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Quantitation of itopride in human serum by high-performance liquid chromatography with fluorescence detection and its application to a bioequivalence study

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Abstract

A new method was developed for determination of itopride in human serum by reversed phase high-performance liquid chromatography (HPLC) with fluorescence detection (excitation at 291 nm and emission at 342 nm). The method employed one-step extraction of itopride from serum matrix with a mixture of *tert*-butyl methyl ether and dichloromethane (70:30, v/v) using etoricoxib as an internal standard. Chromatographic separation was obtained within 12.0 min using a reverse phase YMC-Pack AM ODS column (250 mm × 4.6 mm, 5 μm) and an isocratic mobile phase constituting of a mixture of 0.05% tri-fluoro acetic acid in water and acetonitrile (75:25, v/v) flowing at a flow rate of 1.0 ml/min. The method was linear in the range of 14.0 ng/ml to 1000.0 ng/ml. The lower limit of quantitation (LLOQ) was 14.0 ng/ml. Average recovery of itopride and the internal standard from the biological matrix was more than 66.04 and 64.57%, respectively. The inter-day accuracy of the drug containing serum samples was more than 97.81% with a precision of 2.31–3.68%. The intra-day accuracy was 96.91% or more with a precision of 5.17–9.50%. Serum samples containing itopride were stable for 180.0 days at $-70 \pm 5\,^{\circ}$C and for 24.0 h at ambient temperature ($25 \pm 5\,^{\circ}$C). The method was successfully applied to the bioequivalence study of itopride in healthy, male human subjects.
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Keywords: Itopride; Human serum; Validation; Bioequivalence

1. Introduction

Itopride hydrochloride (*N*-[4-[2-(dimethylamino)-ethoxy]benzyl]-3,4-dimethoxybenzamide hydrochloride) is a novel gastroprokinetic agent which stimulates gastrointestinal motor activity through synergistic effects of dopamine D2 receptor blockade and acetylcholine esterase inhibition [1,2]. Itopride hydrochloride is prescribed for the treatment of gastrointestinal symptoms caused by reduced gastrointestinal motility, feeling of gastric fullness, upper abdominal pain, anorexia, heartburn, nausea and vomiting; non-ulcer dyspepsia or chronic gastritis.

It has been reported [1] that after the administration of single oral dose of 50 mg itopride hydrochloride in healthy adults, the maximum serum concentration (C_{max}) achieved was 0.28 ± 0.02 μg/ml at 0.58 ± 0.08 h with a half-life ($t_{1/2}$) of 5.77 ± 0.33 h. Flavine mono oxygenase (FMO) enzyme is responsible for the extensive metabolism [2] of itopride to N-oxide metabolite by the oxidation of tertiary amine N-dimethyl group. After oral administration, itopride is excreted in the N-oxide form in the urine.

Besides a couple of bioanalytical methods [2,3] not much literature is reported for the determination of itopride in biological samples. Therefore, the aim of the present investigation was to develop a new, sensitive HPLC method for the estimation of itopride in human serum. The method was applied to a bioequivalence study of itopride hydrochloride 50 mg tablets of M/s German Remedies Specialties Ltd.,

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India (a division of Cadila Health Care Ltd., India) versus Ganaton tablet containing 50 mg itopride hydrochloride of M/s Abbott India Ltd., India, in healthy, adult, male, human subjects under fasting condition. The outcome of a study depends upon the reliability, reproducibility and sensitivity of the analytical methodology employed. Therefore, the bioanalytical method was validated in accordance with USFDA guidelines prior to the initiation of the study.

