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Validated RP- HPLC Method for Simultaneous Estimation of Metaxalone and Diclofenac potassium in Combined Dosage Form

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ABSTRACT

A simple, accurate, precise, specific, sensitive, reproducible and Reliable RP- HPLC Method was developed for Quantitative Estimation of Metaxalone and Diclofenac potassium in Pharmaceutical Dosage Form. The developed RP- HPLC method with the mobile phase Methanol: Water (80: 20) and Qualisilgold-C18 (250X4.6mm, 5µm particle size) as stationary phase with a flow rate of 1.0 mL/minute by using λmax 275nm and PDA detector. Proposed method was found to be linear in the concentration range of 8.0 to 80.0 µg/mL for Metaxalone and 1.0 to 10.0 µg/mL for Diclofenac potassium respectively, and the correlation coefficient was found to be 0.9991 for both the drugs. Precision study showed that the % RSD was within the range of acceptable limits (< 2), and the % Recovery was found to be in the range of 99.29%-101.28% for Metaxalone and 99.98%-102.45% for Diclofenac potassium. The proposed method has been validated as per ICH guidelines.

Keywords: RP- HPLC, Metaxalone, Diclofenac potassium, PDA, ICH, Method validation.

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INTRODUCTION

Metaxalone (5-[(3,5-dimethylphenoxy)methyl]-1,3-oxazolidin-2-one) (figure.1), is a central nervous system depressant, It is official in Indian Pharmacopoeia (IP), United States Pharmacopoeia (USP) and British Pharmacopoeia (BP).IP describes non-aqueous titration method for its estimation and Diclofenac potassium (2-[(2,6-dichlorophenyl)amino] benzene acetic acid, mono potassium salt) (figure. 2) is a non steroidal anti- inflammatory agent used in treatment of pain. It is official in United States Pharmacopoeia (USP) and British Pharmacopoeia (BP). USP describes chromatographic method for its estimation. Various methods like Spectrophotometric[10], HPLC[7], stability indicating HPLC[8-9] and HPTLC[11] methods for simultaneous estimation of Metaxalone and Diclofenac with other drug are reported in literature. The combined dosage forms of Metaxalone and Diclofenac available in market for the treatment of Muscle spasm. The present manuscript describes a new, simple, economical, specific, precise, accurate, and reproducible RP- HPLC Method for the simultaneous estimation of Metaxalone and Diclofenac in tablet dosage form.

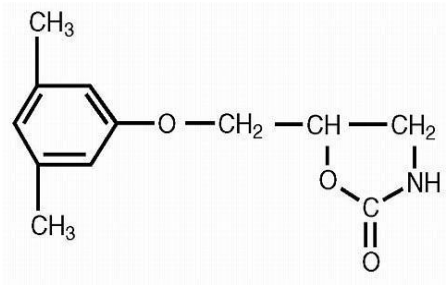

Figure. 1: Structure of Metaxalone

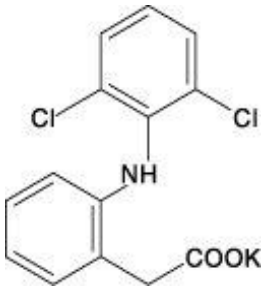

Figure. 2: Structure of Diclofenac potassium

MATERIALS AND METHODS

Metaxalone and Diclofenac potassium were obtained as a gift samples from the Rachem, Hyderabad and Dr. Reddy's labs respectively. Agilent LC-1200 (gradient) chromatograph with PDA detector was used with Ezchrome elite software. Methanol used was of HPLC grade, obtained from Merck chemicals, Mumbai. A commercial tablet formulation (MOBISWIFT D, Ranbaxy Laboratories Ltd) each containing 400mg of Metaxalone, 50mg of Diclofenac potassium were procured from local pharmacy.

Selection of wavelength

UV spectra of Metaxalone and Diclofenac potassium were shown that λmax was found at 278 nm and 280 nm respectively. Isobestic points were at 275 nm and 264 nm. At 275nm Metaxalone and Diclofenac potassium show maximum absorption, so that wavelength is selected for determinations.

