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Highly Sensitive Simultaneous Determination of Oxybutynin and N-Desethyloxybutynin in Human Plasma by LC-MS/MS

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ABSTRACT

Highly sensitive and Rapid LC-MS/MS method for the simultaneous determination of Oxybutynin and major metabolite N-Desethyloxybutynin in human plasma has developed. Deuterium labeled Oxybutynin D_{11} & N-Desethyloxybutynin D_5 were used as Internal standards respectively, Plasma samples are extracted through liquid-liquid extraction with tert-Methyl Butyl Ether, the supernatant was dried and reconstituted in 0.400 mL of Acetonitrile and 2 mM Ammonium Acetate (90:10 V/V) solution and separated on a column Hypurity C18, 100 x 4.6 mm, 5 µ within 3.5 min. Quantification was performed by multiple reaction monitoring, where the acquired mass for Oxybutynin and Metabolite are m/z 358.2→142.2 and 330.3→96.1 respectively. A linear range of 0.049 to 13.965 ng/mL for Oxybutynin and a range 0.249 to 70.255 ng/mL for N-Desethyloxybutynin was tested. This method is very suitable for Bioequivalence study sample analysis for quantification of Oxybutynin and major metabolite N-Desethyloxybutynin in human plasma.

KEY WORDS: Oxybutynin, Oxybutynin & its metabolite, N-Desethyloxybutynin, Oxybutynin in plasma, Oxybutynin by LC-MS/MS.

1. INTRODUCTION

Oxybutynin molecular formula is 4-Diethylaminobut-2-ynyl 2-cyclohexyl-2-hydroxy-2-phenylethanoate with a molecular weight of 357.486 g/mol. Oxybutynin is an anticholinergic medication used to relieve urinary and bladder difficulties, including frequent urination and inability to control urination (urge incontinence), by decreasing muscle spasms of the bladder. It competitively antagonizes the M1, M2, and M3 subtypes of the muscarinic acetylcholine receptor. It also has direct spasmolytic effects on bladder smooth muscle as a calcium antagonist and local anesthetic, but at concentrations far above those used clinically. Oxybutynin is also a possible treatment of hyperhidrosis (hyper-active sweating). Oxybutynin contains one stereo center. Commercial formulations are sold as the racemate. The (R)-enantiomer is a more potent anticholinergic than either the racemate or the (S)-enantiomer, which is essentially without anticholinergic activity at doses used in clinical practice. However, (R)-oxybutynin administered alone offers little or no clinical benefit above and beyond the racemic mixture. The other actions (calcium antagonism, local anesthesia) of oxybutynin are not stereospecific. (S)-Oxybutynin has not been clinically tested for its spasmolytic effects, but may be clinically useful for the same indications as the racemate, without the unpleasant anticholinergic side effects.

Oxybutynin is rapidly absorbed from the gastrointestinal tract following oral administration with maximum plasma concentrations reached in less than 1 hour. First-passage effect is high and less than 10% of the administered dose reaches the circulation unchanged. Oxybutynin is extensively metabolized by the liver, primarily by the cytochrome P450 enzyme system, particularly CYP 3A4 found mostly in the liver and gut wall. Metabolites include phenylcyclohexylglycolic acid, which is pharmacologically inactive, and N-Desethyloxybutynin, which is pharmacologically active. As far as Bioequivalence studies concern, Both Oxybutynin and major active metabolite N-Desethyloxybutynin are to be quantified in plasma, Bioequivalence is based on 90% CI of Oxybutynin whereas the metabolite data will be submitted as supporting evidence of comparable therapeutic outcome.