2. Experimental

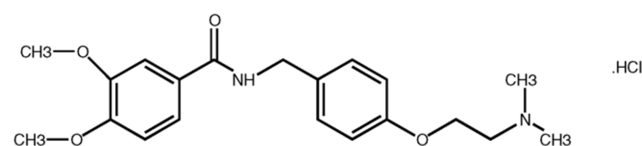
2.1. Chemicals and reagents

Working standards of itopride hydrochloride (Fig. 1) with 99.96% purity was obtained from German Remedies Ltd., Mumbai, India. Etoricoxib (purity 99.56%) working standard was prepared in house (Cadila Health Care Ltd., Ahmedabad, India). Acetonitrile (HPLC grade) was obtained from QUALIGENS Fine Chemicals, a division of Glaxo SmithKline Pharmaceuticals Ltd., Mumbai. Merck–Schuchardt, Germany, supplied tri-fluoro acetic acid. *tert*-Butyl methyl ether (HPLC grade) was supplied by Spectrochem Pvt. Ltd., Mumbai, India. Dichloromethane (HPLC grade) was obtained by Merck (India) Ltd. Human serum was obtained from Gujarat Blood Bank, Ahmedabad, India. HPLC grade water from Millipore's Milli-Q system was used throughout the analysis.

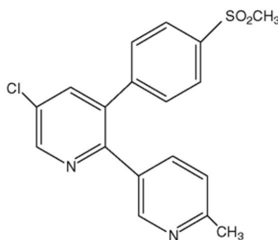
2.2. Stock solutions and working solutions

Stock solution (1.0 mg/ml) of itopride hydrochloride was prepared by dissolving 10.0 mg of itopride hydrochloride working standard in 10.0 ml of diluent (water:methanol, 50:50, v/v). One milliliter of this solution was further diluted to 10.0 ml with the same diluent to obtain a stock solution containing 0.1 mg/ml of the test compound. The stock solution was appropriately diluted with the above diluent to obtain a working solution of calibration standards with concentrations of 140.0, 300.0, 500.0, 1000.0, 2000.0, 3000.0, 5000.0 and 10,000.0 ng/ml. Similarly, the working solutions for quality control standards (450.0, 4500.0 and 8000.0 ng/ml) were also prepared.

In order to prepare the stock solutions (1.0 mg/ml) of internal standard (IS), 50.22 mg of etoricoxib working standard was dissolved in a 50 ml of water:methanol, 50:50 (v/v) mixture. Five milliliters of this solution was further diluted to 10.0 ml with the same diluent and a working solution of 0.5 mg/ml etoricoxib was prepared. All solutions were stored at 2–8 °C.



itopride hydrochloride



etoricoxib

Fig. 1. Structure of itopride and internal standard etoricoxib.

2.3. Calibration standards and quality control (QC) samples

Eight non-zero calibration standards ranging from 14.0 to 1000.0 ng/ml were prepared by adding 50.0 μl of a known working solution of itopride hydrochloride and 50.0 μl of internal standard solution to 450.0 μl of drug free human serum. The quality control (QC) samples were prepared in the manner similar to the calibration standard at three concentration levels: low, medium (mid) and high (45.0, 450.0 and 800.0 ng/ml). During each run, six replicates of each concentration of QC samples were extracted (as per Section 2.4) along with the calibration standards to check that the system performs in control.

2.4. Sample preparation

After adding 50.0 μl of internal standard to 500.0 μl of human serum samples, liquid–liquid extraction (LLE) was performed with the addition of 4.0 ml of extraction solvent (*tert*-butyl methyl ether and dichloro methane, 70:30, v/v). The sample was vortexed for 2.0 min and was allowed to settle for 15.0 min. About 3.0 ml of supernatant was transferred to another test tube and was evaporated to dryness in a thermostatically controlled water-bath maintained at 40 °C under the stream of nitrogen for about 25.0 min. After drying, the residue was reconstituted in 300.0 μl of mobile phase and injected in liquid chromatograph.

2.5. Chromatographic conditions

Chromatographic separation was performed on a Class VP liquid chromatograph equipped with a SIL-HT*c* autosampler, and RF-10AXL fluorescence detector from Shimadzu Corporation, Kyoto, Japan. The data acquisition was carried out by Class VP 6.01 version data system from Shimadzu Corporation, Kyoto, Japan. Seventy-five microliters of reconstituted sample was injected into a YMC-Pack AM ODS column 5 μm (4.6 mm i.d. × 250 mm) maintained at 30 °C with a mobile phase (acetonitrile:0.05% tri-fluoro acetic acid in water, 25:75, v/v) flowing through it at a flow rate of 1.0 ml/min. Itopride and internal standard were detected at

an excitation wavelength of 291 nm and emission wavelength of 342 nm.

