Int. J. Chem. Sci.: 12(4), 2014, 1430-1438
ISSN 0972-768X
www.sadgurupublications.com

DEVELOPMENT OF RP-HPLC METHOD FOR RAPID DETERMINATION OF METAXALONE AND IN BULK AND ITS SOLID ORAL DOSAGE FORM

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ABSTRACT

A simple, sensitive and reproducible reversed phase high performance liquid chromatography (RP-HPLC) coupled with a UV detector method was developed for the quantitative determination of metaxalone (MET) in pharmaceutical dosage forms. The method is applicable to the quantification of related substances and assay of drug product. Chromatographic separation was achieved on a BDS Hypersil C-18 (150 mm x 4.6 mm, 5 µm) column. The optimized isocratic mobile phase consists of a mixture of methanol: water, in the ratio of 90:10 % v/v. The eluted compounds were monitored at 279 nm for MET assay, the flow rate was 1 mL/min, and the column oven temperature was maintained at room temperature. The developed method separated MET from its excipients within 6.0 min. The developed method was validated as per International Conference on Harmonization (ICH) guidelines. The method was validated for specificity, linearity, precision, accuracy, robustness and system suitability. The method was linear in the drug concentration range of 10-60 µg/mL. The precision (% RSD) of six samples was 1.67. The mean recoveries were between 100.96%. The proposed method can be used successfully for routine analysis of the drug in bulk and combined pharmaceutical dosage forms.

Key words: Methanol, Hypersil BDS, Metaxalone and Chromatographic separation.

INTRODUCTION

Metaxalone, chemically 2-[(3, 4-dimethylphenoxy) methyl]-2-oxazolidinone is centrally acting muscle relaxant[1]. Figure 1 shows the chemical structure of Metaxalone. The mechanism of action of metaxalone in humans has not been established, but may be due to general central nervous system depression[2]. Metaxalone has no direct action on the

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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, drug in muscle relaxant therapies for acute lower back pain[4].

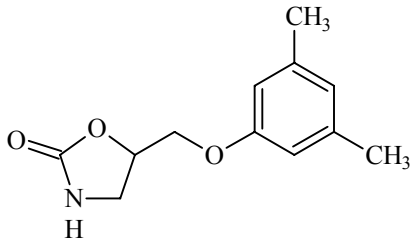

Fig. 1: Chemical structure of Metaxalone

Literature survey revealed that, RP-HPLC[5–9], LC–MS[10], HPTLC[11] and UV spectrophotometricmethods[12-14] were available for the estimation of MET alone or in combination with diclofenac sodium. This present study reports a simple method for the estimation of the Metaxalone by HPLC in bulk drug and in tablet dosage form. The proposed method is validated as per ICH guidelines[15-16].

EXPERIMENTAL

HPLC instrumentation and chromatographic conditions

The HPLC system consisted of a pump (Analytical Technologies, HPLC pump), a manual injector with 20 µ capacity per injection. The UV–vis detector was operated at a wavelength of 279 nm. The software used was N 2000 Chemstation. Column used were Hypersil BDS C18 (250 mm x 4.6 cm, 5 µm) (Thermo scientific, India), Chromatographic separation of MET was achieved at room temperature. The mobile phase consisted of Methanol: water in the ratio of 90:10 v/v at a flow rate of 1.0 mL/min. Before use, the mobile phase was filtered through a 0.22 mm nylon membrane filter and sonicated for 5 min. Injection volume was 20 µ, and the optimum wavelength selected for quantification was 279 nm.

Chemicals and reagents

Pharmaceutical grade metaxalone was suppied as a gift sample by MSN Laboratories Hyderabad, India. Methanol (HPLC grade) was purchased from Merck Chemical Company (India). HPLC grade water was prepared inhouse using Milli-Q water

filtration system. Sodium hydroxide, HCl and H_2O_2 was purchased from SD Fine Chem., Mumbai. All the reagents used were of AR grade.