Kim & Han (2003), reported a sensitive determination of oxybutynin and Desethyloxybutynin in dog plasma by LC-ESI/MS/MS, Sharma P and co reported Parallel achiral-chiral determination of oxybutynin, N-desethyl oxybutynin and their enantiomers in human plasma by LC-MS/MS to support a bioequivalence trial, Agilent posted a application note on Quantitation of Oxybutynin in Rabbit Plasma Using the Varian 1200L LC/MS/MS System, developed by Garteiz, TexMS Analytical Services (2016), ZHU (2012). Reported Determination of Oxybutynin and N-Desethyloxybutynin in Rat Plasma by UPLC-MS/MS, Hughesa (1992), were reported a HPLC method for Measurement of oxybutynin and its N-desethyl metabolite in plasma, Isotope dilution method was reported. Different methods are reported by different scientist but no one reported a direct method to the requirements as per office of generic drugs guidance. In the present study we are reporting a highly sensitive and rapid simultaneous determination of Oxybutynin and its active metabolite N-Desethyloxybutynin in plasma by LC-MS/MS. The developed method is fully compliant to office of generic drugs (OGD) guidance and can be applied to Bioequivalence/pharmacokinetic studies.

2. MATERIALS AND METHODS

Oxybutynin standard was received from VerGo Pharma Laboratories, N-Desethyloxybutynin and deuterium standards were purchased from Clearsynth Labs, India. All working standards were stored in a refrigerator

maintained between 2-8^{0}C, HPLC grade Methanol & Acetonitrile from JT Baker, Extraction solvent and buffer from Rankem were used. Blank plasma for the preparation of CC standards and QC samples has arranged by Clinical Department of VerGo Clinicals. Milli-Q water is from an In-house Milli-Q Gradient was used. Hypurity column was purchased from Themo Fisher Scientific, USA.

Instrumentation and Conditions: Shimazdu HPLC coupled with ABSCIEX Triple Quad API4000 system was used for the analysis. Analyst 1.6.2 is used to operate the LC-MS/MS system. Multiple reaction monitoring was performed in positive polarity with unit resolution and turbo ion spray. m/z 358.2→142.2 for Oxybutynin and 330.3→96.1 for N-Desethyloxybutynin were monitored along with their internal standards with a dwell time of 200ms, while separation was achieved on a Hypurity C18, 100 x 4.6 mm, 5 µ column within 3.5 minutes run time using Acetonitrile and 2 mM Ammonium Acetate (90:10 V/V) solution as mobile phase, isocratic flow, Injection volume was 20µL & 80% Methanol was used as rinsing solution.

Preparation of Calibration Curve Standards and Quality Control samples: 1 mg/mL stock solutions were prepared for analytes and Internal standards from their respective working reference standards & were dissolved in Methanol. All further stock dilutions were performed using 60% Methanol solution. CC & QC spiking solution were prepared using serial dilutions & 5% spiking was performed in screened blank matrix for CCs & QCs. 8 point Linear range from 0.049 to 13.965 ng/mL for Oxybutynin and 0.249 to 70.255 ng/mL for N-Desethyloxybutynin & four (LLOQ, LQC, MQC, HQC) levels of QC's were prepared.

Sample Extraction Procedure: To a aliquot of 0.400mL plasma sample, 20µL internal standard (100.000 ng/mL Oxybutynin D_{11} & 500.000 ng/mL N-Desethyloxybutynin D_5) mixture is added and vortexed, 100 µL of 0.5M Sodium Hydroxide Solution was then added and vortexed. Then the samples are subjected to liquid liquid extraction with 2mL of tert-Butyl Methyl Ether as extraction solvent, samples were vortexed for 5min at 2500 rpm & Centrifuged for 5minutes at 4000 rpm at 5^{0}C. Supernatant is separated using flash-freeze technique and dried at 40^{0}C under nitrogen stream. Dried samples were then reconstituted with 0.400mL of Mobile phase for Acquisition.

3. RESULTS AND DISCUSSION

Developed method was tested for selectivity, carryover, accuracy, precision, linearity, recovery, ruggedness, matrix effect and stabilities.

Autosampler Carry-over: Carry-over tested using equivalent aqueous samples as well as extracted samples. Blank sample was injected immediately after highest standard acquisition. No Carry-over was observed for any analyte or internal standard.

Selectivity/Sensitivity: Selectivity was tested using 8 different donor plasma (K_2EDTA) lots including one Hemolytic and one Hyperlipidemic plasma lot. A blank without internal standard, a blank with internal standard and a LLOQ from each plasma batch were processed and analyzed. Interference at analyte and internal standard retention times in blanks are compared with that of respective LLOQ. Results revealed that the endogenous interferences are within acceptable limits, signal to noise ratio is more than 5 always for all lots. Refer figure 1 to 4 for Blank sample and LLOQ sample chromatograms.

