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Sensitive method for the determination of ibutilide in human plasma by liquid chromatography–tandem mass spectrometry

Lei Tian, Yishi Li*, Yiling Huang, Fuhua Kuang, Feiou Li, Lu Hua

Clinical Pharmacology Center, Fu Wai Hospital, CAMS and PUMC, 167 Beilishi Road, Beijing 100037, PR China

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Abstract

A rapid, selective and sensitive liquid chromatography–tandem mass spectrometry (LC–MS–MS) method was developed and validated for determination of ibutilide in human plasma. The analyte and internal standard sotalol were extracted from plasma samples by liquid–liquid extraction, and separated on a C_{18} column, using acetonitrile–water–10% butylamine–10% acetic acid (80:20:0.07:0.06, v/v/v/v) as the mobile phase. Detection was performed on a triple-quadrupole tandem mass spectrometer by multiple reaction monitoring (MRM) mode via TurboIonSpray ionization (ESI). Linear calibration curves were obtained in the concentration range of 20–10,000 pg/ml, with a lower limit of quantitation of 10 pg/ml. The intra- and inter-day precision values were below 8% and accuracy was within ±3% at all three QC levels. The method was utilized to support clinical pharmacokinetic studies of ibutilide in healthy volunteers following intravenous administration.

Keyword: Ibutilide

1. Introduction

Ibutilide, (±)-*N*-[4-[4-(ethylheptylamino)-1-hydroxybutyl]phenyl]-methanesulfonamide, structurally related to sotalol (Fig. 1), is an investigational class III antiarrhythmic agent in clinical development for the treatment of atrial flutter and fibrillation [1]. Though ibutilide contains a single stereogenic center bearing a secondary alcohol group, the pharmacokinetic and electrophysiologic properties of the individual enantiomers are similar to each other. There was no evidence of racemization of ibutilide in the body. Ibutilide must be infused intravenously because the bioavailability of orally administered ibutilide fumarate is low due to the extensive hepatic metabolism [2].

The use of low intravenous dose (0.005–0.03 mg/kg) and extensive hepatic metabolism resulted in very low plasma concentrations, from pg/ml to low ng/ml values, for ibutilide. Due to lack of sensitive and specific analytical techniques, currently available data regarding the pharmacokinetics of ibutilide are sparse. Information has primarily been acquired from a few studies in man [3–5].

A few HPLC methods have been employed to monitor ibutilide in plasma [6,7]. These methods needed long chromatographic run times and time-consuming sample pretreatments. Hsu described a chiral assay method to quantitate the individual enantiomers of ibutilide in plasma samples, employing a solid-phase extraction, derivatization, column-switching HPLC technique with fluorescence detection. The column-switching system needed five columns and two fluorescence detectors. The limit of quantitation (LOQ) of the method was only 100 pg/ml in 1 ml of plasma.

The need to quantify low plasma concentrations of ibutilide and to better characterize its clinical pharmacokinetic properties after low dose administration compelled us to set up and validate a specific and highly sensitive analytical method. In this paper, we describe a liquid chromatography–tandem mass spectrometry (LC–MS–MS)

* Corresponding author. Fax: +86 10 6835 4535.
E-mail addresses: ittl@fuwaihospital.org, LiYiShi@public3.bta.net.cn (Y. Li).

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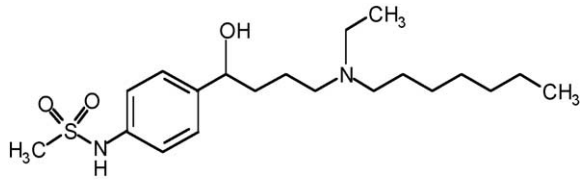
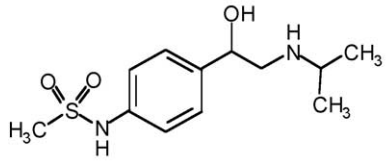


Ibutilide



Sotalol (IS)

Fig. 1. Chemical structures of ibutilide and sotalol (internal standard, IS).

method for this purpose. After validation, this method was successfully applied to phase I clinical studies of ibutilide after single intravenous doses from 0.005 to 0.02 mg/kg, using 0.5 ml plasma sample.

