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SIMPLE AND SENSITIVE RP-HPLC METHOD FOR THEDETERMINATION 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 was developed and validated for the rapid quantification of metaxalone in bulk and its pharmaceutical dosage form. The chromatographic separation was achieved with a reverse phase column C18 (4.6×150mm, 5µm) and the mobile phase consisted of a cetonitrile potassium dihydrogen phosphate buffer methanol in the ratio 40:40:20 v/v, at a flow rate of 1.0ml/min, the injection volume was 10µl with run time of <5min and UV detection was carried out at279.5nm. The method was validated for specificity, linearity, precision, accuracy, robustness and system suitability. The method was linear in the drug concentration range of 20-100µg/ml the precision (RSD) of six samples was performed for repeatability and the intermediate precision (RSD) among six samples preparation was performed and the mean recovery was 99.7% the proposed method can be used successfully for routine analysis of the drug in bulk and pharmaceutical dosage forms.

KEYWORDS

Metaxalone, Acetonitrile and Potassium dihydrogen phosphate buffer.

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INTRODUCTION

Metaxalone has the molecular formula $C_{12}H_{15}NO_3$ and chemical name 5-[(3, 5dimethylphenoxy) methyl]-2-oxazolidinone) (Figure No.1) with a molecular mass of 221.25 g/mol and absorption maxima around 279.5 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 mephenaxal one 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 discomforts3. 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.

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 electro spray 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 prove 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 (20 µg/mL).

MATERIALS AND METHODS

Chemicals and reagents

Metaxalone was kindly gifted by aurobindo Pharma (Hyderabad, India). Skelaxin tablets containing 400 mg of metaxalone, were purchased from Atlanta Pharmacy U.S.A. Purified water was prepared using a Millipore Milli-Q (Bedford, M.A., USA) water purification system. Acetonitrile of HPLC grade, Potassium di hydrogen diphosphate, Methanol HPLC - Grade and NaOH, and Ortho phosphoric acid were purchased from Qualigens India Pvt.Ltd., and Loba chemie India Ltd.,

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 SPD-IOvpShimadzu UV-VIS detector. The output signal was monitored and integrated using Shimadzu CLASS-VP Version 6.12 SP1 software. Symmetry C18 column was used for the separation. The pH of the solution was adjusted by using digital pH meter, model DI 707 and Shimadzu AX-200 Digital Balance. (Digisun electronics, Hyderabad, India).

Chromatographic conditions

The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0 mL/min. The eluents were monitored at 279.5 nm. Although the λmax of metaxalone in the mobile phase is 280 nm, but good resolution, peak area were resulted at 279.5 nm separated metaxalone was confirmed by running the chromatograms of the individual compound under identical conditions. 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

4000mL (40%) of Potassium di hydrogen phosphate buffer and 400 mL of Acetonitrile HPLC grade (40%) and 200ml of methanol 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 diluents.

Preparation of standard drug solutions

Stock solution of metaxalone was prepared by dissolving 25 mg of metaxalone in 25 mL of volumetric flask and dissolved in minimum quantity of methanol and made up to 25ml with methanol. Daily working standard solutions of metaxalone was prepared by suitable dilution of the stock solution with the mobile phase.

Procedure for tablets

20 tablets of formulation (SKELAXIN) containing 400mg of metaxalone was accurately weighed to find out the avg weight and powdered. powdered tablet equivalent to 250mg of metaxalone was transferred n to 25ml volumetric flask, added methanol to dissolve and made up to the volume then the solution was solicited for 15min. after sonication, the solution was filtered through Whitman No 44 filter paper. From the clear solution, further dilution was made to bring a 100µg/ml using methanol. The prepared solution was measured at 279.5nm the amount of metaxalone was determined by using represented data were shown in (Table No.1).

RESULTS AND DISCUSSION

Method development and optimization

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 the chromatographic method was optimized by changing various parameters, such as pH of tailing obtained. Mixture of Acetonitrile (ACN) potassium dihydrogen phosphate buffer (pH adjusted with sodium hydroxide) methanol in the proportions of 40:40:20 (v/v) were tested as a mobile phase with Symmetry C18 column was carried by changing the various pH and flow rates. After several trials, the method was optimized as a mixture of Acetonitrile potassium dihydrogen phosphate buffer and methanol (40:40:20v/v), at pH 5.0 and at flow rate of 1.0 mL/min, at 219 nm for run time of <5 min. These chromatographic conditions achieved satisfactory resolution, retention tailing for metaxalone. The Figure No.2 and 3 shows that chromatogram of metaxalone in bulk and in formulations respectively.