2.6. Validation

2.6.1. Linearity (calibration curves)

Linearity was evaluated using freshly prepared spiked serum samples in the concentration range of 14.0–1000.0 ng/ml. Each calibration curve consisted of a blank sample, a zero sample and eight calibrator concentrations. Five such linearity curves were analyzed. Samples were quantified using the ratio of peak area of itopride to that of IS as the assay parameter. Peak area ratio were plotted against itopride serum concentrations and standard curves were calculated by the equation: $y = mx + c$ using weighted (1/response^2) least square regression. A correlation of more than 0.99 was desirable for all the calibration curves.

2.6.2. Limit of detection and lower limit of quantitation

The limit of detection (LOD) was defined by the concentration with a signal-to-noise ratio of 3. The lowest standard on the calibration curve was to be accepted as the lower limit of quantitation (LLOQ) if it complied the acceptance criteria [4] of exhibiting the analyte response five times that of drug free (blank) processed serum. In addition, the analyte peak in LLOQ sample should be identifiable, discrete and reproducible with a precision of ±20.0% and accuracy within 80.0–120.0%. The deviation of standards other than LLOQ from the nominal concentration should not be more than ±15.0%. It was desirable that a minimum of six non-zero standards, including LLOQ, met the above criteria.

2.6.3. Specificity

Six randomly selected control drug free human serum samples were processed by the similar extraction procedure and analyzed to determine the extent to which endogenous serum components may contribute to the interference at retention time of analyte and internal standard.

2.6.4. Recovery (extraction efficiency) from matrix

Recovery of itopride in serum was evaluated by comparing the mean detector response of different QC samples post-extracted with those prepared by adding compound to post-extracted drug free plasma at the corresponding concentrations. Similarly, the recovery of IS from serum was also evaluated. As per the acceptance criteria [4], the recovery of the analyte need not be 100% but the extent of recovery of an analyte and of IS should be consistent, precise and reproducible.

2.6.5. Accuracy and precision

For determining the inter-day accuracy and precision, replicate analysis of serum samples containing similar concentration of itopride in human serum was performed on the same day. The run consisted of a calibration curve and six replicates of each LLOQ, low, mid and high quality control samples, i.e. precision and accuracy batch. The intra-day accuracy and precision were assessed by analysis of five precision and accuracy batches on different days. The evaluation of precision was based on the criteria [4] that the deviation of each concentration level should not be more than ±15.0% from the nominal concentration except for the LLOQ, for which it should not be more than ±20.0%. Similarly for accuracy, the mean value should not deviate by ±15.0% of the nominal concentration except for the LLOQ where it should not deviate by more than ±20.0% of the nominal concentration.

2.6.6. Stability

2.6.6.1. Long-term stability. To determine the long-term stability of itopride hydrochloride in human serum six aliquots of each, low and high QC samples were kept in deep freezer at $-70 \pm 5\,^{\circ}$C for 180.0 days. The samples were analyzed and concentrations obtained were compared with the nominal values of QC set and all values within ±15.0% qualified the test.

2.6.6.2. Short-term stability. Six aliquots each of the low and high unprocessed QC samples were kept at ambient temperature ($25 \pm 5\,^{\circ}$C) for 24.0 h. After 24.0 h, the samples were processed, analyzed and compared with the theoretical values and the samples were considered stable if the deviation from the nominal concentration was not more than ±15.0%.

2.6.6.3. Autosampler stability. Autosampler stability was determined by analyzing six aliquots each of low and high QC samples that were processed and reconstituted before storing at 4 °C for 30.0 h. Thereafter, samples were analyzed and concentrations were compared with nominal values. A deviation of more than ±15.0% was undesirable.

2.6.6.4. Freeze and thaw stability of frozen samples. Effect of three freeze and thaw cycles on stability of frozen serum samples containing itopride hydrochloride was determined to establish the ruggedness of the method. Six aliquots each of low and high un-extracted quality control samples were stored at $-70 \pm 5\,^{\circ}$C and subjected to three freeze–thaw cycles. After the completion of third cycle the samples were processed, analyzed and results were compared with nominal values. The values were expected to fall within ±15.0% of the theoretical concentration.

