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A LC-MS/MS Quantification of Moxonidine in Plasma

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KEYWORDS
Moxonidine, plasma, LC-MS/MS, positive ionization, validation

ABSTRACT:
Introduction: Moxonidine is a new generation centrally acting antihypertensive drug for the treatment of mild to moderate essential hypertension. It may play a role when thiazides, beta-blockers, ACE inhibitors, and calcium channel blockers are inappropriate or fail to control blood pressure. It demonstrates beneficial effects on the parameters of the insulin resistance syndrome, apparently independent of blood pressure reduction. Moxonidine is a selective imidazoline receptor subtype 1 agonist. This receptor subtype is found in both the rostral ventro-lateral pressor region and the ventromedial depressor region of the medulla oblongata. **Objectives**: quantification of Moxonidine antihypertensive drug in plasma using hyphenated analytical technique LC-MS/MS.
Methods: To determine the plasma concentrations of moxinidine by liquid chromatography in conjunction with a triple quad mass spectrometer (LC-MS/MS) using an internal method. The development of the method was conducted in such a way that sufficient sensitivity was achieved and no short-term and long-term matrix effects affected the performance of the method. Sensitivity was found to be better with positive ionization than with negative ionization.
Results: The sum of multiple daughter ions was used as mass transactions for moxonidine are 242.05/206.1 and 242.05/199.05 where as single mass transaction was used for clonidine are 230.1/213.1. Chromatography was optimized using acetonitrile:buffer (10 mmol ammonium acetate) (75:25) on a Hypurity C8 analytical column, 100 x 4.6 mm. Calibration curve found to be linear over the range 5.004 to 10345.023 pg/ml using 1/x2 as weighting factor.
Conclusions: The method is successfully applied to the analysis of 360 clinical trial samples collected after administration of 25 mg of moxonidine in a two-phase IEC-approved clinical trial. The resulting sample was reanalyzed and the method found is reproducible.

1. Introduction

Moxonidine is a new generation centrally acting antihypertensive drug for the treatment of mild to moderate essential hypertension. It may play a role when thiazides, beta-blockers, ACE inhibitors, and calcium channel blockers are inappropriate or fail to control blood pressure. It demonstrates beneficial effects on the parameters of the insulin resistance syndrome, apparently independent of blood pressure reduction. Moxonidine is a selective imidazoline receptor subtype 1 agonist. This receptor subtype is found in both the rostral ventro-lateral pressor region and the ventromedial depressor region of the medulla oblongata. Moxonidine therefore causes a decrease in the activity of the sympathetic nervous system and thus a decrease in blood pressure. Compared to older centrally acting antihypertensives, moxonidine binds with much greater affinity to the imidazoline I1-receptor than to the α2-receptor[1].The molecular formula of moxonidine is $C_9H_{12}ClN_5O$ with a molecular weight of 241.677 g/mol. Bioequivalence studies under fasting conditions are in accordance with Note CPMP/EWP/QWP/1401/98 on Bioavailability and Bioequivalence Research Guidelines[2]. Moxonidine is rapidly absorbed after oral administration. In humans, 90% of an oral dose is absorbed. Food intake has no effect on the pharmacokinetics of moxonidine. There is no first-pass metabolism by the liver and bioavailability is around 88%. About 7% of moxonidine is bound to human plasma proteins (Vdss = 1.8 ± 0.4 l/kg). Maximum plasma levels of moxonidine are reached 30-180 minutes after administration of the coated tablet.

