WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 4, 1160-1168. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF RP-LC METHOD FOR METAXALONE IN PHARMACEUTICAL FORMULATIONS

Venkateswara Reddy Billa*[1], Prof. Anuradha Vejendla[1], Prof. Ramachandran Dittakavi[2] and Naveen Reddy Seelam[3]

[1,1]*Department of Chemistry, Vignan Degree College, Guntur, Andhra Pradesh, India.

[2]Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur. Andhra Pradesh, India.

[3]Department of Chemistry, Dharma Apparao College, Nuzvid. Andhra Pradesh, India.

Article Received on
02 Feb. 2020,

Revised on 22 Feb. 2020,
Accepted on 13 March 2020,

DOI: 10.20959/wjpr20204-17105

***Corresponding Author**
Venkateswara Reddy Billa
Department of Chemistry,
Vignan Degree College,
Guntur, Andhra Pradesh,
India.

ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid Chromatography assay method has been developed for estimation of Metaxalone in tablet formulations. The separation was achieved by using column Waters Acquity HSS T-3 C_{18} (100 × 2.1 mm, 1.7µm) in mobile phase consisted of pH 4.0 phosphate buffer and methanol in the ratio of (60:40, v/v). The flow rate was 0.8 mL/min^{-1} and the separated Metaxalone was detected using UV detector at the wavelength of 228 nm. Column temperature 25°C and sample temperature ambient and injection volume 10µl. The retention time of Metaxalone, was noted to be 7.05 min respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible and consistent.

KEYWORDS: Liquid Chromatography, Metaxalone, Validation.

1.0 INTRODUCTION

Metaxalone is a muscle relaxant used to relax muscles and relieve pain caused by strains, sprains, and other musculoskeletal conditions. Chemically metaxalone is 5-[(3, 5-dimethylphenoxy) methyl]-1, 3-oxazolidin-2-one.[1] It is a white to almost white, odorless crystalline powder freely soluble in chloroform, soluble in methanol, 96% ethanol and in propylene glycol, but practically insoluble in ether and water.[2] Metaxalone has no direct action on the contractile mechanism of striated muscle, the motor end plate, or the nerve

fiber. There is very limited or inconsistent data regarding the effectiveness and safety of metaxalone.[3] Metaxalone is one of the commonly used muscle relaxant therapies for acute low back pain.[4]

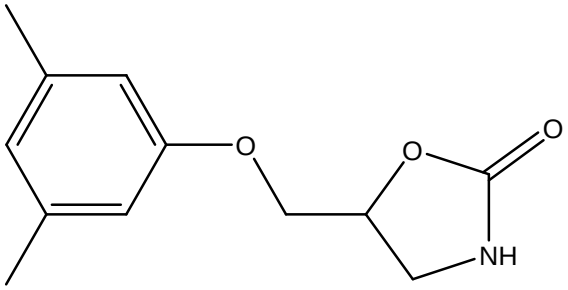

Figure 1.1: Chemical structure of Metaxalone.

Literature survey carried out revealed that several methods have been reported for estimation of metaxalone by using, RP-HPLC Methods[5-6], HPLC[7], RP-UPLC Method[8], LC-MS Method[9], UV Spectrophotometric method[10], UV Derivative Spectrophotometric method[11], HPTLC Method[12] are available to determine metaxalone in tablet dosage form. Although reports are available on stability indicating HPLC methods, the information provided is incomplete as well as results are contrast. Hence we tried to develop stability indicating HPLC method for Metaxalone. The present work describes a simple, stability indicating HPLC method for the determination of Metaxalone in bulk and tablet dosage form according to ICH guidelines.

2.0 Experimental

Chemicals and Reagents

Analytical-grade Potassium dihydrogen orthophosphate, orthophosphoric acid, were from Merck Chemicals Mumbai, India. Methanol and Water, both HPLC-grades, were from Merck Chemicals. Mumbai, India. Millex syringe filters (0.45 µm) were from Millex-HN, Millipore Mumbai, and India.

Instrumentation

Waters 2489 U.V-Visible detector/2695 Separation Module, equipped with Empower^2 software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model) Centrifuge Eppendorf 5810 were use in the present assay.

Preparation phosphate Buffer pH 4.0: Accurately measured quantity of 7.0 gm of Potassium dihydrogen phosphate in 1000 ml of HPLC Grade water and pH was adjusted to 4.0 with dilute orthophosphoric acid solution and degassed. The solution was filtered through 0.45µ filter paper and degassed.

