INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METAXALONE AND DICLOFENAC POTASSIUM IN TABLET DOSAGE FORM

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ABSTRACT

The study describes method development and subsequent validation of RP-HPLC method for simultaneous estimation of metaxalone and diclofenac potassium in combined tablet dosage forms. Chromatographic separation was achieved on an Inertsil ODS 3V (250 mm × 4.6 mm id, 5µm) column using a mobile phase consisting of (30:30:40v/v/v) water: methanol: acetonitrile at a flow rate of1.2ml/min. The detection wavelength is 218 nm. The retention times of metaxalone and diclofenac potassium were found to be 2.610 min and 4.607 min respectively. The developed method was validated as per ICH guidelines .The developed and validated method was successfully used for the quantitative analysis of metaxalone and diclofenac potassium in tablet dosage forms.

Keywords: Metaxalone, Diclofenac potassium, RP-HPLC method, Validation.

INTRODUCTION

Diclofenac Potassium chemically, Potassium [2-[(2,6-dichlorophenyl)amino]phenyl]acetate (Figure.1) is a non-steroidal anti-inflammatory agent (NSAID) that is widely used in pharmaceutical preparations for antipyretic and analgesic actions.

Metaxalone (5-[(3,5-dimethylphenoxy)methyl]-1,3-oxazolidin-2-one) (Figure.2), is a central nervous system depressant, muscle relaxant used to relax muscles and relieve pain caused by strains, sprains, and other musculoskeletal conditions. Its exact mechanism of action is not known, but it may be due to general central nervous system depression. It is considered to be a moderately strong muscle relaxant, with relatively low incidence of side effects.

Literature survey reveals few analytical methods like UV[1, 2], HPLC[3, 4, 5] and HPTLC[6]and stability indicating RP-HPLC[7, 8,9,10]have been reported for estimation of these drugs alone as well as in combination with other drugs in pharmaceutical dosage forms. The present work is aimed to develop and validate a new RP- HPLC method for simultaneous estimation of metaxalone and diclofenac potassium in bulk and pharmaceutical tablet dosage form.

MATERIALS AND METHODS

Equipment

Separation was carried out by using Shimadzu LC20A system equipped with LC20AT pump.SPD 20A Prominence Uv-Visible detector and the peak areas were integrated

by using Spinchrome software CFR. Analysis was carried out on Inertsil ODS 3V (250 x 4.6mm, 5µm) column.

CHEMICALS AND REAGENTS

Methanol, acetonitrile, water are of HPLC grade,orthophosphoric acid are of analytical grade.

Determination of absorption maxima by UV/Visible Spectrophotometer

Accurately weighed and transferred about 100 mg each of metaxalone and diclofenac potassium working standard into a 100 ml volumetric flask separately, then added to it about few ml of methanol and sonicated for 10 minutes to dissolve and diluted up to mark with mobile phase. This produced standard stock solution 1000µg/ml. Further 1ml of above solution was transferred into 10 ml volumetric flask and volume was made up with mobile phase. This produced solutions of concentration 100µg/ml. Finally diluted 1 ml of above solution to 10 ml using mobile phase and mixed well. The concentration of the working solution thus produced is10µg/ml.

The working standard solutions of metaxalone and diclofenac potassium (10µg/ml) were scanned over the range of 190-400nm. Both the drugs showed good response at 218nm and therefore 218 nm is selected for further study. The UV absorption spectrum of metaxalone and diclofenacpotassium is shown in Fig.no.3.

Preparation of solutions

Preparation of Mobile phase

Mobile phase was prepared by mixing water of pH 3.5±0.05 withmethanol and acetonitrile in the ratio 30:30:40 v/v/v. Mobile phase was filtered through 0.45µ membrane filter and sonicated for 10 min to remove dissolved gases before transferring in to the reservoir.

Preparation of standards

Preparation of standard stock solution of metaxalone

Accurately weighed 100 mg of metaxalone standard drug was transferred to 100 ml of volumetric flask. Then it was dissolved by adding a little amount of diluent. Mixed it well, sonicated to remove dissolved gases and to dissolve, finally thevolume was made up to 100 ml with diluent (1000µg/ml).

