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SIMULTANEOUS ESTIMATION OF DICLOFENAC POTASSIUM AND METAXALONE IN BULK AND COMBINED TABLET DOSAGE FORM USING RP-HPLC METHOD

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ABSTRACT A simple, precise and accurate isocratic RP-HPLC stability-indicating assay Method has been developed to determine Diclofenac potassium and Metaxalone in their combined dosage forms. Isocratic separation was achieved on a Phenomenex Luna C18 (250 mm × 4.6 mm i.d., particle size 5 µm) column at room temperature in isocratic mode, the mobile phase consists of Acetonitrile: Phosphate Buffer (40:60% v/v) at a flow rate of 1.2 ml/min, the injection volume was 20 µl and UV detection was carried out at 220 nm. The drug was subjected to acid and alkali hydrolysis, oxidation, photolysis and heat as stress conditions. The Method was validated for specificity, linearity, precision, accuracy, robustness and system suitability. The Method was linear in the drug concentration range of 2–64 µg/ml and 8–256 µg/ml for Diclofenac potassium and Metaxalone, respectively. The precision (RSD) of six samples was 1.03 and 0.67% for repeatability, and the intermediate precision (RSD) among six sample preparation was 0.97 and 0.53% for Diclofenac potassium and Metaxalone, respectively. The mean recoveries were between 99.78–99.92% and 99.99–100.11% for Diclofenac potassium and Metaxalone respectively. The proposed Method can be used successfully for routine analysis of the drug in bulk and combined pharmaceutical dosage forms.

KEYWORDS : Diclofenac potassium , Metaxalone , Stability Indicating, RP- HPLC, RSD

Introduction

Diclofenac Potassium (DP), potassium {2-[(2,6-DPhlorophenyl) amino]phenyl}acetate (Figure 1A), is a non- steroidal anti-inflammatory drug (NSAID) that exhibits anti-inflammatory, analgesic, and antipyretic activities[1]. DP monograph is official in British Pharmacopoeia[2]. Metaxalone (MTX), 5-[(3,5-dimethyl-phenoxy)methyl]-1,3-oxazolidin-2-one (Figure 1B), is a skeletal muscle relaxant[3]. It works by blocking nerve impulses in the brain. MTX is used to relax muscles and relieve pain caused by strains, sprains and other musculoskeletal conditions.

Literature survey shows that some stability indicating RP-HPLC Methods has been reported so far for the simultaneous estimation of both the drugs using different mobile phase combination. This method developed using of Acetonitrile: Phosphate Buffer (40:60%, v/v) has not reported. Some of the reported Methods for DP include UV-spectroscopic Methods[4–7], RP-HPLC Methods [8–11] and LC-MS Method[12]. For MTX the reported Methods are UV-Spectroscopic Method[13], RP-HPLC Method[14,15] and LC-MS Method[16]. So far only two RP-HPLC Methods have been reported for simultaneous estimation of both the drugs [17,18], with one method reported is stability-indicating in nature. The objective of this work was to develop a novel, simple, precise and more accurate with good separation RP-HPLC Method than the previous mentioned methods which can be used as a stability-indicating assay for combination drug product of DP and MTX.

(A) (B)

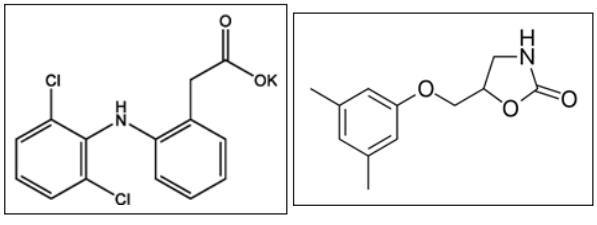

Fig. 1. Chemical structures of (A) Diclofenac potassium and (B) Metaxalone

To establish the stability-indicating nature of the Method, forced degradation of drug substances and drug product was performed under stress conditions (acid, alkali, oxidation, thermal and photolysis), and stressed samples were analyzed by the proposed Method. The Method was also validated according to ICH guideline requirements [19].

