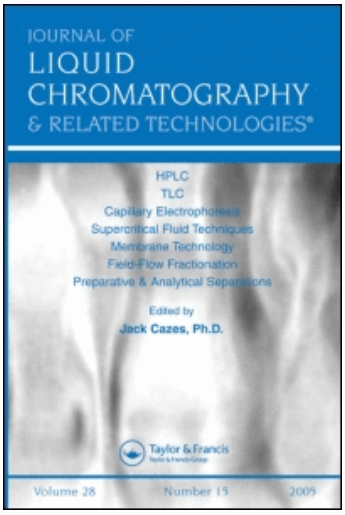

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Borges, Ney Carter do Carmo , Rigato, Hamilton Modesto , de Oliveira, Paulo Renato , Nogueira, Daniele Rubert , Moreno, Ronilson Agnaldo and Dalmora, Sérgio Luiz(2008) 'Liquid Chromatography-Tandem Mass Spectrometry Method for the Determination of Propranolol in Human Plasma and its Application to a Bioequivalence Study', Journal of Liquid Chromatography & Related Technologies, 31: 19, 2927 — 2941

To link to this Article: DOI: 10.1080/10826070802424519

URL: http://dx.doi.org/10.1080/10826070802424519

Journal of Liquid Chromatography & Related Technologies®, 31: 2927–2941, 2008

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802424519

Liquid Chromatography-Tandem Mass Spectrometry Method for the Determination of Propranolol in Human Plasma and its Application to a Bioequivalence Study

Ney Carter do Carmo Borges,[1,2] Hamilton Modesto Rigato,[1,2] Paulo Renato de Oliveira,[4] Daniele Rubert Nogueira,[4] Ronilson Agnaldo Moreno,[1,3] and Sérgio Luiz Dalmora[4]

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Abstract: A rapid, sensitive, and specific method based on liquid chromatography/tandem mass spectrometry (LC-MS/MS) was developed and validated for the determination of propranolol in human plasma using metoprolol as internal standard. The drugs were extracted from plasma by liquid-liquid extraction and separated isocratically on a Phenomenex Synergi Fusion-RP C_{18} analytical column, 4 μm (150 mm × 4.6 mm i.d.) maintained at 30°C, with acetonitrile/water (95/5, v/v): 100 mM ammonium acetate: 100 mM acetic acid (65:15:20 v/v/v) as mobile phase, run at a flow rate of 1 mL min^{-1} (split 1:3). Detection was carried out by positive electrospray ionization (ESI+) in selected reaction monitoring (SRM) mode. The chromatographic separation was obtained within 3.0 min and was linear in the concentration range of 2–150 ng mL^{-1} ($r^2 = 0.9969$). The method was successfully applied for the bioequivalence study of two tablet

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formulations (test and reference) of propranolol 80 mg after single oral dose administration to 36 healthy human volunteers, using an open, randomized, two period crossover design with one week wash out interval.The geometric means ratios of C_{max} and $AUC_{(0-\infty)}$ were 99.77 and 103.70%, respectively, with both the confidence intervals between 90.12–112.92% demonstrating the bioequivalence of the two formulations.

Keywords: Bioequivalence, Liquid chromatography-tandem mass spectrometry method, Liquid-liquid extraction, Pharmacokinetics, Propranolol, Validation

INTRODUCTION

Propranolol (Figure 1) is a competitive β receptor antagonist and remains the prototype to which other β antagonists are compared.[1] It has equal affinity for β_1 and β_2 adrenergic receptors, thus, it is a nonselective β adrenergic receptor antagonist. When access to beta-receptor sites is blocked by propanolol, the chronotropic, inotropic and vasodilator responses to β-adrenergic stimulation are decreased proportionally. The β adreno receptor antagonists are largely and successfully prescribed to patients with arterial hypertension and other cardiovascular diseases.[2,3]

