SIMULTANEOUS ESTIMATION AND QUANTIFICATION OF OXYBUTYNIN AND ITS ACTIVE METABOLITE N-DESMETHYL OXYBUTYNINE WITH SENSITIVE HPLC-MS/MS VALIDATED METHODS IN HUMAN PLASMA

Sheeba Nair1*, Bhavesh Dasandi1, Dharmesh Parmar1, Shivprakash1 and Dr. Denish Karia2

1Synchron Research Services Private Limited, Synchron House, B/h Mondeal Park, Nr. Gurudwara, S-G Highway, Ahmedabad, India.
2Assistant Professor, Department of Chemistry, Patel J. D. K Science College, Borsad-388540 (Gujarat), India.

*Corresponding Author: Dr. Sheeba Nair

Article Received on 04/03/2019 Article Revised on 24/03/2019 Article Accepted on 14/04/2019

ABSTRACT
A simple and specific method for simultaneous determination of Oxybutynin and N-Desmethyl Oxybutynine in human plasma by liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed and validated. The analytes and the deuterated internal standard were extracted from 200 μL plasma by liquid phase extraction. Detection and quantitation was done by multiple reaction monitoring in positive ionization with Q3 LCMS-8050, Shimadzu. Mass parameters 358.11/142.10 and 329.78/95.98 and 369.48/142.08 m/z on a triple quadrupole mass spectrometer were chosen for analysis of Oxybutynin and N-Desmethyl Oxybutynine. Linearity was established in human plasma covering the concentration range 75.051 pg/mL to 7400.849 pg/mL for Oxybutynine and 75.163 pg/mL to 7411.923 pg/mL for N-Desmethyl Oxybutynine. Correlation coefficient was consistently greater than 0.98 for Oxybutynin and N-Desmethyl Oxybutynine using (Trientine-D4 and N1-Acetyl trienteine trihydrochloride D4) as internal standards. Chromatographic analysis was carried out on column Synergi Hydro-RP 80 A (4.6 x 50 mm) 4S μ with a flow rate of 0.4 mL/min, at 40˚C temperature. A Gradient elution method was applied using (A) Methanol 58% and (B) 0.1% Formic acid buffer (42%). The method was applied to support a bioequivalence study with reference scaled study design of 5 mg tablet formulation in 32 healthy Indian subjects.

KEYWORDS: Oxybutynine and N-Desmethyl Oxybutynine, LC-MS/MS, Validation, ICH.

1. INTRODUCTION
This research paper relates to development and validation of LCMS methods for estimation of Oxybutynin and N-Desmethyl Oxybutynine in API. The IUPAC name of Oxybutynin is 4-(diethylamino)but-2-ynyl 2-cyclohexyl-2-hydroxy-2-phenylacetate and IUPAC name of N-Desmethyl Oxybutynine is 2-[4-[4-[di(phenyl)methoxy]piperidin-1-yl]butanoyl]phenyl]-2-methylpropanoic acid. Oxybutynin is an antimuscarinic drug with a great selection for the muscarinic receptors of the bladder. It is used in the management of urinary frequency, urgency, and incontinence in detrusor instability and in the treatment of nocturnal enuresis. Oxybutynine molecular formula is C22H31NO3HCL with average molecular weight 357.49 g/mol. N-Desmethyl Oxybutynine molecular formula is C20H27NO3HCL with average molecular weight 329.43 g/mol.[1,2]
There are published analytical methods available in the literature reported in different species plasma with different analytical techniques, reported determination of oxybutynine alone or with Desethyl oxybutynine.\(^{[3,9, 13, 14, 16-23]}\) Determination in dog plasma by LC-ESI/MS/MS, by Kim & Han (2003)\(^{[8]}\), a Parallel achiral-chiral determination of oxybutynin, N-desethyl oxybutynin and their enantiomers in human plasma by LC-MS/MS by Sharma P and co.\(^{[4]}\). Quantitation of Oxybutynin in Rabbit Plasma Using the Varian 1200L LC/MS/MS System, developed by Garteiz, TexMS Analytical Services (2016).\(^{[23]}\) Hughesa (1992), were reported a HPLC method for Measurement of oxybutynin and its N-desethyl metabolite in plasma, Isotope dilution method\(^{[1,6]}\) and enantio separation by RP-HPLC method\(^{[8]}\) and detection by spectrophotometry, chemometry and HPTLC in presence of its degradation product and additives in different pharmaceutical dosage form\(^{[7]}\) was also reported. Reported highly sensitive and rapid simultaneous determination of Oxybutynin and its active metabolite N-Desethyloxybutynin in plasma by LC-MS/MS by (Vivek Anand PA*1) (2016).\(^{[15]}\) Spectrophotometric and spectrofluorimetric analysis of Oxybutynine in Pharmaceuticals via Reaction with Mixed Acids Anhydrides was reported with respect to application to Content Uniformity Testing.\(^{[8]}\) Gas Chromatographic Mass Spectrometric Analysis of Plasma Oxybutynin Using a Deuterated Internal Standard was also reported.\(^{[15]}\)

