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Development and validation of a UPLC-ESI-MS/MS method for the determination of N-butylscopolamine in human plasma: Application to a bioequivalence study

Wagner Alex Jann Favreto,* Ana Maria Pugens Pinto, Josélia Larger Manfio, Karina Graziella Fiametti, Maycon Fernando Percio and Mauricio Bedim dos Santos

A sensitive and fast ultra performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS) method for measurements of N-butylscopolamine in plasma was developed and validated. A single protein precipitation was proposed for the clean up of the plasma and N-methylhomatropine was added as internal standard (IS). The analyses were carried out using a C_{18} column and mobile phase of acetonitrile: 5 mM ammonium acetate + 0.1% formic acid (90:10, *v/v*). The triple quadrupole mass spectrometer equipped with an electrospray source in positive mode, was set up in selective reaction monitoring, to detect precursor → product ion 360.0 → 194.0 *m/z* and 290.3 → 138.0 *m/z* transitions, for N-butylscopolamine and IS, respectively. The method was linear in 0.03 (lower limit of quantitation; LLOQ) – 10.00 ng/ml range for N-butylscopolamine. Satisfactory selectivity, linearity, precision, accuracy, and robustness were obtained for the UPLC-ESI-MS/MS method. The proposed method was successfully applied to a pharmacokinetic study of healthy human volunteers; the results showed that the two scopolamine butylbromide formulations tested are not bioequivalent in rate and extent of absorption. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: N-butylscopolamine; UPLC-ESI-MS/MS; plasma; protein precipitation; bioequivalence

Introduction

Butylscopolamine, also known as scopolamine butylbromide, butylhyoscine, and hyoscine butylbromide, is a quaternary ammonium compound. A semi-synthetic derivative of scopolamine is a kind of tropane alkaloid separated from various solanaceous species, such as the roots of Chinese traditional medicine *Anisodus tanguticus* (Maxim.) Pascher. Scopolamine has widespread physiological activities such as spasmolytic, anaesthetic, acesodyne, and ophthalmic effects. In recent years, more pharmacological activities of scopolamine have been investigated and widely noticed. Compared with comprehensive investigations of its therapeutic purpose, the study of its metabolism *in vivo* or *in vitro* is limited, although the metabolic study of scopolamine plays an important role in the development of new drugs and their clinical applications.[1] A number of analogs have been synthesized, the most common being homatropine, ipratropium, oxitropium, flutropium, and *N*-butylscopolamine. These derivatives have similar structures and are generally used for anti-cholinergic purposes. Analysis of these compounds in pharmaceutical preparations is, therefore, of special interest.[2]

The pharmacokinetic parameters of N-butylscopolamine bromide following oral administration are generally highly variable; the compound has limited bioavailability because of its high first-pass metabolism. The maximum drug concentration occurs approximately 0.5 h after oral administration.[3,4]

Capillary electrophoresis methods have been developed for the separation and determination of tropane alkaloids and scopolamine derivatives in plants.[2,5] High performance liquid chromatography (HPLC) methods have been reported for the determination of scopolamine in plant and pharmaceutical samples.[6,8] A pharmacokinetic study has been based on gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS).[9–12] In the GC-MS/MS method, the scopolamine and the internal standard (IS) mexiletine were extracted from serum by using a single step liquid/liquid extraction; however, after that, a derivatization step was necessary.[9] In the method reported by Oertel *et al*., the scopolamine and atropine (IS) were extracted and cleaned up by using solid-phase extraction (SPE). [10] This type of extraction has a high variability and is more expensive than liquid/liquid human plasma extraction. Chen *et al*. applied the LC-MS technique to identify the metabolites of scopolamine in rat, by using liquid/liquid extraction, with ethyl acetate;

* *Correspondence to: Wagner Alex Jann Favreto, Biocinese – Centro de Estudos Biofarmacêuticos Ltda, Av. Cirne Lima, 1541- Vila Becker, 85.902-400 – Toledo– PR, Brazil. E-mail: wajann8@hotmail.com*

