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Analytical Method Development And Validation Of Simultaneous Estimation Of Diclofenac Sodium And Misoprostol In Bulk And Pharmaceutical Dosage Forms By Rp-Hplc Method

M Chandrika[1], Dr. P V Madhavi Latha[2], K Suvarna[3], Dr. P Uma Devi[4]

1,2,3,4 Viswanadha Institute of Pharmaceutical Sciences

Abstract — A quick, sensitive, and accurate RP-HPLC approach has been employed to identify and measure Diclofenac Sodium and Misoprostol using a Waters PDA-detected HPLC mode. An Inertsil -ODS C18 (250 x 4.6 mm, 5) column was utilized for separation of Misoprostol and Diclofenac Sodium with flow rate of 1.0 ml/min. The mobile phase was degassed Methanol and Acetonitrile (75:25) with the detection wave length of 230nm.

Key words: Diclofenac Sodium, Misoprostol, RP-HPLC

I. INTRODUCTION

Diclofenac sodium + Misoprostol (brand name Arthrotec®) is a combination drug used to relieve pain and inflammation associated with conditions such as osteoarthritis and rheumatoid arthritis, while also preventing gastric ulcers caused by NSAID therapy. Diclofenac sodium (6-7) and Misoprostol (8) are classified as a nonsteroidal anti-inflammatory drug (NSAID) and a prostaglandin E1 analog, respectively. Diclofenac sodium works by inhibiting the cyclooxygenase (COX-1 and COX-2) enzymes, leading to decreased synthesis of prostaglandins responsible for inflammation, pain, and fever. Misoprostol works by mimicking the action of prostaglandin E1 on the gastric mucosa, increasing mucus and bicarbonate secretion and enhancing mucosal blood flow, which protects the stomach lining and reduces the risk of gastric and duodenal ulcers associated with NSAID use. This combination is administered orally, usually with food, to minimize gastrointestinal irritation. It is commonly prescribed for patients who require long-term NSAID therapy but are at high risk of developing gastrointestinal complications. The dual-action formulation improves overall treatment compliance by combining pain relief and gastric protection in a single tablet.

II.MATERIALS AND METHODS

Preparation of Stock solution: 100 mg of Diclofenac sodium and 100 mg of Misoprostol API standards were accurately weighed and are transferred into two separate 100 ml volumetric flasks, dissolved in mobile phase, then sonicated for 20 minutes to obtain 1000µg/ml.

Preparation of working standard solution: From the above standard stock solution, 4 ml from each solution were transferred into 100ml volumetric flasks, made up to the volume with mobile phase to get 40µg/ml of Diclofenac sodium and Misoprostol.

III. RESULTS AND DISCUSSION

Method validation: Validation parameters include specificity, linearity, range, accuracy, precision, limit of detection, limit of quantification, robustness and assay (1-5).

Specificity: Specificity is the ability to assessing equivocally the analyte in the presence of components which may be expected to be present. Typically, these components include impurities, degradants, matrix etc. Blank solution and standard solutions of Diclofenac Sodium (40µg/ml) and Misoprostol (40µg/ ml) were injected into the HPLC system. The peak purity data of Diclofenac Sodium and Misoprostol were compared. There should not be any interference at the retention time of the main peaks (9-18).

Linearity: Linearity for the drugs Diclofenac Sodium and Misoprostol was determined by preparing the standard solutions at six concentrations levels in the range of 20-70µg/ml for Diclofenac Sodium and 20-70µg/ml for Misoprostol from stock solution. The linearity charts of Diclofenac Sodium and Misoprostol were shown in the figure no 2&3. The correlation coefficient was found to be 0.9997 and 0.9993 for Diclofenac Sodium and Misoprostol respectively. Linearity results were tabulated in table 2.

Accuracy: Accuracy was performed by spiking known amounts of standard solution to sample solution at three different concentrations levels (50%, 100%, 150%) and there by analysed for %RSD which should not be more than 2.0. The % recovery was calculated and the results were reported in table no. 3 & 4.