2.6.6.5. Effect of dilution with drug free matrix. Although the range of a calibration curve is generally based on the reported C_{max} considerations, still there are instances that some of the samples will need to be diluted if their concentrations lie above the upper limit of linearity range. In such cases, reanalysis of those samples after appropriate dilution with drug free matrix is recommended. Therefore, it becomes mandatory to study the effect of dilution on the integrity of samples during method validation. In order to determine the effect

of dilution, known concentrations of itopride hydrochloride working solutions were added to drug free serum to obtain concentrations beyond the linearity range, i.e. 2000.0 and 4000.0 ng/ml. These samples were diluted four times with drug free serum to bring them within the linearity range of the present method and analyzed. The acceptance criteria was ±15.0% of the nominal value.

2.6.6.6. Solution stability. For determining the solution stability of itopride, working solutions (4500.0 ng/ml) were kept at 2–8 °C for 180.0 days. Thereafter, the mean detector response of itopride from three replicate chromatographic runs was compared to that of freshly prepared solutions of the same concentration. The samples qualified the criteria of stability if the deviation was within ±2.0%.

2.7. Bioequivalence study design

The above method was applied to compare the single dose oral relative bioavailability and to establish bioequivalance of 50 mg itopride hydrochloride of M/s German Remedies Specialties Ltd., India versus reference product Ganaton tablet containing 50 mg of itopride hydrochloride of M/s Abbott India Ltd., India, in healthy, adult, male, human subjects under fasting condition.

The study was conducted using an experimental design [5] of open label, balanced, randomized, two-treatment, two-sequence, two-period, single dose, crossover, bioequivalence study in healthy adult, male, human subjects under fasting conditions. The study design is depicted in Fig. 2. The subjects were pre-informed of the purpose, protocol and risk of the study. Fourteen subjects were enrolled for the study but one subject withdrew the consent before the initiation of the study and therefore the study was conducted on thirteen participants only. All subjects gave written informed consent and the protocol was approved by the local ethics committee. The study was conducted strictly in accordance with the current Good Clinical Practices (GCP) International Conference on Harmonization (ICH) and Indian Council of Medical Research (ICMR) and USFDA guidelines [5]. Blood samples were withdrawn at pre-dose 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 40.0, 60.0 min and 2.0, 4.0, 8.0, 12.0, 18.0 and 24.0 h after the oral administration of the dose. Serum was separated and stored at −70 ± 5 °C until analyzed.

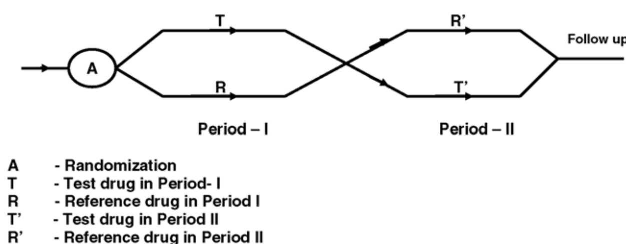


Fig. 2. Study design.

The pharmacokinetic parameters namely, maximum serum concentration (C_{max}), time point of maximum serum concentration (T_{max}), area under the serum concentration–time curve from 0.0 h to the last measurable concentration ($AUC_{0–t}$), area under the serum concentration–time curve from 0.0 h to infinity ($AUC_{0–\infty}$), elimination rate constant (λ_Z) and half-life of drug elimination during the terminal phase ($t_{1/2}$) were estimated for itopride in test and reference formulations. These pharmacokinetic parameters were estimated using non-compartmental analysis of WinNonlin Professional Software version 4.0.1 (Pharsight Corporation, USA).

The comparison of the pharmacokinetic parameters and analysis of variance (ANOVA) was carried out using SAS® Release 8.2 (SAS Institute Inc., USA) for un-transformed and ln-transformed pharmacokinetic parameters. ANOVA model included sequence, subject nested into sequence, period and formulation effects. Subject nested into sequence was be used as error term for checking the significance of sequence. These effects were considered to be statistically significant at 0.05 level of significance. Least square means for un-transformed and ln-transformed pharmacokinetic parameters C_{max}, $AUC_{0–t}$ and $AUC_{0–\infty}$ were computed.