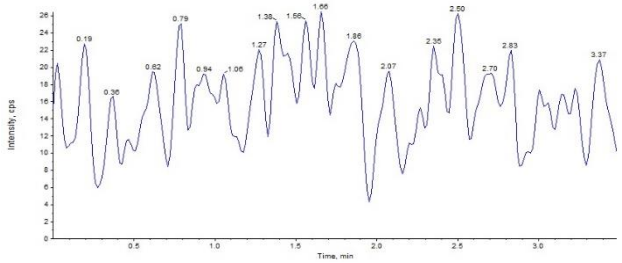

Figure.1.Blank sample chromatogram for Oxybutynin

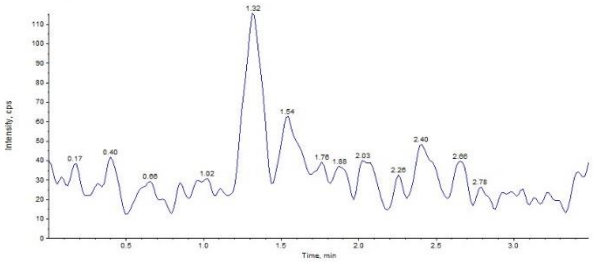

Figure.2.Blank sample chromatogram for N-Desethyloxybutynin

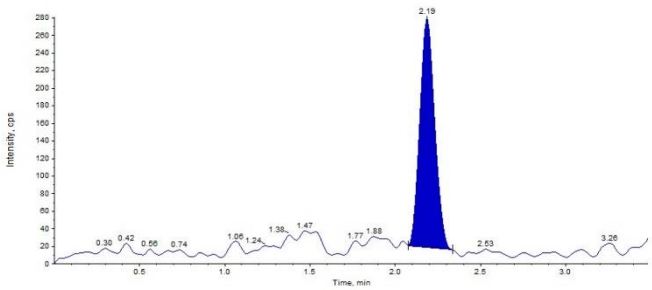

Figure.3.LLOQ chromatogram for Oxybutynin

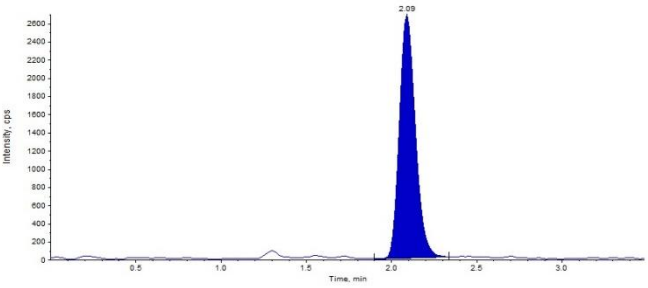

Figure.4.LLOQ chromatogram for N-Desethyloxybutynin

Linearity: Linearity was tested using three Calibration curves processed by two different analysts on different days. Oxybutynin was found linear over the range 0.049 to 13.965 ng/mL & N-Desethyloxybutynin found linear over the range 0.249 to 70.255 ng/mL with weighting factor $1/x^2$. Regression found more than 0.98 for both analytes. Refer Figure 5 & 6 for Calibration curves.