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The average plasma elimination half-life is 2.2-2.3 hours and the renal half-life is 2.6-2.8 hours[3]. Under the OGD, a fasted single-dose bioequivalence study will be conducted for new generics in a healthy population to enter the US market. A highly sensitive (5 pg/ml LOQ) and selective, rapid (3 min), MRM method was developed and validated for the determination of moxonidine in human plasma (K2EDTA as an anticoagulant) by simple isocratic liquid chromatography with 10 microliter injection tandem mass spectrometry, the method includes sample processing for solid phase extraction with very low matrix effects and highly selective and fast, Clonidine used as internal standard. The method was successfully validated and applied to the analysis of ~360 bioequivalence samples. Moxonidine is a low molecular weight basic polar drug. Basic functional groups are active for extraction and ionization. Due to the polar drug, we had problems with the matrix effect, but clonidine (ISTD) compensates for them, the IS normalized matrix factor is within limits. Recovery found to be ~40% due to matrix effects, but recovery is consistent and reproducible. Selectivity, precision and accuracy were found to be acceptable. There is no method published in the public domain for the estimation of moxonidine in plasma by LC-MS/MS with 5 pg/ml and 3 minutes. Minxia M. He et al. described a method with a high duration gradient LC program for 27 min for the range of 0.05 to 8 ng/ml CC with a solid phase extraction method using an unusual trifluoroacetic acid elution solvent on a mass spectrometer operated with APCI and sample was concentrated to 150 microliters and 125 microliters injection volume[4] , this method involves a long duration with many complex steps such as gradient LC, trifluoroacetic acid, APCI, 125 microliter injection. Stephen D Wise et al published a method that includes APCI with 0.25 ng/ml LOQ[5], Luhia zhao et al recently reported LC-MS[6]· SIM methods are not sensitive and selective, LC-MS/MS methods have more advantages over SIM Others reported methods is the estimation of moxonidine in tablet dosage form, which have no direct comparison with the current method [7-15].

Validation of the method performed as of ICH guidelines[16]. Akash Shelke et al reported For the analysis of drugs in pharmaceutical formulation this method is successful [17] . Mohammed E. Abdel-Hamid et al reported proposed route of drug administration which permits accurate drug placement and collection of serum samples[18]. Umadevi et al reported this process is useful for quality control of bulk and pharmaceutical formulations[19]. Dilip Chandak et al reported Method applicable for routine analysis containing the drugs in combination[20]. Ankita Shinde et al reported the drugs were detected using UV detector,with flow rate 10 ml[21]. Swapna goday et al reported the extraction of sample in plasma by liquid – liquid extraction technique[22].

2. MATERIALS AND METHODS

Materials

Moxonidine and clonidine (ISTD) standards are obtained from VLS, Mumbai, India, Chemicals purchased from JT Baker Inc. USA. A pressurized solid phase extraction line from Orochem, USA was used for processing. Bond elute Plexa (1CC 30mg) cartridges procured from Agilent technologies, USA, while blank plasma was received from in-house clinical department. The study protocol was reviewed and approved by the Ethics Committee of Nirmala College of Pharmacy, AP, India.

Methods

Stock solutions of 1 mg/ml moxonidine and clonidine were prepared in methanol, further stock dilutions and additional solutions were prepared in 50% methanol in water and then stored in a refrigerator. 5% buffer solutions were added to blank plasma to archive the desired concentration of CC and QC sample. An 8-point calibration curve is selected in the range of 5 to 10,000 pg/ml, while 4 quality control levels are included in the CC range. Enriched samples are stored at -70°C until analysis.

Sample Extraction Procedure

Add 50 µL of IS Diluent to samples except blank where add 50 µL of Diluent, aliquot 0.500 mL of sample and vortex, add 0.500 mL of water and vortex, condition SPE cartridges with 1000 mL of methanol followed by 1000 mL of water, Fill prepared sample into the SPE cartridges, wash the cartridges twice with 1000 mL of water followed by twice with 1000 mL of 5.00% methanol in water and dry the cartridges for ~2 min. Elute samples with 0.200 ml mobile phase.

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Centrifuge all samples at 4000 rpm for 5 min at 4.0 °C, transfer samples to auto sampler vials for analysis

3. RESULTS

Method development was initiated, aqueous and extracted CC standards (K2EDTA as anticoagulant) (5 pg/ml to 10 ng/ml) using the LLE (Ethyl Acetate with Alkalization) technique and CC was found to be acceptable with a LOQ area of ~2000 on API4000Qtrap The mobile phase used was acetonitrile and 10 mmol of ammonium acetate in a ratio of 80:20 with 30 µl fed onto an Inertsil ODS 150 mm column, MRM was 242.05/206.1. The procedure was tested and found to be acceptable for a short time, but the effectiveness of the method was found to be lower for a long time (more than 50 injections). LLE was replaced by SPE and was found to be suitable with no drop in response, but the LLOQ response is between 1000 and 2000, so we considered introducing an additional daughter ion to use the sum of multiple ions to obtain an area for Std 1 of at least 2000.

