma sample 36 h after administration of a 5 mg oral dose to a healthy male volunteer. Peak I, iptakalim; Peak II, sildenafil.

below which the inter-day R.S.D. exceeded 20%. Recoveries of IPT at the three QC concentrations and of I.S. at 500 ng/ml were determined by comparing peak areas of spiked plasma samples relative to those of blank plasma spiked after extraction. Stability during three freeze–thaw cycles, on storage in room temperature for 6 h and on storage in polypropylene tubes at −20 °C for 30 days was assessed by analyzing high, medium and low QC samples ($n = 6$). Bench-top stability of extracted samples in autosampler vials for 12 h was also evaluated.

2.7. Application of method

The method was applied to a single oral dose study of IPT (5 mg tablet) in 12 healthy male volunteers who were signed the consent before clinical trial and the clinical trial was approved by the ethics committee of Chinese PLA general hospital. Blood samples (2 ml) were collected by venepuncture before and at 0.17, 0.33, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12, 18, 24 and 36 h post dosing. Following centrifugation at 3000 × *g* for 10 min, plasma samples were stored in polypropylene tubes at −20 °C and analyzed within 30 days.

3. Results and discussion

3.1. Mass spectrometry/chromatography

Initially, ESI was chosen over atmospheric pressure chemical ionization because of demonstrably better sensitivity. The most intense product ions in the MS–MS spectra were *m/z* 60.0 and 85.0 for IPT and *m/z* 283.2 and 100.2 for the I.S. (Fig. 1). The fragment ions at *m/z* 85.0 and *m/z* 100.2 were associated with considerable noise in blank plasma leading to the selection of MRM transitions at *m/z* 144.1 → 60.0 and *m/z* 475.3 → 283.2 for quantitation of IPT and I.S., respectively.

To optimize chromatographic conditions, different analytical columns, mobile phase compositions and injection solvents were evaluated. Reversed phase C_8 (Zorbax eclipse XDB-C_8, 4.6 mm × 150 mm, 5 μm) and C_{18} (Zorbax extend-C_{18}, 4.6 mm × 150 mm, 5 μm) columns did not retain the analytes but an amino column (Restek, 4.6 mm × 150 mm, 5 μm) gave suitable retention. The isocratic mobile phase composition giving maximum peak responses and symmetrical chromatographic peaks was a mixture of acetonitrile–water (55:45, v/v, water containing 0.5% formic acid), which was pumped at a flow-rate

Table 1
Precision and accuracy for the determination of iptakalim in human plasma (data are based on analysis of QC samples ($n = 6$) on 3 different days)

Nominal concentration (ng/ml)	Calculated concentration (ng/ml)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)	Relative error (%)
1.00	0.97	4.42	4.15	−3.43
30.0	30.5	2.67	10.5	1.74
80.0	79.2	3.56	11.8	−1.06

Table 2
Stability studies for iptakalim

Stability experiments	Storage condition	Nominal concentration (ng/ml)	Mean found concentration (ng/ml)	No. of replicates	Relative error (%)	Coefficient of variation (%)
Process (extracted sample)	Autosampler (room temperature, 12 h)	1.00	1.00	6	0.48	2.73
		30.0	30.7		2.39	2.12
		80.0	79.9		−0.13	2.17
Freeze/thaw stability	After third freeze/thaw cycle at −20 °C	1.00	1.02	6	1.67	2.76
		30.0	30.6		1.89	1.56
		80.0	82.2		2.71	2.10
Long-term stability in human plasma	For 30 days at −20 °C	1.00	1.01	6	0.67	2.32
		30.0	30.3		1.00	3.34
		80.0	80.4		0.54	1.75
Room temperature stability in human plasma	Room temperature 6 h	1.00	0.99	6	−0.67	2.75
		30.0	29.6		−1.33	3.08
		80.0	80.1		0.13	1.98

of 0.8 ml/min, and the column oven temperature maintained at 30 °C. Under these optimum conditions, analyte and I.S. were free of interference from endogenous substances and gave retention times of 1.46 and 1.47 min, respectively. The cycle time of only 2.4 min allowed a sample throughput of 180–200 samples per day.

During investigation of liquid–liquid extraction, it was found that IPT was lost during evaporation of the organic phase despite good recovery of the internal standard. It was found that addition of 0.5 ml 1% formic acid followed by gentle evaporation of the volatile solvents gave good recovery in a matrix suitable for direct injection into the LC/MS system.

