International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

PLASMA PROTEIN BINDING STUDY OF METAXALONE WITH HUMAN PLASMA BY RP-HPLC

Kasture Veena S, Mhaske Sharayu D*, Pathan Mallika A, Waman Nitesh, Ajage Rohit K

Department of Quality Assurance Techniques, Sanjivani College of Pharmaceutical Education and Research, Kopargaon, MS, India-423603

***Corresponding author e-mail:** sharayumhaske@yahoo.com

ABSTRACT

The HPLC method was developed and validated to quantify Metaxalone binding with human plasma. Metaxalone was extracted from spiked plasma by simple protein precipitation with methanol. The seperation was performed on RP-C18 column with mobile phase of HPLC grade methanol at a flow rate of 1 ml/min. The peak of Metaxalone was monitored at 272 nm with UV-VIS detector. The calibration curve was found to be linear at concentration range of 1-8 µg/ml. Regression was found to be 0.995 . The interday and intraday precisions SD and RSD were 0.576 and 0.515, 1.851% and 1.648%. respectively. The recovery of Metaxalone from formulation was 96.97±2.14% and from plasma was found to be 84%. Plasma protein binding was found to be 16.90±1.67. The developed method was validated as per ICH guidelines.The simple, rapid, sensitive and reproducible HPLC method was developed for estimation of Metaxalone plasma protein binding.. The proposed method can be used for the pharmacokinetic and bioequivalence studies of Metaxalone.

Keywords: Metaxalone, HPLC method development, Plasma Protein binding, Human plasma.

INTRODUCTION

Metaxalone is 5-[(3,5 dimethylphenoxy)methyl]-2-oxazolidinone with molecular formula $C_{12}H_{15}NO_3$ and molecular weight of 221.2425, a skeletal muscle relaxant drug marketed by King pharmaceuticals. Skeletal muscle relaxants have been used for either treatment of spasticity or for treatment of musculoskeletal conditions. Metaxalone is used to relax muscles and relieve pain caused by strains, sprains, and other musculoskeletal conditions. Its exact mechanism of action is not known but it may be due to general central nervous system depression.[1,2] Protein binding of Metaxalone is not known[1]. Literature survey revealed that only few methods were reported for quantification of Metaxalone in plasma by liquid chromatography.[3] These methods reported the recovery of Metaxalone from plasma but no method reported about binding of Metaxalone to human plasma.

Since the protein binding study was not much reported for Metaxalone, in present study, the human plasma protein binding of Metaxalone was studied. The developed chromatographic seperation method is useful for pharmacokinetic and bioequivalence studies of Metaxalone containing formulations.[4]

MATERIALS AND METHODS

Instruments

1. HPLC, SHIMADZU (isocratic) with LC-20 AD pump, SPD-20A detector system and spinchrom software
2. Cyclomixer, 101 Remi
3. Centrifuge, Remi
4. Sonicator, Sidilu ultrasonics-1.5L50H

Materials

Standard Metaxalone was obtained as a gift sample from Angelini pharmaceuticals Ltd. and HPLC grade methanol was obtained from Research lab fine chem industry, Mumbai. Drug free human plasma was purchased from local blood bank of Kopargaon.

Tablets of Metaxalone (FLEXURA 400 Sun pharma Sikkim), were procured from local market of Kopargaon.

Preparation of standard stock solution: Stock solution of Metaxalone was prepared in methanol at a concentration of 100 µg/ml and was kept at 5-10^0 C. Stock solution was diluted with methanol to obtain the concentrations of 1-8 µg/ml.

Preparation of Quality Control Standards: Lowest quality control standards (LQC), median quality control standards (MQC) and highest quality control standards (HQC) were prepared by spiking 0.25 ml drug free plasma with Metaxalone to give samples containing 3, 5, 8 µg/ml of Metaxalone respectively and were stored at 4^0 C till analysed. [5]

Chromatographic conditions:[6] The chromatographic seperation of Metaxalone was done using RP-C18 (250mmx4.6mm), 5µ column(Phenomenex). The mobile phase was HPLC grade methanol. Flow rate was maintained at 1ml/min. Total run time of analysis was 10 min.The peaks were determined using UV-VIS detector set at a wavelength of 272 nm. Retention time was found to be 2.9-3 min. Injection volume was 20 µl. All the procedures were performed at ambient temperature. The mobile phase was selected after trial and error basis in which the composition of methanol, water and acetonitrile were tried but sharp peak was not resulted. The asymmetry of peak was minimum with pure methanol.