Experimental

Chemicals and Reagents

Raw materials DP and MTX (purity > 99.5%) were procured from Aarthi Drugs (Mumbai, India) and Ra Chem Pharma (Hyderabad, India) respectively. Acetonitrile (Merck Ltd., Mumbai, India) was of HPLC grade. Analytical grade of Potassium dihydrogen orthophosphate, sodium hydroxide, hydrochloric acid and hydrogen peroxide were procured from S.D. Fine Chem. Ltd., Mumbai, India. The water used for reagents, mobile phase and HPLC was prepared by using Borosilicate glass double distillation Water Purification System, India. The combined tablet formulation (M Sure-D) containing 50 mg of DP and 400mg of MTX was taken for analysis which is manufactured at Veras Pharmaceuticals Pvt. Ltd., Vizianagaram.

HPLC Instrumentation and chromatographic conditions

Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC-20AD Prominence Series HPLC pumps, with a 20 µl sample injection loop (manual) and SPD 20A Prominence series UV Visible detector. The output signal was monitored and integrated using Shimadzu LC solutions Software. A Phenomenex Luna C18 (250 mm × 4.6 mm i.d., particle size 5 µm) was used for separation. Chromatographic analysis was carried out at ambient temperature on the column using the Acetonitrile: Phosphate Buffer (40:60% v/v) as mobile phase at a flow rate of 1.2 ml/min in isocratic mode. Phosphate buffer was prepared by dissolving 6.8 g of potassium dihydrogen orthophosphate in 1000 ml of distilled water. The mobile phase prepared was filtered by Tensil make glass filtration kit using 0.45 µm membrane filter paper (Pall Corporation). Afterwards the mobile phase was ultrasonicated (Sonica, India) for 15 minutes to degas prior to use. The UV detection wavelength was selected at 220 nm. Water bath (NSW, India) and UV Chamber (Advance Research, India) were used for forced degradation study of the drugs. Analytical balance, Shimadzu, Model-ATX-224 of sensitivity 0.1 mg was used to weigh the chemicals and reagents.

Preparation of Standard and Sample Solution

Standard stock solutions for both the drugs were prepared separately by dissolving 50 mg of the both the drugs in mobile phase up to 50 ml. Initially 20 ml of the mobile phase was added to the drugs and ultrasonicated for 5 minutes to dissolve completely. Finally, the volumes were made up to the mark with mobile phase, which gave 1000 µg/ml solutions of each drug respectively. From this a mixed standard stock solution was prepared so that the drugs DP and MTX were in the ratio equal to that of the marketed formulation (1:8) available. Working standard solutions of DP and MTX were prepared in the concentration ratio of 1:8.

Twenty tablets of sample M Sure-D tablets were weighed accurately and finely powdered. A quantity of tablet powder equivalent to 25 mg of DP and 200 mg of MTX was accurately weighed and transferred into

a 25 ml volumetric flask, containing 10 ml of mobile phase and ultrasonicated for 20 min; the volume was made up to the mark and mixed well. The solution was filtered through a 0.45 µm membrane filter to ensure the free of particulate matter. The filtered solution was appropriately diluted with the mobile phase for analysis as already described. The amount of drug present in the sample solution was calculated by using the calibration curves.

Method validation

Specificity

The specificity of the Method was determined by checking the interference of any of the possible degradation products generated during the forced degradation study of the drugs. The forced degradation of the drug was carried out with 0.1 N HCl, 0.1 N NaOH, 3% v/v hydrogen peroxide, heat (80°C) and photolysis (365 nm) for determining the stability nature of the drugs. The degradation samples were prepared by taking suitable aliquots of the drug and drug product solutions, and then undertaking the respective stress testing procedures for each solution. After the fixed time period the treated test solutions were diluted up to the mark with mobile phase. For every stress condition three solutions were prepared as 8 µg/ml of DP, 64 µg /ml of MTX and drug product solution containing 8 µg/ml of DP along with 64 µg/ml of MTX. The specific stress conditions are described as follows.

Acid degradation condition:

Acid degradation was carried out by adding 2 ml of 0.1N HCl, and after 45 minutes neutralizing the mixture by adding 2 ml of 0.1N NaOH.

Alkali degradation condition.

Alkali degradation was carried out by adding 2 ml of 0.1N NaOH, and after 45 minutes neutralizing the mixture by adding 2 ml of 0.1N HCl.

Oxidative degradation condition:

Oxidative degradation was performed by exposing the drug to 2 ml of 3% (v/v) H_2O_2 for 45 minutes.

