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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DICLOFENAC AND MISOPROSTOL IN COMBINED DOSAGE FORM

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ABSTRACT

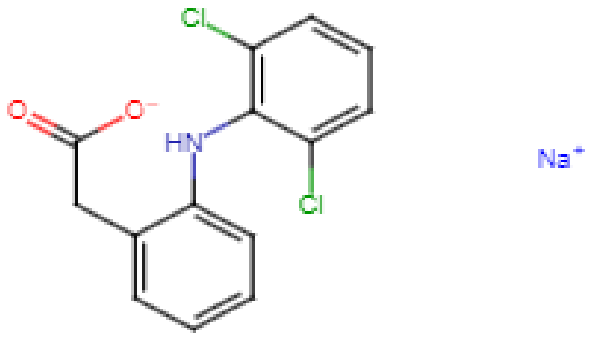
HPLC method has been used for simultaneous determination of Diclofenac Sodium and Misoprostol in formulation. This method is based on HPLC separation of the two drugs on the Thermo Hypersil BDS–C18 (250 mm × 4.6 mm, 2.5 µ) from Agilent with isocratic conditions and simple mobile phase containing methanol: 0.05%OPA water pH 2.7 (70: 30) at flow rate of 0.7 mL/min using UV detection at 235 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 200-400 µg/mL for Misoprostol respectively. The method was validated for precision, robustness and recovery. The limit of detection (LOD) and limit of quantitation (LOQ) was 0.26µg/mL and 0.78µg/mL for Diclofenac Sodium and 1.87µg/mL and 5.6µg/mL Misoprostol respectively. Statistical analysis showed that the method is repeatable and selective for the estimation of Diclofenac Sodium and Misoprostol.

KEYWORDS: HPLC, Diclofenac sodium, Misoprostol, Analysis of formulated drug.

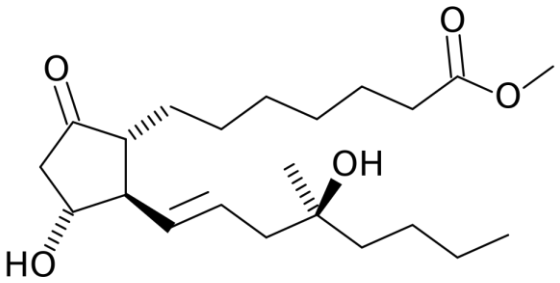
INTRODUCTION

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 worked by inhibiting prostaglandin synthesis by inhibition of cyclo oxygenase (COX), inhibiting DNA synthesis.[1]



Misoprostol, methyl-7-((1R, 2R, 3R)-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]



Literature review reveals that many 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 the 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 the other drugs[10], HPTLC method for quantitation of Diclofenac and Misoprostol in combination with other drugs has been

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 with Methanol as solvent. Thus the present study was undertaken to develop and validate a simple, sensitive, accurate, precise and reproducible RP-HPLC method for Diclofenac Sodium and Misoprostol in methanol as solvent as per International Conference of Harmonization guidelines.[11] The method has been successfully applied for the determination of the studied Diclofenac Sodium and Misoprostol in commercial dosage forms. Statistical comparisons of the results with the reference methods show excellent results and indicate no significant difference in accuracy and precision. Hence proposed method was precise, accurate and cost effective. This method can be applicable for quantitative determination of the titled drug with respect to assay from their new commercial formulation in quality control laboratories.

MATERIALS AND METHODS

Materials

The bulk drug of Diclofenac Sodium was provided by Cadila Ltd Ahmadabad as gift sample and Misoprostol was provided from Wockhardt Pharmaceuticals Ltd. Aurangabad as gift sample. All chemicals and reagents used were of HPLC grade and were purchased from USV LTD, Mumbai, India.

Instrumentation

The HPLC system consisted of a Pump (model Systronics --138), SYS LC—138 pump with sampler programmed at 20 µL capacity per injection was used. The detector consisted of UV/ VIS (Shimadzu UV-1800 double beam spectrophotometer) model operated at a wavelength of 234 nm. Data was integrated using Systronics –138 system. The column used was Thermo Hypersil BDS– C18 (100 mm × 4.6 mm, 2.5 µ) from AGILENT.

Preparation of Standard Stock Solutions

Standard stock solution of concentration 500 µg/mL of Diclofenac Sodium and 2000 µg/mL of Misoprostol was prepared using methanol. From the standard stock solution, the mixed standard solutions were prepared using Methanol to contain 5 µg/mL of Diclofenac Sodium and 20 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 (500 µg/mL of Diclofenac Sodium and 2000 µg/mL of Misoprostol) was injected in HPLC.

For HPLC method optimization different ratios of Methanol and 0.05 % OPA Water pH 2.7 were tried. But it was found that Methanol and 0.05 % OPA Water pH 2.7 in the ratio 70: 30 v/v, at flow rate 0.7 mL/min gives acceptable retention time (tR), plates and good resolution for Diclofenac Sodium and Misoprostol (Figure 2).