Extraction procedure for plasma protein binding study [7] Appropriate quantity of metaxalone solution was spiked with 0.25 ml of plasma to produce 1-8 µg/ml. The mixture was vortexed for 2 min. and kept overnight. On next day the mixure was centrifuged at 4000 rpm for 30 min. The supernatant solution was seperated from settled plasma. 10ml of methanol was added to settled plasma and was vortexed for 30 min. for complete extraction of Metaxalone from plasma. The PTFE syring filter was firstly preconditioned with methanol. and extracted Metaxalone solution was passed through 0.45 µ PTFE Syringe filter. The resultant filtrate of 20 µl was injected into HPLC system for analysis.

HPLC method validation[8]

System suitability parameters: The AUC of respective concentrations, theoretical plates (Efficiency), peak symmetry (Tailing factor) was recorded.

Linearity: Dilutions of standard solution of metaxalone in the range of 1-8 µg/ml were prepared by taking suitable aliquots of standard solutions in different 10 ml of volumetric flasks and diluting upto the mark with methanol. 20 µl of the resultant was injected, at each time into the column at flow rate of 1ml/min. the standard solution was monitored at 272 nm. A plot of peak area over concentration was constructed. Regresssion of the plot was computed by least square regresssion method of analysis.

Precision: Precision of analytical method was studied by multiple injection of homogeneous sample. 5 replicates of 3 ppm were prepared and injected for precision at same flow rate of 1 ml/min. the SD and %RSD were calculated.

Robustness: Robustness was studied by changing parameters like change in flow rate. The flow rate was kept 0.8 ml/min. The SD and %RSD were calculated.

Ruggedness: Analyst was changed for ruggedness study. The SD and %RSD were calculated

LOD and LOQ: Limit of detection and limit of quantitation of method was calculated by formula given below

LOD=3.3x SD / Slope

LOQ=10x SD / Slope

Accuracy:Accuracy of the method was studied using the standard addition method. The % recovery was determined at three different levels (50%, 100% and 150%).

RESULTS AND DISCUSSION

Develpoment and Validation of HPLC method

The Metaxalone peak was appeared at 2.9-3.0 min. (Figure 1). The system suitability parameters for developed HPLC method was stated in table 1. The HPLC method was developed and validated for analysis of Metaxalone plasma binding. Calibration curve showed linear relationship between concentration and area at 272 nm in concentration range of 1-8µg/ml. The regression was found to be 0.995. The regression equation was y= 12.488x-1.9667 (Figure 2). Standard deviation and % RSD for precision was found to be 0.481 and 1.532% respectively. Standard deviation and % RSD for interday and intraday precision was found to be 0.576, 1.851% and 0.515 1.648% respectively. The standard deviation and %RSD for ruggedness and robustness were found to be 0.476, 1.533% and 0.569, 1.817% respectivly (Table 2). Limit of detection was found to be 0.152µg/ml and limit of quantitation was found to be 0.461 µg/ml. Accuracy

was found to be 96.97% (Table 3). The values were within the limit specified by ICH guidelines. Hence method was found to be precise, rugged and robust. [8]

Plasma protein binding study of Metaxalone:

Percentage of plasma protein binding was calculated by:

$$\%\text{protein binding} = \frac{\text{Area of bound drug} \times 100}{\text{Area of standard drug}}$$

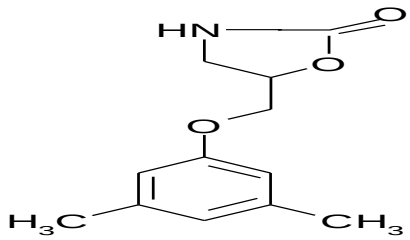
Plasma protein binding of Metaxalone was found to be 16.90±1.67%.

CONCLUSION

Metaxalone is poorly water soluble muscle relaxant drug of which protein binding is not reported in literature . In the proposed study Metaxalone binding with human plasma (in vitro) was studied. The plasma binding was quantified by validated isocratic HPLC method. Plasma protein binding of Metaxalone was found to be 16.90%. The developed method was sensitive, accurate, precise bioanalytical method. The proposed method canbe successfully applied for pharmacokinetic and bioequivalence studies.

ACKNOWLEDGEMENTS

We wish to express our sincere thanks to Dr. Kasture Sanjay B. Principal, Sanjivani College of Pharmaceutical Education and Research, Kopargaon for providing necessary facilities.