Propranolol is a highly lipophilic substance and is almost completely absorbed following oral administration. However, most of the drug is metabolized in the liver during its first passage through the portal circulation, on average, about 25% reach the systemic circulation.[4] The mean peak plasma concentrations between 15–180 ng mL^{-1} are reached in 2–3 hours after a 80 mg dose.[2] There is great inter-individual variation in the presystemic clearance of propranolol by the liver, which contributes to significant variability in plasma concentrations (approximately 20 fold) after oral administration of the drug, as well, to the wide range of doses required to produce clinical efficacy.[5]

Propranolol is extensively metabolized to 4-hydroxyl-propranolol followed by conjugation with glucuronic acid.[6] The elimination half life ($T_{1/2}$) of propranolol has been reported ranging from 3 to 6 hours or approximately 3.9 hours. Propranolol has a large volume of distribution (4 L kg^{-1}) and readily enters the Central Nervous System. Approximately, 90% of the drug is bound to plasma proteins.[4]

A reversed-phase liquid chromatography (RP-LC) method was developed with fluorimetric detection in the linear range of 3.13–100 ng mL^{-1} and applied for the determination of propranolol in human plasma.[7] The RP-LC with UV detection was developed for the determination of propranolol in animal plasma.[8] Also, the RP-LC was partially validated and applied for the bioequivalence study of propranolol tablets in human plasma with the linear range from 15–180 ng mL^{-1} and retention time of

9.67 min.[9] A chromatography/tandem mass spectrometry (LC-MS/MS) method using electrospray ionization in positive ion mode was validated for simultaneous quantitation of ten antiarrhythic drugs, including propranolol, in human plasma samples after extraction and deproteinization with acetonitrile. The method was suggested with potential application to clinical research in the linear range from 2–400 ng mL^{-1} with the run time of 7.5 min.[10] The LC-MS method was also developed and validated, with liquid-liquid extraction, for the determination of three beta-blockers including propranolol in rabbits biofluids and tissues with the limit of quantitation of 50 ng mL^{-1}.[11] The LC-MS/MS method was also applied to quantify other drugs, using propranolol as internal standard, in clinical pharmacokinetic studies.[12,13]

The aim of the present work was to develop and validate a simple, fast, precise, and accurate LC-MS/MS method, using liquid-liquid extraction, for the pharmacokinetic analysis of propranolol in human plasma supporting the bioequivalence study of two pharmaceutical formulations.

EXPERIMENTAL

Chemicals and Reagents

The test and reference formulations containing 80 mg of propranolol were manufactured by the Pharmaceutical Company Prati Donaduzzi, Batch A (Brazil) and Sigmapharma, Batch 05F215, (Brazil), respectively, within their shelf life period. Propranolol reference substance (Batch 1005) was purchased from Farmacopeia Brasileira (Brazil). Metoprolol tartarate (internal standard, IS) (Batch 0603024001) was obtained from Galena Química e Farmaceutica Ltda (Brazil). HPLC grade acetonitrile and tert-butyl methyl ether were obtained from Tedia (Fairfield, CT, USA). All chemicals used were of pharmaceutical or special analytical grade. For all the analyses, ultrapure water was purified using an Elix 3 coupled to a Milli-Q Gradient A10 system (Millipore, Bedford, MA, USA). All solutions were filtered through a 0.22 μm membrane filter (Millipore, Bedford, MA, USA).

Apparatus and Chromatographic Conditions

The LC-MS/MS method was performed on a Shimadzu LC system (Shimadzu, Kyoto, Japan) equipped with a SCL-10A_{VP} system controller, LC-10 AD_{VP} pump, DGU-14A degasser, and CTO-10 AD_{VP} column oven. A triathlon autosampler (Spark, Emmen, The Netherlands) was used. The experiments were carried out on a reversed phase Phenomenex (Torrance, USA) Synergi Fusion-RP C_{18} analytical column (150 mm × 4.6 mm I.D., with a particle size of 4 μm and pore size of 100 Å) maintained at 30°C.

