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Validated HPLC Method for Simultaneous Quantitation of Diclofenac Sodium and Misoprostol in Bulk Drug and Formulation

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ABSTRACT

HPLC method has been described for simultaneous determination of Diclofenac Sodium and Misoprostol in formulation. This method is based on HPLC separation of the two drugs on the Thermo Hypersil BDS–C_{18} (250 mm × 4.6 mm, 5.0 µ) from Germany with isocratic conditions and simple mobile phase containing acetonitrile: water (85: 15) at flow rate of 1 mL/min using UV detection at 220 nm. This method has been applied to formulation without interference of excipients of formulation. The linear regression analysis data for the calibration plots showed a good linear relationship over the concentration range of 50-100 µg/mL for Diclofenac Sodium and 0.2-0.4 µg/mL for Misoprostol respectively. The mean values of the correlation coefficient, slope and intercept were 0.9952 ± 1.27, 80433 ± 1.1 8 and 187960 ± 1.82 for Diclofenac Sodium and 0.9975 ± 0.78, 862734 ± 1.21 and 4750.9 ± 1.09 for Misoprostol respectively. The method was validated for precision, robustness and recovery. The limit of detection (LOD) and limit of quantitation (LOQ) was 1 µg/mL and 2 µg/mL for Diclofenac Sodium and 0.03 µg/mL and 0.1 µg/mL Misoprostol respectively. Statistical analysis showed that the method is repeatable and selective for the estimation of Diclofenac Sodium and Misoprostol.

Keywords: Diclofenac Sodium**;** Misoprostol; HPLC; Validation.

INTRODUCTION

Diclofenac Sodium, 2-(2-(2,6-dichlorophenylamino)phenyl)acetic acid (Figure 1a) is taken to reduce inflammation and as an analgesic reducing pain in conditions such as arthritis or acute injury. It can also be used to reduce menstrual pain, dysmenorrhea. Diclofenac works by

inhibiting prostaglandin synthesis by inhibition of cyclooxygenase (COX), inhibiting DNA synthesis. [1]

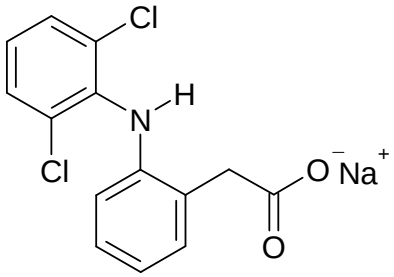

Figure 1(a) Structure of Diclofenac Sodium

Misoprostol, methyl-7-((1*R*,2*R*,3*R*)-3-hydroxy-2-((*S*,*E*)-4-hydroxy-4-methyloct-1-enyl)-5-oxocyclopentyl)heptanoate (Figure 1b) is used for the prevention of non-steroidal anti-inflammatory drug (NSAID) induced gastric ulcers, for early abortion, to treat missed miscarriage, and to induce labor. Misoprostol inhibits gastric acid secretion by a direct action on the parietal cells through binding to the prostaglandin receptor. The activity of this receptor is mediated by G proteins which normally activate adenylate cyclase. The indirect inhibition of adenylate cyclase by Misoprostol may be dependent on guanosine-5-triphosphate (GTP). [2]

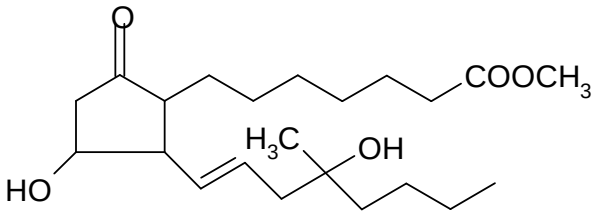

Figure 1 (b) Structure of Misoprostol

Literature review reveals that methods have been reported for analysis of Diclofenac Sodium and Misoprostol, stability indicating HPLC method for Diclofenac in raw materials and solid dosage form [3], RP-HPLC method for determination of Diclofenac in combination with other drugs [4, 5, 6] and few bioanalytical methods are also reported [7, 8]. Stability Indicating HPLC Assay Method for Misoprostol [9], RP-HPLC method for determination of Misoprostol in combination with other drugs [10], HPTLC method for quantitation of Diclofenac and Misoprostol in combination with other drugs is also reported.

To date, there have been no published reports about the simultaneous quantitation of Diclofenac Sodium and Misoprostol by HPLC in bulk drug and in tablet dosage form. This present study reports for the first time simultaneous quantitation of Diclofenac Sodium and Misoprostol by HPLC in bulk drug and in tablet dosage form. The proposed method is validated as per ICH guidelines.