Preparation of standard stock solution of diclofenac potassium

Accurately weighed 100 mg of diclofenac potassium standard drug and transferred to a 100 ml of volumetric flask. Then it was dissolved by adding a little amount of diluent. Mixed it well, sonicated the solution and volume was made up to 100 ml with diluent.

Preparation of Working mixed Standard Solution

From the above stock solution 0.8ml of MET solution and 0.1ml of DCLF solution was transferred to 10 ml of volumetric flask and was made up to the mark with mobile phase. The working standard solution produced contains 80µg/ml of MET and 10µg/ml of DCLF.

Preparation of Sample Solution

Twenty tablets were weighed accurately and average weight was recorded. 116.4mg of powder equivalent to 40mg of MET and 5mg of DCLF was weighed accurately and transferred to 50ml volumetric flask and dissolved with mobile phase and sonicated for 10mins and filtered through 0.45µ membrane filter and volume made up to with mobile phase. The produced sample stock solution was (800µg/ml of MET and 100µg/ml of DCLF). From this stock solution 0.8ml of solution was transferred to 10ml volumetric flask and made upto volume with mobile phase.

Chromatographic conditions

A reverse phase column Inertsil ODS 3V (250 x 4.6mm, 5µm particle size)], equilibrated with mobile phase (water: methanol: Acetonitrile in the ratio of 30:30:40v/v/v) was used. Mobile phase flow rate was maintained at 1.2ml/min and effluents were monitored at 218nm. The sample was injected using 20 microlitre manual sample injector and run time was 6 mins.

Procedure

Under optimized chromatographic conditions 20 µl of each standard of linearity range was injected and chromatograms were recorded. Typical chromatogram of metaxalone and diclofenac potassium is given in fig.4.

METHOD VALIDATION

System suitability

The system suitability studies were done for parameters like theoretical plates, tailing factor, retention time, resolution by injecting the standard solution in to the optimized chromatographic system for six times and the results are given in the table no 1.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of compounds that may be expected to be

present, such as impurities, degradation products and matrix components.
The specificity of the method was assessed by comparing the chromatograms obtained from the drug standards with that of obtained from the drug in tablet and blank. The results are presented in the table 2.

Linearity
Linear calibrations plots of the proposed method were obtained over concentration ranges of 48-112µg/ml for metaxalone (48,64,80,96,112µg/ml)and 6-14µg/ml for diclofenac (6,8,10,12,14µg/ml).each solution was prepared in triplicate. Regression coefficientwas found to be 0.998for both the drugs (fig. 5and 6)**.**Standard curve had a reliable reproducible over the standard concentrations across the calibration range. All back calculated concentrations did not differ from the theoretical value as no single calibration standard point was dropped during the validation.

Accuracy
Thestandard addition method was used to demonstrate the accuracy of the proposed method. For this purpose, known quantities of metaxaloneand diclofenac potassium were supplemented to the previously analyzed sample solution and then experimental and true values compared. Three levels of solutions were made corresponding to 80, 100 and 120% of nominal analytical concentration (80µg/mlMTX, 10 µg/ml for DCLF). Standard preparation & Sample preparations was injected into the HPLC and % RSD forMetaxaloneand Diclofenac potassium peaks in Standard preparation was calculated and tabulated in table 3.

Recovery
The recovery of an analytical method should be established across its range. The study was performed by making three different standard concentrations 80%, 100% and120 % of known amounts of studied drugs. The recovery was found for metaxalone80%-99.09%, 100%-99.99% and 120%- 100.81% and for diclofenac potassium 80%- 99.61%, 100%- 120.61% and 120%- 100.26%.

Precision
For precision same concentration solution i.e. 80µg/ml of metaxalone and 10µg/ml of diclofenac potassium solution was injected 6 times and observed for any peculiar change in the areas and % RSD was calculated for each drug.

System Precision
For system precision study the standard solution replicates was injected repeatedly for six times and was observed. The standard deviation values were found to be 37.98 and 7.859 for metaxalone and diclofenac potassium and the %RSD values were 1.11 and 1.05 for metaxalone and diclofenac potassium and the results are tabulated in the table 4.