Biocinese–Centro de Estudos Biofarmacêuticos Ltda, Av. Cirne Lima, 1541-Vila Becker, 85.902-400 - Toledo– PR, Brazil

however, a chromatographic method with both sensitivity and specificity is needed to measure plasma N-butylscopolamine levels.[1]

Ultra performance liquid chromatography (UPLC), a novel advance in rapid, sensitive, and high-resolution liquid chromatography, offers the possibility of significantly increased efficiency of the chromatographic separation through the utilization of columns packed with smaller diameter particles (1.8 μm) that can withstand higher pressures compared to conventional packing materials.[13]

The aim of the work described in this paper was to validate a simple, fast, precise, accurate, and reproducible UPLC-MS/MS method for determination of N-butylscopolamine in human plasma. The method uses protein precipitation.

Protein precipitation is widely used in downstream processing of biological samples, such as plasma, blood, and urine. This process serves to concentrate and fractionate the target product from various contaminants. The underlying mechanism of precipitation is to alter the solvation potential of the solvent and thus lower the solubility of the solute by addition of a reagent. Protein precipitation, which is an improvement over previously published procedures, showed to be applicable for the simultaneous determination of seven natural and semisynthetic tropane alkaloids in plasma: atropine (d-hyoscyamine/l-hyoscyamine), cocaine, homatropine, ipratropium, littorine, N-butylscopolamine, and scopolamine measuring within a linear range of three orders of magnitude (0.05–50 ng/ml plasma).[22] This procedure also showed to be applicable to the determination of N-butylscopolamine in bioequivalence studies using acetonitrile as precipitating agent.

Experimental

Chemical and reagents

- N-butylscopolamine bromide – Reference standard 100.0% (European Pharmacopoeia), view Figure 1A.
- N-methylhomatropine bromide – Reference IS 99.9% (US Pharmacopeia), view Figure 1B.
- Acetonitrile – LC grade (Carlo Erba, Milan, Italy).
- Methanol – LC grade (Carlo Erba, Milan, Italy).
- Formic acid – ACS for Analysis (Carlo Erba, Milan, Italy).
- Ammonium acetate – ACS for Analysis (Carlo Erba, Milan, Italy).
- Water – Obtained from Gehaka system (Gehaka, Kansas City, KS, USA).
- Anticoagulant – Vacutainer® tubes with sodium heparin (Becton & Dicskson, New Jersey, NJ, USA).

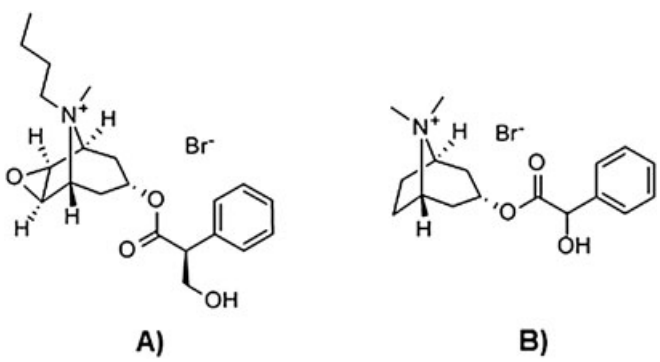

Figure 1. Chemical structure of (A) N-butylscopolamine bromide and (B) N-methylhomatropine bromide.

Apparatus and analytical conditions

The UPLC system utilized was a Waters Acquity system (Milford, MA, USA). The experiments were carried out on a reversed phase BEH Waters (Milford, MA, USA) C_{18} column (150 X 2.1 mm i.d.; 1.7 μm) and a C_{18} Kit Security Guard Cartridges was used to protect the analytical column. The UPLC system was operated at 40 C. The flow rate of the mobile phase under isocratic condition was kept at 0.5 ml/min; 66.7% of column eluate was diverted to waste, and 33.3% was allowed to reach the mass spectrometer. The autosampler was set at 5 C and the injection volume was 7.5 μl. Mobile phase consisted of acetonitrile: 5 mM ammonium acetate + 0.1% formic acid (90:10, *v/v*). The mobile phases were filtered through a 0.2 μm membrane filter (Millipore, Bedford, MA, USA).