Metaxalone

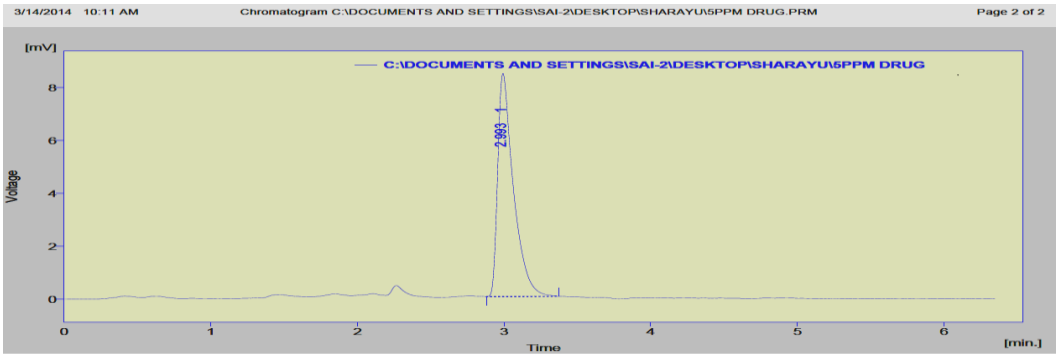


Figure 1: Chromatogram of Standard 5 ppm Metaxalone

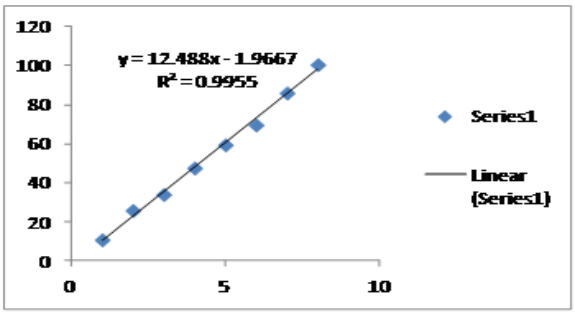


Figure 2: Calibration curve of Metaxalone at 272 nm (Concentration vs. Area)

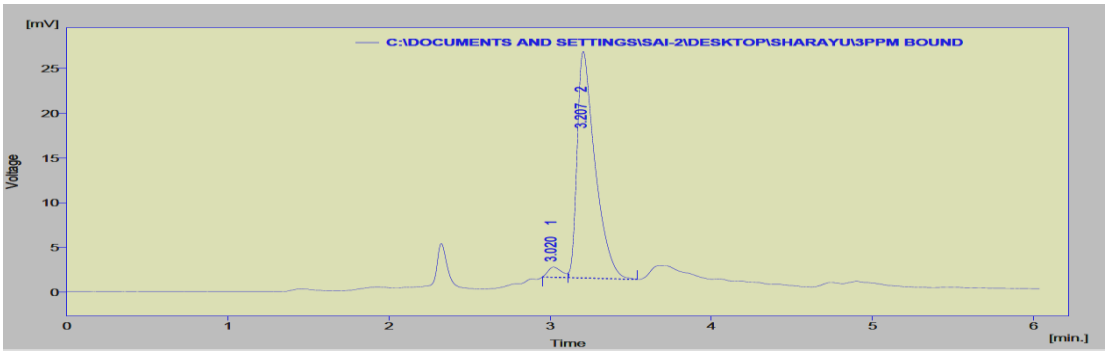


Figure 3: Chromatogram of Metaxalone bound to plasma (3ppm)

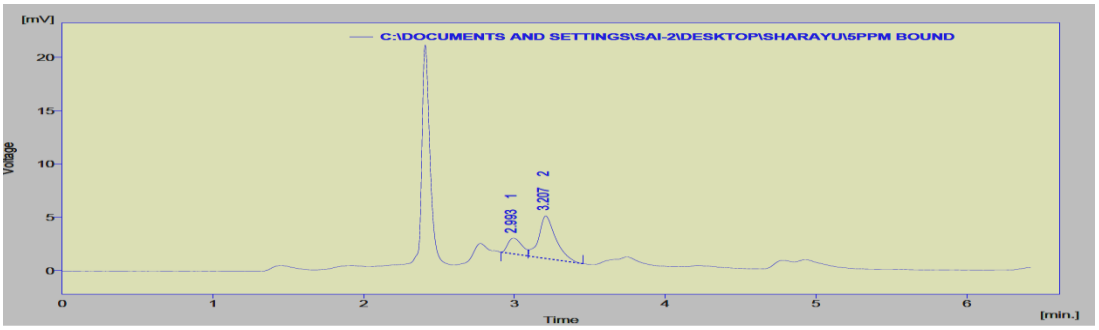


Figure 4: Chromatogram of Metaxalone bound to plasma (5ppm)