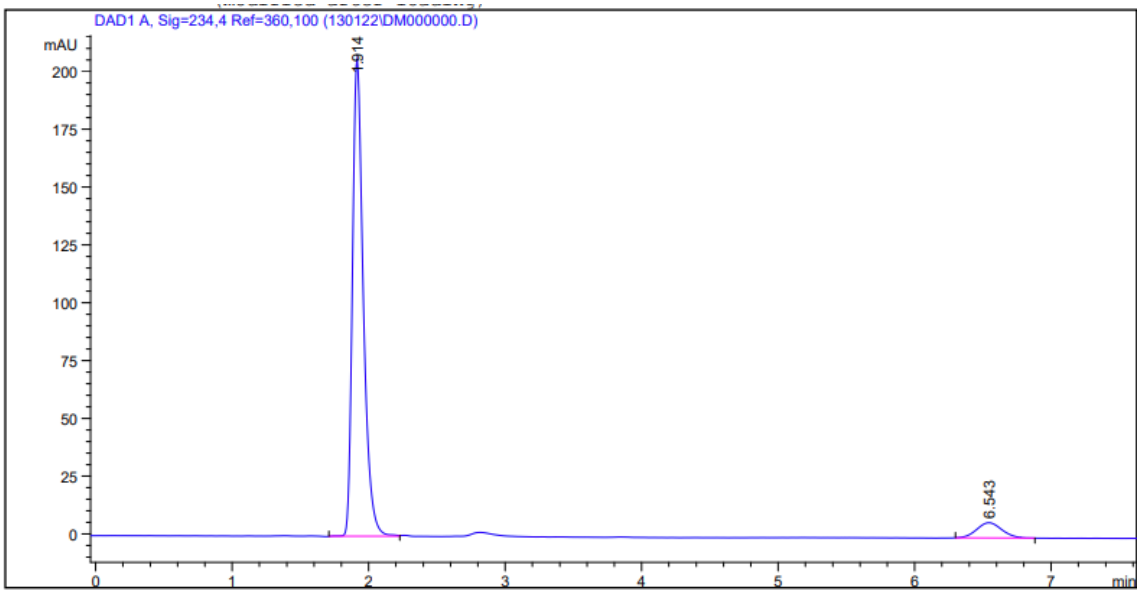


Figure 2: HPLC chromatogram of standard Diclofenac Sodium and Misoprostol (500 µg/mL and 2000 µg/mL).

VALIDATION OF THE METHOD

Validation of the optimized HPLC method was carried out with respect to the following parameters are as follows:

Linearity and range

The mixed standard stock solution (500 µg/mL of Diclofenac Sodium and 2000 µg/mL of Misoprostol) was further diluted to get Diclofenac Sodium and Misoprostol concentration in the range of 5-20 µg/mL and 10-40 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 10, 15, 20 µg/mL for Diclofenac Sodium and 40, 60, 80 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 analytes 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 methanol in the mobile phase and solvents from different lot were taken. Robustness of the method was done at concentration levels 25 µg/mL and 100 µg/ mL for Diclofenac Sodium and Misoprostol respectively.

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: Arthotec 50, Label claim: 50 mg Diclofenac and 200 mcg Misoprostol per tablet), twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 5 mg of Diclofenac Sodium and 200 mcg Misoprostol was transferred into a 10 mL volumetric flask containing 10 mL methanol, sonicated for 30 min and diluted upto 10 mL with methanol. The resulting solution was

centrifuged for 5 min and the drug content of the supernatant was determined (500 and 2000 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 5 and 20 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 230 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 Methanol: 0.05% OPA water pH 2.7 (70: 30, v/v) are given below.

Table I: Precision study.

Concentration (µg/mL)	Mean ± SD, Amount found (%)	
	Intraday Precision	Interday Precision
Diclofenac Sodium		
10	259.28±2.42, 99.05%	260.14±5.68, 99.39%
15	384.74±2.45, 98.74%	386.48±8.52, 99.20%
20	517.47±3.53, 100.01%	518.23±3.99, 100.16%
Misoprostol		
40	2057.56±10.21, 99.63%	2057.75± 3.44, 99.64%
60	3042.10±7.07, 98.62%	3046.38± 8.37, 98.76%
80	4070.82±13.85, 99.20%	4074.31± 4.34, 99.29%

Linearity

Diclofenac Sodium and Misoprostol showed good correlation coefficient (r2 = 0.999 for Diclofenac Sodium and 0.999 for Misoprostol).The mean values of the slope and intercept were 25.55 and 6.012 for Diclofenac Sodium and 50.96 and 26.64 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 0.26µg/mL and 0.78µg/mL for Diclofenac Sodium and 1.87µg/mL and 5.6µg/mL Misoprostol respectively.