Preparation of mobile phase

900 mL of HPLC grade methanol was placed in a 1000 mL volumetric flask and 100 mL HPLC grade water was added to it. The solution was mixed, filtered through the membrane filter and sonicated for 5 mins. Mobile phase was freshly prepared daily. The same was used as diluents for further analysis.

Preparation of stock solution

100 mg of pharmaceutical grade MET was weighed and dissolved in mobile phase in a 100 mL volumetric flask. The final volume was made upto the mark with the same to get 1 mg/mL primary stock solution. This solution was sonicated for 5 mins. From the above stock solution 3 mL was further diluted with the mobile phase in a 100 mL volumetric flask. The final volume was made upto the mark with the same. The required working standards for linearity were prepared from the primary stock solution.

Preparation of working standards (Assay of dosage forms)

198.8 mg of tablet powder was dissolved in 100 mL of mobile phase in a 100 mL volumetric flask. The solution was sonicated for 5 mins and filtered if necessary. 3 mL aliquot was further diluted to 100 mL with the mobile phase to get 30 µg/mL concentration. 20 µL each of stock and working standards were injected into the HPLC system.

Validation of HPLC method

The proposed RP-HPLC method was validated as per ICH guidelines.

RESULTS AND DISCUSSION

Method development

The UV absorption spectrum of MET shows absorption maxima at 279 nm; the response at 219 nm was higher than that of 279 nm, but detection wavelength of 279 nm was selected for chromatographic monitoring to reduce the base-line noise at 279 nm and to get the better response than from monitoring at 219 nm. For HPLC method development, initially the mobile phase used was acetonitrile and water at different proportions, but the peak shape was not good, so actonitrile was replaced by Methanol. Different proportions of

methanol: water was prepared. A combination of 90: 10 v/v of methanol: water shows good resolved peak in standards and in the dosage form samples, which resulted in a tailing factor of 1.62. Finally the optimized mobile phase composition for MET was methanol: water in the ratio of 90: 10 v/v at a flow rate of 1.0 mL/min. A model chromatogram for the standard was shown in Fig. 2.

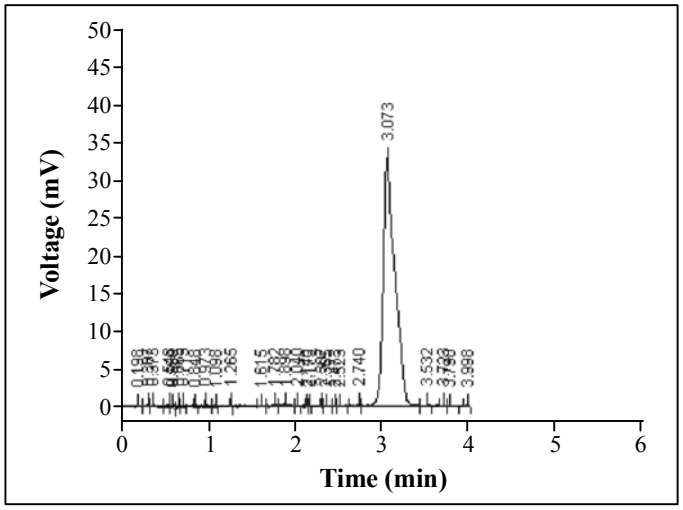

Fig. 2: Representative chromatogram of Metaxalone

Method validation

To confirm the suitability of the method for its intended purpose, the method was validated in accordance with ICH guidelines[15], for system suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and specificity.

System suitability

System suitability test was an integral part of method development and has been used to ensure adequate performance of the chromatographic system. Retention time (R_t), capacity factor (k), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 30 mg/mL. All the results were within the acceptable limits.

Linearity

MET showed linearity in the concentration range of 10-60 µg/mL. The regression equation obtained was $Y = 3841 x + 69745$ and $R^2 = 0.998$.

