Quantitative Estimation of Metaxalone in rat serum using Ultrafast Liquid Chromatography

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ABSTRACT

Metaxalone is skeletal muscle relaxant used for treating skeletomuscular pains. A new and reliable liquid chromatography method was developed and validated for estimation of metaxalone in wistar rat serum. The analyte was extracted from wistar albino rat serum using diethyl ether as extraction solvent. A C-18 column (250mm×4.6mm, 5µm) using methanol: water (80:20, % v/v) flowing at 1.0mL/min, with detection at 225nm was the optimized chromatographic condition. Metaxalone shows a retention time of 4.4min and was well resolved from serum components as well as internal standard vilazodone (6.3min) showing method linearity at 50-3200ng/mL with acceptable accuracy and precision. The newly optimized method was validated for various parameters with values in accordance to federal guidance. The current method was found to show reliable and reproducible recovery of analyte and has the potential for application in pharmacokinetic, bioavailability and bioequivalence studies.

KEYWORDS: Metaxalone, Liquid-Liquid Extraction, Ultrafast Liquid Chromatography, Bioanalysis, Stability.

1. INTRODUCTION

Metaxalone, 5-[(3,5-dimethylphenoxy)methyl]-1,3-oxazolidin-2-one (Figure 1) is skeletal muscle relaxant. Only one LC and several LC-MS methods are reported to estimate metaxalone in biological samples 2-7. However, application of LC-MS/MS for routine routine bioanalysis purpose is calls for solving additional challenges and high expenses. Hence, HPLC is a better option than LC-MS/MS with regard to affordability, versatility, handling and maintenance during bioanalytical estimation process. However, in the reported HPLC methods the authors have observed few critical issues like use of a highly toxic extraction solvent, fails to establish a chromatogram displaying resolution among analyte and internal standard, rather shows separate chromatograms for both of them, at many instances it is ambiguous to identify the source of plasma obtained (either rabbit or rat), use of very high volume of rat plasma etc. which calls for developing a new efficient and reliable bioanalytical chromatographic method. In view of all these aspects in this current study the authors tried to resolve the above mentioned issues and attempted to develop and validate new bioanalytical method free of any drawbacks to estimate metaxalone in rat serum.

2. MATERIAL AND METHODS

Reagents and Standards
Pure standard drug of metaxalone (purity>98%) was received as gift sample from Sun Pharmaceuticals Ltd., India. HPLC grade methanol and diethyl ether were purchased from Merck Ltd., Mumbai, India. HPLC grade water was obtained using TKA Water Purifier, Germany.

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Instrumentation and chromatographic conditions
A SHIMADZU Prominence Series UFLC equipped with binary pumps and a photo diode array (PDA) detector was used for the purpose. A reversed phase column (250×4.6 mm, 5 µm) was used for chromatography. The mobile phase contained methanol and water in a composition of 80:20%, v/v flowing at 1.0mL/min, with detection at 225nm. A cyclomixer and centrifuge (Remi, India) were used for preparing bioanalytical samples.

Preparation of Stock Solution and Calibration Curve
Around 5mg of metaxalone was dissolved in mobile phase placed in 5ml volumetric flask and working standard solutions within 50-3200ng/mL of metaxalone were prepared using the above solution. The peak areas at each point (n=3) were used to prepare the calibration curve.

Collection of Rat Serum and Bioanalytical Sample Preparation
Whole blood was collected from the retro orbital plexus of Wistar albino rats. Afterwards, the blood was allowed to clot, centrifuged at 10,000 rpm for around 10min and the obtained serum content was stored at -20°C. Around 50 µL of standard metaxalone solution was spiked into 200 µL of serum. A volume of 1000 µL of diethyl ether was added to the spiked samples for extraction of analyte. The typical extraction process consisted of vortexing for 30 s, followed by 5min of centrifugation at 3500 rpm (4°C). Suitable aliquot (500 µL) of supernatant organic portion was withdrawn, followed by evaporation to dryness on a water bath (50°C). Suitable aliquot (500 µL) of supernatant organic portion was withdrawn, followed by evaporation to dryness on a water bath (50°C). Prior injections in to the UFLC, the dried residues were freshly reconstituted with the mobile phase. The research study was approved as per IAEC Approval No. 82/Chairman IAEC,RIPS, Berhampur-760010.

Bioanalytical Method validation

Accuracy and Precision
Bioanalytical method accuracy and precision was assessed at four concentration levels inclusive of LLOQ (50ng/mL) and three quality control samples with replicate analysis (n=6).The three concentration levels were 100ng/mL (low QC:LQC), 400ng/mL (mid QC:MQC) and 1600ng/mL (high QC:HQC). The intra and inter-day studies were carried out on same day and three different days, respectively. The method trueness (±15% for three QC levels and ±20% at LLOQ) and preciseness (% relative standard deviation ±15% for three QC levels, except ±20% at LLOQ) was calculated based on the results obtained from UFLC analysis.