Robustness
Robustness is generally done by changing the parameters like flow rate and organic phase of the mobile phase.The results are shown in the following data is given in the table No.5.

Limit of detection (LOD)
The LOD for this method was found to be 1.81µg/ml and 0.12µg/ml for metaxalone and diclofenac potassium respectively.

Limit of quantitation (LOQ)
The LOQ for this method was found to be 5.48µg/ml and 0.37µg/ml for metaxalone and diclofenac potassium respectively.

Ruggedness
Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions.
It is checked that the results are reproducible under differences in conditions, analysts and instruments and hence the proposed method was found to be rugged.

Assay
Twenty micro liters of Standard and sample solutions were injected separately in to the chromatographic system and the peak areas for the analyte peaks were measured. The % content of each drug was calculated.

RESULTS AND DISCUSSION
To develop a new RP-HPLC method, several mobile phase compositions were tried. A satisfactory separation with good peak symmetry was obtained with Inertsil ODS 3V(4.6 x 250 mm, 5 µm) column using mobile phase containing water:methanol:acetonitrile (30:30:40 v/v /v)at a flow rate of 1.2ml/min. Quantification was achieved with UV detection at 218 nm based on peak area. The retention time for metaxalone and diclofenac potassium were found to be 2.610min and 4.607min, respectively. The optimized method was validated as per ICH guidelines. The System suitability parameters observed by using this optimized conditions were reported. A linearity range of 48-112µg/ml with correlation coefficient 0.998 was established for

metaxaloneand 6-14µg/ml with correlation coefficient 0.998 was established for diclofenac potassium.The precision of the proposed method was carried in terms of the repeatability and the %RSD values of metaxalone was found to be 0.47% and of diclofenac potassium was found to be 0.75% and reveal that the proposed method is precise. The LOD and LOQ values formetaxalone were1.81µg/ml, 5.48µg/ml respectively and for diclofenac potassium were found to be 0.12µg/ml, 0.37µg/ml. The study of robustness in the present method shows no significant changes either in the peak area or Rt. The results of analysis of commercial formulation indicated that there is no interference due to common formulation excipients with the developed method. Therefore, the proposed method can be used for routine analysis of these two drugs in their combined pharmaceutical dosage form.

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of metaxalone and diclofenac potassium from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of metaxalone and diclofenac potassium in pure form and its dosage form and also can be used for dissolution or similar studies.

ACKNOWLEDGEMENT

The authors are thankful to Active Pharma Labs, Hyderabad, India for providing sample of metaxalone and diclofenac potassium for research. The authors express heartful thanks to Principal and Management of Malla Reddy Institute of Pharmaceutical sciences for allowing us to use the lab facilities to carry out the project.