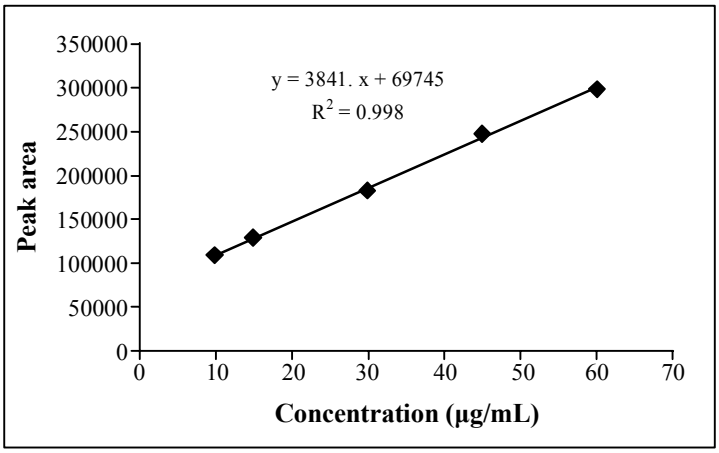

Fig. 3: Calibration curve of Metaxalone

Limits of detection and quantification

The LOD was defined as the lowest concentration of MET resulting in a signal-to-noise ratio of 3:1 and LOQ was expressed as a signal-to-noise ratio of 10:1. The LOD and LOQ obtained were 0.75 and 2.48 µg/mL, respectively.

Accuracy

Accuracy of the method was determined by performing the recovery experiments. Known amount of the standard at 50%, 100% and 150% levels was fortified to the degradation sample. Peak area of the standards was calculated by the difference of peak area between fortified and unfortified samples. Six replicate samples of each concentration level were prepared and the percentage recovery at each level (n = 6) was determined for MET, the results obtained are in good agreement with the added amounts. The results are shown in Table 1.

Table 1: Results of accuracy

Concentration (µg/mL)	Peak area*	µg/mL added*	µg/mL found*	Recovery*	% Mean Recovery*	Mean Recovery*
15	78879.25	14.92	14.72	98.65	97.56	
30	166618.95	29.84	30.15	101.02	102.52	100.96
45	241926.56	44.77	46.57	104.03	102.79	

* Mean of 6 replicates

Precision

Intraday and interday precision was evaluated by injecting six different replications of 30 µg/mL of MET. For intra-day variation, sets of six replicates of the optimized concentrations was analyzed on the same day; for inter- day variation, six replicates was analyzed on six different days. The intra-day and inter-day precision (% RSD) was found to be less than 2%. The results was shown in Table 3, indicating that the method was precise.

Table 2: Results of Precision

S. No.	Peak area	
	Interday	**Intraday**
1	159254.15	159144.15
2	165387.78	163287.78
3	161616.72	162116.72
4	157449.09	154549.09
5	150787.42	160237.42
6	167816.55	159878.55
Mean	160751.95	159897.79
SD	2680.79	2980.79
% RSD	1.67	1.86

Specificity

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. The chromtogram of specificity for the HPLC method is illustrated in Fig. 2, where complete separation of MET was noticed in the presence of excipients.

Assay

The developed HPLC method was applied for the analysis of MET in tablet dosage form. The mean % purity of the tablets was found to be 100.04%. The results of Assay are shown in Table 3.

Table 3: Results of the assay

S. No.	Peak area	% Purity
1	159254.15	99.57
2	160387.78	100.28
3	161616.72	101.04
4	157449.09	98.44
5	160787.42	100.53
6	160016.55	100.04
Average		99.98
SD		0.90
%RSD		0.90

CONCLUSION

The proposed HPLC method was proved to be simple, accurate, precise, specific and selective for quantitative analysis of MET. Hence, this can be useful as anassay method for the determination of metaxalone in bulk and pharmaceutical dosage forms.

ACKNOWLEDGEMENT

Authors are thankful to MSN laboratories, Hyderabad, AP for providing the API of MET as a gift sample. Authors are also thankful to management of Balaji College of Pharmacy, Anantapur (AP) for providing the necessary facilities to carry out this research project.

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Revised : 29.05.2014 *Accepted : 31.05.2014*