Mobile phase preparation

Mixed phosphate buffer (pH 4.0) and Methanol in the ratio of 60:40% V/V.

Diluent preparation

Mix Methanol and Water in the ratio of (85:15 %v/v) and Sonicated for about 5 minutes for degas the diluent.

Standard preparation

Accurately transferred 100mg of Metaxalone working standard into a 100 mL volumetric flask and about 70 mL of diluent added, then sonicated to dissolve it completely and the volume was made up to the mark with the same solvent (Stock solution). Further pipette 5.0 ml of the above stock solution into a 50mL volumetric flask and diluted up to the mark with diluent. Further pipette 3.0 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Preparation

Accurately transferred the sample equivalent to 100 mg of Metaxalone into a 100 mL volumetric flask. About 70 mL of diluent added and sonicated to dissolve it completely and the volume is made up to the mark with diluent. Mixed well and filtered through 0.45µm filter. Further pipette 5.0 ml of the above stock solution into a 50mL volumetric flask and diluted up to the mark with diluent. Further pipette 3.0 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Chromatographic conditions

Chromatographic analysis was performed on Acquity HSS T-3 C18 (100 × 2.1 mm, 1.7µm) (Make: waters) column. The mobile phase consisted of pH 4.0 phosphate buffer and Methanol in the ratio of 60:40%v/v. The flow rate was 0.8 mL/min, column oven temperature 25°C, the injection volume was 20µL, and detection was performed at 228 nm using a photodiode array detector (PDA).

3.0 RESULTS AND DISCUSSION

Method development

Spectroscopic analysis of compound Metaxalone showed that maximum UV absorbance (λmax) at 228 nm respectively. To develop a suitable and robust LC method for the determination of Metaxalone, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Inertsil ODS 3V with the following different mobile phase compositions like that Buffer and acetonitrile in the ratio of 40:60 v/v 50:50 v/v & 55:45. It was observed that when Metaxalone standard was injected, Peak Tailing, not satisfactory.

For next trial Acquity HSS T-3 C18 (100 × 2.1 mm, 1.7µm) column used and the mobile phase composition were changed slightly. The mobile phase composition was buffer and methanol in the ratio of 60:40 v/v. respectively as eluent at flow rate 0.8 mL/min. UV detection was performed at 228nm. The retention time of Metaxalone is 2.45 minutes and the peak shape was good. The chromatogram of Metaxalone standard using the proposed method is shown in **(Fig: 1.2)** system suitability results of the method are presented in **Table-1.1.**

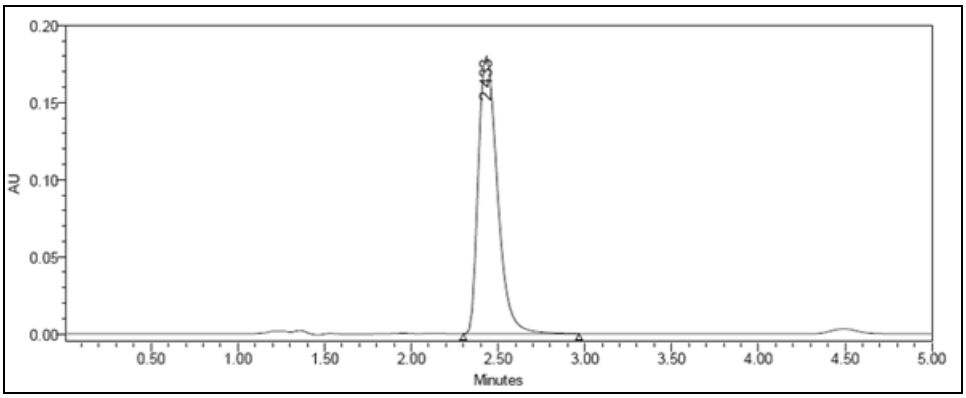

Figure 1.2: Chromatogram showing the peak of Metaxalone.

4.0 Method validation

The developed RP-LC method extensively validated for assay of Metaxalone using the following parameters.

4.1 Specificity

Preparation of blank solution

Mix Methanol and Water in the ratio of (85:15%v/v) and Sonicated for about 5 minutes for degas the diluent.

Preparation of Placebo solution

Placebo solution was prepared in duplicate by weighing the equivalent amount of excipients present in the finished drug product and analysed as per proposed method. Interference due to placebo was evaluated for each of the placebo preparations.

Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution **(Fig: 1.3)** showed no peak at the retention time of Metaxalone peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Metaxalone in Metaxalone tablets. Similarly chromatogram of placebo solution **(Fig: 1.4)** showed no peaks at the retention time of Metaxalone peak. This indicates that the placebo used in sample preparation do not interfere in estimation of Metaxalone in Metaxalone tablets.

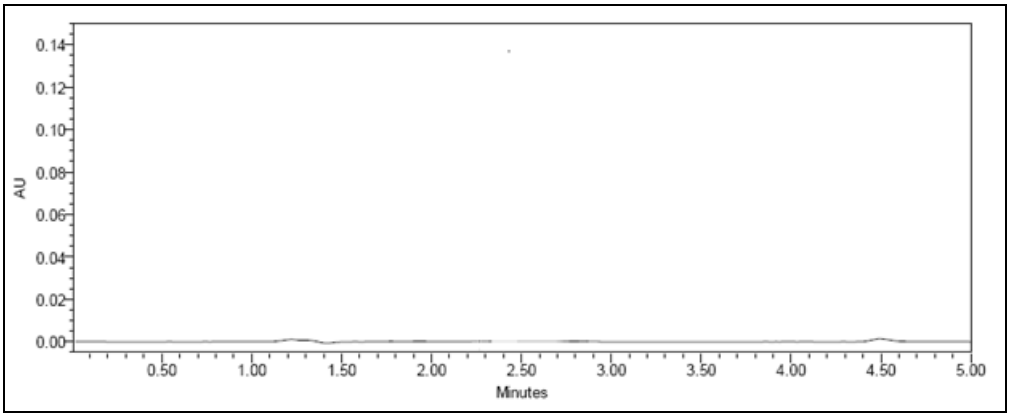

Fig: 1.3: Chromatogram showing the no interference of diluent for Metaxalone.

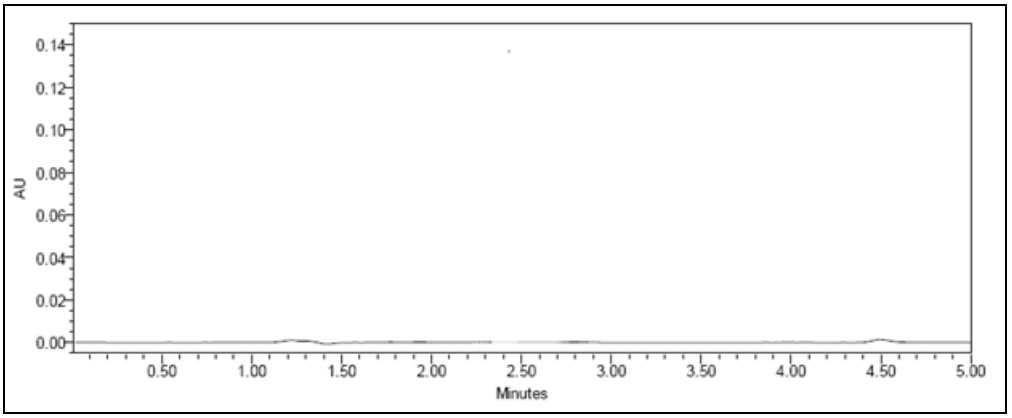

Fig: 1.4 Chromatogram showing the no interference of placebo for Metaxalone.

Table 1.1: System suitability parameters for Metaxalone by proposed method.

Name of the Compound	Retention Time	Theoretical plates	Tailing factor
Metaxalone	2.433	9287	1.3

4.2 Method precision

The precision of test method was evaluated by doing assay for six samples of Metaxalone tablet as per test method. The content in mg and % label claim for Metaxalone for each of the test preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The data were shown in **Table: 1.2**

Table: 1.2 Method precision data for Metaxalone.

No. of injections	Metaxalone
	% assay
Preparation 1	99. 0
Preparation 2	98.9
Preparation 3	98.6
Preparation 4	98.7
Preparation 5	98.6
Preparation 6	99.8
Average	**98.9**
%RSD	**0.5**

4.3 Linearity of detector response

The linearity of an analytical method is its ability to obtain test results which has a definite mathematical relation to the concentration of analyte. The linearity of response for Metaxalone was determined in the range of 30% to 165% (10µg/ml to 50µg/ml for Metaxalone). The calibration curve of analytical method was assessed by plotting concentration versus peak area and represented graphically. The correlation coefficient was found to be 0.9992. Therefore the HPLC method was found to be linear standard curve were calculated and given in **Figure: 1.5** to demonstrate the linearity of the proposed method. From the data obtained which given in **Table: 1.3** the method was found to be linear within the proposed range.