All the reported method has a longer run time and a high limit of quantitation. A detailed summary of chromatographic methods developed for quantitation of Oxybutynine and its active metabolite is listed in Table 1.

In the present work a sensitive, simple and rapid LC-MS/MS method was developed, optimized and validated for the simultaneous determination of Oxybutynin and its metabolite N-Desethyl Oxybutynine in human plasma. Advantages of this method include shorter runtime that is needed to achieve high throughput analysis, required for clinical, pharmacokinetic and bioequivalence studies and a very sensitive lower limit of quantitation and range ie. 75.051 pg/mL to 7400.849 pg/mL. In addition to simple sample preparation steps with less sample volume and lower limit of quantitation achieved that permit applicability of the proposed method to estimate lower concentrations of the studied drug in human plasma.

Different parameters such as linearity, range, precision, accuracy, ruggedness and robustness, limit of detection (LOD) and limit of quantification (LOQ) were used for a full validation of the method. The results were found to be acceptable as per the guidelines of International Conference on Harmonization (ICH).

The method was successfully applied for bioequivalence study in 32 healthy indicant subjects with replicated design, required accuracy and precision.

2. EXPERIMENTAL
2.1. Chemicals and reagents
Oxybutynin chloride working standard and N-Desethyl Oxybutynine working standard Oxybutynin D11 HCL, working standard were obtained from Vivian life Sciences (p) Ltd. (Mumbai India). Water used in the entire analysis was prepared using Milli - Q water purification system from Millipore (Banglore India). Blank Human Plasma (in K3EDTA was obtained from Supratech Micropath (Ahmedabad India) and was stored in -70 oC until use. Formic acid (GR Grade), Methanol [HPLC Grade], N-Hexane [HPLC Grade], Iso amyl alcohol (GR Grade) were obtained from Merck Specialties Pvt. Ltd (Mumbai India). The detection was done using LCMS instrument Nexera X2 highest pressure UHPLC. [LCMS-8050, Shimadzu] and lab solutions for data processing.

2.2. Instrumentation and chromatographic conditions
The chromatography was performed on Nexera X2 UHPLC system (Shimadzu LCMS 8050) with cooling auto-sampler and column oven enabling temperature control of the analytical column. The column utilized was Synergi 4µ Hydro-RP 80 A (4.6 x 50 mm) at 40°C temperature. A Gradient elution method was applied using (A) Methanol 58% and (B) 0.1% Formic acid buffer (42%). Detection was done by Q3 LCMS-8050, Shimadzu with ESI (+) ion mode for Oxybutynin and N-Desmethyl Oxybutynine. The auto-sampler was maintained at 10°C and the injection volume was 20µL. Total run time for each sample analysis was 3.5mins. Quantitation was achieved by MS/MS detection electrospray ionization (ESI) source operating in the positive ionization was used for multiple reaction monitoring (MRM) mode using Shimadzu LCMS-8050 mass spectrometer. The MS conditions optimized for quantification are summarized in Table 2. Lab solution software was used for instrument control and data acquisition.