Sensitivity, Selectivity and Recovery
Method sensitivity as limit of detection (LOD) was determined based on signal-to-noise ratio(S/N=3). The lowest and highest concentrations in the linearity curve were designated as lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ), respectively. Method selectivity was assessed using serum samples (n=6) by evaluating interference due to serum components at the analytes retention time. A comparison based assessment of amount of metaxalone recovered from rat serum at LLOQ to that of standard metaxalone solution was also performed.

Stability studies and system suitability
Stability studies at conditions such as freeze-thaw (three cycles), short-term (up to 5h), long-term (up to 14th day) and stock solution (up to 5h at ambient condition and 14days at -20°C) stability were performed. The study concentrations consisted of LQC, MQC and HQC. Further, average recovery was calculated, to assess stability of metaxalone in serum. The results (%RSD) obtained for stressed and recovered metaxalone at the above levels was compared with the initial content of analyte. The typical chromatographic system suitability parameters such as retention time (Rt), plate number (N), resolution (Rs) and tailing factor (T) were assessed from the precision study of metaxalone at LLOQ.

3.RESULTS AND DISCUSSION

UFLC method development studies
According to the physicochemical property of analyte an LC method utilizing C-18 phase was found befitting for chromatography of metaxalone. Various compositions of mobile phase (50:50, 70:30, 80:20, v/v) and flow rates such as 0.8 and 1.0mL were tested to check response of metaxalone. A composition of methanol: water at 80:20, v/v flowing at 1.0mL/min, and detection at 225nm gave best peak shape. Metaxalone shows a retention time at 4.4min, which is well distant from the retention of I.S. Vilazodone was selected as the I.S. which shows retention at 6.3min with suitable resolution. Afterwards; bioanalytical method validation was performed as per federal guidelines.

Bioanalytical Method Validation
This was proved to be linear over 50-3200 ng/mL for metaxalone (R²= 0.999) Results of regression studies advocated for method aptness for the purpose. Table 1 demonstrates the bioanalytical method trueness and preciseness. Accuracy (intra-day and inter-day) of the method was acceptable (87.33% to 93.66%).

<table>
<thead>
<tr>
<th>Days</th>
<th>Concentration (ng/mL)</th>
<th>Average Concentration recovered (ng/mL)</th>
<th>Accuracy (% RE)</th>
<th>Precision (% RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra</td>
<td>LLOQ:50</td>
<td>45.5</td>
<td>-9.5</td>
<td>2.39</td>
</tr>
<tr>
<td></td>
<td>LQC:100</td>
<td>93.66</td>
<td>-6.34</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>MQC:400</td>
<td>373.41</td>
<td>-6.64</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>HQC:1600</td>
<td>1492.5</td>
<td>-6.71</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>LLOQ:50</td>
<td>43.66</td>
<td>-12.66</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>LQC:100</td>
<td>91.47</td>
<td>-8.52</td>
<td>1.57</td>
</tr>
<tr>
<td></td>
<td>MQC:400</td>
<td>360.58</td>
<td>-9.85</td>
<td>1.57</td>
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<tr>
<td></td>
<td>HQC:1600</td>
<td>1451.11</td>
<td>-9.3</td>
<td>2.16</td>
</tr>
</tbody>
</table>

Table 1. Accuracy and precision data of the method
The RSD values within 2% for the various parameters investigated, suggested optimum system suitability for estimation of metaxalone present in rat serum.

4. DISCUSSION

The new method consisted of a reversed phase UFLC along with a new mobile phase apt for the bioanalytical estimation purpose. Use of an I.S. ensured better quantification of analyte with recoveries >90%. Validation studies were pivotal for establishing method appropriacy and reliability. A linear and sensitive liquid chromatographic method free from any drawbacks was developed for estimating metaxalone in rat serum. Method accuracy and precision in both intraday and inter-day pattern produced optimum and reproducible results advocating method reliability. Further, the stability studies for metaxalone under vivid stress conditions indicated stability aspect of metaxalone during the study period.

5. CONCLUSION

A new and reliable bioanalytical UFLC method was developed and validated for quantitative estimation of metaxalone in wistar albino rat serum. Bioanalytical method validation studies indicated method suitability for estimation of metaxalone in rat serum. Hence, the present liquid chromatographic method is apt (recovery > 90%) for estimating metaxalone in biological matrix. Furthermore, the developed bioanalytical method projects its applicability for bioavailability and bioequivalence studies of metaxalone in different biological fluids.

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CONFLICTS OF INTEREST

The authors do not have any conflict of interest.

REFERENCE