Selection of Mobile phase

Trial 1 Mobile phase: 70 methanol: 30 water, pH: 6 with phosphate buffer, Detection wavelength: 275 nm, Drawback: Co-elution of peaks

Trial 2 Mobile phase: 80 methanol: 20 water pH: 4 with phosphate buffer, Detection wavelength: 275 nm Drawback: No baseline separation,

Trial 3 Mobile phase: 80 methanol: 20 water Detection wavelength: 275 nm, pH: adjusted to 3 with phosphate buffer: both peaks eluted with adequate separation and meet all system suitability parameters, so trail 3 is selected for determinations. The chromatogram was shown in figure. 3

Preparation of standard drug solutions

Stock solution of Metaxalone and Diclofenac potassium were prepared by dissolving 10 mg of each in separate 10 ml volumetric flasks with small quantity of methanol. The mixture was sonicated for 15 min and then makes up to the volume with methanol. From the stock solution 100 µg/ml of Metaxalone and Diclofenac potassium were prepared by pipette out 1ml of stock solution into 10 ml flask and make up to volume with mobile phase (Methanol 80: water 20).

Chromatographic conditions

The mobile phase consisting of methanol and water in the ratio of 80: 20 was filtered before use through a 0.45µm membrane filter and degassed in an ultrasonicator for 10 min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0 ml/min and the injection volume was 20 µL. The column temperature was maintained at 25°C. The eluent were monitored at 275 nm.

Calibration of standards

Two separate calibration plots were constructed for each component. Concentration range of 8-80 µg/ml for Metaxalone and 1-10 µg/ml for Diclofenac potassium were prepared for each component by pippeting different volumes of each stock solution and made up to the mark with mobile phase (Methanol 80: water 20).

Method validation[6]

The developed method was validated as per International Conference on Harmonization (ICH) guidelines**.** The validation parameters are,

Specificity

For determining specificity of the method, a tablet dosage form was analyzed. The chromatograms were examined to determine if compounds of interest co-elute with each other or with any additional excipients peaks**.** Injections of the marketed product revealed the absence of

interferences with the elution of the drug. These results demonstrate that there was no interference from other materials in the tablet formulation therefore, confirm the specificity of the method.

Linearity

The linearity of calibration curves in pure solution was checked over the ranges of 8-80 µg/ml for Metaxalone and 1-10 µg/ml for Diclofenac potassium. the calibration curves were linear in the studied range and equations of the regression analysis were obtained y = 19671x+5471.2 for Metaxalone and y = 100286x+224427 for Diclofenac potassium and the linearity plots were shown in figure no. 3 & 4. The mean± Standard Deviation (SD), slope, intercept, and correlation coefficient of standard curves (N=3) were calculated and the results were shown in table no. 1 and 2.

Precision

The system precision was studied by six replicate measurements of single concentration or three replicate measurements of three different concentrations and the results were shown in table no. 11. To assess the precision of the method, the intraday (3 times) and Interday (3 days) measurements of two drugs were completed with computation of % RSD for replicate samples (n=3) using concentrations of 16, 32, 48 µg/ml of Metaxalone and 2, 4, 6 µg/ml for Diclofenac potassium. Both intraday and inter day results were calibrated with standard curve concurrently prepared on the day of analysis and were shown in table no. 5.

Accuracy (Recovery study)

Accuracy of the method was determined by recovery experiments. To the formulation, the reference standards of the drug were added at the level of 50%, 100%, 150%. The recovery studies were carried out 3 times and the % Recovery and % RSD of the recovery of Metaxalone and Diclofenac potassium were calculated and the results were shown in table no. 3.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD was expressed by establishing the minimum level at which the analyte can be reliably detected. LOQ was considered as the lowest concentration of analyte in standards that can be reproducibly measured with acceptable precision. The LOD and LOQ were calculated by standard deviation of y-intercept and slope of the linearity curves. The results were shown in table no. 8.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and

S = slope of the calibration curve

Robustness

The Robustness of the method was evaluated by analyzing the system suitability standards and evaluating system suitability parameter data after varying pump flow rate, mobile phase ratio, pH and detection wavelength. None of the alterations caused a significant change in peak area RSD, USP tailing factor and Theoretical plates. The results were shown in table no. 6.

System suitability

It is defined as tests to measure the method that can generate result of acceptable accuracy and precision. The system suitability was carried out after the method development and validation have been completed. For this, parameters like Plate number (N), Resolution (R), tailing factor, Capacity factor, HETP, Peak symmetry of samples were measured. The results were shown in table no. 7.

