ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF OXACEPROL IN HUMAN PLASMA BY USING UV-VISIBLE SPECTROPHOTOMETER

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Abstract: Osteoarthritis is commonly treated with oxaceprol. An easy, quick, accurate, and exact technique for estimating oxaceprol is required since 1 billion people worldwide suffer from rheumatoid arthritis each year. Utilizing a UV-visible spectrophotometer, procedures are developed and then validated. Only a few techniques for estimating oxaceprol have been described, but after reviewing the literature, we developed a method with a wide linearity range and low DL and QL values. Methanol: ACN was used as the solvent in the development of this procedure. The chosen wavelength for examination in this approach was discovered to be 215nm. To do the spectrum study, a double beam spectrophotometer with UV and visible light was employed.In the UV spectrophotometer, the absorbance of several series of dilutions was measured at a wavelength of 215 nm. Oxaceprol concentration was plotted on x-axis & absorbance was taken on y-axis to plot Calibration curve. All the validation parameters were validated as per ICHQ2 (R1) guidelines.

Keywords: *Osteoarthritis, UV-visible spectrophotometer, linearity, calibration curve, methanol.*

INTRODUCTION

Oxaceprol is a type of the group of medicaments known as non-steroidal anti-inflammatory and antirheumatic drugs (NSAIDs) that are used to treat osteoarthritis. Osteoarthritis is a typical kind of arthritis in which the cartilage that protects the ends of the bones erodes over time as a result of wear and strain. Joint stiffness and pain are typical symptoms. Age-related increases in osteoarthritis risk are seen.

Oxaceprol prevents the production of certain chemical mediators that cause pain, redness, swelling, and other unpleasant symptoms. hence offering treatment from inflammatory diseases of the joints and bones. By preventing the buildup of leukocytes (white blood cells) in the afflicted region or joints, it exhibits anti-inflammatory and analgesic (pain alleviation) effect. Additionally, symptoms like pain, edoema, and stiffness are lessened by this impact. Among the negative effects of oxaceprol include stomach discomfort, nausea, vomiting, diarrhoea, constipation, bloating, indigestion, headache, dizziness, and skin rashes. Typically, these adverse effects are minor and transient. It was approved in 2013. Oxaceprol is highly soluble in water, acetonitrile, methanol, DMSO, etc.

Figure 1: Structure of Oxaceprol

Absorption spectroscopy includes UV-visible spectroscopy. It focuses on the investigation of UV and visible radiation absorption. The visible spectrum extends from 400 to 800 nm, while the UV spectrum from 200 to 400 nm. For coloured compounds, visible light is employed, whereas colorless molecules are exposed to UV radiation.

MATERIAL AND METHODOLOGY

Chemicals

Active pharmaceutical ingredient of oxaceprol was gifted by a pharmaceutical company, distilled water, Acetonitrile, methanol, ammonia solution, formic acid, DMSO.

Instruments

Double beam UV/Visible spectrophotometer. For analysis, standard cuvettes with a 10 mm route length are employed. Weighing the drug sample was done with an electronic analytical balance.

Extraction Procedure

The plasma extraction was done by protein precipitate method. 100μl of plasma was pipetted out and to this 400μl of acetonitrile was added. The solution was then vortexed for 10 minutes. It was transferred to centrifugation tube and centrifuged for 10 minutes at 10,000rpm. The supernatant was separated out and used for further analysis.

Stock Solution Preparation

1mg of pure drug of oxaceprol was weighed and transferred in clean and dry vol. flask small quantity of DMSO solvent is added to dissolve the drug, it was then made upto the mark with the diluent. The resultant concentration was 1000ppm.The calibration concentrations of 100, 200, 300, 400, 500, 600, 700, 800 ppm were prepared by using diluent methanol: ACN (2:1) it was then made up to 10 ml with distilled water. The plasma then spiked with the respective concentrations.

METHOD VALIDATION

Validation is the procedure of confirming that the requirements for a particular specified application are met by inspection and the presenting of objective evidence. Validation of analytical procedure was performed according to ICH Q2R1.

The technique was verified for a number of factors, including oxaceprol specificity, linearity, accuracy, precision, robustness, and limit of detection (LOD).

Specificity: It is performed by scanning both blank and the standard drug solution in range 200-400nm.

Linearity: Linearity and the range of Oxaceprol was determined from the plotted calibration curve. The correlation coefficient (r^2) along with the equation (y= mx+c) was obtained from the graph plotted using the regression analysis. The range in which the drug shows linear response was also noted.

LLOQ: Lower Limit of Quantification is the lowest concentration of the standard calibration curve which can be quantified with good accuracy and precision.

ULOQ: Upper Limit of Quantification is the highest concentration of the standard calibration curve which can be quantified with good accuracy and precision.

Accuracy and Precision: Accuracy and precision of the bioanalytical method developed is validated according to the M10 guidelines using the within-run and between-run data. For within-run accuracy and precision 5 replicates of the different QC concentration levels are analysed.

Whereas, for the between-run accuracy and precision, each QC concentration levels are analysed at 3 analytical runs for two days. Accuracy and Precision of the method was determined by analysing replicates of concentrations at LLOQ, 3LLOQ, <75% of ULOQ and at ULOQ i.e., at 100, 300, 700, 1000µg/ml. The accuracy and precision iscalculated by calculating the %RSD [1-14].