The LC system was operated isocratically using a mobile phase of acetonitrile/water (95:5, v/v): 100 mM ammonium acetate: 100 mM acetic acid (65:15:20, v/v/v). This was filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA, USA) and run at a flow rate of 1.0 mL min^{-1} (split 1:3). The injection volume was 30 μL for both standard and samples. Under these conditions, typical standard retention times were 1.6 min for propranolol and 1.8 min for metoprolol (IS), and a back pressure value of approximately 1100 psi was observed. The temperature of the autosampler was kept at 5°C and the run time was 3.0 min. The triple quadrupole mass spectrometer (Micromass, Manchester, UK), model Quattro LC, equipped with an electrospray source using a crossflow counter electrode run in positive mode (ESI+), was set up in selected reaction monitoring (SRM) mode, monitoring the transitions $260 > 116.1$ and $268 > 116.1$, for propranolol and IS (Figure 1), respectively. For the mass spectrometer conditions optimization, a mixed standard solution containing propranolol and IS was directly introduced and the following parameters were selected: nebulizer gas (nitrogen), cone gas and desolvation gas set at 120, 30, and 520 L h^{-1}, respectively. Capillary voltage, extractor voltage, RF lens voltage, and source temperature were 3.0 kV, 3 V, 0.2 V, and 500°C, respectively. The dwell time was set at 0.2 seconds; the collision gas pressure (argon) was 2.3×10^{-3} mbar. The collision energy was 18 V, and the cone voltage was 33 and 35 V for propranolol and IS, respectively. Data acquisition and analysis were performed using the software Masslynx (v 3.5).

Standard Solutions and Calibration Curves

The stock solution of propranolol was prepared by weighing 10 mg of reference material into a 10 mL volumetric flask and diluting to volume with methanol:water (90:10, v/v), obtaining a concentration of 1 mg mL^{-1}. Metoprolol (IS) stock solution was also made at a final concentration of 1 mg mL^{-1} using methanol:water (90:10, v/v). The prepared stock solutions were stored at 2–8°C protected from light. Analytical curves of propranolol were prepared by spiking blank plasma at concentrations from 2 to 150 ng mL^{-1}. The quality control (QC) samples were prepared in blank plasma at concentrations of 5 (low), 60 (medium), and 120 ng mL^{-1} (high), and then divided in aliquots that were stored at −80°C until analysis. The spiked plasma samples (standards and quality controls) were extracted on each analytical batch along with the unknown samples.

Plasma Extraction

For the analysis of propranolol, 300 μL of each plasma sample was transferred to a 15 mL glass tube, followed by addition of 50 μL of IS solution