2.3. Preparation of Stock solutions calibration standards and quality control samples
A stock solution of Oxybutynin hydrochloride and N-Desmethyl Oxybutynine (1000 µg/mL) was prepared by dissolving requisite amount in methanol. Working solutions were prepared by diluting the stock solution with methanol. The stock and working solutions were stored at 2-8 degree C. Stock solution (1000 µg/mL) of Oxybutynin hydrochloride and N-Desmethyl Oxybutynine were prepared by dissolving approximately 3.0mg of Oxybutynin hydrochloride and N-Desmethyl Oxybutynine to 3mL methanol to produce the solution of 1000 µg/mL of oxybutynine and N-Desmethyl Oxybutynine. Dilute 0.20 mL of this solution to 10.0mL with diluent to produce drug dilution of 20 µg/mL. Stock and working solutions were stored in Refrigerator at -
20°C. Calibration standards (CSs) and quality control (QCs) samples were prepared by spiking blank plasma with working solutions. The concentration of CSs in the range of 7400.849-75.051 pg/mL for oxybutynine and 7411.923 – 75.163 pg/mL for N-Desmethyl Oxybutynine. QC samples were prepared at five levels (76, 220, 950, 3545, 5908 for Oxybutynin hydrochloride and 76, 221, 951, 3550, 5916, 7414 for N-Desmethyl Oxybutynine) are given in Table 2.

2.4 Sample extraction procedure
To an aliquot of 0.5 mL of spiked plasma/subject samples, 25 μL of working solution was added and vortexed to mix it. Add mL mixture of N-hexane and Isopropyl alcohol (99:1) and vortex for 05 minutes. Then centrifuged for 2000 RPM for 5 mins at 5 degree c to separate the two layers. Transferred the organic layer in pre-labelled test tube and added 0.3mL reconstitution solution and vortexed for 5 mins. Then centrifuge for 2000 RPM for 5 mins at 5 degree C. Transfer the acid layer, transfer it into vial and inject 20 μL of samples using LC-MS/MS system.

2.5. Method Validation

The method was validated to meet the acceptance criteria of industrial guidance for the bioanalytical method validation. The method was validated for selectivity, linearity, precision, accuracy, matrix effect, recovery and stability.[10-12]

2.6 Pharmacokinetic Application

The application of the method was demonstrated by a bioequivalence study in 36 healthy subjects using a single dose of (Oxybutynine hydrochloride capsules 5 mg of an Indian Pharmaceutical Company) under fasting conditions. Each volunteer was judged to be in good health through medical history, physical examination and routine laboratory tests. The design of study comprised of a randomized, open label, single dose, two treatments, three periods, three sequence crossover bioequivalence study. The ethics committee approved the protocol and the volunteers provided informed written consent to participate in the study according to the principles of the Declaration of Helsinki. The study was conducted strictly in accordance with guidelines laid down by International Conference on Harmonization and USFDA. Plasma concentration–time data of Oxybutynin and N-Desmethyl Oxybutynine was analyzed by non-compartmental method using Kinetica Version 4.4 (Thermo USA). Regarding AUC0–t, AUC0–∞ and Cmax, bioequivalence was assessed by means of an analysis of variance (ANOVA) and calculating the standard 90% confidence intervals (90% CIs) of the ratios test/reference (logarithmically transformed data).

3. RESULT AND DISCUSSION

3.1 LC-MS/MS method development
There are multiple methods reported, however all have higher range of quantitation and more run time. The newly developed method have a much sensitive quantification of LLOQ (75.051 pg/mL) with minimum run time of 3.5 mins. The method was successfully applied for bioequivalence study in healthy indicant subjects with a reference replicated model of three period study.