MATERIALS AND METHODS

Materials

Wockhardt Pharmaceuticals Ltd. Aurangabad, India, kindly supplied pure drug sample of Diclofenac Sodium as a gift sample of Batch No.: DS88654 and Misoprostol of Batch No.: MT11836. It was used without further purification and certified to contain 99.85 % (w/w) for Diclofenac Sodium and 99.92 % (w/w) for Misoprostol on dried basis. All chemicals and reagents used were of HPLC grade and were purchased from Merck Chemicals, India.

Instrumentation

The HPLC system consisted of a Pump (model Jasco PU 2080), Intelligent LC pump with sampler programmed at 20 µL capacity per injection was used. The detector consisted of UV/ VIS (Jasco UV 2075) model operated at a wavelength of 220 nm. Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. The column used was Thermo Hypersil BDS–C_{18} (250 mm × 4.6 mm, 5.0 µ) from Germany.

Preparation of Standard Stock Solutions

Standard stock solution of concentration 5000 µg/mL of Diclofenac Sodium and 20 µg/mL of Misoprostol was prepared using water. From the standard stock solution, the mixed standard solution were prepared using acetonitrile to contain 50 µg/mL of Diclofenac Sodium and 0.2 µg/mL of Misoprostol. The stock solution was stored at 2-8 °C protected from light.

Optimization of HPLC Method

The HPLC procedure was optimized with a view to develop a simultaneous assay method for Diclofenac Sodium and Misoprostol respectively. The mixed standard stock solution (50 µg/mL of Diclofenac Sodium and 0.2 µg/mL of Misoprostol) was injected in HPLC.

For HPLC method optimization different ratios of acetonitrile and water were tried but it was found that acetonitrile: water in the ratio 85: 15 v/v, at flow rate 1 mL/min gives acceptable retention time (t_R), plates and good resolution for Diclofenac Sodium and Misoprostol (Figure 2).

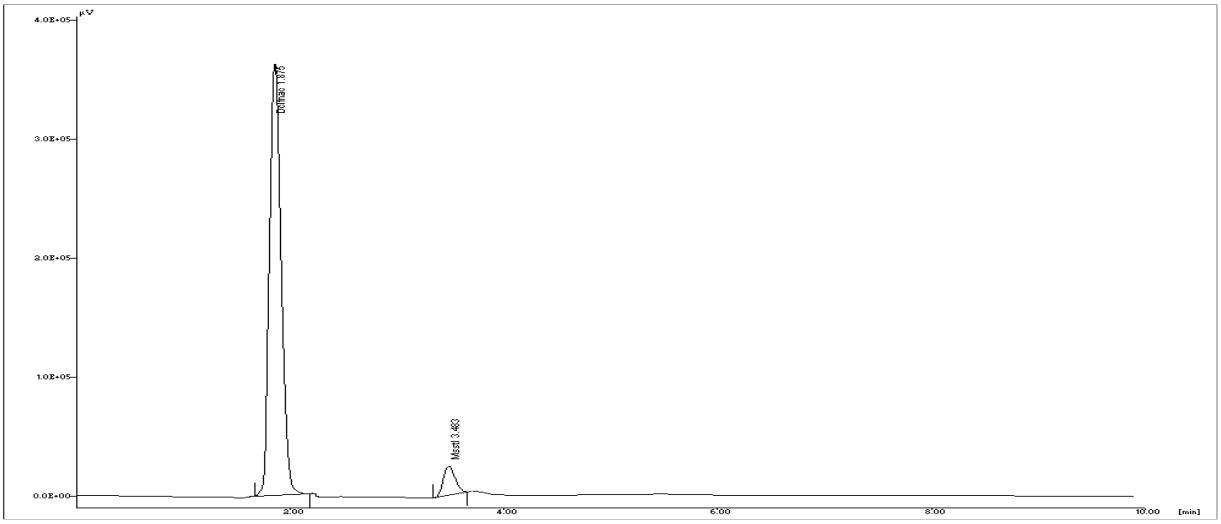

Figure 2 HPLC chromatogram of standard Diclofenac Sodium and Misoprostol (50 µg/mL and 0.2 µg/mL)

Validation of the method

Validation of the optimized HPLC method was carried out with respect to the following parameters.

Linearity and range

The mixed standard stock solution (5000 µg/mL of Diclofenac Sodium and 20 µg/mL of Misoprostol) was further diluted to get Diclofenac Sodium and Misoprostol concentration in the range of 50-100 µg/mL and 0.2-0.4 µg/mL respectively. Linearity of the method was studied by injecting six concentrations of the drug prepared in the mobile phase in triplicate into the LC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations 60, 80, 100 µg/mL for Diclofenac Sodium and 0.24, 0.32, 0.40 µg/mL for Misoprostol six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Limit of detection and limit of quantitation

Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. To determine the LOD and LOQ, serial dilutions of mixed standard solution of Diclofenac Sodium and Misoprostol was made from the standard stock solution. The samples were injected in LC system and measured signal from the samples was compared with those of blank samples.