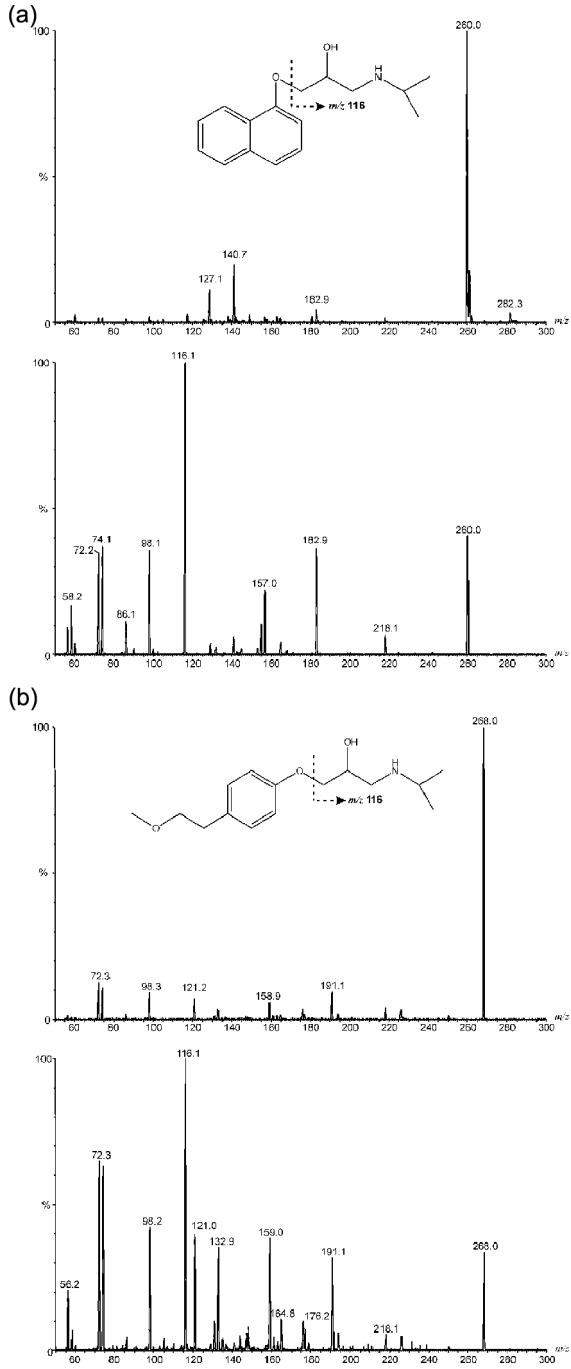

Figure 1. Full scan positive ion mass spectra in upper trace and product ion spectra in lower trace, and chemical structures with the proposed fragmentation pathway of propranolol (a) and metoprolol (b).

(300 ng mL^{-1}). All samples were mixed by vortex agitation for 30 s. Then, twenty microliters of ammonium hydroxide: water (1:1) was added into the tube. After vortexing for 30 s, a 4 mL aliquot of extraction solvent, tert-butyl methyl ether, was added using a Dispensette Organic (Brand GmbH, Wertheim, Germany) and vortex mixed for 90 s. The tubes were centrifuged for 15 min at 2700 rpm and the organic layer was filtered through a Millex GV 0.45 μm filter unit (Millipore, Bedford, MA, USA) into 15 mL conical tubes and evaporated under a nitrogen stream while immersed in a 40°C water bath. The residues were reconstituted with 300 μL of mobile phase. The samples were transferred to autosampler vials and 30 μL was injected into the LC-MS/MS system.

Validation of the Bioanalytical Method

The method was validated by the determination of the following parameters: specificity, linearity, range, recovery, accuracy, precision, lower limit of quantitation (LLOQ), and stability studies, following the bioanalytical method validation guidelines.[14,15] To evaluate the matrix effects, three replicates of low, medium, and high QC samples were spiked, each one with six samples of blank human plasma from different sources. The mean peak areas of each QC were compared to the mean peak areas of the neat standard (propranolol and IS dried and reconstituted in mobile phase) at the same concentrations.

Specificity was assessed using six blank human plasma samples, randomly selected, from different sources (including haemolysed and lipemic plasma), that were subjected to the extraction procedure and chromatographed to determine the extent to which endogenous plasma components could interfere in the analysis of propranolol or the IS. The results were compared to a solution containing 2 ng mL^{-1} of propranolol.

The analytical curves were constructed from a blank sample (plasma sample processed without IS), a zero sample (plasma processed with IS), and eight concentrations of propranolol, including the LLOQ, ranging from 2 to 150 ng mL^{-1}. The peak area ratio of the drug to the IS against the respective standard concentrations was used for plotting the graph and the linearity evaluated by a weighed (1/x) least square regression analysis. The acceptance criteria for each calculated standard concentration was not more than 15% deviation from the nominal value, except for the LLOQ, which was set at 20%.

The recovery was evaluated by the mean of the response of three concentrations of propranolol (5, 60, and 120 ng mL^{-1}), each one with addition of 50 ng mL^{-1} of the IS, dividing the mean of the extracted sample by the mean of the unextracted sample (spiked with the extracted blank plasma residues) at the same concentration level. To eliminate

the matrix effects, a comparison of the extracted to the unextracted sample was performed, giving the true recovery.

To evaluate the inter-day precision and accuracy, QC samples were analyzed together with one independent analytical standard curve for 3 days, while intra-day precision and accuracy were evaluated through analysis of the QC samples in six replicates in the same day. Inter- and intra-day precision were expressed as relative standard deviation (RSD). The evaluation of precision and accuracy was based on the criteria[15] that the RSD of each concentration level should be within ±15% of the nominal concentration.

The lowest standard concentration on the analytical curve should be accepted as the limit of quantitation if the following conditions are met: the analyte response at the LLOQ should be at least five times the response compared to blank response and analyte peak (response) should be identifiable, discrete, and reproducible with a precision of 20% and accuracy of 80–120%. The limit of detection (LOD) was defined by the concentration with a signal-to-noise ratio of 3.