Assay of Mobiswift-D Tablets

Twenty tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 40 mg of Metaxalone and 5 mg of Diclofenac potassium was extracted with methanol in a 100 ml volumetric flask and 50 ml of methanol was added to the same. The flask was sonicated for 10 min and volume was made up to the mark with methanol. The above solution was filtered using whatman filter paper, 0.8 ml of above solution was transferred into a 10 ml volumetric flask and the volume was made up to the mark with mobile phase to obtain 32 µg/ml of Metaxalone and 4 µg/ml of Diclofenac potassium. The solution was injected under above chromatographic conditions and peak area was measured. The assay chromatogram was shown in figure. 4. The assay procedure was made triplicate (n=3) and weight of sample taken for assay was calculated. The percentage of drug found in formulation, mean and standard deviation in formulation were calculated. The results were shown in table no. 4.

RESULTS AND DISCUSSION

The present work was aimed at developing new validated RP-HPLC Method for Metaxalone and Diclofenac potassium. In the present work the RP-HPLC method development was done for Metaxalone and Diclofenac potassium by using MeOH: Water (80: 20) as mobile phase and detection was performed at 275 nm. The system suitability parameters[2] like Plate number (N), Resolution (R), tailing factor, Capacity factor, HETP, Peak symmetry of samples were within the acceptance limits. Linearity curves were obtained between peak area and concentrations of Meta and Diclo in the concentration ranges of 8-80 µg/ml and 1-10 µg/ml, with R^2 value 0.9991 respectively. The linearity of the calibration curve was validated by the high values of correlation

coefficient of regression. The RSD values for Intraday and Interday precision of Meta and Diclo were found to be less than 2%. LOD and LOQ values for Meta were found to be 79ng/ml and 242ng/ml respectively. LOD and LOQ values for Diclo were found to be 9ng/ml and 30ng/ml respectively. These data show that method is sensitive for the determination of Meta and Diclo. The recovery experiment was performed by the standard addition method. The recoveries of Meta and Diclo were found to be in the range of 99.29-101.28% and 99.98.-102.45% respectively. The results of recovery studies indicate that the proposed method is highly accurate. The proposed validated RP-HPLC method was successfully applied to combined dosage form (tablet). Hence the proposed method can be used as alternative method to already reported methods and provide a wide choice for the routine determination of the above mentioned drugs.