Flow injection method with standard solution was used in order to optimize ESI conditions during tuning. Full scan mass spectra were acquired for positive mode for Oxybutynin and N-Desmethyl Oxybutynine with their labeled internal standards respectively. Oxybutynin and N-Desmethyl Oxybutynine showed higher responses in positive ionization mode. The major fragments ions of these molecules were observed in the product ion scan of the collision cell. Based on the analytes properties deuterated analogs of Oxybutynin and N-Desmethyl Oxybutynine (Oxybutynine D11 hydrochloride) were chosen as internal standards. Oxybutynin and N-Desmethyl Oxybutynine ionized efficiently in positive mode. In mass spectrometry, the parameters including temperature, flow rate of drying gas, collision gas (medium), nebulizing gas and desolvation temperature were optimized to obtain maximum response of the fragmentation. The optimized multiple reaction monitoring (MRM) conditions of mass spectroscopy applied in the method development are the transitions of m/z 358.11/142.10 and 329.78/95.98 and 369.48/142.08 m/z on a triple quadrupole mass spectrometer for Oxybutynin, N-Desmethyl Oxybutynine and Oxybutynine D11 hydrochloride respectively, with a scan time of .150 sec per transition. The disolivation temperature was 400°C.

A variety of mobile phases and columns were tried to develop a suitable method for the simultaneous estimation of Oxybutynin and N-Desmethyl Oxybutynine. Chromatographic conditions were optimized by using different ratios of combinatins of mobile phases by employing various columns to achieve good resolution and increase the signal of analytes. It was found that gradient mobile phase consisted of 420 mL of 0.1% formic acid buffer with 580 mL of methanol at a flow rate of 0.4 mL/min could achieve this purpose and was finally adopted as the mobile phase. The retention time Oxybutynin was 2.58 mins and that of N-Desmethyl Oxybutynine was 2.48 mins and Oxybutynin D11 was around 2.50 mins. The total chromatographic run time was 3.5 min. The reinjection reproducibility of (%CV) in the measurement of retention time was ≤ 0.4%.

3.2 Assay validation results
The results of system suitability, autosampler and column carryover, ruggedness and dilution integrity suggest acceptable assay performance as evident from the data presented in table 2. The selectivity of the method is evident from the chromatograms of double blank plasma, plasma spiked with Oxybutynin and Oxybutynin D11 at different concentration level and subject sample at Cmax level. No interface due to
endogenous compound was observed at the retention time of Oxybutynin and Oxybutynin D11. Further more none of the commonly used medications by human volunteers interfered at their retention times. The calibration curves showed good linearity over the established concentration range of 75.051 pg/mL to 7400.849 pg/mL for Oxybutynine and 75.163 pg/mL to 7411.923 pg/mL for N-Desmethyl Oxybutynine (r2=0.98). The mean value of slope, intercept, accuracy, and precision date in the measurement of calibration concentrations are shown in Table 1. The intra-day precision (%CV) ranged from 0.6%-4.8% and 0.9%-4.5% and accuracy was within 98.2%-103.1% and 95.7%-104.4% for Oxybutynine and N-Desmethyl Oxybutynine respectively. Similarly for inter-day experiments, the precision varied from 1.3%-3.4% and 2.7%-4.1% and accuracy was within 99.1%-100.6% and 98.4%-100.7% for Oxybutynine and N-Desmethyl Oxybutynine respectively (table 2).

The mean extraction recovery and IS-normalized matrix factor (MFs) for Oxybutynine and N-Desmethyl Oxybutynine are presented in Table 3. Highly precise extraction recovery in the range of 100.04% - 104.21% was obtained across QC level for oxybutynine. The mean recovery was 95.95% and 96.90% for Oxybutynine and N-Desmethyl Oxybutynine respectively. The presence of unmonitored, co-eluting compounds from the matrix can be directly impact the overall performance of a validated method. It is necessary to evaluate MFs to assess the matrix effect. Matrix effect was also checked in lipemic and hemolyzed plasma samples together with normal K3 EDTA plasma. This was determined by examining the precision (%cv) values of the slopes of the calibration curves prepared from eight different plasma lots, which included six K3 EDTA, one lipemic and one hemolyzed plasma samples.