Method Validation

The proposed method was validated accordance to ICH guidelines [17], for system suitability, linearity, and precision, and 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 diluents to yield concentrations of 20-100 µg/mL. A 20 µL aliquot was injected in to the analytical column. The resultant peak areas of the drug were measured. Calibration curve was plotted between peak areas 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 No.4.

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 No.1 and 2.

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 were found to be 0.3µg/ml and 0.9µg/ml respectively.

Table No.1: Recovery Studies for Metaxalone Formulation by UV-Method

S.No	Concentration used for estimation (%)	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered (µg/ml)	% Recovery	S.D.	% RSD
1	75	49.83	25	74.7	24.87	99.48	0.9828	0.9867
2	75	49.83	25	75.01	25.18	100.72		
3	75	49.83	25	74.39	24.56	98.24		
4	100	49.83	50	99.66	49.8	99.61		
5	100	49.83	50	99.06	49.23	98.46		
6	100	49.83	50	100.31	50.48	100.96		
7	125	49.83	75	124.33	74.5	99.33		
8	125	49.83	75	123.86	74.03	98.7		
9	125	49.83	75	125.56	75.73	100.97		

Table No.2: Quantification of Formulation: Skelaxin by HPLC Method

S.No	Expected amount (mg/tab)	Amount found (mg/tab)	Percentage obtained	Average (%)	S.D.	% RSD
1	400	401.72	100.43	99.99	0.3656	0.3656
		400.80	100.20			
		399.76	99.94			
		399.20	99.80			
		397.60	99.40			
		400.80	100.20			