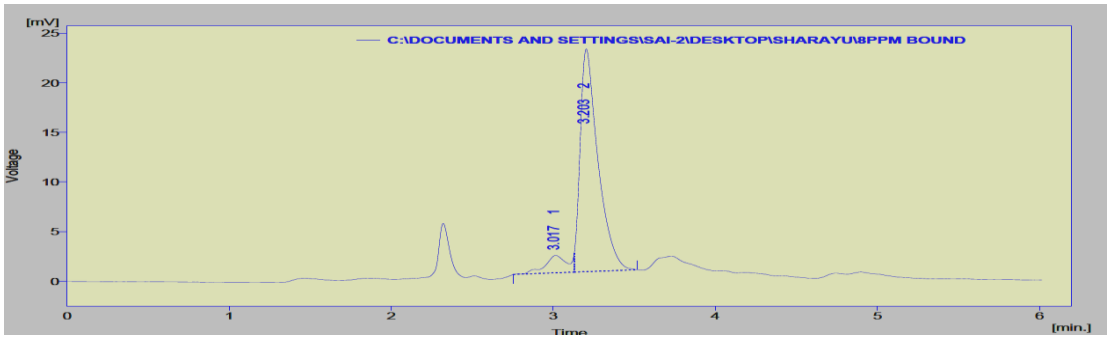


Figure 5: chromatogram of Metaxalone bound to plasma (8 ppm)

Table 1:Results of system suitability parameters

Sr. no.	Concentration (ppm)	Retention Time	Asymmetry	Efficiency
1.	2	2.987	1.95	4942
2.	3	2.980	1.95	4920
3.	4	2.993	1.95	4964
4.	5	2.987	1.95	4942
5.	6	3.017	2.0	5042

Table 2: Results of Precision by HPLC

Parameters	SD	%RSD
Precision	0.481	1.532%
Interday Precision	0.576	1.851%
Intraday Precision	0.515	1.648%
Ruggedness	0.476	1.533%
Robustness	0.569	1.817%

Table 3: Results of accuracy by HPLC

Sr No	Concentration (ppm)	Mean Area	Area of standard	%Recovery
1.	1ppm test+2ppm standard (n=3)	32.160	33.972	94.66%
2.	1ppm test+3ppm standard (n=3)	46.331	47.597	97.34%
3.	1ppm test+4ppm standard (n=3)	58.787	59.432	98.91%

Table 4: Summary of HPLC Method validation

Sr no.	Parameter	Observation
1.	Linearity range	1-8 µg/ml
2.	Slope	12.488
3.	Intercept	1.9667
4.	Correlation Coefficient	0.995
5.	Precision	SD-0.481 %RSD-1.532
6.	Interday Precision	SD-0.576 %RSD-1.851
7.	Intraday Precision	SD-0.515 %RSD-1.648
8.	Ruggedness	SD-0.476 %RSD-1.533
9.	Robustness	SD-0.569 %RSD-1.817
10.	Accuracy	96.97%
11.	LOD	0.152µg/ml
12.	LOQ	0.461µg/ml

Table 5: Results for plasma protein binding of Metaxalone

Sr. no.	Concentration (ppm)	Mean area of bound drug	Area of std. drug	% Binding
1.	3 (n=3)	6.637	35.362	18.76%
2.	5 (n=3)	9.760	62.907	15.51%
3.	8 (n=3)	16.546	100.585	16.44%

REFERENCES

1. DrugBank, Open Data Drug and Drug Target Database approved by FDA, http://www.drugbank.ca/drugs/DB00660.
2. Wikipedia: The Free Encyclopedia, http://en.wikipedia.org/wiki/Metaxalone
3. Karthikeyan Kandasamy, Vasantharaju Surenahalli Gowdra, Hariprabhu Nammalvar, Arulkumaran Kottur S Govindarajan. J. Bioanal Biomed, 2012; S6:1-7.
4. Pavan Balabathula, Dileep R Janagam, Nivesh K Mittal, Bivash Mandal, Laura A Thoma and George C Wood. J. Bioequiv Availab, 2013; 5(3): 121-4.
5. Guidance for Industry Bioanalytical Method Validation, U.S. Department of Health and Human Services FDA, CDER, CVM, BP 2001; 2-19.
6. Khan Imran, Saraf Madhusudan Natvarlal, Sayyed Nazim. Int. J. ChemTech Res, 2011; 3(4):2025-32.
7. Dhaval S. Thakar, Alice Varghese . Int. J. Pharm. Biosci. Technol, 2013; 1(1):20-6.
8. ICH Q2B: Guidance for Industry: Validation of Analytical procedures:Methodology, U. S. Department of Health and Human services Food and Drug Administration, CDER, CBER , 1996; 1-10.