4.DISCUSSION

Blank chromatograms were presented as **Figure 1 and 2**.

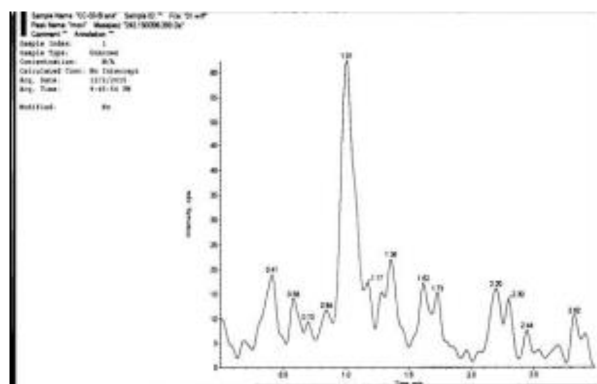

Figure 1. Moxonidine Blank Chromatogram

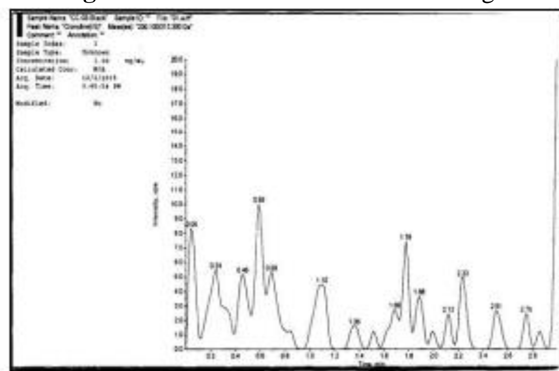

Figure 2. ISTD Blank Chromatogram

The selectivity of the method is tested using 6 batches of plasma processed as a blank and LLOQ and the response of the blank is compared to that of the LLOQ, no endogenous or exogenous interferences were found in the retention times. The LLOQ chromatogram is shown in **Figure 3**.

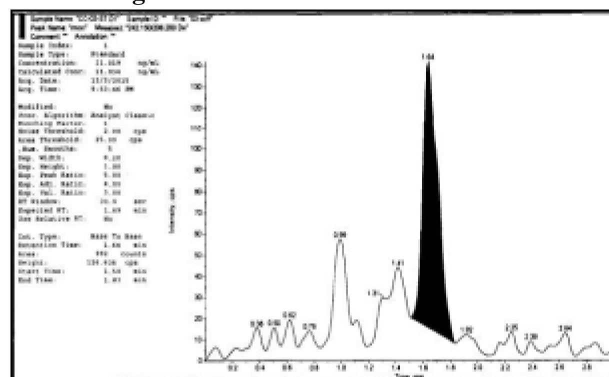

Figure 3. Moxonidine LLOQ Chromatogram

The matrix factor for this method was determined using LQC and HQC level samples prepared in post-extracted blanks and compared to unextracted pure samples, the matrix factor found to be between 0.85 to 1.15 with a maximum internal standard normalized matrix factor %CV of 11.5. Intra-day and inter-day precision and accuracy along with robustness were tested by analyzing three batches of precision and accuracy. Intra and Inter Accuracy and precision found to be acceptable. Method recovery was found to be reproducible and consistent over the full range of the calibration curve. ULOQ and ISTD chromatograms were presented as **Figure 4 and Figure 5**, respectively.

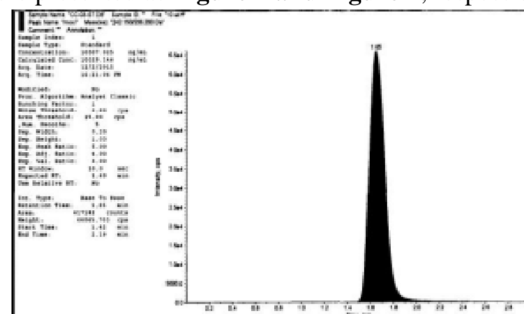

Figure 4. Moxonidine ULOQ Chromatogram

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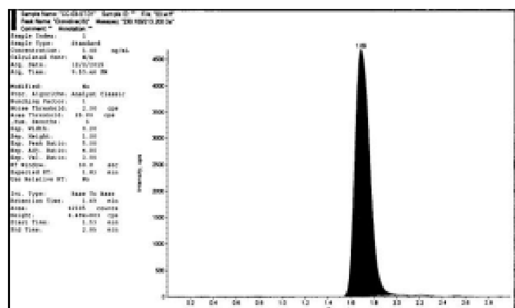