2. Experimental

2.1. Reference compounds and chemicals

Ibutilide fumarate was provided by Honghui Pharmaceutical Corp. (Beijing, China) with the purity of 99.1%. Sotalol hydrochloride (internal standard, IS) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and its purity was 99.8%. HPLC grade acetonitrile, methyl-*tert*-butyl ether and isopropanol were all obtained from Fisher (Fair Lawn, NJ, USA). All other chemicals and reagents were of either HPLC- or analytical-grade and were used without any further purification. Deionized water was generated in-house with a Milli-Q Gradient system (Millipore, Bedford, MA, USA) and was used throughout the study.

2.2. Instrumentation

An Agilent 1100 system (Wilmington, DE, USA) consisting of a vacuum degasser, a binary pump, a column oven and an autosampler was used for solvent and sample delivery. Chromatography was carried out on a Nova-Pak C_{18} column (150 mm × 3.9 mm, 5 μm, Waters, Milford, MA, USA), using a mobile phase of acetonitrile–water–10% butylamine–10% acetic acid (80:20:0.07:0.06, v/v/v/v). The flow-rate was isocratic at 1.0 ml/min, and the effluent from the liquid chromatography was split in a post-column T connection in order to get a liquid flow to the TurboIonSpray interface of about 100 μl/min. The column temperature was maintained at 30 °C.

An AB MDS Sciex (Concord, Ontario, Canada) API 4000 triple-quadrupole mass spectrometer equipped with a TurboIonSpray ionization (ESI) source was used for mass analysis and detection. The mass spectrometer was operated in the positive mode. For optimizing the MS conditions, a solution containing 400 ng/ml of ibutilide and the internal standard was used, delivered by a Harvard syringe pump (Harvard Apparatus, South Natick, MA, USA) at a constant flow-rate of 10 μl/min. The nebulizer and TurboIonSpray gases (nitrogen) were both set at a value of 40 (instrument units). The optimized TurboIonSpray voltage and temperature were set at 5400 V and 400 °C, respectively. Nitrogen was also used as curtain gas and collision gas. The curtain gas was set at 20 (instrument units) and the collision gas flow setting was 4 (instrument units). Quantitation was performed using multiple reaction monitoring (MRM) of the transition m/z 385 → m/z 367 for ibutilide and m/z 273 → m/z 255 for the internal standard respectively, with a dwell time of 200 ms per transition. The optimized collision energy of 25 eV was used for the analyte and 17 eV for the internal standard. The mass spectrometer was operated at unit mass resolution (peak width at half-height set at 0.7 Da) for both Q1 and Q3.

2.3. Preparation of standard and quality control solutions

Stock solutions of ibutilide and the internal standard were prepared by dissolving the accurately weighted standard compounds in methanol to give final concentrations of 1 mg/ml (calculated as free base). The stock solution of ibutilide was then successively diluted with methanol to achieve standard working solutions at concentrations of 200, 400, 1000, 2000, 5000, 10,000, 20,000, 50,000 and 100,000 pg/ml. The standard solutions at concentrations of 1000, 5000 and 20,000 pg/ml were used as quality control (QC) solutions (low, medium, high). The extraction solvent containing 10 ng/ml internal standard was prepared by diluting the 1 mg/ml stock solution of sotalol with the mixture of methyl-*tert*-butyl ether–isopropanol (5:1, v/v).

The standard working solutions (50 μl) were used to spike blank plasma (0.5 ml) either for calibration curves or for quality control samples in prestudy validation and during the pharmacokinetic study.

All the solutions were stored at 4 °C and were brought to room temperature before use.