Analyte and IS were detected on a triple-quadrupole mass spectrometer (Quattro Premier XE Atmospheric Pressure Ionization, Waters Micromass, Milford, MA, USA) equipped with electrospray ion source, operating in the positive ion mode. MassLynx software version 4.1 was used to control all parameters of LC and MS. Quantitation was performed using selective reaction monitoring (SRM) mode to study precursor → product ion transitions for N-butylscopolamine (m/z 360.0 → 194.0) and for N-methylhomatropine (m/z 290.3 → 138.0). Source-dependent parameters optimized were gas 1 (nebulizer gas): 50 l/h; gas 2 (heater gas): 600 l/h flow; ion spray voltage: 3500 V; temperature: 450 °C. Compound-dependent parameters set were declustering potential (DP), 40 V (N-butylscopolamine) and 40 V (N-methylhomatropine); collision energy (CE), 25 V (N-butylscopolamine) and 35 V (N-methylhomatropine). Entrance and exit potential was 1 V for both analyte and IS. Argon was used as collision-induced dissociation (CID) gas was set at 4.0×10^{-3} bar and the electron multiplier was set at 900 eV. The MS was maintained at unit resolution and dwell time was set at 0.4 s.

Preparation of stock solutions, calibration standards, and quality control (QC) samples

The stock solutions of N-butylscopolamine and IS were prepared by dissolving accurately weighed amounts in methanol to give a final concentration of 1000 μg/ml and 500 ng/ml respectively. The standard stock solution of N-butylscopolamine was diluted with methanol to obtain a series of working solutions from 3–1000 ng/ml concentration range. All the solutions were stored at 4–8 C and were left in room temperature before use. Blank human blood was collected into a heparin vacutainer from healthy and drug-free volunteers. After centrifugation, blank plasma was collected and stored at −20 C until use. The calibration standards and QC samples were prepared by spiking blank plasma with working solution. Calibration standards were made at 0.03, 0.10, 0.25, 0.50, 1.00, 2.50, 5.00, and 10.00 ng/ml for N-butylscopolamine. The QC samples were prepared in pooled plasma, with the concentrations of 0.09 (low), 4.50 (medium), and 9.00 ng/ml (high) for N-butylscopolamine. They were divided in aliquots that were stored at −20 C until analysis.

Plasma preparation procedure

Five hundred μl of the spiked plasma was transferred to a 15-ml glass tube, followed by addition of 50 μl of IS solution (50 ng/ml of N-methylhomatropine in methanol). Then, acetonitrile was added using Dispensette Organic (Brand GmbH, Wertheim, Germany)

in a 4000 µl aliquot of protein precipitation agent. The tubes were vortex-mixed for 1.5 min, and then centrifuged (Eppendorf 5804 R, rotor A-4-44 with 17 cm of radius) for 10 min at 5000 g at 4 °C. The supernatant liquid was transferred into 15-ml conical glass tubes and evaporated under nitrogen stream while immersed in a 45 °C water bath. Each sample was reconstituted with 100 µl of mobile phase. The samples were transferred to autosampler vials and 7.5 µl was injected into the UPLC system.

Bioanalytical method validation

The method was validated by the determination of the following parameters: matrix effects, selectivity, linearity, range, recovery, accuracy, precision, LLOQ, and stability studies.[14]

Selectivity and matrix effect

The selectivity was assessed using six blank human plasma samples, randomly selected, from different sources (including haemolyzed and lipemic plasma), that were subjected to the precipitation procedure and analyzed to determine the extent to which endogenous plasma components could interfere in the analysis of the N-butylscopolamine and IS. The results were compared to a solution (LLOQ) containing 0.03 ng/ml (N-butylscopolamine) and 50.00 ng/ml (N-methylhomatropine).