Average matrix factor values (matrix factor = response of post-spiked concentrations/ response of neat concentrations) obtained for Oxybutynine and N-Desmethyl Oxybutynine were 0.96 (CV 2.1%, n = 6+2) and 0.94 (CV 2.1%, n = 6+2) at LQC and 1.0 (CV 2.1%, n = 6+2) and 1.0 (CV 3.0%, n = 6+2) for HQC, respectively, where as on ISs it was found to be 0.96 (CV 5.1%, n = 6+2) for Trientine D4 & 1.0 (CV 5.0%, n = 6+2) for N1-Acetyl Trientine D4 at tested concentration of 500 ng/mL& 100 ng/mL respectively. There were no significant matrix effects observed for any of the analytes or the ISs. Dilution integrity for Oxybutynin was evaluated by preparing DI samples with about 2 times the concentration of the high QC. Take 0.89 mL volume from drug stock (2008341.884 pg/mL) and spike of this solution in plasma to get final conc. 11871.616 pg/mL. This was diluted to 1/2nd and 1/10th, processed as per standard test method and analyzed against a calibration curve.

The accuracy of both the dilutions (1/2nd and 1/10th) were 104.9% and 94.0% respectively Oxybutynin and 103.2% and 91.8% for N-Desethyl Oxybutynin, which is within the acceptance range of %cv <15%. The precision of the samples for both the dilutions (1/5th and 1/10th) were 1.1% and 1.2% for Oxybutynin and 0.9% and 1.3% for D-Desethyl Oxybutynin, which is within the acceptance range of %cv <15%.

Stock solutions kept for short term and long term stability as well as spiked plasma solutions showed no evidence of degradation under all the studied conditions. The stability of the analytes in human plasma under different temperature and timing conditions was evaluated. QC samples were subjected to long-term storage conditions (-20°C), and to freeze–thaw stability studies. All the stability studies were conducted at two concentration levels with six determinations for each. Stability was assessed for 154 days. For process stability, the results indicated that the difference in the back-calculated concentration are stable at least for 102 hrs at 10°C in the auto-sampler.

For bench top stability, the results allowed us to conclude that both analytes are stable for at least 6:18 h at room temperature in plasma samples. The % mean ratio for the Low and High QCs were 102.10% and 98.62% for Oxybutynin and 96.29% and 96.61% for N-Desethyl Oxybutynin. Freeze and thaw stability results indicated that the repeated freeze and thawing (five cycles) did not affect the stability of Oxybutynin and N-Desmethyl Oxybutynine.