The stability of propranolol in human plasma was evaluated after each storage period and related to the initial concentration as zero cycle (samples that were freshly prepared and processed immediately). The samples were considered stable if the deviation (expressed as percentage bias) from the zero cycle was within ±15%. The freeze-thaw stability of propranolol was determined at low, medium, and high QC samples ($n = 3$) over three freeze thaw cycles within 3 days. In each cycle, the frozen plasma samples were thawed at room temperature for 2 h and refrozen for 24 h. After completion of each cycle the samples were analyzed and the results compared with that of the zero cycle. The short term stability was evaluated using three aliquots each of the low, medium, and high unprocessed QC samples kept at room temperature ($25 \pm 5°C$) for 8 h, and then analyzed. For the processed sample stability, three aliquots each one of the low, medium, and high QC samples were processed and placed into the autosampler at 5°C and analyzed after 24 and 48 h. For the long term stability analysis of propranolol, three aliquots of each QC samples were frozen at −80°C for 107 days and then analyzed.

Bioequivalence Study

The study was an open, randomized, two period crossover design with a one week washout interval between the doses. Thirty six healthy volunteers of both sexes, aged between 18 and 50 years, and within 15% of the ideal body weight were selected by clinical evaluation and laboratory tests. The clinical protocol was approved by the local Ethics Committee and the volunteers given written informed agreements to participate in

the study. During each period, a single oral dose of propranolol (1 tablet 80 mg) was given with 200 mL of water after an overnight fast of at least 8 hours. The dose chosen for the study was selected because it is clinically relevant in hypertension, cardiac ischemia, arrhythmias, and other cardiovascular diseases, and it was expected to produce measurable plasma levels for a sufficient portion of the terminal elimination phase. Blood samples were collected at 0 h (predose) and at: 0.25, 0.5, 0.75, 1.0, 1.33, 1.67, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 14.0, and 24.0 hours post dosing. The samples were centrifuged immediately (at 3000 rpm for 15 minutes at 4°C), and the plasma separated and kept frozen at −80°C in labeled cryogenic tubes until assayed.

Pharmacokinetics and Statistical Analysis

The pharmacokinetic parameters of propranolol, namely: the maximum plasma concentration (C_{max}) and time point of maximum plasma concentration (T_{max}) were obtained directly from the measured data. The area under the propranolol plasma concentration time curve ($AUC_{(0\text{-}t)}$) was computed using the linear trapezoidal rule, whereas, the area under the concentration plasma – time curve from time 0 to the infinity ($AUC_{(0\text{-}\infty)}$) was calculated as the sum of $AUC_{(0\text{-}t)}$ and Ct/ke, where t was the time of the last measurable concentration (Ct) and ke was the elimination rate constant. The 4-hydroxypropranolol metabolite was not assessed for the purpose of the study as recommended.[16,17]

The pharmacokinetic parameters of propranolol were estimated with softwares R2.2, Microsoft Excel, Tinn-R, Win-Edit, Scientific Work Place, and Equivtest.

In general, a multiplicative model was assumed for concentration dependent parameters, implying a logarithmic normal distribution, whereas an additive model with normal distribution of non-transformed data was assumed for time related parameters. The pharmacokinetic parameters that describe the early and total exposure to propranolol (C_{max}, $AUC_{(0\text{-}\infty)}$, and T_{max}) were derived from the individual plasma concentration time profiles and subjected to statistical analysis. Comparison of the secondary kinetic parameters ($AUC_{(0\text{-}t)}$, k_e, $T_{1/2}$) was only descriptive.

After logarithmic transformation, $AUC_{(0\text{-}\infty)}$ and C_{max} values were subjected to analysis of variance (ANOVA), including the terms for subjects, treatment (sequence), and time period; the residuals of which were then tested for normality, as described by Chow and Liu.[18] For the evaluation of bioequivalence, the point estimates and 90% confidence interval (C.I.) for the relative difference between the test and reference formulations (test/reference) in each subject were constructed, using the residual mean square error obtained from the multifactorial ANOVA.