Two one-sided 90.0% confidence intervals for both the un-transformed and ln-transformed ratios of the means, of test and reference formulations were constructed using root mean square error computed by PROC GLM. Intra-subject variability and power were calculated for un-transformed and ln-transformed pharmacokinetic parameters using root mean square error computed by PROC GLM. Power was calculated in order to obtain the probability of detecting a difference greater than or equal to 20.0% of the reference least squares mean.

Based on the statistical results of 90.0% confidence intervals for the ratios of the means of ln-transformed pharmacokinetic parameters namely, C_{max}, $AUC_{0–t}$ and $AUC_{0–\infty}$ bioequivalence was to be concluded if the 90.0% confidence interval fell within the range of 80.0–125.0% [6] for C_{max}, $AUC_{0–t}$ and $AUC_{0–\infty}$.

3. Results and discussion

3.1. Bioanalytical method validation

3.1.1. Linearity

All calibration curves were found to be linear over the calibration range of 14.0–1000.0 ng/ml. The mean correlation coefficient was 0.9993. The data is exhibited in Table 1.

3.1.2. Limit of detection and lower limit of quantitation

The LOD was 1.0 ng/ml. The lower limit of quantitation was 14.0 ng/ml (lowest standard level) with coefficient of variation of 3.19% and accuracy of 100.16%. The upper limit

Table 1
Mean concentration ($n = 5$) for calibration standards in human serum

Nominal concentration (ng/ml)	Mean concentration found (ng/ml)	CV (%)	Accuracy (%)	n
14.0	14.02	3.19	100.16	5
30.0	30.48	4.10	101.62	5
50.0	51.12	2.68	102.23	5
100.0	100.21	1.31	100.21	5
200.0	199.33	0.70	99.66	5
300.0	293.67	2.57	97.89	5
500.0	492.75	0.84	98.55	5
1000.0	999.56	2.30	99.96	5
Mean correlation	0.9993	0.077	–	5

Table 2
Recovery (extraction efficiency) of itopride from human serum

QC samples	Nominal concentration (ng/ml)	Mean recovery (%)	CV (%)	n
Low	45.0	67.17	3.58	6
Mid	450.0	66.04	2.34	6
High	800.0	69.12	4.30	6

of quantitation was 1000.0 ng/ml with coefficient of variation of 2.30% and accuracy 99.96%. Results are presented in Table 1.

3.1.3. Specificity

There was no significant interference at the retention times for itopride or internal standard from six different batches of drug free human serum used for analysis.

3.1.4. Recovery (extraction efficiency) from matrix

The mean recovery for itopride in human serum ranged between 66.04 and 69.12% and data are presented in Table 2. Although the IS was structurally unrelated, still the mean recovery for internal standard was 64.57%, which was satisfactory for the assay. Moreover, the chromatographic parameters of IS were also suitable for the assay (Fig. 3).

Table 3
Inter-day and intra-day accuracy and precision of itopride in human serum

QC samples	Nominal concentration (ng/ml)	Mean concentration found (ng/ml)	CV (%)	Accuracy (%)	n
Inter-day					
LLOQ	14.0	14.10	3.68	100.70	6
Low	45.0	44.69	2.83	99.32	6
Mid	450.0	442.78	2.55	98.40	6
High	800.0	782.47	2.31	97.81	6
Intra-day					
LLOQ	14.0	14.11	9.50	100.76	6
Low	45.0	44.75	6.33	99.44	6
Mid	450.0	436.08	6.31	96.91	6
High	800.0	779.33	5.17	97.42	6

3.1.5. Accuracy and precision

The recovery for inter-day accuracy was between 97.81 and 100.70% with the precision of 2.31–3.68% in human serum. Results are presented in Table 3. Intra-day accuracy was between 96.91 and 100.76% with the precision of 5.17–9.50% (Table 3).

3.1.6. Stability

3.1.6.1. Long- and short-term stability. Itopride was stable at -70 ± 5 °C for 180.0 days in human serum. The percent accuracy of itopride was 98.91 and 99.06% with a bias of −1.10 and −0.94% at the concentrations of 45.0 and 800.0 ng/ml, respectively. At ambient temperature (25 ± 5 °C), itopride was found to be stable over 24.0 h in human serum. The percent accuracy of was 94.51 and 93.01% with a bias of −5.49 and −6.99 % at the concentration of 45.0 and 800.0 ng/ml, respectively. Results are presented in Table 4.