Table: 1.3 Linearity studies for Metaxalone by proposed method.

S.No	Metaxalone		
	Linearity concentration (%)	Concentration (µg/ml)	Average area response
1	30	10	264840
2	65	20	491451
3	100	30	690307
4	130	40	873311
5	165	50	1065958
Correlation coefficient:			0.9992
R^2			0.9984
Slope (m):			19840.96
Intercept (Y):			81944.6

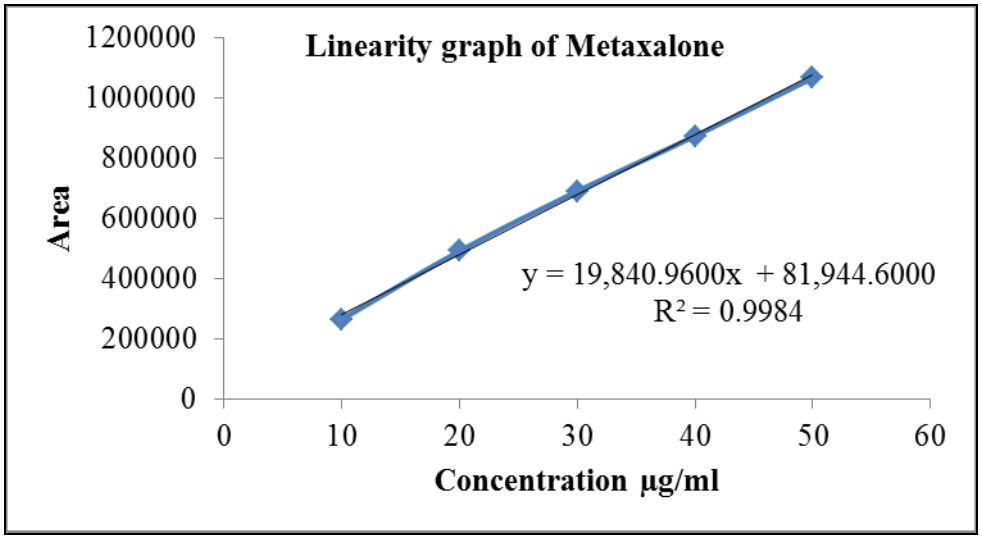

Figure 1.5: Calibration curve for Metaxalone.

4.4 Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of Metaxalone, analyzed as per the proposed method. The mean percentage recovery for 50%, 100%, 150% level was found to be 100.33, 100.36 and 100.32. %RSD was found to be 0.51, 0.79 and 0.46 respectively. They are within the acceptance limits. Therefore, the HPLC method for the determination of assay of Metaxalone in formulation was found to be accurate. The data obtained which given in **Table: 1.4** the method was found to be accurate.

Table: 1.4 Recovery studies for Metaxalone by proposed method.

S.No	% Recovery results of Metaxalone		
	50%	100%	150%
Preparation-1	100.89	99.74	100.85
Preparation-2	100.21	100.1	100.12
Preparation-3	99.89	101.25	99.99
Mean	**100.33**	**100.36**	**100.32**
SD	**0.51**	**0.79**	**0.46**
%RSD	**0.51**	**0.79**	**0.46**

5.0 CONCLUSION

An RP-HPLC method for estimation of Metaxalone was developed and validated as per ICH guidelines. A simple, accurate and reproducible reverse phase HPLC method was developed for the estimation of Metaxalone in bulk drugs and formulations. The optimized method consists of mobile phase pH 4.0 phosphate buffer and Methanol in the ratio of 60:40%v/v with Waters Acquity HSS T-3 C_{18} (100 × 2.1 mm, 1.7µm) column. The retention time of Metaxalone was found to be 2.45 minutes. The developed method was validated as per ICH Q2A (R1) guideline. The proposed HPLC method was linear over the range of 10µg/ml to 50.0µg/ml, the correlation coefficient was found to be 0.9992. Relative standard deviation for method precision was found to be 0.50.

We have developed a fast, simple and reliable analytical method for determination of Metaxalone in pharmaceutical preparation using RP-LC. As there is no interference of blank and placebo at the retention time of Metaxalone. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and Linearity. It allows reliably the analysis of Metaxalone in its different pharmaceutical dosage forms.

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