The bioequivalence between the two formulations was evaluated based on the 90% C.I. transformed back for the geometric mean ratios of $AUC_{(0\text{-}\infty)}$ and C_{max}, which are recommended within the 80–125% interval according to the guidelines.[16,17]

RESULTS AND DISCUSSION

To obtain the best chromatographic conditions, different columns and mobile phases consisting of acetonitrile-water or methanol-water were tested to provide sufficient selectivity and sensitivity in a short separation time. Modifiers such as formic acid, acetic acid, and ammonium acetate were tested. The best signal was achieved using acetonitrile/water (95/5, v/v): 100 mM ammonium acetate: 100 mM acetic acid (65:15:20, v/v/v) as mobile phase with a flow rate of 1.0 mL min^{-1} (split 1:3) in a C_{18} analytical column. The protonated molecular ions $[M + H]^+$ of propranolol and IS observed on the full scan mass spectra were m/z 260 and 268, respectively. The MS-MS transition $260 > 116.1$ and $268 > 116.1$ were selected since the ion scan product with m/z 116.1 for both compounds presented a higher abundance and stability for propranolol and IS. Figure 1 shows the proposed fragmentation for propranolol and metoprolol. The coupling of LC with MS-MS detection in the SRM mode showed high specificity because only the ions derived from the analytes of interest were monitored.

Matrix effects and the possible ionization suppression or enhancement of propranolol and internal standard were examined (Figure 2). A sample of human pooled blank plasma was extracted by the extraction procedure. The reconstituted extract was injected into the LC system while the mixture of the analyte and internal standard was being infused. In this system, no ion suppression was observed as a depression of the MS signal.

Linearity was evaluated by six determinations of eight concentrations in the range of 2–150 ng mL^{-1}. The values of the determination coefficient ($r^2 = 0.9969$, $y = 0.0205x + 0.0053$) indicated significant linearity of the analytical curves for the method. The LLOQ was evaluated in an experimental assay and was found to be 2 ng mL^{-1} with precision and accuracy lower than 20%. Comparison of the blank and spiked human plasma (2 ng mL^{-1}) chromatograms indicated that no significant interferences were detected from endogenous substances. The low retention times of 1.6 and 1.8 min allow rapid determination of the drug, which is an important advantage.

Propranolol in human plasma was directly extracted with tert-butyl methyl ether by liquid–liquid extraction. The mean extraction recoveries for the three concentration levels of the QC samples were 103.03% for