3.1.6.2. Autosampler stability. In the autosampler at 4 °C, reconstituted samples of itopride were stable for 30.0 h after sample processing. Percent accuracy of reconstituted samples after 30.0 h was 96.73 and 91.60% with a bias of −3.26 and −8.40% at the two concentration levels studied (Table 4).

Table 4
Summary of stability of itopride in human serum

Stability	Nominal concentration (ng/ml)	Mean concentration found (ng/ml)	CV (%)	Accuracy (%)	Bias (%)	n
Long-term (180.0 days)	45.0	44.51	2.63	98.91	−1.10	6
	800.0	792.45	3.38	99.06	−0.94	6
Short-term (24.0 h)	45.0	42.53	1.97	94.51	−5.49	6
	800.0	744.05	1.22	93.01	−6.94	6
Autosampler (30.0 h)	45.0	43.53	1.19	96.73	−3.26	6
	800.0	732.82	1.69	91.60	−8.40	6
Freeze–thaw	45.0	41.70	6.29	92.67	−7.34	6
	800.0	749.59	3.73	93.70	−6.30	6
Effect of dilution	2000.0 diluted to 500.0 ng/ml	490.01	1.54	98.00	1.98	3
	4000.0 diluted to 1000.0 ng/ml	995.80	2.94	99.58	0.42	3