Robustness of the method

To evaluate robustness of a HPLC method, few parameters were deliberately varied. The parameters included variation of flow rate, percentage of acetonitrile in the mobile phase and solvents from different lot were taken. Robustness of the method was done at three different concentration levels 60, 80, 100 µg/mL and 0.24, 0.32, 0.4 µg/mL for Diclofenac Sodium and Misoprostol respectively.

Specificity

The specificity of the method towards the drug was established through study of resolution factor of the drug peak from the nearest resolving peak.The peak purity of Diclofenac Sodium and Misoprostol was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E). Effect of excipients of formulation was studied for whether it interfered with the assay.

Accuracy

Accuracy of the method was carried out by applying the method to drug sample (Diclofenac Sodium and Misoprostol combination tablet) to which know amount of Diclofenac Sodium and Misoprostol standard powder corresponding to 80, 100 and 120 % of label claim had been added (Standard addition method), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

Analysis of a marketed formulation

To determine the content of Diclofenac Sodium and Misoprostol in conventional tablet (Brand name: Arthrotec 50, Label claim: 50 mg Diclofenac and 0.2 mg Misoprostol per tablet), twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 50 mg of Diclofenac Sodium and 0.2 mg Misoprostol was transferred into a 50 mL volumetric flask containing 30 mL water, sonicated for 30 min and diluted upto 50 mL with water. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (1000 and 4 µg/mL for Diclofenac Sodium and Misoprostol respectively). Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45-micron filter (Millipore, Milford, MA). The above stock solution was further diluted to get sample solution of 50 and 0.2 µg/mL for Diclofenac Sodium and Misoprostol respectively. A 20 µl volume of sample solution was injected into HPLC, six times, under the conditions described above. The peak areas were measured at 220 nm and concentrations in the samples were determined using multilevel calibration developed on the same HPLC system under the same conditions using linear regression equation.

RESULTS AND DISCUSSION

The results of validation studies on simultaneous estimation method developed for Diclofenac Sodium and Misoprostol in the current study involving acetonitrile: water (85: 15, *v/v*) are given below.

Table I : Precision studies

Concentration (µg/mL)	Measured concentration ± SD, RSD (%)	
	Repeatability (n= 6)	Intermediate precision (n= 6)
Diclofenac Sodium		
60	59.229 ± 95406, 1.926	59.985 ± 76634, 1.528
80	78.586 ± 59108, 0.908	79.189 ± 76837, 1.170
100	98.258 ± 77023, 0.951	98.018 ± 106690, 1.321
Misoprostol		
0.24	0.243 ± 899, 0.419	0.244 ± 1985, 0.919
0.32	0.325 ± 2985, 1.045	0.325 ± 2177, 0.763
0.40	0.406 ± 3165, 0.899	0.404 ± 2348, 0.664

Linearity

Diclofenac Sodium and Misoprostol showed good correlation coefficient (r^2 = 0.9952 for Diclofenac Sodium and 0.9975 for Misoprostol) in given concentration range (50-100 µg/mL for Diclofenac Sodium and 0.20-0.40 µg/mL for Misoprostol). The mean values of the slope and

intercept were 80433 ± 1.18 and 187960 ± 1.82 for Diclofenac Sodium and 862734 ± 1.21 and 4750.9 ± 1.09 for Misoprostol respectively.

Precision

The results of the repeatability and intermediate precision experiments are shown in Table I. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2 %, respectively as recommended by ICH guidelines.

LOD and LOQ

Signal-to-noise ratios of 3:1 and 10:1 were obtained for the LOD and LOQ respectively. The LOD and LOQ were found to be 1 µg/mL and 2 µg/mL for Diclofenac Sodium and 0.03 µg/mL and 0.1 µg/mL Misoprostol respectively.

Robustness of the method

Each factor selected (except columns from different manufacturers) was changed at three levels (−1, 0 and 1). One factor at the time was changed to estimate the effect. Thus, replicate injections (n = 6) of mixed standard solution at three concentration levels were performed under small changes of three chromatographic parameters (factors). Insignificant differences in peak areas and less variability in retention time were observed (Table II).