2.4. Sample preparation

An aliquot of plasma (0.5 ml) was mixed with 50 μl methanol, and then extracted with 3 ml of methyl-*tert*-butyl ether–isopropanol (5:1, v/v, containing 10 ng/ml internal standard) by vortexing for 3 min. The organic and aqueous phases were separated by centrifugation at 3000 × *g* for 10 min. The upper organic phase was transferred to another glass tube and was evaporated to dryness at 45 °C under a gentle stream of nitrogen. The residue was dissolved in 200 μl

of the mobile phase, and vortex mixed. A 50 μl aliquot of the solution was injected onto the LC–MS–MS system for analysis.

2.5. Data acquisition and analysis

Data were collected and analyzed by Analyst 1.3.1 software (AB MDS Sciex). Calibration curves were constructed by plotting peak area ratios of the analyte and the internal standard against the analyte's concentrations. The weighted ($1/x^2$) linear least-squares regression line was fitted over the 500-fold concentration range. Drug concentrations in the unknown and quality control samples were calculated by interpolation from the calibration curves.

2.6. Method validation

The calibration curves for the determination of ibutilide were prepared by analyzing spiked plasma. The spiked plasma samples at three concentration levels (low, medium and high concentrations) were used as QC samples and analyzed by LC–MS–MS system.

During prestudy validation [8], the calibration curves were defined in four runs based on triplicate assays of the spiked plasma samples, and QC samples were determined in replicates ($n = 6$) on the same run. Overall assay performance was assessed by calculating the accuracy and intra- and inter-run precision of QC samples analyzed. During routine analysis each analytical run included a set of calibration samples, a set of QC samples in duplicate and unknowns.

3. Results and discussion

3.1. LC–MS–MS

Atmospheric pressure ionization (including APCI and ESI) MS is a proven technique for the rapid and sensitive determination of drugs and metabolites. Its sensitivity depends on the nature of the analyte and the mobile phase composition. The ESI interface was used and good sensitivity and linearity were obtained in the experiment. An APCI interface has been tested and no obvious improvement was observed. In most cases, an internal standard labelled with a stable isotope would be preferable for quantitation in complex matrices by LC–MS–MS analysis [9]. A stable isotope labelled internal standard was not available in our case. Therefore, sotalol, an analog of ibutilide, was utilized as internal standard, as it would show the similar behaviour as the analyte in the entire sample extraction, chromatographic elution and mass spectrometric detection.

To obtain higher sensitivity and specificity, multiple reaction monitoring (MRM) mode was used to detect ibutilide and the internal standard. The fragmentation behavior of the $[M + H]^+$ ion of ibutilide (*m/z* 385) is strongly dependent on the collision energy. A major fragment ion at *m/z* 367 was formed using 25 eV collision energy (Fig. 2A). When higher collision energy was used, this ion was further fragmented, forming fragmentation ions of *m/z* 144 and *m/z* 224. The absolute intensities of those product ions were considerably lower than that of the ion at *m/z* 367 obtained using the lower collision; the peak-area obtained using the *m/z* 385 → *m/z* 144 transition at 40 eV was about two times lower than that obtained using the *m/z* 385 → *m/z* 367 transition at 25 eV. Thus, MRM was performed by monitoring the transition of $[M + H]^+ \rightarrow$ *m/z* 367 (formed by loss of neutral H_2O from the $[M + H]^+$ ion) for ibutilide using 25 eV collision energy. The internal standard showed the $[M + H]^+$ ion at *m/z* 273 as the base peak in the full scan Q1 mass spectrum. As for ibutilide, the product ion spectrum of *m/z* 273 (IS) also showed a major fragment ion at *m/z* 255, indicating the loss of neutral H_2O from the $[M + H]^+$ ion at *m/z* 273 (Fig. 2B). Additional tuning of the ESI source parameters for the transitions *m/z* 385 → *m/z* 367 and *m/z* 273 → *m/z* 255 further improved the sensitivity.