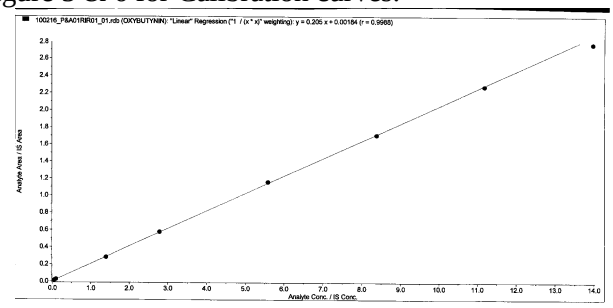

Figure.5.Calibration Curve for Oxybutynin

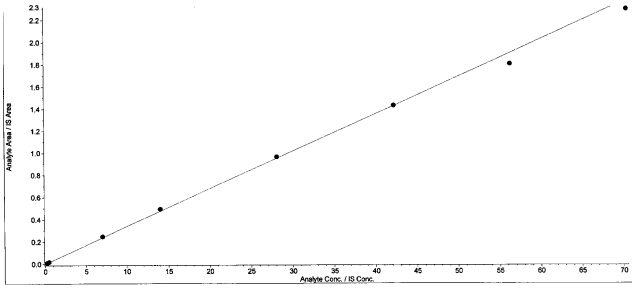

Figure.6.Calibration Curve for N-Desethyloxybutynin

Precision & Accuracy: Precision and Accuracy was evaluated using three batches processed by two different analysts on two different days to cover intra & inter day. Each Precision and Accuracy batch contains 6 replicates of 4 levels of QC's (LLOQQC, LQC, MQC, HQC) over the calibration range. LLOQQC at 100-105% of LLOQ, LQC at 2.7 times to LLOQ, MQC at 45% of ULOQ & HQC at 76% of ULOQ. Results were back calculated using y=mx+c on the respective calibration curve. Precision and Accuracy results for intra and inter day were found well within acceptable limits. Inter-day Precision ranged from 5.4% to 10.4% whereas the Inter-day accuracy ranged from 89.7% to 92.3% for Oxybutynin. Inter-day Precision ranged from 4.8% to 6.7% whereas the Inter-day accuracy ranged from 92.9% to 98.9% for Metabolite.

Extraction Recovery: Absolute Recovery of the method was evaluated by comparing the area response of analyte and internal standard of extracted samples with that of pure authentic aqueous samples. Recovery was performed using three QC levels (LQC, MQC, HQC). 6 replicates of these three QC levels were processed and acquired along with equivalent aqueous samples. Recovery found consistent and reproducible. Recoveries are 57%, 51%, 76%, 71% for Oxybutynin, Oxybutynin D_{11}, N-Desethyloxybutynin & N-Desethyloxybutynin D_5 respectively.

Matrix Effect: The developed method is based on ionization technique hence evaluation of matrix effect from endogenous compounds is highly recommended. Matrix effect was evaluated at LQC & HQC levels using 6 individual donor plasma lots including one Hemolytic and one Hyperlipidemic plasma lot. Two Blank samples from each lot were subjected to sample extraction up to drying step and then respective QC was spiked. Drug spiked in Post extracted blanks of 6 LQC and 6 HQC along with equivalent aqueous samples were acquired. Internal standard normalized matrix factor was then calculated. %CV for IS normalized Matrix factor found 4.92 at LQC level whereas 0.56 at HQC level for Oxybutynin. % CV for IS normalized Matrix factor found 2.50 at LQC level whereas 1.05 at HQC level for Oxybutynin. This experiment concluded that the developed method is not showing significant matrix effect either as ion supersession or ion enhancement in the ESI source.

Stability: Bench top stability, freeze thaw stability, auto sampler stability and long term stability in plasma were evaluated using 6 replicates of two levels of QC's (LQC & HQC) for each stability evaluation. Stability samples were subjected to required conditions and then processed along with freshly prepared calibration curve standards. Back calculated concentrations were then compared with respective nominal concentrations. Stabilities experiments found well within acceptable limits.

Acceptance limits; Selectivity/Carryover: The Response of interfering peak at analyte retention time if any, should be ≤20% of analyte response observed in respective LLOQ, whereas it is ≤5% for ISTD.

Calibration Curve: At least 75% with minimum of six calibration curve standards including LLOQ and ULOQ should be within ±15% of nominal concentration, except for LLOQ, where it should be ±20% of the nominal value, two consecutive standards should not exclude, The correlation coefficient (r^2) should be ≥0.98.

Precision: The within - batch and between batch precision for the QC samples should be <15% except LLOQ QC where it should be <20%.

Accuracy: The within- batch and between-batch % nominal should be within ±15% of the nominal value for QC samples except LLOQ QC where it should be within ±20%.

Recovery: The % CV of mean recovery at high, medium & low QC levels for analyte (s) should be ≤20%.

Matrix effect: The matrix effect is nullified at LQC and HQC concentrations for each lot if the IS normalized matrix factor CV is ≤15%.