The matrix effects were evaluated according to the literature.[15–20] To evaluate, 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 samples were compared to the mean peak areas of the neat references (N-butylscopolamine and IS dried and reconstituted in mobile phase) at the same concentrations.

Calibration curves

The calibration curves were constructed from a blank sample (a plasma sample processed without IS), a zero sample (a plasma sample processed with IS), and seven concentrations including the LLOQ, ranging from 0.03 to 10.00 ng/ml for N-butylscopolamine. The blank and the zero samples were used to check any kind of interference, from volunteer plasma, for example or another type of mistake from analytical process. The ratio of the peak area of the drug to that of the IS versus the respective standard concentrations was used for plotting the graph, and the linearity was evaluated by a weighted ($1/x^2$) least-squares 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%.

Recovery

The analytical recovery was calculated (Equation (1)) by comparing chromatographic peak areas from unprecipitated standard samples and from precipitated standard samples at three different concentrations 0.03, 4.50 and 9.00 ng/ml for N-butylscopolamine and 50.00 ng/ml for the IS. The unextracted standard was prepared with samples of plasma where the addition of IS occurs after plasma extraction procedure. The extracted sample was prepared with normal plasma extraction procedure.

$$\text{Recovery } \% = (\text{extracted mean}/\text{unextracted mean}) * 100 \quad (1)$$

Lower limit of quantitation (LLOQ) and limit of detection (LOD)

The lowest standard concentration on the calibration curve should be accepted as the limit of quantification 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%.[21] The lowest detected concentration LOD should be identifiable, but it doesn't need to be reproducible. It should be accepted as the LOD if the following conditions are met: the analyte response at the LOD should be at least three times the response compared to blank response and analyte peak (response).[21]

Accuracy and precision

To evaluate the inter-day precision and accuracy, the QC samples were analyzed together with one independent calibration standard curve for three days, while intra-day precision and accuracy were evaluated through analysis of validation control samples at three different concentrations in six replicates in the same day. Inter- and intra-day precision were expressed as relative standard deviation (RSD). The accuracy was expressed as the percent ratio between the experimental concentration and the nominal concentration for each sample. The evaluation of precision was based on the criteria that the deviation of each concentration level should be within ± 15%, except for the LLOQ, that should be within ± 20%.[21] Similarly for accuracy, the mean value should not deviate by ± 15% of the nominal concentration except the LLOQ where it should not deviate by ± 20% of the nominal concentration.

Robustness

The robustness was evaluated when control samples were analyzed through altered chromatography parameters. These alterations correspond to mobile phase concentration (90:10 to 85:15 *v/v*), mobile phase flux (0.5 ml/min to 0.45 ml/min) and column temperature (40 °C to 35 °C). The extraction parameters were checked during the method development in accordance with % recovery, and then the best condition was chosen.

Stability

The samples were stored at −20 °C. The freeze-thaw stability of N-butylscopolamine samples was determined at low and high QC samples (n = 3) over three freeze-thaw cycles within three days. In addition, at room temperature 22 °C, short (7 h) and processed sample stability (54 h) were also studied. Three aliquots each of the low and high QC samples were frozen at −20 °C for 94 days. The samples were analyzed and the results were compared with those of zero cycle. The samples were considered stable if the deviation (expressed as percentage bias) from the zero cycle was within ± 15%.