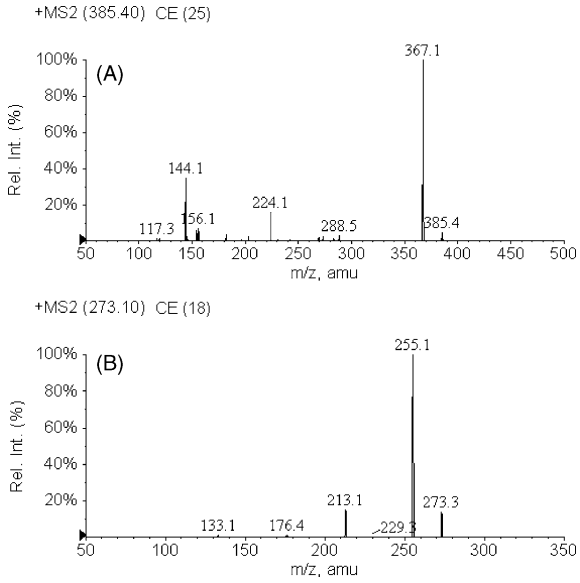


Fig. 2. Product ion mass spectra of (A) ibutilide and (B) sotalol with each protonated molecule $[M + H]^+$ as precursor ion.

The chromatographic conditions were optimized with respect to peak symmetry and analytical cycle time. A chromatographic separation is necessary to minimize ion suppression effects from endogenous components of the plasma. It was found that the butylamine and acetic acid in the mobile phase had no significant effect on the sensitivity of the analyte under ESI conditions. The added amount of the two modifiers could effect the retention time of the analyte and the internal standard significantly, e.g. the retention time of ibutilide changed from 10 to 1.7 min if the added amount of 10% butylamine and 10% acetic acid increased from 10 to 100 μl in 100 ml mobile phase. Therefore, the mobile phase consisting of acetonitrile–water–10% butylamine–10% acetic acid (80:20:0.07:0.06, v/v/v/v) was chosen to obtain good chromatographic peak shape and to keep the analyte and the in-