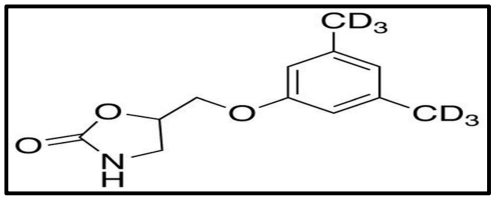

Figure No.1: Chemical structure of Metaxalone

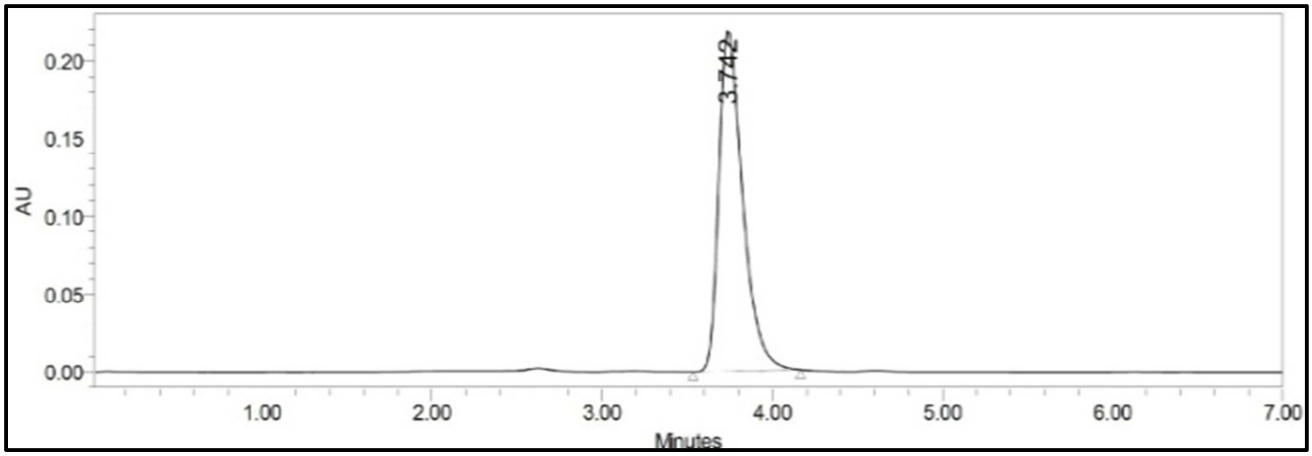

Figure No.2: Typical Chromatogram of Metaxalone Standard

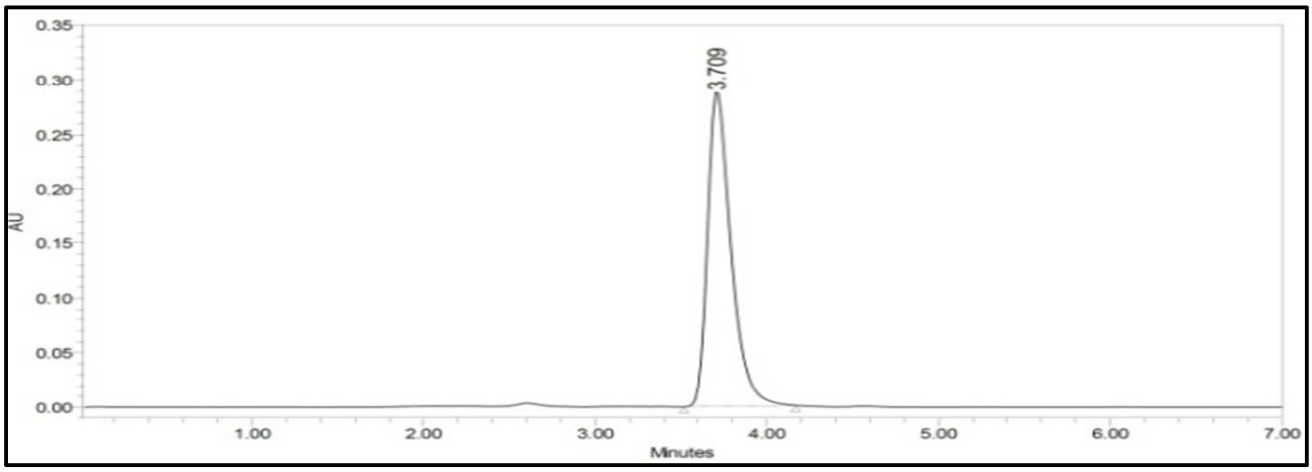

Figure No.3: Typical chromatogram of Metaxalone tablet

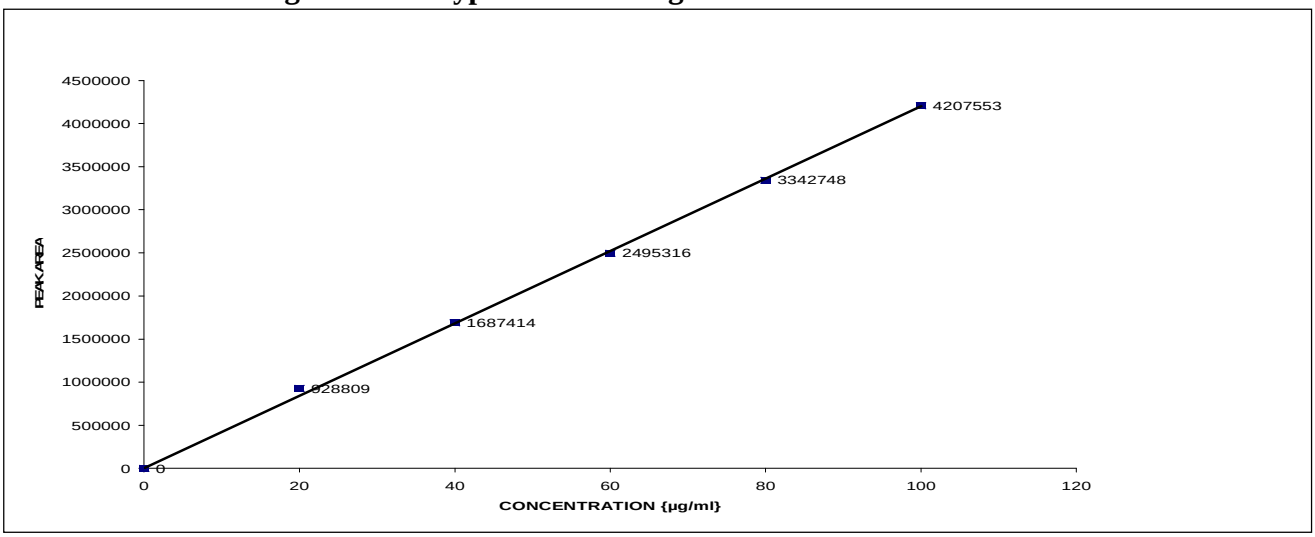

Figure No.4: Linearity Curve of Metaxolone

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 20-100 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 99.7%, which shows that the method is suitable for routine quality control analysis of Metaxolone.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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