Bioequivalence study

Twenty-four healthy volunteers, 12 male and 12 female, ranging in age from 19 to 47 years (mean ± SD, 28.8 ± 7.9 years), in weight from 53.4 to 92.0 kg (mean ± SD, 68.5 ± 11.2 kg), and in height from 153 to 185 cm (mean ± SD, 169.0 ± 0.1 cm), and

within 15% of their ideal body weight, were enrolled. The clinical protocol was approved by the local Ethics Committee and the volunteers gave written informed consent to participate in the study. Volunteers were healthy and had no history of heart, kidney, neurological or metabolic diseases; no history of drug hypersensitivity; were not undergoing any pharmacological treatment; and female volunteers were not pregnant. The study was an open, randomized, two-period, two-group crossover trial with a one-week wash-out interval. During the first period, volunteers from group A received two tablets with 10 mg dose of Buscopan® (reference product), while volunteers from group B received two tablets with 10 mg dose of N-butylscopolamine bromide (test product). During the second period, the procedure was repeated on the groups in reverse. The tablets were administered to the volunteers in the morning, after an overnight fast, with 200 ml of water. Volunteers received standard lunch and afternoon snacks, respectively, 5 and 8 h after drug administration. Volunteers did not ingest any alcoholic drink, coffee or other xanthine-containing drinks during the trial. Furthermore, they did not take any other drug, one week before the study and during its execution. Blood samples were collected at 0:00 (pre-dose); 0:15; 0:30; 0:45; 1:00; 1:20; 1:40; 2:00; 2:20; 2:40; 3:00; 3:30; 4:00; 4:30; 5:00; 6:00; 8:00; 10:00; 12:00; 14:00; 24:00; 36:00 h after dosing. The samples were centrifuged and the plasma was stored at −20 °C until N-butylscopolamine extraction and quantification.

Statistical analysis and pharmacokinetic parameters

The maximum plasma concentration C_{max} and the time to reach it (t_{max}) were obtained directly from the plasma data.[14] The area under the plasma concentration-time curve from 0–36 h (AUC_{0-t}) was derived using the trapezoidal method and the $AUC_{0-\infty}$ was calculated as $AUC_{0-t} + C/k_e$, with C being the last measured concentration and k_e was estimated from the terminal slope of the plasma concentration-time curve after logarithmic transformation and application of linear regression, while the elimination half-life $t_{1/2}$ was calculated using ln $2/k_e$. Bioequivalence was assessed calculating the standard 90% CIs of the ratio test/reference (T/R) for the parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$.[21] Pharmacokinetics parameters and statistical analysis were calculated by non-compartmental pharmacokinetics modelling with the software WinNonlin®, version 5.0.1.

Results and discussion

The coupling of UPLC with MS/MS detection showed high selectivity because only the ions derived from the analytes of-interest were monitored by multiple reaction monitoring (MRM) mode, where the chromatograms show x axis: time in minutes and y axis: total ion count and the comparison of the chromatograms of the blank and spiked human plasma indicated that no interferences was detected from endogenous substances (Figure 2A). Typical chromatograms obtained from the proposed ultra performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS) method, with the resolution of the symmetrical peak corresponding to N-butylscopolamine and IS are shown in Figure 2B. The low retention times of N-butylscopolamine and IS, 0.76 and 0.76 min, respectively allow a rapid determination of the drugs, which is an important advantage for the routine analysis (Figure 2C).