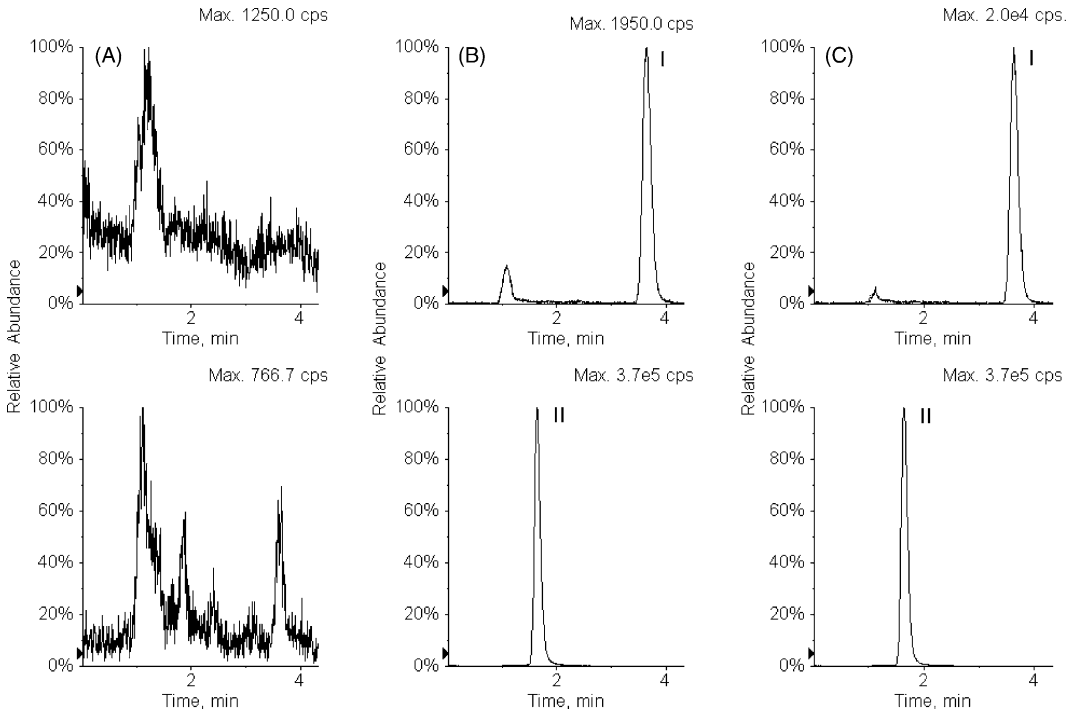


Fig. 3. Representative chromatograms of plasma extracts obtained by MRM mode: (A) blank plasma sample; (B) blank plasma sample spiked with 20 pg/ml ibutilide and 60 ng/ml sotalol (internal standard); (C) plasma sample from a volunteer 4.0 h after intravenous administration of 0.02 mg/kg ibutilide fumarate (ibutilide, 354 pg/ml). Peaks I and II refer to ibutilide and the internal standard, respectively.

ternal standard at suitable retention time (3.6 and 1.7 min, respectively).

3.2. Method validation

3.2.1. Assay specificity

The coupling of LC with MS–MS detection in the MRM mode has high specificity because only ions derived from the analytes of interest are monitored. Comparison of the chromatograms for the blank and spiked human plasma matrices (Fig. 3) indicated that no interferences were detected from endogenous substances with the analyte and internal standard. The nominal retention times for ibutilide and the IS were 3.6 and 1.7 min, respectively.

Matrix effects from "unseen" co-eluting endogenous substances provide another possible source of problems regarding assay specificity. The matrix effect was evaluated by comparing the peak areas of the compounds prepared from six different blank plasma samples with those of the corresponding standard solutions. The peak areas from the six reconstituted samples had a coefficient of variation of 6.2 and 7.3% for ibutilide and IS respectively, and the relative error was 7.8 and 6.7% compared with those from standard solutions. The results indicated that no co-eluting endogenous substances could influence the ionization of ibutilide and IS significantly.

3.2.2. Linearity of calibration curve and lower limit of quantitation

Linear calibration curves with correlation coefficients greater than 0.995 were obtained over the concentration range of 20–10,000 pg/ml for ibutilide in human plasma. A typical equation of the calibration curves was as follows, with the standard error (S.E.) of 2.44×10^{-4} and 4.64×10^{-6} for the intercept and slope:

$$y = 2.86 \times 10^{-3} + 3.43 \times 10^{-4}x, \qquad r = 0.9994$$

The lower limit of quantitation (LLOQ), defined as the lowest concentration analyzed with accuracy within ±15% and a precision ≤15%, was 10 pg/ml for determination of ibutilide in plasma ($n = 6$).

3.2.3. Precision and accuracy

Precision and accuracy of the assay were determined by replicate analyses ($n = 6$) of QC samples at three concentrations, by performing the complete analytical runs on the same day and also on four consecutive days. The intra- and inter-day precision were less than 8% for each QC level of ibutilide. The accuracy, assessed by calculating the percentage deviation observed in the analysis of QC samples and expressed in the relative error (R.E.), was within ±3% at all three QC levels. The results are summarized in Table 1.

3.2.4. Extraction recovery

The extraction recoveries of ibutilide were determined by comparing peak areas obtained from extracted plasma samples with those found by direct injection of standard solution at the same concentration, using the same autosampler equipped with the same loop. The results showed that

Table 1
Precision, accuracy and LLOQ results for ibutilide in human plasma extracts (in prestudy validation)

Added C (pg/ml)	*n*	Found C (pg/ml)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)	Relative error (%)
100	24	101	6.0	7.0	1.0
500	24	505	4.5	5.1	0.9
2000	24	1949	3.3	4.4	−2.6
10.0	6	10.2	7.3	–	2.1

the mean extraction recoveries of ibutilide were 72.6 ± 3.9, 73.1 ± 3.0, and 73.3 ± 1.9% at concentrations of 100, 500, and 2000 pg/ml, respectively. The mean extraction recovery of the internal standard sotalol was 71.3 ± 5.8%.