Thermal degradation condition:

Thermal degradation was performed by heating the drug at 80ºC on a thermostatically controlled water bath for 45 minutes.

Photolytic degradation condition:

Photolytic degradation was carried out by exposing the drug content to UV light (365nm) inside a UV chamber for 180 minutes.

Linearity

Six point calibration curves were obtained in a concentration range from 2 to 64 µg/ml for DP and 8 to 256 µg/ml for MTX. Calibration curves were plotted by taking the average peak area (n=3) on y-axis and concentration (µg /ml) on x-axis for DP and MTX separately.

Precision

The repeatability (intra-day precision) of the Method was ascertained from the peak areas obtained by actual determination of six replicates of a fixed amount of drug. For intermediate precision (inter-day precision) of the Method the above same procedure was carried out by a different analyst on a different day under similar experimental conditions. The percent RSD values were calculated for each type of precision study.

Accuracy

To check the accuracy of the proposed Method, recovery studies were carried out at 80, 100 and 120% of the test concentration. The recovery study was performed five times at each level. The amount of DP and MTX present in the sample were calculated using the calibration curves.

Robustness

The robustness of the Method was studied by very deliberately changing the composition of mobile phase and by determining the solution stability of the sample solution at 25 ± 2°C for 24 h. The Method was also evaluated for different system suitability parameters like Retention time, Theoretical plate, Asymmetry, HETP, capacity factor and Resolution.

Limit of detection and Limit of quantitation

The limit of detection and limit of quantitation were separately determined based on the Signal to Noise ratio. For limit of detection the S/N ratio was taken as 3:1. For limit of quantitation the S/N ratio was taken as 10:1.

Results and Discussion

Optimization of the Chromatographic conditions

Optimization of mobile phase was carried out based on resolution, tailing factor and theoretical plates obtained for DP and MTX. During the trial runs both the drugs were tested with different mobile phase compositions like acetonitrile: water, acetonitrile: 0.1 M Ammonium Acetate, Methanol: water at various compositions and flow rates. The mobile phase consisting of Acetonitrile: Phosphate Buffer (40:60% v/v) at a flow rate of 1.2 ml/min was selected which gave sharp, well-resolved peaks for DP and MTX. The average retention times for DP and MTX were 6.801 and 8.380 minutes, respectively. The asymmetry for DP and MTX were 1.38 and 1.30, respectively. UV spectra of DP and MTX in mobile phase ratio showed that both the drugs absorbed UV radiations appreciably at 220 nm and also gives higher peak areas and height compared to other wavelength at 254 nm or 275 nm. so 220 nm was selected as the detection wavelength. The separation was carried out at room temperature. Fig. 2 represents the chromatograms of standard solution and sample solution of marketed tablet formulation respectively.

Figure (A)

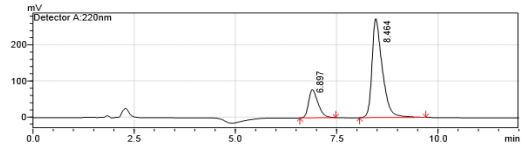

Figure (B)

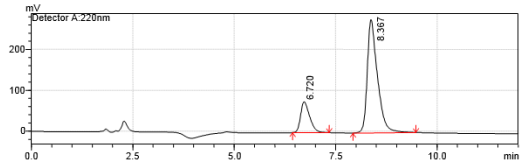

Fig. 2. Chromatograms of (A) combined standard solution and (B) Sample solution of drugs in combined dosage form of marketed formulation

Specificity

Specificity of the method was ascertained by checking any interference due to excipients or degradation products. No extra peaks were obtained either from the excipients used in the drug product or from the stress conditions applied on the drugs and drug product. Hence, there was no interference with the drug peaks. In Acid (38.5%), alkali (20.6%) and oxidation (14.70%) conditions DP shows some significant degradation than compared to thermal (3.90%) and photolytic (3.10%) stress conditions. But the drug MTX was found to be relatively more stable with very less degradation (1.20–5.50%) in all the applied stress conditions than compared to DP.

Linearity

The two six point calibration curves were found to be linear over a concentration range of 2–64 µg/ml and 8–256 µg/ml for DP and MTX, respectively. The linear regression equations were $y = 15328\,x + 22892$ and $y = 74283\,x - 20112$ with correlation coefficients 0.9998 and 0.9998 for DP and MTX, respectively.