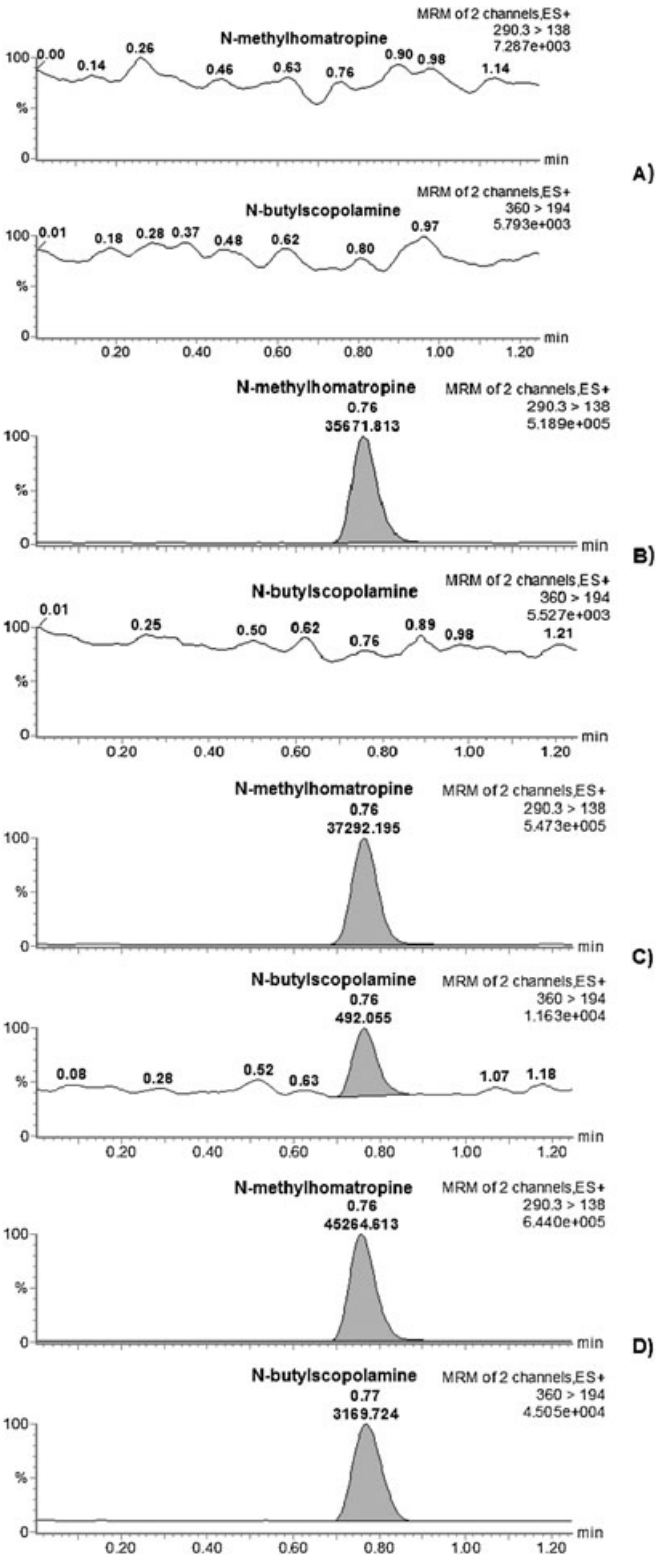

Figure 2. MRM ion chromatograms of (A) Blank normal pooled human plasma, (B) Blank normal pooled human plasma added of IS, (C) LLOQ sample (0.03 ng/ml), and (D) A representative volunteer sample time 4 h. The y-axis represents: relative intensity. Chromatographic conditions are described in text.

Matrix effects were examined and the RSD of the mean peak areas of N-butylscopolamine and IS were < 4.96%, indicating low difference in ionization efficiency using different plasma

samples. Besides, the results were higher than 93.88%, suggesting that the ion suppression by endogenous components was not interfering in the repeatability of the method.

The linearity was determined by six determinations of eight concentrations in the range of 0.03–10.00 ng/ml (N-butylscopolamine). The value of the determination coefficient (r = 0.9960) indicated significant linearity of the calibration curve for the method. The LLOQ evaluated in an experimental assay was 0.03 ng/mL (signal to noise > 5:1) and LOD was found to be 0.01 (signal to noise > 3:1) ng/ml.

The validation results demonstrated good precision and accuracy (Tables 1 and 2), in the linearized range presented, its RSD values were less than 15% for precision and among 85–115% for accuracy, in accordance with regulatory guidelines.[14] The data portrays that the method possesses adequate chromatography robustness (Table 3).