3.9. Application to a Bioequivalence Study
Blood samples were obtained following oral administration of Oxybutynine Hydrochloride capsules 5 mg into K3EDTA vacutainer solution as an anticoagulant. Plasma was separated by centrifuging the blood using an eppendorf centrifuge at 4000 rpm for 5 min and stored frozen at −20±5°C until analysis. An aliquot of 0.5mL of thawed plasma samples were spiked with IS and processed. Plasma concentration–time data of Oxybutynin and N-Desmethyl Oxybutynine was analyzed by non-compartmental method using Kinetica Version 4.4 (Thermo USA). Regarding AUC0–t, AUC0–∞ and Cmax, bioequivalence was assessed by means of an analysis of variance (ANOVA) and calculating the standard 90% confidence intervals (90% CIs) of the ratios test/reference (logarithmically transformed data). Fig. 12 and 13 shows the mean plasma concentration-time curves for the two formulations. Pharmacokinetic parameters derived from these curves are presented in Table 5.
Table 1: Comparative assessment of chromatographic methods developed for analysis of oxybutynine and desmethyl oxybutynine in plasma (1997-2007).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Detection technique</th>
<th>Extraction procedure</th>
<th>Sample volume</th>
<th>Linear range (ng/mL)</th>
<th>Retention time; run time</th>
<th>Application</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LC-MS/MS</td>
<td>LLE Oxybutynin and N-Desethyl oxybutynin</td>
<td>400µL human plasma</td>
<td>0.249 - 70.255 ng/mL</td>
<td>3.5 min</td>
<td>Pharmacokinetic studies of Oxybutynine in human</td>
<td>[3]</td>
</tr>
<tr>
<td>2</td>
<td>Spectrophotometric and spectrofluorimetric</td>
<td>Oxybutynin HCl; reaction of OXB with malonic acid anhydride in acetic acid anhydride</td>
<td>-</td>
<td>4–40 µg/mL and 0.5–6 µg/mL</td>
<td>-</td>
<td>Pharmaceuticals via Reaction with Mixed Acids Anhydrides: Application to Content Uniformity Testing</td>
<td>[6]</td>
</tr>
<tr>
<td>3</td>
<td>RP-HPLC</td>
<td>Oxybutine Chloride on dried bases</td>
<td>10µl</td>
<td>80 ppm to 120 ppm</td>
<td>13.71 min; 40 min;</td>
<td>Oxybutynin Chloride by RP-HPLC Analytical Technique</td>
<td>[8]</td>
</tr>
<tr>
<td>4</td>
<td>LC–MS/MS</td>
<td>LLE</td>
<td>300 µl human plasma</td>
<td>0.050–10.0 ng/mL for oxybutynin and 0.500–100 ng/mL for N-desethyl oxybutynin.</td>
<td>3.13 min; 4.0 min</td>
<td>Bioequivalence study.</td>
<td>[4]</td>
</tr>
<tr>
<td>5</td>
<td>MS/MS with multiple reaction monitoring (MRM) mode</td>
<td>LLE</td>
<td>1 mL dog plasma</td>
<td>0.2 ng/mL-200.0 ng/mL</td>
<td>running time of 2.0 min</td>
<td>Pharmacokinetic study in dog plasma.</td>
<td>[5]</td>
</tr>
<tr>
<td>6</td>
<td>GC-MS</td>
<td>capillary (GC-MS) using deuterated internal standard</td>
<td>Human plasma</td>
<td>0.5-20 ng/ml</td>
<td>Retention time 3 min</td>
<td>Analysis of Plasma Oxybutynin Using a Deuterated Internal Standard</td>
<td>[19]</td>
</tr>
<tr>
<td>7</td>
<td>Lc-Ms/Ms</td>
<td>LLE</td>
<td>500 µl Rabbit Plasma</td>
<td>0.1 ng to 5.0 ng</td>
<td></td>
<td>Quantitation of Oxybutynin in Rabbit Plasma</td>
<td>[23]</td>
</tr>
<tr>
<td>8</td>
<td>HPLC</td>
<td>-</td>
<td>200 µl Human Plasma</td>
<td>0.5 and 5 ng/ml</td>
<td>17 min; 23 min</td>
<td>Pharmacokinetic studies in young, elderly and frail elderly volunteers, Xenobiotica (1992)</td>
<td>[16]</td>
</tr>
<tr>
<td>9</td>
<td>Lc-Ms/Ms</td>
<td>LLE</td>
<td>200 µl Human Plasma</td>
<td>75.051 pg/mL to 7400.849 pg/mL oxybutynine and 75.163 pg/mL to 7411.923 pg/mL for N-Desmethyl Oxybutynine</td>
<td>Oxybutynin was 2.58 mins and that of N-Desmethyl Oxybutynine was 2.48 mins; 3.5 mins total run time</td>
<td>Our newly developed method</td>
<td></td>
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Table 2: Method performance and linearity parameters for Oxybutynine.

<table>
<thead>
<tr>
<th>Assay Performance</th>
<th>Linearity Assessment</th>
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<tr>
<td>System suitability</td>
<td>Linearity range (ng/mL)</td>
</tr>
<tr>
<td>Precision (%cv)</td>
<td>75.051 pg/ml to 7400.849 pg/ml</td>
</tr>
<tr>
<td>Accuracy (%cv)</td>
<td>0.4%/1.3% for retention time/area response 97.7%/101.5%</td>
</tr>
<tr>
<td>System performance</td>
<td>Calibration Standards (ng/mL)</td>
</tr>
<tr>
<td>Signal to Noise ratio (S/N ratio)</td>
<td>Quality control samples (ng/mL)</td>
</tr>
<tr>
<td>≥10</td>
<td>7400, 6660, 5548, 3700, 1110, 369, 150, 75</td>
</tr>
<tr>
<td>Method ruggedness:</td>
<td>Weighing factor</td>
</tr>
<tr>
<td>Precision (%cv)</td>
<td>1/2</td>
</tr>
<tr>
<td>Accuracy (%cv)</td>
<td>1.3-3.4%</td>
</tr>
<tr>
<td>Dilution integrity</td>
<td>Correlation coefficient (r²)</td>
</tr>
<tr>
<td>Precision (%cv)</td>
<td>≥0.98</td>
</tr>
<tr>
<td>Accuracy (%cv)</td>
<td>0.3%-2.9</td>
</tr>
<tr>
<td>1.1%-1.2%</td>
<td>97.8%-102.3</td>
</tr>
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</table>