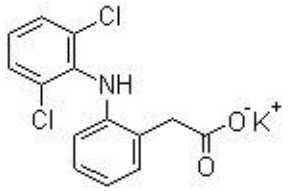

Fig. 1: Chemical structure of diclofenac potassium

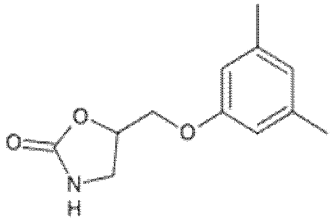

Fig. 2: Chemical structure of metaxalone

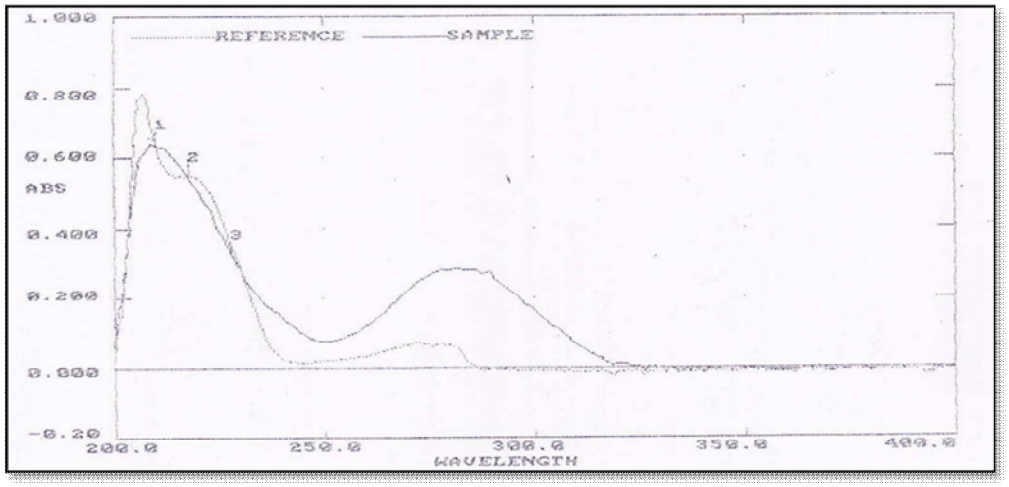

Fig. 3: Overlaid UV- spectrum of metaxalone and diclofenac potassium

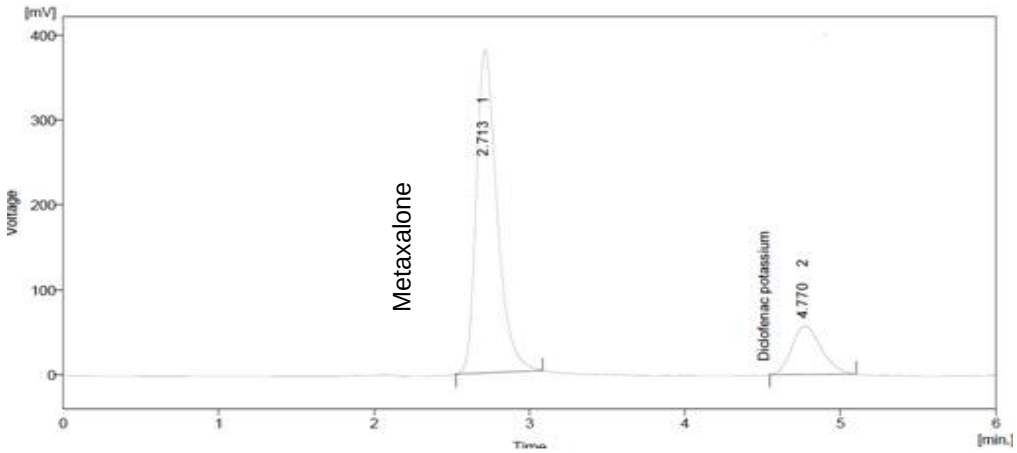

Fig. 4: A typical chromatogram of metaxalone and diclofenac potassium

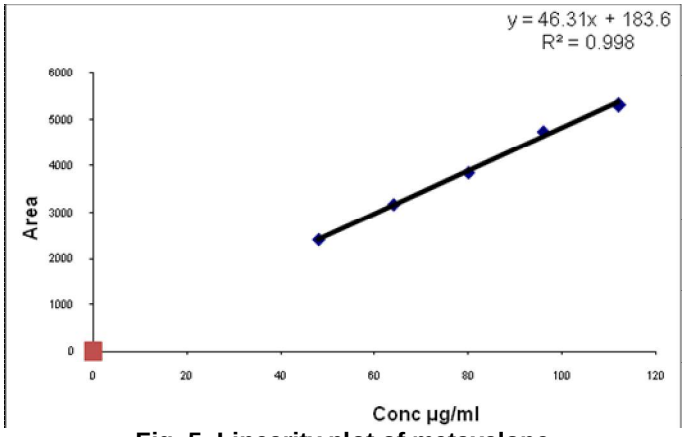

Fig. 5: Linearity plot of metaxalone

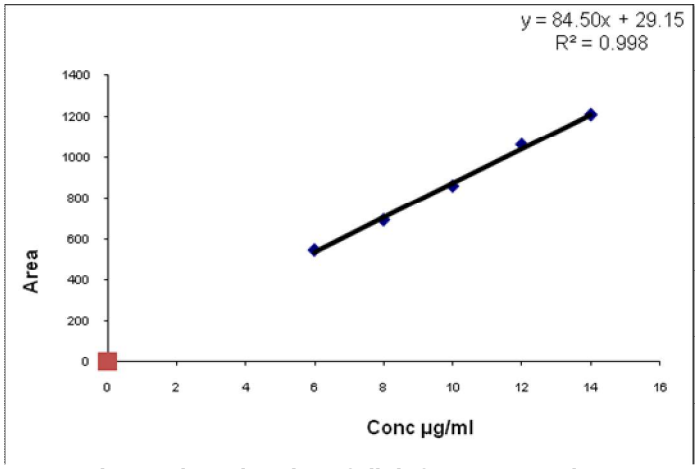

Fig. 6: Linearity plot of diclofenac potassium

Table 1: System suitability parameters

S.NO	Parameter	Drug	Observed Value
1	Theoretical plates*	Metaxalone	3123
		Diclofenac potassium	2939
2	Tailing factor*	Metaxalone	1.3
		Diclofenac potassium	1.4
3	Retention time (min)*	Metaxalone	2.610
		Diclofenac potassium	4.607
4	Resolution*	-------	7.049

*n=6 Average of six determinations

Table 2: Results for Specificity of the method

Compound name	Retention time
Blank	--
Metaxalone	2.713
Diclofenac potassium	4.770

Table 3: Data of Accuracy recovery studies for two drugs at spiked concentration

Sample	Accuracy level (n=3)	standard addition	Formulation	% of recovery	Avg.% of recovery
Metaxalone	80%	64µg/ml	16 µg/ml	99.09%	99.63% SD=48.47 %RSD=1.96
	100%	80 µg/ml	16 µg/ml	99.99%	
	120%	96 µg/ml	16 µg/ml	100.81%	
Diclofenac potassium	80%	8µg/ml	2 µg/ml	99.61%	100.15% SD=7.54 %RSD=1.14
	100%	10µg/ml	2µg/ml	100.61%	
	120%	12µg/ml	2 µg/ml	100.26%	

Table 4: Precision data for metaxalone and diclofenac potassium

S.NO	METAXALONE		DICLOFENAC POTASSIUM	