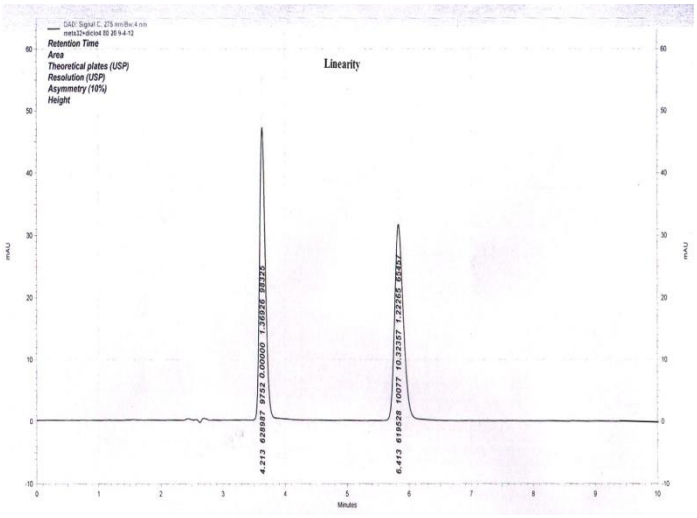

Figure. 3. Optimized chromatogram for Metaxalone and Diclofenac

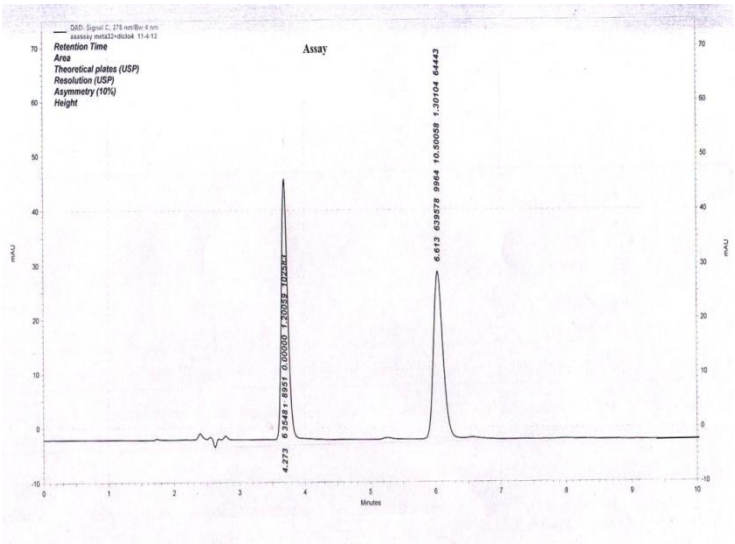

Figure. 4. Assay chromatogram of Metaxalone and Diclofenac

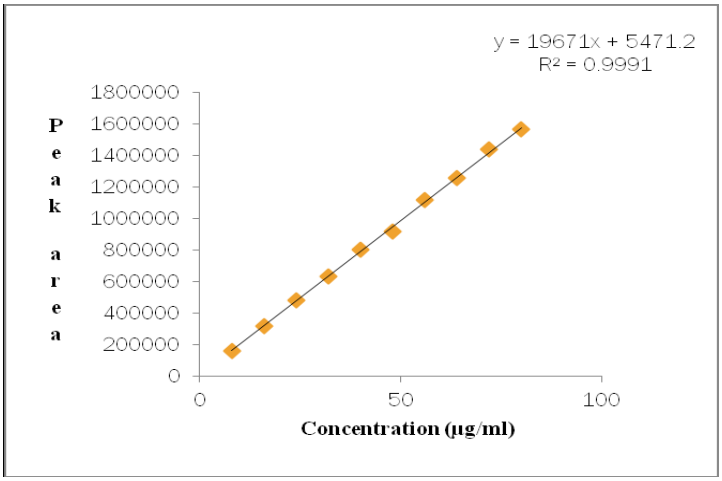

Figure. 5. Linearity plot of Metaxalone

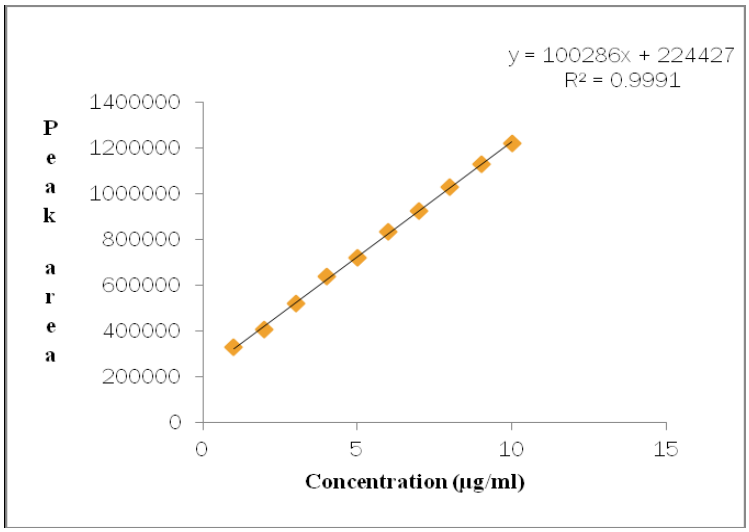

Figure. 6. Linearity plot of Diclofenac potassium

Table 1. Linearity data for Diclofenac potassium

S. No	Concentration of diclo (µg/ml)	peak area (mean ± SD)	%RSD
1	1	332678 ± 1732	0.52
2	2	407292 ± 2581	0.63
3	3	519698 ± 4713	0.8
4	4	639823 ± 3698	0.57
5	5	722453 ± 2586	0.73
6	6	834153 ± 5463	0.65
7	7	927590 ± 8524	0.91
8	8	1028688 ± 6932	0.67
9	9	1128169 ± 9635	0.67
10	10	1219470 ± 12658	1.03
Regression equation		Y = 100286x+224427	
Intercept		224427	
Slope		100286	
Correlation coefficient		0.9991	

Table 2 Linearity data for Metaxalone

S. No	Concentration of Meta (µg/ml)	peak area (mean ± SD)	%RSD
1	8	161880 ± 837	0.51
2	16	320852 ± 2998	0. 93
3	24	479616 ± 3414	0.71
4	32	633876 ± 3671	0.57
5	40	805896 ± 6348	0.78
6	48	918139 ± 7463	0.81
7	56	1121358 ± 6547	0.58
8	64	1260336 ± 9871	0.78
9	72	1441339 ± 9126	0.63
10	80	1566827 ± 10523	0.67
Regression equation		Y = 19671x+5471.2	
Intercept		5471.2	
Slope		19671	
Correlation coefficient		0.9991	