Precision: The precision of the analytical method was studied by injecting six replicates of standard containing 40µg/ml of Diclofenac Sodium and 40µg/ml of Misoprostol which were injected into HPLC system. The % RSD was calculated and the results were reported in the table no.5 & 6.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The limit of detection was defined as the concentration which yields a signal - to – noise ratio 3:1 whereas the limit of quantification was calculated to be the lowest concentration that could be measured with signal - to – noise ratio10:1. LOD and LOQ were calculated from slope and standard deviation. The results were tabulated in table no. 7.

Robustness: The smallest deliberate changes in method like change in flow rate are made but there were no predictable changes in the results and are in the range as per ICH guidelines. Conditions like decrease in flow rate (0.8 ml/min), increase in flow rate (1.2 ml/min) was maintained and samples were injected in duplicate

manner. System suitability parameters were not much affected and all the parameters were passed. % RSD was found to be within the limits and results were tabulated in table no.8.

Assay: Assay was conducted on marketed formulation and mean % assay was found. The results were tabulated in table no. 9.

Table1: Optimized Chromatographic conditions

Parameters	Method
Stationary Phase(column)	Inertsil -ODS C_{18}(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol and Acetonitrile (75:25)
Flow rate (ml/min)	1.0 ml/min
Run time(minutes)	8 min
Temperature in the column	Ambient
Volume of injection (µl)	20
Detection Wavelength	230 nm
Drug RT (min)	4.132 min for Diclofenac Sodium and 6.334 for Misoprostol.

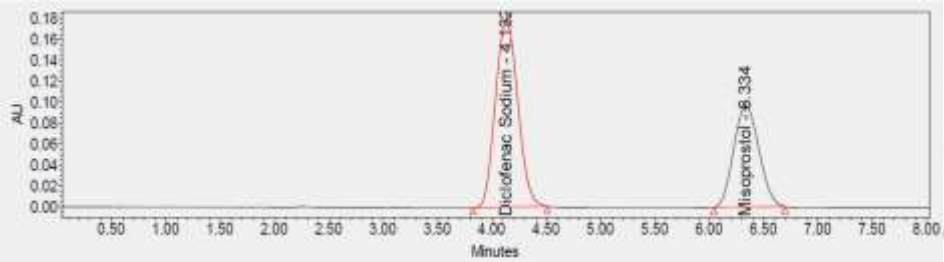

Figure 1: Optimized chromatogram

Table 2: Linearity data of Diclofenac Sodium and Misoprostol

Diclofenac Sodium		Misoprostol	
Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area
20	1209800	20	688747
30	1897414	30	987625
40	2557360	40	1293818
50	3140290	50	1631846
60	3737363	60	1984436
70	4419691	70	2340192