Robustness of the method

Each factor selected (except columns from different manufacturers) was changed. 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	Level	Mean± SD	RSD%	
Flow Rate (mL/min):				
	DF	**MP**	**DF**	**MP**
0.8ml/ min	578.69 ± 2.16	4425.71 ± 0.67	0.37%	0.02%
0.6ml/ min	774.48 ± 1.32	5953.91 ± 20.13	0.17%	0.34%
% of Methanol in the Mobile Phase (v/v):				
69	673.8 ± 2.26	5084.8 ± 1.22	0.34%	0.02
71	664.27 ± 2.88	5063.30 ± 1.26	0.43%	0.02
Wavelength Changes:				
233	626.5 ± 0.86	4821.6 ± 1.51	0.14%	0.03%
235	686.43 ± 1.44	5314.37 ± 1.67	0.21%	0.03%

[a] **Three factors were slightly changed**

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).

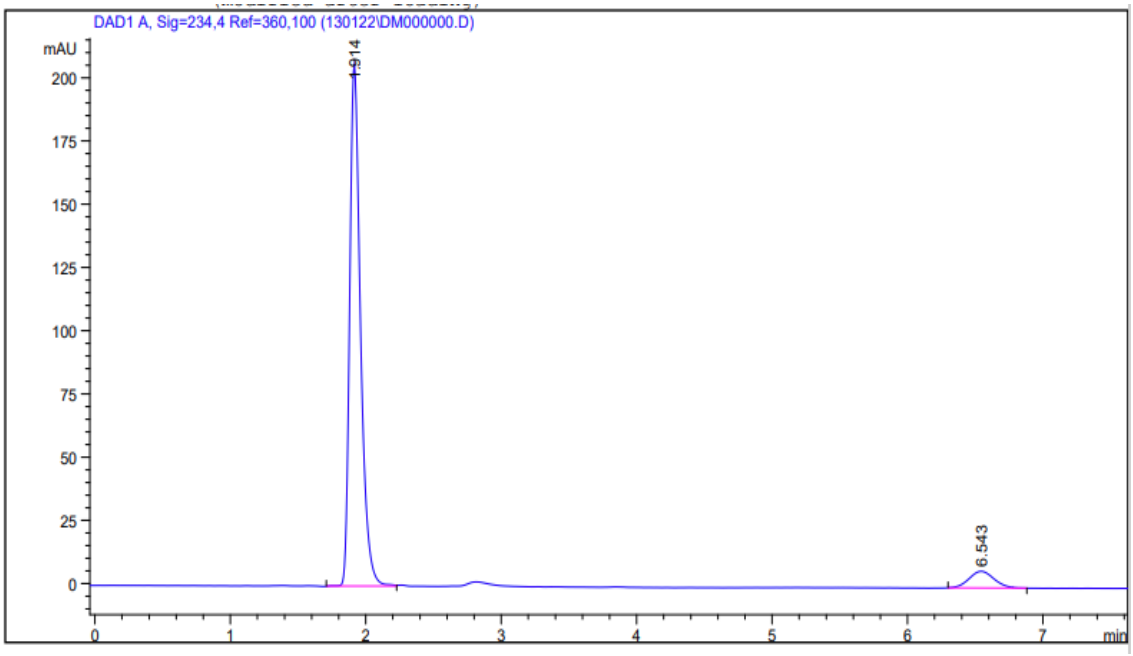
Amount added (mg)	Total amount (mg)	Amount Recovered (mg) ± % RSD	% Recovery
Diclofenac Sodium			
5mg	4 (80%)	3.98 ± 0.540	99.53%
5mg	5 (100%)	4.96 ± 0.308	99.28%
5mg	6 (120%)	6.02 ± 0.508	100.33%
Misoprostol			
20mg	16 (80%)	15.98 ± 0.119	99.85%
20mg	20 (100%)	19.88 ± 0.239	99.39%
20mg	24 (120%)	23.93 ± 0.365	99.69%

Analysis of a formulation

Experimental results of the amount of Diclofenac Sodium and Misoprostol in tablets, expressed as a percentage of label claims 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 a formulation.

Sample	Label claimed	%Label claimed± SD	%RSD
Arthotec	Diclofenac Sodium -50mg	99.77 ± 0.685	0.687%
	Misoprostol- 200mg	99.70 ± 0.096	0.096%



CONCLUSION

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

The drug was analyzed by HPLC method using Thermo Hypersil BDS– C18 (100 mm × 4.6 mm, 2.5 µ) from AGILENT with isocratic conditions and simple mobile phase containing Methanol: 0.05 % OPA Water pH 2.7 (70: 30) at flow rate of 0.7 mL/min using UV detection at 235 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 200- 400 µg/mL for Misoprostol. LOD was found to be 0.26 µg/mL and LOQ was found to be 0.78 µg/mL for Diclofenac Sodium and LOD was found to be 1.87µg/mL and LOQ was found to be 5.6µg/mL for Misoprostol.

Statistical analysis proves that this 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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