Different organic extraction solvents were evaluated in the experiment, including ethyl acetate, methyl-*tert*-butyl ether and the mixture of the above two solvents with isopropanol. The mixture of methyl-*tert*-butyl ether–isopropanol (5:1, v/v) proved to be the most efficient to extract ibutilide from human plasma and had small variation of extraction recoveries over the concentration range.

3.2.5. Stability

The stability of ibutilide in human plasma was investigated under different storage and process conditions. The analytes were found to be stable (R.E. < 5%) in human plasma after three freeze (−20 °C)–thaw (room temperature) cycles. The analytes were also shown to be stable after 24 h of storage in reconstitution solutions at room temperature (R.E. < 6%). The good stability of ibutilide simplifies the precautions needed for laboratory manipulations during the analytical procedures.

3.3. Application of the analytical method in pharmacokinetic studies

In our study, 20 healthy subjects were randomized to receive a single intravenous dose of 0.005, 0.01 or 0.02 mg/kg of ibutilide fumarate over 10 min. Ibutilide plasma concentrations were determined up to 24 h by the LC–MS–MS method described above. Fig. 4 shows mean plasma concentration–time curves of ibutilide after administration. After intravenous infusions, the plasma concentrations rapidly declined with elimination half-life varied from 6.9 to 8.1 h, which was similar to the results reported [4,5]. The pharmacokinetics of ibutilide are linear with respect to the dose of ibutilide over the dose range of 0.005–0.02 mg/kg.

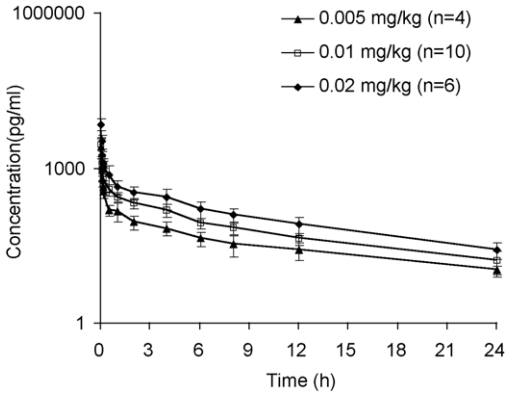


Fig. 4. Mean plasma concentration–time profiles of ibutilide after a single intravenous dose of 0.005, 0.01 or 0.02 mg/kg of ibutilide fumarate over 10 min to healthy volunteers.

4. Conclusion

The optimized method was validated to guarantee a reliable determination of ibutilide in human plasma. The method has a lower limit of quantitation of 10 pg/ml and has been shown to be sensitive, selective and reproducible. The short chromatographic cycle time (4.2 min) allowed high-throughput analysis with minimal matrix interference. The method developed has been shown to be successful in applications supporting clinical studies.

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References

[1] J.T. VanderLugt, S.K. Gaylor, L.K. Wakefield, G.L. Jungbluth, R.R. Walters, G.G. Kabell, Clin. Pharmacol. Ther. 49 (1991) 188.
[2] K.A. Ellenbogen, H.F. Clemo, B.S. Stambler, M.A. Wood, J.T. VanderLugt, Am. J. Cardiol. 78 (Suppl. 8A) (1996) 42.
[3] C.L. Hsu, R.R. Walters, Chirality 8 (1996) 18.
[4] G.L. Jungbluth, J.T. VanderLugt, G.G. Kabell, R.R. Walters, Pharm. Res. 7 (Suppl. 9) (1990) 211.
[5] G.L. Jungbluth, A.A. Della-Coletta, J.T. VanderLugt, Pharm. Res. 8 (Suppl. 10) (1992) 249.
[6] C.L. Hsu, R.R. Walters, J. Chromatogr. 550 (1991) 621.
[7] C.L. Hsu, R.R. Walters, J. Chromatogr. B 667 (1995) 115.
[8] R. Causon, J. Chromatogr. B 689 (1997) 175.
[9] A. Gusev, Quantitation in complex matrices by LC/MS/MS, 47th ASMS, Dallas, 1999.