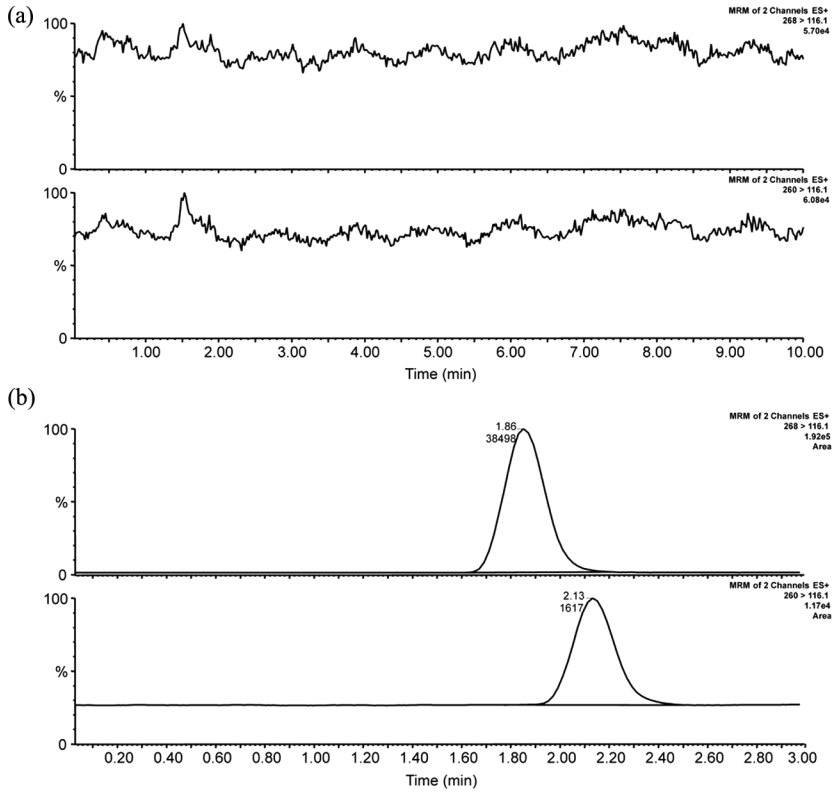

Figure 2. Ion suppression evaluation. (a) Blank plasma sample; (b) Blank plasma spiked with propranolol (LLOQ, 2 ng mL^{-1}) and 50 μL of metoprolol (IS, 300 ng mL^{-1}).

propranolol and 94.25% for the internal standard, metoprolol, showing the method suitability (Table 1).

The intra-day accuracy of the method was within 101.09 and 109.91% with a precision of 2.49–4.30%. The inter-day accuracy was within 99.53 and 101.83% with RSD of 6.77–9.29% (Table 2). The data show that the method possesses adequate repeatability and reproducibility.

Propranolol was stable in neat plasma for up to 8 h at room temperature (short-term) and also after three freeze thaw cycles, demonstrating that human plasma samples could be thawed and refrozen without compromising the integrity of the samples. Plasma samples were stable for at least 107 days at −80°C (long-term) as shown in Table 3. The results demonstrated that extracted samples could be analyzed after being kept in the autosampler for at least 48 h with acceptable precision and accuracy.

Table 1. Recovery of propranolol and metoprolol from human plasma after the extraction procedure

Nominal concentration (ng mL^{-1})	Recovery (mean ± RSD[a] %)	
	Propranolol[b]	Metoprolol[b]
5	108.91±6.84	92.49±11.60
60	101.60±7.05	95.93±7.14
120	98.58±6.36	94.32±10.22
Mean	103.03±5.16	94.25±1.83

[a]RSD = Relative standard deviation.
[b]Mean of six replicates.

Table 2. Inter-day and intra-day precision and accuracy for the determination of propranolol in human plasma

Nominal concentration (ng mL^{-1})	RSD[a] (%)		Accuracy (%)	
	intra-day[b]	inter-day[c]	intra-day[b]	inter-day[c]
5	2.49	9.29	101.09	100.70
60	3.97	8.83	109.91	99.53
120	4.30	6.77	107.04	101.83

[a]RSD = Relative standard deviation.
[b]Mean of six replicates.
[c]Mean of three days.

Table 3. Long term stability of propranolol in human plasma

Nominal concentration (ng mL^{-1})	Fresh samples (zero cycle)			107 days samples (kept at −80°C)		
	Concentration[a] (ng mL^{-1})	RSD[b] (%)	Bias[c] (%)	Concentration[a] (ng mL^{-1})	RSD[b] (%)	Bias[c] (%)
5	4.96	4.44	−0.80	5.12	3.31	2.40
60	55.33	6.67	−7.78	61.47	4.88	2.45
120	120.6	5.52	0.50	127.03	3.83	5.85

[a]Mean of three replicates.
[b]RSD = Relative standard deviation.
[c]Bias = (measured concentration–nominal concentration/nominal concentration)× 100.

Table 4. Mean pharmacokinetic parameters for propranolol, after a single 80 mg oral dose administration of test and reference formulations to 36 healthy volunteers

	Test		Reference	
Parameter	Mean	SD[a]	Mean	SD[a]
C_{max} (ng mL^{-1})	70.46	40.58	70.73	40.76
T_{max} (h)	1.57	0.78	1.59	0.74
K_e (h-1)	0.19	0.05	0.18	0.05
$T_{1/2}$ (h)	3.90	1.17	4.12	1.13
$AUC_{(0\text{-}t)}$ (ng mL^{-1} h)	411.51	215.29	409.68	248.11
$AUC_{(0\text{-}\infty)}$ (ng mL^{-1} h)	442.18	220.64	438.39	252.38

[a]SD = Standard deviation.