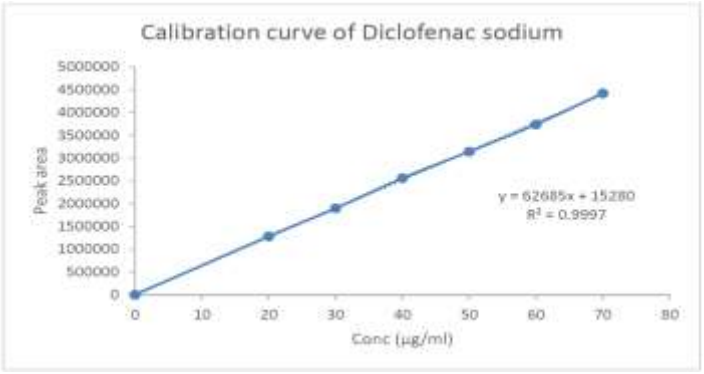

Figure 2: Calibration Curve of Diclofenac Sodium

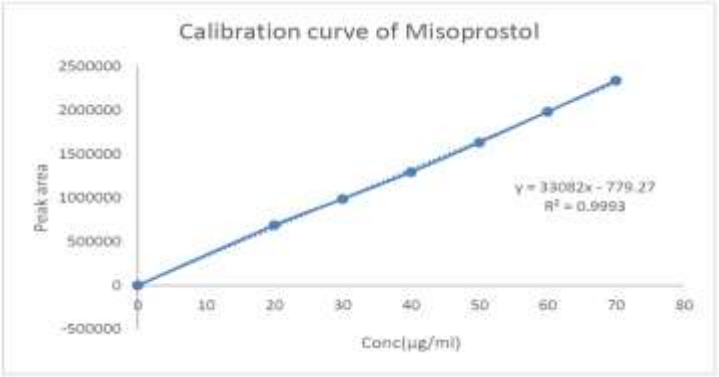

Figure 3: Calibration Curve of Misoprostol

Table 3: Accuracy data of Diclofenac Sodium

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recover y	Statistical Analysis of % Recover y
50% - 1	20	19.95	99.81	99.91
50% - 2	20	19.94	99.86	
50% - 3	20	19.92	99.82	0.74
100 % - 1	40	39.92	98.95	99.94
100 % - 2	40	39.83	99. 86	
100% - 3	40	39.78	99.13	0.763
150% - 1	60	59.97	99.96	100.07
150% - 2	60	60.02	100.08	
150% - 3	60	60.01	100.01	0.357

Table 4: Accuracy data of Misoprostol

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recover y	Statistical Analysis of % Recover y
50% - 1	20	19.92	99.91	99.98
50% - 2	20	19.82	99.39	
50% - 3	20	20.02	100.12	0.981
100 % - 1	40	39.92	99.84	99.98
100 % - 2	40	40.01	100.05	
100% - 3	40	40.05	100.04	0.756
150% - 1	60	59.96	98.96	99.95
150% - 2	60	59.96	99.91	
150% - 3	60	59.99	99.95	0.98

Table 5: System precision data of Diclofenac Sodium and Misoprostol

S. No	Peak areas of Diclofenac Sodium	Peak areas of Misoprostol
1	2559862	1308674
2	2553428	1316572
3	2548340	1317964
4	2550671	1318762
5	2553218	1316754
Mean	2553104	1315745
SD	4312.88	4053.619
% RSD	0.168927	0.308085

Table 6: Method precision data of Diclofenac Sodium and Misoprostol

S. No	Peak areas of Diclofenac Sodium	Peak areas of Misoprostol
1	2556372	1318867
2	2550354	1316854
3	2552234	1316872
4	2551341	1316759
5	2553421	1317584
Mean	2552394	1318052
SD	2246.83	1156.822
% RSD	0.088028	0.087768

Table 7: LOD and LOQ data of Diclofenac Sodium and Misoprostol

Drug Name	LOD (µg/ml)	LOQ (µg/ml)
Diclofenac Sodium	0.11	0.33
Misoprostol	0.19	0.59

Table 8: Robustness data of Diclofenac Sodium and Misoprostol

S No	Drug Name	Condition	Peak area	% RSD
1	Diclofenac Sodium	Decreased Flow rate of 0.8 ml/min	2533608	0.082
2		Increased Flow rate of 1.2 ml/min	2553280	0.077
3	Misoprostol	Decreased Flow rate of 0.8 ml/min	1304762	0.169
4		Increased Flow rate of 1.2 ml/min	1324893	0.288

Table 9: Assay data of Diclofenac Sodium and Misoprostol

S. No	Peak area of Diclofenac Sodium	% Assay	Peak area of Misoprostol	% Assay
1	2546342	100.84	1313642	99.28
2	2543675		1312461	
3	2546634		1312642	
4	2540372		1314672	
5	2543232		1310864	
6	2541643		1313427	

IV. CONCLUSION

The developed RP-HPLC method was validated as per ICH guidelines. All the system suitability parameters were within the range as stated by ICH guidelines. Interference peaks were not observed in blank, standard and sample chromatogram. Hence simple, precise and accurate, sensitive, specific and robust method was developed and validated. This can be used in quality control department with respect to routine analysis.

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