Figure 5. ISTD Chromatogram

Table 1.Precision and Accuracy results

Description	LLOQQC	LQC	MQC	HQC
Nominal	11.009	29.658	4252.66	7650.291
Observed	11.159	28.603	4340.51	7848.344
% Accuracy	101.2	98.4	101.5	101.6
% Precision	10	5	8	2

Application to bioequivalence study sample analysis

The method described above was successfully used to analyze a bioequivalence study consisting of 12 volunteers administered 25 mg of moxonidine according to an IEC approved protocol. Screening was performed to select volunteers according to inclusion and exclusion criteria, volunteers were dosed after a 10-hour fast in a cross-over study design. Blood samples are taken at regular intervals. A total of 360 samples were taken before and after moxonidine administration, the plasma was separated and stored at -70°C. These samples were analyzed according to the calibration curve together with the QC samples. The CC and QC during the study analysis showed accuracy and precision within the acceptable limits specified in the EMA Bioanalytical Method Validation Guidelines. Study analysis is performed in a GLP-regulated environment.A non-compartmental pharmacokinetic analysis is performed, the middle plot is shown as **Figure 6.**

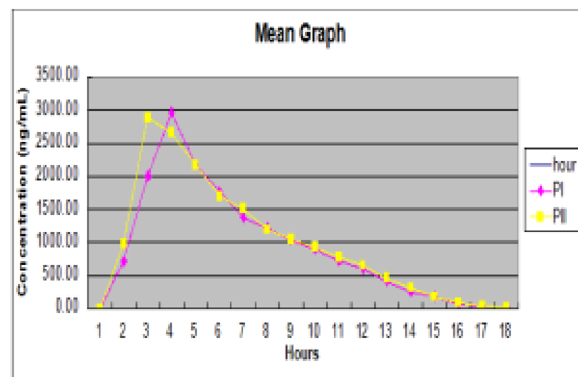

Figure 6.Pharmacokinetic mean graph for Moxonidine Tablets 25 mg test (PI) vs Reference formulation (PII)

5.CONCLUSION

Moxonidine is a low molecular weight basic polar drug. Basic functional groups are active for extraction and ionization. Because of the drug polar in nature, we got matrix effect problems but clonidine (ISTD) is compensating those, IS normalized matrix factor is within limits. Method validation found acceptable. Method can be used for intended purpose. Successfully developed and validated a simple, rapid Bioanalytical method for the estimation of Moxonidine in plasma by LC-MS/MS. Sample extraction using solid phase extraction while separation was achieved on hypurity C8 column, acquired in MRM mode and quantified using analyst 1.6.2 software. Method found selective, no endogenous or exogenous interfences found either at analyte or internal standard retention times.Matrix factor ratio found around 1 whereas recoverys found more than 40 % with within 20% CV. Signal to noise ratio found always more than 5. Precision and accuracy, ruggedness and all stability experiments found acceptable. Method is successfully applied to analyze unknown samples upto 360 and found reproducibility as well.

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AUTHORS CONTRIBUTION STATEMENT

We have assured that “all authors have read and approved the manuscript.” All the authors have equal contribution and participation in this research work.

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CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this research work.