The mean pharmacokinetic parameters after a single 80 mg oral dose administration of test and reference products to thirty six healthy volunteers, and the sex differences of the parameters detected are presented in Tables 4 and 5, respectively.

No period effect was observed in the pharmacokinetic parameters studied (data not shown). The curve of the mean propranolol plasma concentration versus time obtained after a single oral dose of each propranolol formulation is shown in Figure 3.

Table 5. Geometric mean of the individual $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}\infty)}$ and C_{max} ratios (test/reference formulation), the respective 90% confidence intervals (CI) and power

Test/Reference	Geometric	90% CI	Power	Intra-subject (RSD[a] %)
Parametric $n = 36$				
$AUC_{(0\text{-}t)}$ % ratio	103.50	94.36–113.57	0.99	23.51
$AUC_{(0\text{-}\infty)}$ % ratio	103.70	95.19–112.92	0.99	21.68
C_{max} % ratio	99.77	90.12–110.45	0.97	25.94
Men–Parametric $n = 18$				
$AUC_{(0\text{-}t)}$ % ratio	101.70	85.24–121.23	0.69	30.76
$AUC_{(0\text{-}\infty)}$ % ratio	102.00	86.48–120.40	0.74	28.83
C_{max} % ratio	95.60	78.57–116.31	0.60	34.45
Women–Parametric $n = 18$				
$AUC_{(0\text{-}t)}$ % ratio	104.70	97.23–112.71	0.99	12.67
$AUC_{(0\text{-}\infty)}$ % ratio	104.40	97.67–111.53	0.99	11.28
C_{max} % ratio	103.40	94.53–113.09	0.99	15.39

[a]RSD = Relative standard deviation.

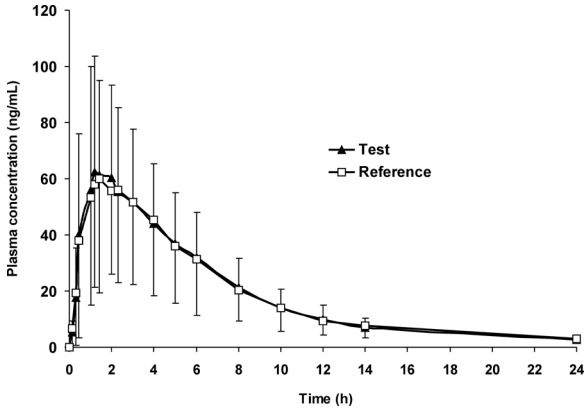

Figure 3. Mean plasma concentration – time profile of propranolol after a single 80 mg oral dose administration to 36 healthy volunteers.

At any of the evaluation times, the mean values and the concentrations of propranolol showed non significant differences between the individual subjects studied after the administration of each of the 2 formulations. The mean $C_{max,}$ obtained at 1.5 hours, were 70.45 ng mL^{-1} and 70.72 ng mL^{-1} for test and reference formulations, respectively.

Further statistical analysis of pharmacokinetic variables that described the early and total exposure to propranolol showed point estimates of the geometric means ratios of C_{max} and $AUC_{(0\text{-}\infty)}$ (propranolol test vs. propranolol reference) to be 99.77% (90% CIs: 90.12–110.45) and 103.70% (90% CIs: 95.19–112.92), respectively. For median T_{max} values, no significant differences ($p = 5\%$) were found between the two formulations evaluated.

CONCLUSION

A simple, fast, and accurate LC-MS/MS method, with a single step liquid-liquid extraction procedure, has been developed and validated for the determination of propranolol in human plasma supporting a pharmacokinetic and bioequivalence study. The statistical analysis demonstrated that none of the parameters accepted for drug bioavailability ($AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}\infty)}$, C_{max}) were significantly different between the treatments for the single dose data. Moreover, it indicated that the two pharmaceutical products showed similar bioavailability profiles and therefore are considered bioequivalent with regard to the extent and rate of absorption and, interchangeable as well, for clinical and therapeutic purposes.

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Received April 11, 2008
Accepted May 2, 2008
Manuscript 6324