Manfio *et al*. proposed an LC-MS/MS method and prior liquid/liquid extraction for dichloromethane, linear in the range of 0.1–40 ng/ml, for N-butylscopolamine determination in serum, the recovery in their study was 69% for N-butylscopolamine, lower than comparing to this present study.[12] The results of protein precipitation of the developed method, using acetonitrile as a precipitation agent, allowed mean recoveries of N-butylscopolamine (94%) and IS (84%) at the specified concentration levels, confirming the suitability of the method for the plasma samples (Table 4). For the extraction, different organic solvents and mixtures were also evaluated, including ethyl acetate, diethyl ether, dichloromethane, and hexane; however, the recovery was worse than achieved with acetonitrile.

Table 1. Intra-day precision and accuracy for the determination of N-butylscopolamine in human plasma

Quality control (QC)	RSD (%)	Accuracy (%)
Low	5.8	105.6
Medium	3.8	108.9
High	9.5	98.7

QC: 0.09 (low), 4.50 (medium), 9.00 ng/ml (high).

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

Quality control	RSD (%)	Bias[a] (%)
Low	4.8	11.1
Medium	5.8	8.9
High	1.6	−1.3

[a]Bias = ((measured concentration - nominal concentration) / nominal concentration)) x 100.
QC: 0.09 (low), 4.50 (medium), 9.00 ng/ml (high).

Table 3. Robustness for the determination of N-butylscopolamine in human plasma

Quality Control	Mobile phase concentration		Mobile phase flux		Column temperature	
	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)
Low	7.4	105.6	0.6	111.1	6.6	108.7
Medium	3.0	106.9	10.1	104.8	6.4	105.4
High	12.8	96.2	1.0	106.4	2.3	93.7

Table 4. Recovery of N-butylscopolamine and N-methylhomatropine (IS) after the precipitation procedure

QC	Recovery (%) (mean ± RSD%)	
	N-butylscopolamine[a]	IS[a]
Low	91.4 ± 6.6	87.2 ± 9.5
Medium	95.8 ± 9.5	80.5 ± 6.3
High	95.0 ± 8.2	85.2 ± 8.7
Mean	**94.1 ± 2.5**	**84.3 ± 4.1**

[a]Mean of six replicates
QC: 0.09 (low), 4.50 (medium), 9.00 ng/ml (high).

As shown in Table 5, the plasma samples were stable for at least 94 days at −20 C (long-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. N-butylscopolamine is stable in neat plasma for up to 7 h at room temperature (short-term). The results demonstrated that precipitated samples could be analyzed after keeping in the autosampler for at least 54 h at room temperature 22 °C with an acceptable precision and accuracy.

The validated method was successfully used to quantify N-butylscopolamine in human plasma samples for the purpose of stabilizing the bioequivalence of a 10-mg formulation tablet in 24 healthy volunteers. Average values of plasmatic concentration with standard deviation versus time curves after administration of Buscopan® (reference product) and N-butylscopolamine bromide (test product) to 24 healthy volunteers are shown in Figure 3. Table 6 shows the average values of pharmacokinetic parameters after administration of reference and test products to 24 healthy volunteers.

The T_{max} difference from that presented by Renner may be caused by delayed absorption due to the tablet coating. The 90% confidence intervals for the ratio of C_{max} (79.55–135.61%), $AUC_{0\text{-}t}$ (96.79–171.36%) and $AUC_{0\text{-}\infty}$ (59.54–195.89%) values for the test and reference products are out the 80–125% interval proposed by FDA and ANVISA requirements.[1] The reference product has a rate and an extent of absorption higher than the test product. Although the samples of these products present

Table 5. Summary of stability of N-butylscopolamine in human plasma

Stability	RSD (%)	Bias (%)[a]
Short-term[c] 7 h	1.9	5.1
	1.7	−2.5
Autosampler 54 h	1.9	5.9
	1.2	−3.6
Three freeze-thaw cycles	1.9	2.9
	2.3	−0.3
Long-term[b] 94 days	13.7	0.5
	3.4	−9.8

[a]Bias = (measured concentration - nominal concentration / nominal concentration) x 100
[b]Long term = stability for at least 94 days at −20 C
[c]Short term = stability in neat plasma for up to 7 h at room temperature