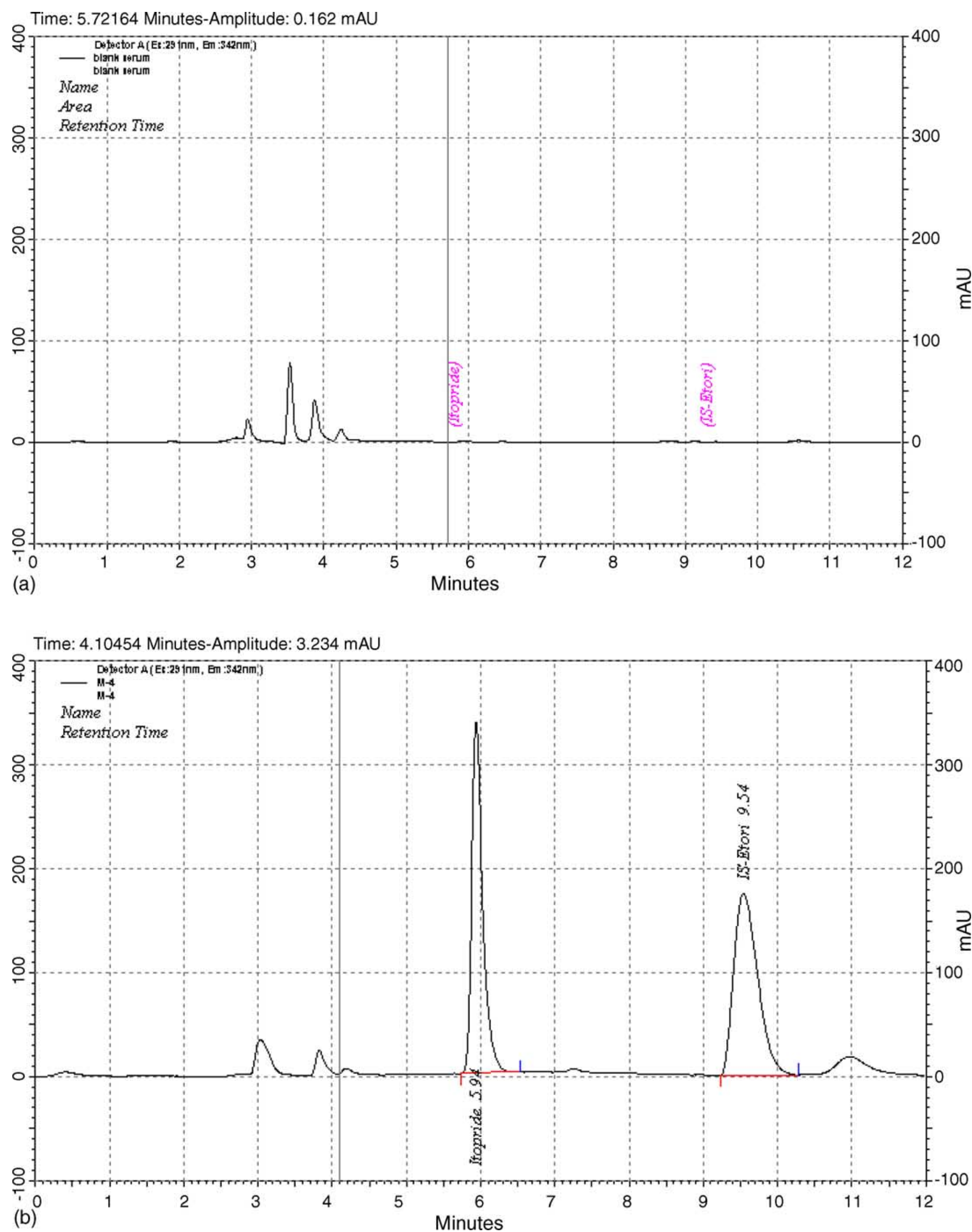


Fig. 3. (a) Representative HPLC chromatogram of blank serum; (b) representative HPLC chromatogram of mid QC plasma sample containing itopride (450.0 ng/ml) and internal standard—etoricoxib (50.0 μg/ml).

3.1.6.3. Freeze–thaw stability. Serum samples of itopride were found to be stable even after subjecting to three freeze–thaw cycles. The percent accuracy was 92.67 and 93.70% with deviation of −7.34 and −6.30% from nominal concentration as shown in Table 4.

3.1.6.4. Effect of dilution with drug free matrix. After four folds dilution of serum samples of known concentrations there was no effect on their integrity and the values were within the acceptance criteria (Table 4).

3.1.6.5. Solution stability. Working solutions of itopride and internal standard were found to be stable for 180.0 days at 2–8 °C and complied the acceptance criteria.

3.2. Statistical evaluation of pharmacokinetic parameters

The pharmacokinetic comparison between the two formulations was made in terms of extent (AUC_{0-t} and $AUC_{0-\infty}$) and rate (C_{max} and T_{max}) of absorption. The mean pharma-

Table 5
Mean pharmacokinetic parameters and 90.0% confidence interval for itopride, after the administration of an oral dose of 50 mg of test and reference formulations to healthy human volunteers

Pharmacokinetic parameters	Reference formulation (mean ± S.D.)	Test formulation (mean ± S.D.)	90% Confidence limit (%)
T_{max} (h)	0.98 ± 0.48	1.03 ± 0.46	–
C_{max} (ng/ml)	287.04 ± 69.26	294.06 ± 66.23	97.96–107.28
AUC_{0-t} (ng h/ml)	1006.96 ± 285.96	1017.51 ± 282.24	96.46–106.32
$AUC_{0-\infty}$ (ng h/ml)	1092.20 ± 304.71	1091.84 ± 299.97	95.89–104.62
$t_{1/2}$ (h)	0.26 ± 0.06	0.26 ± 0.06	–
λ_Z (1/h)	2.72 ± 0.63	2.72 ± 0.56	–

cokinetic parameters for the test and reference formulation are presented in Table 5.

3.2.1. Rate of absorption

The mean C_{max} for the reference and test formulation were 287.04 ± 69.26 and 294.06 ± 66.23, respectively (Table 5). The two one-sided 90.0 % confidence interval for the ratio of the ln-transformed means of C_{max} was found to be 97.96–107.28%. This interval was within the acceptance limit of 80.0–125.0%, required for the conclusion of bioequivalence. Sequence, period and formulation effect for both un-transformed and ln-transformed pharmacokinetic parameter C_{max} was statistically insignificant.

The intra-subject variability for ln-transformed data was 6.4%. The mean T_{max} for reference and test formulations were 0.98 ± 0.48 h and 1.03 ± 0.46 h (Table 5), respectively.

