SIMPLE AND SENSITIVE RP-HPLC METHOD FOR THE DETERMINATION OF METAXALONE IN BULK AND ITS PHARMACEUTICAL FORMULATION

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ABSTRACT
A simple, highly sensitive, precise and accurate high-performance liquid chromatographic method with Diode Array detection (DAD) was developed and validated for the rapid quantification of Metaxalone (CAS Registry No, 1665-48-1) in bulk and its pharmaceutical dosage form. The chromatographic separation was achieved with a reverse phase column Symmetry C8 (4.6 x 150mm, 3.5 µm, Make: XTerra) and the mobile phase consisted of Acetonitrile: Potassium di hydrogen phosphate Buffer pH 7 adjusted with NaOH in the ratio of 65:35 v/v, at a flow rate of 0.6 mL/min, the injection volume was 20 µl with run time of 7 mins and UV detection was carried out at 219nm. The method was validated for specificity, linearity, precision, accuracy, robustness and system suitability. The method was linear in the drug concentration range of 20–60 µg/ml. The precision (RSD) of six samples was 0.22% for repeatability, and the intermediate precision (RSD) among six-sample preparation was 0.11%. The mean recovery was 99.4%. The proposed method can be used successfully for routine analysis of the drug in bulk and pharmaceutical dosage forms.

Keywords: Metaxalone, Diode Array detection, Acetonitrile, Potassium di hydrogen phosphate Buffer pH7.
INTRODUCTION
Metaxalone has the molecular formula C12H15NO3 and chemical name 5-[(3,5dimethylphenoxy)methyl]-2-oxazolidinone) (Figure 1) with a molecular mass of 221.26 g/mol and absorption maxima around 280 nm. Metaxalone belong to the BCS class II of centrally acting skeletal muscle relaxant drug with antispasmodic effect [1]. Metaxalone belongs to non benzodiazepine antispasmodics with a structure similar to mephenaxalone nucleus [2]. Metaxalone (skelaxin) got FDA approval in 1962 by King Pharmaceuticals mainly for the treatment of acute, painful and musculoskeletal conditions like fractures, dislocations, and trauma to tendons and ligaments and other measures for the relief of discomforts [3]. The mode of action of the metaxalone is clearly unknown but hypothesized as CNS depressant drug which causes skeletal muscle relaxation and sedation [4]. It acts through inhibiting interneuronal activity and blocking polysynaptic reflex pathways at spinal cord and at descending reticular formation in brain but leaving monosynaptic pathways intact like other similar class of skeletal muscle relaxants [5,6]. Metaxalone directly does not cause any relaxant effect on tense skeletal muscles or on the contractile mechanism of striated muscle, the motor end plate or the nerve fiber in humans.

Figure 1: Chemical structure of Metaxalone

Literature survey has revealed that there only few methods were reported for the determination of metaxalone in plasma by liquid chromatography. Methods reported in the literature for the estimation of metaxalone in bulk and biological fluids include soft ionization interfaces like electrospray ionization (ESI) for determining metaxalone (LC-MS/MS) [10], ultraviolet spectroscopy with LC Chromatography method (HPLC-UV) [11], UV spectroscopic method [12] gas chromatography with flame ionization detection [13,14], gas chromatography with mass detection [15,16].

From the literature survey, reported methods were mainly designed for human biological samples typically above 0.2 mL of human plasma reveals the usage of high quantity of
sample in terms of volume, high solvent consumption and tedious sample processing includes control of the factors like pH, extraction solvent, evaporating temperature which is highly time consuming and laborious analysis. Under the scope of this view, aim of our research work is to develop a highly specific, reliable and sensitive method for metaxalone determination in bulk and its dosage forms that proves to be of immense importance for conducting regular quality control analysis efficiently in terms of less sample volume, short run time, less tedious processing and sensitive analysis. Hence, a highly sensitive isocratic Rp-HPLC-DAD method was developed and validated according to the ICH guidelines [17] for quantifying metaxalone in bulk and its pharmaceutical dosage form at a concentration range (40 ng/mL).