4. CONCLUSION

Simultaneous determination of Oxybutynin and major active metabolite N-Desethyloxybutynin in human plasma by LC-MS/MS was developed using a simple extraction method and chromatographic method conditions. Method is highly selective & sensitive, reproducible & rapid. By using this method, ~200 project samples can be analyzed per day because of the shorter runtime which is very important when large number of samples are being analyzed. Method involves simple liquid-liquid extraction with lesser chromatographic solvent volumes which is cost effective. Method demonstrated greater selectivity, sensitivity, reproducibility. The method is free from endogenous interferences; recovery is reproducible and consistent throughout the linearity range. Regression was above 0.98. Developed method is very suitable for the quantitation of Oxybutynin and major metabolite in volunteer samples who dosed with Oxybutynin formulations.

REFERENCES

EMC, Oxybutynin hydrochloride 5 mg tablets, SPC, 2016.

FDA.Gov, Draft guidance on Oxybutynin chloride, Office of Generic Drugs, 2013.

Garteiz D, Quantitation of Oxybutynin in Rabbit Plasma Using the Varian 1200L LC/MS/MS System, Agilent LC-MS application note, Number 5, June 2003.

Guay DR, Plasma Concentrations of Desethyloxybutynin (DEO), The Primary Active Metabolite of Oxybutynin (OXY), Are Inversely Related to Salivary Production, Clinical Pharmacology & Therapeutics, 73 (2), 2003, P49.

Hughesa K.M, Langa J.C.T, Lazarea R, Gordonb D, Stantonb S.L, Malone-Leec J, & Geraintd M, Measurement of oxybutynin and its N-desethyl metabolite in plasma, and its application to pharmacokinetic studies in young, elderly and frail elderly volunteers, Xenobiotica, 22 (7), 1992, 859-6.

Kim H & Han SB, Sensitive determination of oxybutynin and Desethyloxybutynin in dog plasma by LC-ESI/MS/MS, J Pharm Biomed Anal., 31 (2), 2003, 341-9.

Nour E Wagieha, Maha A. Hegazyb, Abdelkawyb M, Eglal A. Abdelaleem, Quantitative determination of oxybutynin hydrochloride by spectrophotometry, chemometry and HPTLC in presence of its degradation product and additives in different pharmaceutical dosage forms, Talanta, 80 (5), 2010, 2007–2015.

Ragaa E Sheikh, Ayman A. Gouda, Lameess I Mahfouz, Spectrophotometric determination of oxybutinine hydrochloride by ion-pair extraction in pharmaceutical preparations, International journal of pharmacy and pharmaceutical sciences, 7 (6), 2015.

Sharma P, Patel DP, Sanyal M, Guttikar S, Shrivastav PS, Parallel achiral-chiral determination of oxybutynin, N-desethyl oxybutynin and their enantiomers in human plasma by LC-MS/MS to support a bioequivalence trial, J Pharm Biomed Anal., 88, 2014, 81-91.

Shodhganga, Chapter 3- Oxybutynin, Inflibnet, Gandhi Nager, 2010.

Sonia T. Hassib, Awatef E. Farag, Marianne A Mahrouse, and Eman A Mostafa, Spectrophotometric Determination of Oxybutynin Hydrochloride via Charge- Transfer Complexation Reaction, IJPS, 2 (4), 2013, 2024-2033.

Srikanth K, Emmanuel K.A, and Ramesh Raju K, Spectrophotometric determination of oxybutynin chloride through ion-association complex formation, Rasayan J.Chem, 3 (1), 2010, 179-187.

Suman Avula, Naveen Babu K, Ramana MV, Validated RP - HPLC method for the estimation of oxybutynin in formulation, Pharmacophore, 2 (2), 2011, 156-162.

Walash MI, Belal F, El-Enany N, Elmansi H, Determination of oxybutynin in pharmaceuticals via reaction with mixed acids anhydrides: application to content uniformity testing, J Fluoresc., 21 (2), 2011, 715 - 22.

Wikipedia, Oxybutynin, encyclopedia, 2016.

ZHU Zhuang-Zhi, WU Yu-Bo, LUO Hua-Fei, Determination of Oxybutynin and N-Desethyloxybutynin in Rat Plasma by UPLC-MS/MS, CJPH, 43 (2), 2012, 116-119.