3.2. Method validation

3.2.1. Specificity

Representative chromatograms obtained for blank human plasma, blank plasma spiked with I.S., blank plasma spiked with IPT at the limit of quantitation (0.5 ng/ml) and I.S., and a study sample containing a low concentration of IPT are shown in Fig. 2. No interference from endogenous plasma substances with analyte or I.S. was detected.

Percent nominal concentrations estimated by analysis of spiked plasma samples prepared using six different lots of plasma were within acceptable limits (100–107%) indicating that no co-eluting endogenous species interfered with the ionization of the analyte and I.S.

3.2.2. Linearity, precision and accuracy

Good linearity was obtained in the concentration range of 0.5–100 ng/ml ($r > 0.99$). The lower limit of detection of the method was 0.05 ng/ml and the LLOQ was 0.5 ng/ml. Intra- and inter-day precision and accuracy results (Table 1) gave satisfactory results in that all R.S.D. and R.E. were <15%.

Table 3
Recoveries of IPT ($n = 6$) and I.S.

Compound	Concentration (ng/ml)	Recovery (%) (mean ± S.D.)
IPT	1.0	76.7 ± 3.9
	30.0	79.0 ± 1.3
	80.0	74.2 ± 5.1
I.S.	500.0	87.5 ± 3.1

3.2.3. Stability and recovery

Stability testing during three freeze–thaw cycles, on storage at −20 °C for 30 days, on storage in room temperature for 6 h and on storage in autosampler vials for 12 h gave concentrations within acceptable limits (±15% of nominal concentrations, Table 2). The extraction recoveries of IPT and I.S. were shown in Table 3.

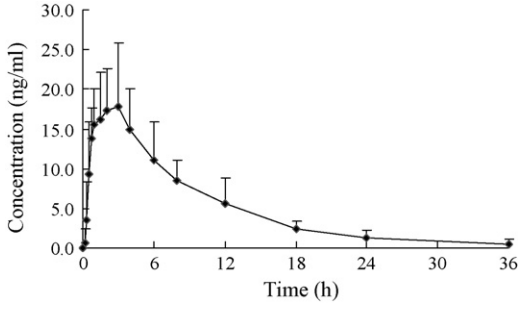

Fig. 3. Mean plasma concentration–time profile of iptakalim after administration of a 5 mg tablet. Data are mean ± S.D. for 12 healthy male volunteers.

3.3. Application of the method

The LC–ESI/MS method was successfully applied to a pharmacokinetic study of IPT following a single oral 5 mg dose to human volunteers ($n = 12$). The mean concentration–time profile is shown in Fig. 3. Mean values of pharmacokinetic parameters were: C_{max} 21.6 ± 6.4 ng/ml, t_{max} 1.83 ± 0.86 h, $t_{1/2}$ 11.3 ± 5.1 h, and area under the plasma concentration–time curve 178.6 ± 63.3 ng h/ml.

4. Conclusions

A rapid, sensitive and reliable LC–MS/MS method for the quantitation of IPT in human plasma has been successfully developed and applied to a clinical pharmacokinetic study. The method involves a simple one-step liquid–liquid extraction procedure and gives an LLOQ of 0.5 ng/ml using only 0.1 ml of plasma. The cycle time of <2.5 min per sample is suitable for high-throughput analysis.

References

[1] H. Wang, Drug Dev. Res. 58 (2003) 65.
[2] H. Wang, C.L. Long, Y.L. Zhang, J. Pharmacol. Exp. Ther. 312 (2005) 1326.
[3] H. Wang, Y.L. Zhang, X.C. Tang, H.S. Feng, G. Hu, Mol. Pharmacol. 66 (2004) 1160.
[4] H. Wang, C.L. Long, Z.B. Duan, C.G. Shi, G.D. Jia, Y.G. Zhang, Cardiovasc. Res. 73 (2007) 497.
[5] Z.Q. Zhang, S.L. Yuan, J.Z. Qiao, X.Y. Wang, R.F. Yang, J.X. Ruan, J. Instrum. Anal. 20 (2001) 17.
[6] D. Projean, T. Minh Tu, J. Ducharme, J. Chromatogr. B 787 (2003) 243.
[7] J.Y. Zhang, D.M. Fast, A.P. Breau, J. Chromatogr. B 787 (2003) 333.