Table 3: Extraction recovery and matrix factor for Oxybutynine.

<table>
<thead>
<tr>
<th>Quality control level (pg/mL)</th>
<th>Oxybutynine</th>
<th>N-Desmethyl Oxybutynine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean of Matrix factor (%CV)</td>
<td>ISTD normalized Matrix factor(%CV)</td>
</tr>
<tr>
<td>HQC</td>
<td>0.89 (2.0)</td>
<td>1.01 (5.7)</td>
</tr>
<tr>
<td>LQC</td>
<td>0.92(2.4)</td>
<td>1.04 (6.5)</td>
</tr>
<tr>
<td>ISTD</td>
<td>0.89(4.8)</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5: Pharmacokinetic parameters of Oxybutynine and N-Desmethyl Oxybutynine following oral administration of one Oxybutynine Hydrochloride capsules 5 mg.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Geometric Mean (% CV) Arithmetic Mean ± SD</th>
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<tbody>
<tr>
<td></td>
<td>Oxybutynine</td>
</tr>
<tr>
<td></td>
<td>Test Formulation (n=36)</td>
</tr>
<tr>
<td>Cmax (ng/mL) (%cv)</td>
<td>17.955±10.171 (56.65)</td>
</tr>
<tr>
<td>AUC(0-t) (ng.h/mL)</td>
<td>72.023±39.350 (54.64)</td>
</tr>
<tr>
<td>AUC(0-inf) (ng.h/mL)</td>
<td>90.920±51.279 (56.60)</td>
</tr>
<tr>
<td>Tmax (h)*</td>
<td>1.64±1.24 (75.37)</td>
</tr>
</tbody>
</table>
Fig No. 1: A Representative Regression Analysis of a Calibration curve.
Fig No. 2: A Representative Chromatogram of Aqueous Standard.
Fig No. 3: A Representative Chromatogram of Double Blank.

Fig No 4: A Representative Chromatogram of Blank.
Fig No. 5: A Representative Chromatogram of ULOQ Sample.
Fig No. 6: A Representative Chromatogram of LLOQ Sample.

Fig No. 7: A Representative Chromatogram of LLOQ QC Sample.
Fig No 8: A Representative Chromatogram of LQC Sample.
Fig No. 9: A representative Chromatogram of MQC-02 sample.

Fig No. 10: A representative Chromatogram of MQC-01 sample.
Fig No. 11: A representative Chromatogram of HQC sample.

Fig 12: Mean plasma concentration-time semilog plots of oxybutynine after administration of (test and reference) oxybutynine formulation to 32 healthy Indian subjects.
4. CONCLUSIONS
The described LC-MS/MS methods provide fast, sensitive and selective procedure for the simultaneous determination of Oxybutynin and N-Desmethyl Oxybutynin in human plasma. The analytes in this method were free from ion suppression effects generated by matrix. From the results of all the validation parameters, we can conclude that the present method can be useful for bioequivalence studies with desired precision and accuracy. The method can be useful for the analysis of larger number of samples as it uses a sample extraction procedure without derivatization and requires low sample volume is highly selective and has a short assay time.

Conflicts of interest
The author declare no conflict of interests.

ACKNOWLDEGMENTS
The author wish to thank the management of Synchron research services Pvt. Ltd., Ahmedabad to carry out this research.

Appendix A. Supporting information
Supplementary data associated with this article can be found in the online version.

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5. Sensitive determination of oxybutynin and desethyl oxybutynin in dog plasma by LC-ESI/MS/MS.
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