Table II: Robustness testing[a] (n = 3)

Factor[a]	Level	Retention time	Retention factor	Asymmetry
Diclofenac Sodium				
A: Flow rate (mL/min)				
0.9	-1	1.94	0.22	1.19
1.0	0	1.87	0.25	1.15
1.1	+1	1.80	0.28	1.11
Mean ± SD (n = 3)		1.87 ± 0.07	0.25 ± 0.03	1.15 ± 0.04
B: % of acetonitrile in the mobile phase (v/v)				
84	-1	1.92	0.23	1.18
85	0	1.87	0.25	1.15
86	+1	1.82	0.27	1.12
Mean ± SD (n = 3)		1.87 ± 0.05	0.25 ± 0.02	1.15 ± 0.03
C: Solvents of different lots				
First lot		1.87	0.25	1.15
Second lot		1.85	0.26	1.17
Mean ± SD (n = 3)		1.86 ± 0.01	0.25 ± 0.01	1.16 ± 0.01
Misoprostol				

A: Flow rate (mL/min)				
0.9	-1	3.52	0.40	1.15
1.0	0	3.48	0.39	1.11
1.1	+1	3.44	0.37	1.07
Mean ± SD (n = 3)		3.48 ± 0.05	0.39 ± 0.01	1.11 ± 0.04
B: % of methanol in the mobile phase (v/v)				
84	-1	3.54	0.41	1.16
85	0	3.48	0.39	1.11
86	+1	3.42	0.36	1.06
Mean ± SD (n = 3)		3.48 ± 0.06	0.38 ± 0.02	1.11 ± 0.05
C: Solvents of different lots				
First lot		3.48	0.39	1.11
Second lot		3.50	0.40	1.10
Mean ± SD (n = 3)		3.49 ± 0.01	0.39 ± 0.01	1.10 ± 0.01

[a] Three factors were slightly changed at three levels (-1, 0, 1)

Specificity

The peak purity of Diclofenac Sodium and Misoprostol was assessed by comparing their respective spectra at the peak start, apex and peak end positions i.e., r (S, M) = 0.9989 and r (M, E) = 0.9989. A good correlation (r = 0.9997) was also obtained between the standard and sample spectra of Diclofenac Sodium and Misoprostol respectively. Also, excipients from formulation were not interfering with the assay.

Recovery Studies

As shown from the data in Table III good recoveries of the Diclofenac Sodium and Misoprostol in the range from 99 to 101 % were obtained at various added concentrations.

Table III : Recovery studies (n = 6)

Label claim (mg/tablet)	Amount added (mg)	Total amount (mg)	Amount Recovered (mg) ± % RSD	% Recovery
Diclofenac Sodium				
50	40 (80%)	90	90.87 ± 0.96	100.96
50	50 (100%)	100	99.31 ± 1.01	99.31
50	60 (120%)	110	111.26 ± 0.78	101.14
Misoprostol				
0.2	0.16 (80%)	0.36	0.363 ± 1.16	100.83
0.2	0.20 (100%)	0.40	0.406 ± 1.40	101.50
0.2	0.24 (120%)	0.44	0.441 ± 0.98	100.22

Analysis of a formulation

Experimental results of the amount of Diclofenac Sodium and Misoprostol in tablets, expressed as a percentage of label claim were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present. The drug content was found to be 99.93 % for Diclofenac Sodium and 100.75 % for Misoprostol. Two different lots of Diclofenac Sodium and Misoprostol combination tablets were analyzed using the proposed procedures as shown in Table IV.

Table IV: Analysis of commercial formulation

Diclofenac Sodium (50 mg)	Diclofenac Sodium found (mg per tablet)	
	Mean ± SD (n= 6)	Recovery (%)
1st Lot	49.85 ± 1.06	99.70
2nd Lot	50.08 ± 0.94	100.16
Misoprostol (0.2 mg)	Misoprostol found (mg per tablet)	
	Mean ± SD (n= 6)	Recovery (%)
1st Lot	0.202 ± 1.16	101.00
2nd Lot	0.201 ± 1.04	100.50

CONCLUSION

HPLC method was developed and validated as per ICH guidelines. UV detection allowed an accurate quantitation of chromophoric compounds.

The drug was analysed by HPLC method using Thermo Hypersil BDS–C_{18} (250 mm × 4.6 mm, 5.0 µ) from Germany with isocratic conditions and simple mobile phase containing acetonitrile: water (85: 15) at flow rate of 1 mL/min using UV detection at 220 nm. The procedure has been evaluated for the linearity, accuracy, precision and robustness in order to ascertain the suitability of the analytical method. The method was also applied to marketed samples. It has been proved that the method is selective and linear between concentration range 50-100 µg/mL for Diclofenac Sodium and 0.20-0.40 µg/mL for Misoprostol. LOD was found to be 1 µg/mL and LOQ was found to be 2 µg/mL for Diclofenac Sodium and LOD was found to be 0.03 µg/mL and LOQ was found to be 0.10 µg/mL for Misoprostol.

Statistical analysis proves that the method is suitable for the analysis of Diclofenac Sodium and Misoprostol as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Diclofenac Sodium and Misoprostol and also for its estimation in plasma and other biological fluids.

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