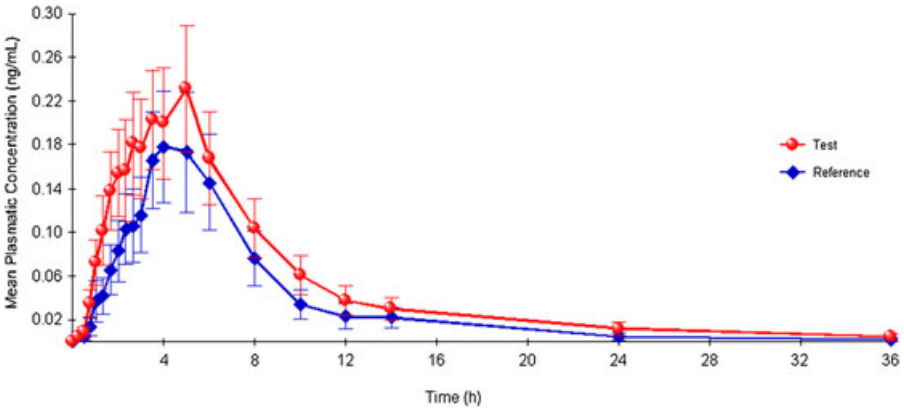

Figure 3. Average values of plasmatic concentrations with standard error after administration of reference product (Buscopan, 10 mg) and test product (N-butylscopolamine bromide, 10 mg) to 24 healthy volunteers.

Table 6. Pharmacokinetic parameters after administration of reference (Buscopan® 10 mg) and test (N-butylscopolamine bromide 10 mg) products to 24 healthy volunteers

Statistics	Buscopan® 10 mg					N-butylscopolamine bromide 10 mg				
	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-t} (ng h/ml)	$AUC_{0-\infty}$ (ng h/ml)	$t_{(1/2)el}$ (h)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-t} (ng h/ml)	$AUC_{0-\infty}$ (ng h/ml)	$t_{(1/2)el}$ (h)
Average	0.29	4.26	1.41	3.91	22.72	0.33	4.11	2.12	3.41	19.79
S.D.	0.23	1.48	1.63	7.57	60.66	0.30	1.76	2.59	3.72	60.00
C.V. (%)	77.52	34.71	115.81	193.59	266.94	91.41	42.90	122.33	109.20	303.11

the same dissolution profile *in vitro*, when they were analyzed *in vivo*, between them a significant difference was observed. The bioequivalence is primarily dependent upon on similar bioavailability which is determined by solubility, permeability and metabolism. The solubility might be studied by *in vitro* dissolution tests; on the other hand, *in vivo* permeability is a challenge. Several factors may affect the drug absorption like drug permeability through intestinal cells, carrier-transport processes, gut pH and mobility, stability of drug, blood flux, and others. For orally delivered drugs, absorption is a predominant factor in systemic bioavailability; in addition, it is essential that researchers have an accurate method to predict the *in vivo* permeability.[16]

Conclusion

A simple and fast UPLC-ESI-MS/MS method for the determination of N-butylscopolamine in human plasma was developed and validated allowing it determination in the 0.03–10.00 ng/ml range. The assay is rapid, the analysis time is only 1.2 min, and this method involves a single-step protein precipitation procedure using N-methylhomatropine as IS, where about 500 samples can be easily prepared and analyzed in one working day. The results of the validation studies show that the optimized method possesses selectivity, sensitivity, linearity, precision, and accuracy over the entire range of significant therapeutic plasma concentrations.

Moreover, the proposed method was successfully applied to a pharmacokinetic study of healthy human volunteers; the results showed that the two scopolamine butylbromide formulations studied are not bioequivalent in rate and extent of absorption.

It is important to mention that the analyses are easy and fast, which are desirable characteristics, so new studies of extractive processes and new tools for analysis instruments can contribute more sensitively and technically for the development of the pharmacokinetic studies.

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