	Retention time	Area	Retention time	Area
Injection-1	2.610	3372.256	4.607	745.545
Injection-2	2.653	3442.361	4.637	759.211
Injection-3	2.633	3415.109	4.610	749.139
Injection-4	2.630	3460.835	4.603	740.051
Injection-5	2.653	3442.361	4.637	759.211
Injection-6	2.610	3372.356	4.607	745.545
Average	2.6315	3417.530	4.617	744.784
Standard Deviation	0.0193	37.984	0.016	7.859
%RSD	0.73	1.11	0.34	1.05

Table 5: Robustness data for the proposed method

Condition	variation	Average area (n=3)		%RSD	
		MTX	DCLF	MTX	DCLF
Flow rate	Less flow 1 ml/min	4119.859	874.3013	0.018469	0.089512
	Actual Flow 1.2ml/min	3372.921	724.881	0.027792	0.064773
	More Flow 1.4 ml/min	3117.891	661.367	0.114167	0.003131
Wavelength	Less 216 nm	3805.097	791.747	0.089512	0.089512
	Actual 218nm	3372.921	724.881	0.027792	0.064773
	More 220nm	3692.697	817.0027	0.091538	0.064495

Table 6: Ruggedness data for proposed method

S.No.	Metaxalone		Diclofenac potassium	
	Analyst 1 (Average Area)	Analyst 2 (Average area)	Analyst 1 (Average area)	Analyst 2 (Average area)
1.	3372.256	3430.887	725.545	743.436
2.	3369.203	3430.886	725.543	742.478
3.	3371.261	3429.892	723.541	743.439
4.	3373.254	3432.886	725.539	743.437
5.	3373.258	3429.883	724.541	748.437
6.	3375.541	3432.869	724.549	742.481
Average	3372.462	3431.217	724.559	743.951
Standard Deviation	1.951369	1.242867	0.74464	2.051276
%RSD	0.057	0.036	0.102	0.275

Table 7: Results of Analysis of Commercial formulation:

Formulation	Labeled Claim(mg)	% Recovery by proposed method
FLEXURA D	Metaxalone-400mg	99.20
	Diclofenac potassium 50mg	99.30

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