MATERIALS AND METHODS

Chemicals and reagents
Metaxalone was kindly gifted by aurobindopharma (Hyderabad, India). Flexura tablets (Sun Pharma) containing 400 mg of metaxalone, were purchased from local market. Purified water was prepared using a Millipore Milli-Q (Bedford, M.A., USA) water purification system. Acetonitrile of HPLC grade was purchased from Burdick and Jackson (Muskagon, MI, USA), Potassium di hydrogen phosphate A.R. grade and NaOH of A.R. grade were purchased from local suppliers.

Instrumentation
Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC10AT and LC10AT VP series HPLC pumps, with a 20 µL Injection of sample loop (manual) and Diode Array Detector. The output signal was monitored and integrated using Shimadzu CLASS-VP Version 6.12 SP1 software. Symmetry C8 (4.6 x 150mm, 3.5 µm, Make: XTerra) column was used for the separation. The pH of the solution was adjusted by using digital pH meter, model DI 707 (Digisun electronics, Hyderabad, India).

Chromatographic conditions
The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 0.6 mL/min. The eluents were monitored at 219 nm. Although the λmax of metaxalone in the mobile phase is 272 nm, but good resolution, peak area were resulted at 219 nm. The column temperature was maintained ambient throughout the experiment. The identification of the
separated metaxalone was confirmed by running the chromatograms of the individual compound under identical conditions.

**Preparation of mobile phase**

350mL (35%) of Potassium di hydrogen phosphate buffer and 650 mL of Acetonitrile HPLC grade (65%) was mixed and pH was adjusted to 7.0 with Sodium hydroxide, degas in ultrasonic water bath for 5 minutes. Filtered through 0.45 µ filter under vacuum filtration. Mobile phase was used as diluent.

**Preparation of standard drug solutions**

Stock solution of metaxalone was prepared by dissolving 25 mg of metaxalone in 25 mL of volumetric flask containing 20 mL diluent. The solution was sonicated for about 5 min and then made up to volume with the same. Daily working standard solutions of metaxalone was prepared by suitable dilution of the stock solution with the mobile phase.

**Procedure for tablets**

20 Tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 25 mg of metaxalone was extracted with mobile phase in a 25 mL volumetric flask using ultra sonicator. This solution was filtered through Whatman No 1 filter paper. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously determined. All determinations were carried out in six replicates. The represented data were shown in (Table 1).

**RESULTS AND DISCUSSION**

**Method development and optimization**

The chromatographic method was optimized by changing various parameters, such as pH of the mobile phase, organic modifier and buffer used in the mobile phase and composition of mobile phase. Several mobile phases were tested until good resolution, retention time and tailing obtained. Mixture of acetonitrile (ACN): potassium dihydrogen phosphate buffer (pH 7 adjusted with sodium hydroxide) in the proportions of 40:60, 45:55, 50:50, 60:40 and 65:35 (v/v) were tested as a mobile phase with Symmetry C8 (4.6 x 150mm, 3.5 µm, Make: XTerra) column. Increasing the composition of organic modifier decrease in retention time, the peak shape of drug was poor and shoulder peak was observed (60:40 v/v ACN: Buffer). Decreasing the composition of organic modifier increase in retention time (40:60 v/v ACN: Buffer). The mobile phase composition of 60:40 v/v ACN: Buffer, resolution, retention time
were good but tailing factor is high. The method was optimized with the mobile phase composition of acetonitrile and phosphate buffer 65:35 (v/v). Buffer molarity of 10, 20 and 50 mM was tested. There were no significant changes in the chromatographic response and peak shape with change in buffer molarity. After several trials, the method was optimized as a mixture of acetonitrile: potassium dihydrogen phosphate buffer (pH 7) (65:35 v/v), at a flow rate of 0.6 mL/min, at 219 nm for run time of 7 min. These chromatographic conditions achieved satisfactory resolution, retention tailing for metaxalone. The Figure 2 & 3 shows that chromatogram of metaxalone in bulk and in formulations respectively.