3.2.2. Extent of absorption

The mean AUC_{0-t} and $AUC_{0-\infty}$ for the reference and test formulation are presented in Table 5. The two one-sided 90.0% confidence interval for the ratios of the ln-transformed means of AUC_{0-t} and $AUC_{0-\infty}$ was found to be 96.46–106.32% and 95.89–104.62% (Table 5). These intervals were within the acceptance limits of 80.0–125.0%, required for the conclusion of bioequivalence. The intra-subject variability for ln-transformed AUC_{0-t} and $AUC_{0-\infty}$ was 6.9 and 6.2%, respectively. Sequence, period and formulation effect for both un-transformed and ln-transformed pharmacokinetic parameters, AUC_{0-t} and $AUC_{0-\infty}$ were statistically insignificant.

3.2.3. Power

The power of the test for un-transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ was found to be 100.0, 100.0 and 100.0%, respectively and for ln-transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ was found to be 100.0, 100.0 and 100.0%, respectively. This shows that the probability of detecting a difference greater than or equal to 20% of reference least square mean was 1.0. In other words, the test was capable of detecting the difference between test and reference formulation with maximum assurance.

These observations confirm that the test formulation (50 mg itopride hydrochloride of M/s German Remedies Specialties Ltd., India and reference formulation Ganaton tablet containing 50 mg itopride hydrochloride of M/s Abbott India

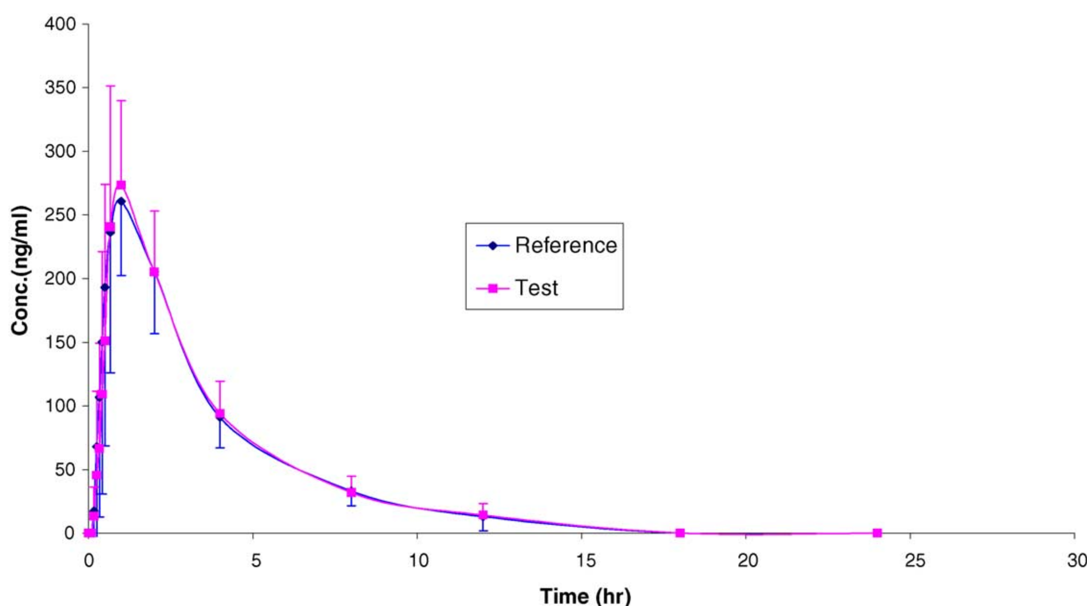


Fig. 4. Mean serum concentration vs. time graphs of itopride after administration of test and reference formulations to healthy, adult, male and human subjects under fasting condition.

Ltd., India were bioequivalent in terms of rate and extent of absorption. The mean concentration versus time graphs for the two formulations are shown in Fig. 4. In addition, there were no reports of any adverse events during the conduct of the study.

4. Conclusions

The present investigation describes a simple, sensitive and selective bioanalytical method for the estimation of itopride in serum samples. The method involved a single step liquid–liquid extraction procedure followed by chromatographic separation on a reversed phase HPLC system equipped with a fluorescence detector. The method was validated and it satisfied the requirement of linearity, recovery, accuracy, precision and stability for a bioequivalence study. The statistical analysis of pharmacokinetic parameters confirmed that the test formulation was bioequivalent with the reference in terms of rate and extent of absorption.

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