CALCULATIONS

% Recovery

Absorbance before Centrifugation of 400µg/ml: 0.8943
Absorbance after Centrifugation of 400µg/ml: 0.7321
% Recovery = 83%

RESULTS AND DISCUSSION

The blank and the prepared dilutions of the pure Oxceprol were scanned in the UV range of 200-400nm. A sharp peak was obtained at λmax215nm, but for the blank no peak was obtained. This shows that the method

developed is Specific to the sample.The drug obeyed the Beer-Lambert's law in the calibration curve range of 100-1000 µg/ml where the linear response of the drug was recorded. Correlation coefficient was found to be 0.999.

Linearity

Table1: Linearity of Baricitinib spiked in the plasma sample

	Calibration	Curve
S. No.	**Concentration (ppm)**	**Absorbance**
1	100	0.0462
2	200	0.2926
3	300	0.5962
4	400	0.8943
5	500	1.2091
6	600	1.4929
7	700	1.8132
8	800	2.2963

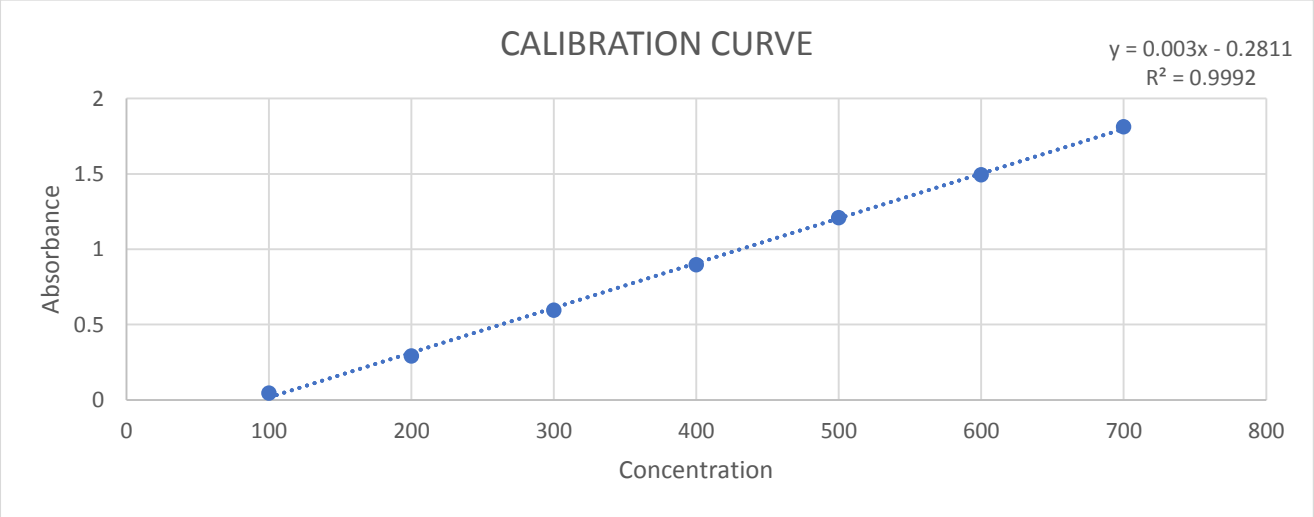

Figure: 2 Calibration curve of Oxaceprol

Repeatability

Table 2: Repeatability of Oxaceprol

QC Concentration levels	Within-run data	Between-run data
100 µg/ml	5 replicates	3 replicates for 2 days
300 µg/ml	5 replicates	3 replicates for 2 days
700 µg/ml	5 replicates	3 replicates for 2 days
1000 µg/ml	5 replicates	3 replicates for 2 days
Standard Deviation	0.006137236	0.003029442
% RSD	0.963182142	0.537389225

Accuracy

Table 3: Accuracy data of Oxaceprol

QC Concentration levels	Within-run data	Between-run data
100 µg/ml	5 replicates	3 replicates for 2 days
200 µg/ml	5 replicates	3 replicates for 2 days
700µg/ml	5 replicates	3 replicates for 2 days
1000 µg/ml	5 replicates	3 replicates for 2 days
Standard Deviation	0.00351782	0.005128382
% RSD	0.754209672	0.521934166

LLOQ: LLOQ from the calibration curve was found to be 100µg/ml.

ULOQ: ULOQ from the calibration curve was found to be 1000µg/ml.

The accuracy and precision were also calculated for the method developed. The data related to the within-run and between-run accuracy and precision is calculated. The %RSD for the precision was calculated to be 0.963185%. The recovery % of drug from the plasma sample was obtained as 83 %.

CONCLUSION

The method which is proposed in the above study was found to be simple and economical. Determination of oxaceprol in formulations were good agreement with their respective label claims without any interferences of excipients or additives. The calibration curve was plotted and found to be linear. All validation parameters were determined and found to be within the limits.

ACKNOWLEDGEMENT

We are thankful to our Principal, Prof. M. Sumakanth and Faculty of Department of Pharmaceutical Analysis of RBVRR Women's College of Pharmacy for giving us this opportunity to perform this research work.

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