![Figure 2: Typical Chromatogram of Metaxalone Active Pharmaceutical Ingredient](image1)

![Figure 3: Typical chromatogram of Metaxalone tablet](image2)

**Method validation**

The proposed method was validated accordance to ICH guidelines [17], for system suitability, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and specificity. For system suitability, six replicates of standard sample were injected and studied the parameters like plate number (N), tailing factor (k), and relative retention time (α), HETP, capacity factor (kI), plates per meter and peak symmetry of samples and the results were found to be within the specified limits as per the guidelines.
**Linearity**

The linearity of this method was evaluated by linear regression analysis, which was calculated by least square method. Calibration standards were prepared by spiking required volume of working standard (100 µg/mL) solution into different 10 mL volumetric flasks and volume made with the diluent to yield concentrations of 20-60 µg/mL. A 20 µL aliquot was injected into the analytical column. The resultant peak areas of the drug was measured. Calibration curve was plotted between peak area of drug against concentration of the drug. The results show there was an excellent correlation between peak area and analyte concentration. The linearity results are presented in Figure 4.

![Figure 4: Linearity of Metaxalone](image)

**Accuracy (recovery studies) and precision**

The accuracy (Recovery studies) was performed at the concentrations of 20µg/ml, 40µg/ml and 60µg/ml, respectively. The precision of the method was performed with the 100 % concentration i.e., 40µg/ml. The accuracy and precision samples of metaxalone in bulk and its tablets were within acceptable limits (n = 6). The results of the method validation studies presented in Table 1 and 2.

**Table 1: Results of Accuracy (Recovery Studies)**

<table>
<thead>
<tr>
<th>% Concentration (at specification Level)</th>
<th>Area</th>
<th>Amount Added (mg)</th>
<th>Amount Found (mg)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>1464008</td>
<td>5.25</td>
<td>5.32</td>
<td>101.4%</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>2652370</td>
<td>9.80</td>
<td>9.65</td>
<td>98.5%</td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td>3897020</td>
<td>14.4</td>
<td>14.1</td>
<td>98.4%</td>
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</table>
Table 2: Results of Precision (Assay)

<table>
<thead>
<tr>
<th>Injection</th>
<th>Area</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection-1</td>
<td>2731408</td>
<td>100.06</td>
</tr>
<tr>
<td>Injection-2</td>
<td>2729670</td>
<td>99.85</td>
</tr>
<tr>
<td>Injection-3</td>
<td>2733815</td>
<td>100.00</td>
</tr>
<tr>
<td>Injection-4</td>
<td>2737467</td>
<td>99.87</td>
</tr>
<tr>
<td>Injection-5</td>
<td>2735220</td>
<td>100.05</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>99.97</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td></td>
<td><strong>0.102</strong></td>
</tr>
<tr>
<td><strong>%RSD</strong></td>
<td></td>
<td><strong>0.102</strong></td>
</tr>
</tbody>
</table>

Limits of detection and quantification

Limit of detection (LOD) and limit of quantification (LOQ) was calculated based on the standard deviation of the response and the slope. LOD and LOQ was found to be 0.02µg/ml and 0.06µg/ml respectively.

Conclusion:

Since merits of LC method compare to other techniques are well recognized, a highly sensitive, specific and reproducible isocratic LC method with diode array detection method is more valuable. In addition, along with method development, the method is also validated to quantify the concentration range of 20-60 µg/mL of metaxalone in bulk and tablet samples and requires only 20 µL of sample volume. The RP-HPLC method presented here fulfils the criteria generally required for the assays. This assay has sufficient sensitivity, selectivity and recovery above 98%, which shows that the method is suitable for routine quality control analysis of Metaxolone.

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REFERENCES