Table 3 Recovery report of Metaxalone and Diclofenac potassium

Conc. of standard	Recovery level	Amount of drug added (µg/ml)	Total amount of drug (µg/ml)	Amount found (µg/ml) n=3 (mean±SD)	% recovery	%RSD
	50%	16	48	48.21±0.40	100.44±0.84	0.82
Metaxalone	100%	32	64	64.01±0.48	100.01±0.76	0.74
(32 µg/ml)	150%	48	80	79.97±0.53	99.96±0.67	0.66
	50%	2	6	6.09±0.066	101.55±1.11	1.08
Diclofenac	100%	4	8	8.06±0.64	100.78±0.80	0.79
(4µg/ml)	150%	6	10	10.17±0.075	101.7±0.75	0.73

Table 4. Assay report of *Mobiswift-D* (Metaxalone and Diclofenac potassium)

Formulation *Mobiswift-D*	Labeled claim (mg)	Peak area mean±SD (n=3)	Amount found (mg) mean±SD n=3	Assay	%RSD
Metaxalone	400 (32µg)	633517±4794	399.15±3.055	99.78%	0.76
Diclofenac potassium	50 (4µg)	635150±3982	51.19±0.500	102.38%	0.97

Table 5 Intraday and Interday precision data of Metaxalone and Diclofenac potassium

Drug	Concentration (µg/ml)	Peak (peak area±SD) n=3		%RSD	
		Intraday	Interday	Intraday	Interday
Metaxalone	16	312298±2554	310592±4878	0.81	1.57
	32	633276±4226	633519±6046	0.66	0.95
	48	919543±7913	917293±6809	0.86	0.74
Diclofenac potassium	2	406188±7274	402749±6125	1.79	1.52
	4	637562±4759	636240±3616	0.74	0.57
	6	836241±5264	837921±4609	0.62	0.55

Table 6. Robustness data of Metaxalone and Diclofenac potassium

Parameter	Modification	Retention time (mean±SD) n=3		Asymmetry	
		Metaxalone	Diclofenac	Metaxalone	Diclofenac
Flow rate (ml/min)	0.8	5.34±0.04	8.25±0.03	1.29	1.27
	1.0	4.28±0.02	6.552±0.04	1.26	1.18
	1.2	3.55±0.03	5.5±0.06	1.34	1.27
Mobile phase (MeOH: water)	78:22	4.44±0.07	7.307±0.02	1.26	1.27
	80:20	4.27±0.02	6.552±0.04	1.26	1.18
	82:18	4.113±0.05	6.047±0.03	1.29	1.28
Wavelength (nm)	278	4.267±0.07	6.607±0.05	1.29	1.37
	275	4.247±0.02	6.552±0.04	1.26	1.18
	280	4.247±0.09	6.56±0.03	1.34	1.28

Table 7. System suitability data of Metaxalone and Diclofenac potassium

Parameter	Metaxalone	Diclofenac potassium	Acceptance criteria
Retention time (min)	4.247	6.552	6-8
Peak area (mean±SD) n=3	919543±9196	836241±8101	-
Plate count	10234	11450	>2000
Tailing factor	1.00	0.96	≤2.0
Asymmetry (10%)	1.26	1.24	0.9-1.2
Resolution	--	10.48	>1.5
Capacity factor	5.43	6.24	1-10

Table 8. Summary of the method

Parameter	Metaxalone	Diclofenac potassium
Retention time	4.247 min	6.552 min
Run time	10 min	
Assay	99.78%	102.38%
Specificity	Response of analyte with respect to blank is well resolved	
Linearity	8-80 µg/ml	1-10 µg/ml
LOD	79 ng/ml	9 ng/ml
LOQ	242 ng/ml	30 ng/ml
System precision	% RSD <2	% RSD <2
Precision	% RSD <2	% RSD <2

CONCLUSION

RP-HPLC Method was developed for the Simultaneous Estimation of Metaxalone and Diclofenac potassium. The HPLC used was Agilent 1200 (Gradient) PDA detector with Rheodyne injector of 20µL volume and column Qualisil gold C18 (250X4.6mm, 5µm). The mobile phase comprised of MeOH: Water in the ratio of 80:20 v/v and flow rate of 1ml/min with detection at 275nm produced peaks for Metaxalone and Diclofenac potassium in the chromatogram with retention times of 4.247 and 6.552 respectively. The HPLC method was validated for various parameters like Linearity, Assay, Accuracy, Precision, Robustness,

Specificity, LOD and LOQ as per ICH guidelines. The proposed method was applied for determination of Metaxalone and Diclofenac potassium in marketed formulation. The Assay results confirmed to the label claim of the formulation. Hence the proposed method was found to be satisfactory and could be used for the routine analysis of Metaxalone and Diclofenac potassium in their marketed tablet dosage formulations.

ACKNOWLEDGEMENT

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