Refrences

1. http://wikipedia\moxonidine, encyclopedia, 2016 .
2. http://EMA, Note for guideline on the investigation of BA/BE, 2000 .
3. http://emc, Moxonidine Specific product characteristics, 2012 .
4. Minxia M. He, Trent. Abraham, Thomas J. Lindsay, Hans C. Schaefer, Isabelle J. Pouliquen, Chris Payne: The American Society for Pharmacology and Experimental Therapeutics, Vol.31, no.3, 2003, 334-342.
5. Stephen D Wise, Clark Chan, Hans G Schaefer, Minxia M He, Isabelle J Pouliquen, Malcolm I Mitchell: Br J Clin Pharmacol, 2002, 54(3), 251–254.
6. Luhua Zhao, Li Ding, Xin Wei: Journal of Pharmaceutical and Biomedical Analysis 2006, 40(1):95-9.
7. Milovanović S, Otašević B, Zečević M, Zivanović L, Protić A: J Pharm Biomed Anal. 2012, 5(59), 151-156.
8. Rajendra Kakde, Kamlesh Gadpayale, M. Obaid Qureshi: International Journal of PharmTech Research, 2012,4(1); 358-363.
9. http://EMA, Moxonidine, public assessment report, 2009 .
10. CUI, Han-ming, SUN, Hui-min, WANG, Wei, TIAN, Song-jiu: HPLC Determination of Dissolution of Moxonidine Hydrochloride Tablets, Chinese, Journal of Pharmaceutical Analysis, 2003,July7; 23(4), 315-316.
11. Milovanović S, Otašević B, Zečević M, Zivanović L, Protić A: Development and validation of reversed phase high performance liquid chromatographic method for determination of moxonidine in the presence of its impurities, J Pharm Biomed Anal, 2012, Feb 2; 59 (1),151-156.
12. Lena Brynne, John L McNay, Hans G Schaefer, Karl Swedberg, Curtis G Wiltse, and Mats O Karlsson: Pharmacodynamic models for the cardiovascular effects of moxonidine in patients with congestive heart failure, Br J Clin Pharmacology, 2001, Jan 1;51(1),35–43.
13. Rudolph M, Janssen W, Strassner M: Determination of moxonidine (BDF 5895) in plasma by gas chromatographynegative ion chemical ionization mass spectrometry, J Pharm Biomed Anal,1992 May 5 ;10(5),323-328.
14. Chazova I, Almazov VA, Shlyakhto E: Moxonidine improves glycaemic control in mildly hypertensive, overweight patients: a comparison with metformin, Diabetes Obes Metab,2006 Jul 7;8(4):456-65.
15. Slavica Filipic, Milica Elek, Marija Popović, Katarina Nikolic, and Danica Agbaba: Development of Hydrophilic Interaction Liquid Chromatography Method for the Analysis of Moxonidine and Its Impurities, Journal of Analytical Methods in Chemistry, 2016,Oct 10;20(16),
16. Rabindra K Nanda, Amol A Kulkarni, Meenal N Ranjane , Poonam N. Ranjane. :A Stability-indicating HPTLC method for estimation of Nadifloxacin in topical cream. Res. J. Topical and Cosmetic Sci, 2010, Jan1; 1(1): 25-29
17. Akash Shelke, Someshwar Mankar, Mahesh Kolhe. Development and Validation of RP-HPLC Method for estimation of Secnidazole in API and Pharmaceutical Dosage Form. . Research J. Science and Tech, 2021,Apr 4; 13(2):100-4.
18. Mohammed E. Abdel-Hamid, Leyla H. Sharaf, Oludotun A. Phillips, Elijah O. Kehinde, Alice Babu. A Rapid liquid Chromatography-Tandem Mass Spectrometry Method for the Determination of Ciprofloxacin in Rabbits Serum Following Intra-gastric Dosing: Application to Pharmacokinetics Study. Research J. Pharm. and Tech,2012, May 5; 5(5): 624-631.
19. Umadevi, Mohibul Hoque1, Ramya Sri. S. Quantitative Estimation of Roxithromycin and Ambroxol in Bulk and Tablet Dosage Forms by RP-HPLC Method. Research J. Science and Tech., 2023, Dec12; 15(1):1-7.
20. Dilip Chandak, Pushpendra Sharma. Development and Validation for Simultaneous Estimation of Gabapentin, Mecobalamin and Alpha Lipoic Acid in Tablet Formulation. Research J. Science and Tech. 2020, Feb 2; 12(1):74-78.

JCHR (2024) 14(1), 877-882 | ISSN:2251-6727

21. Ankita Shinde, G.B. Gajeli, Sneha Ubale, Vinod Matole. Simultaneous HPLC Method Development and Validation of Bilastine and Montelukast in Bulk and Formulation. Research J. Pharm. and Tech, 2021, Nov 9; 14(11):6061-5.
22. Swapna Goday, Prameelarani Avula. Development and validation of a LC-ESI-MS/MS based bioanalytical method for dapagliflozin and saxagliptin in human plasma. Ind. J. Pharm. Edu. Res,2018,oct1;52(4): S277-86.