Precision

The Method was found to be precise as the % RSD values for repeatability and intermediate precision studies were well below 2%, confirming that the Method was precise. The results are shown in Table1.

Tab. 1.Precision of the Method

Precision Type	Concentration (µg/ml)		Peak Area[a] ± Standard Deviation		% RSD	
	DP	MTX	DP	MTX	DP	MTX
Repeatability (Intraday, n=6)	8	64	1255099 ± 12201	4846163 ± 25944	0.97	0.53
Intermediate Precision (Interday, n=6)	8	64	1253942 ± 12976	4832102 ± 32458	1.03	0.67

[a]Mean of six determinations.

Accuracy

Accuracy of the method was determined by recoveries of DP and MTX by standard addition methods. The average recoveries were 99.78–99.92% for DP and 99.99–100.11% for MTX, respectively. The values show high levels of accuracy of the Method. The result of accuracy study is shown in Table 2.

Tab. 2. Accuracy of the Method

Recovery Type (%)	Amount added (µg/ml)		% Recovery[a] ± Standard Deviation		% RSD	
	DP	MTX	DP	MTX	DP	MTX
80	6.4	51.2	99.78 ± 0.26	100.11 ± 0.70	0.26	0.70
100	8.0	64.0	99.90 ± 0.30	100.03 ± 1.03	0.30	1.03
120	9.6	76.8	99.92 ± 0.20	99.99 ± 0.32	0.20	0.32

[a]Mean of six determinations.

Robustness

Robustness of the Method was ascertained by deliberately changing the mobile phase composition to Acetonitrile: Phosphate Buffer (38:62, v/v) by increasing percentage of buffwe, the retention time for DP and MTX were observed to be 6.920 and 8.640 minutes, respectively. Similarly, when the percentage of Acetonitrile was increased in Acetonitrile: Phosphate Buffer (42:,58 v/v) the retention time for DP and MTX were found to be 6.642 and 8.235 minutes, respectively. The solution stability study shows that DP and MTX solutions were stable for 24 h at ambient conditions without any significant degradation of the analyte. The results for system suitability parameters were also found to be satisfactory. The obtained results for robustness study and system suitability parameters are shown in Table3.

Tab. 3.Robustness of the Method

i)

Mobile Phase	Standard Condition (Ratio)	Modified Conditions	Retention Times		Resolution
			DP	MTX	
Acetonitrile: Phosphate Buffer	40:60 (v/v)	38:62 (v/v)	6.920	8.640	3.520
		42:58 (v/v)	6.642	8.235	3.480

ii)

Solution Stability	Initial Time	Final Time	% Recovery	
			DP	MTX
	0 Hour	24 hours	99.10	99.85

iii)

System suitability Parameters	DX	MTX
Retention Time (min)	6.801	8.380
Theoretical Plates	4100	5509
Height equivalent to Theoretical Plates (HETP)	36.579	27.225
Resolution	-	3.539
Peak Asymmetry	1.12	1.25
Tailing Factor	1.406	1.529
Capacity factor (k')	0.000	0.231

Limit of detection and Limit of quantitation

The limit of detection values for DP and MTX were 0.15 µg/ml and 0.20 µg/ml, respectively. The limit of quantification values for DP and MTX were 0.5 µg/ml and 0.85 µg/ml, respectively.

Analysis of combined tablet dosage form

The developed Method was applied for determination of DP and MTX in their combined tablet dosage form. The higher percentage of recovery and non interference of the formulation excipients in retention time of the drugs show the selectivity of the Method for estimation of both drugs in their combined dosage forms. The result of assay (n=3) for both drugs yielded 100.20% (S.D.=±0.88) and 99.85% (S.D.=±0.25) for DP and MTX, respectively, from the combined tablet dosage form.

Conclusion

A validated stability-indicating RP-HPLC Method has been developed for determination of DP and MTX in their combined tablet dosage form. The results obtained by the stress degradation conditions of the drugs show that the Method is specific and stability-indicating. The drug MTX was found to be more stable to degradation under different stress conditions than compared to DP. The Method was found to be simple, accurate, precise and sensitive. The Method was successfully applied for the determination of both drugs in combined tablet dosage form. In the future, this Method may be applied for routine analysis of both the drugs in API, formulations, dissolution studies, bioavailability and pharmacokinetic studies.

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