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Developoment of validated stability indicating assay method for simultaneous estimation of diclofenac sodium and misoprostol in their combined dosage form

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Abstract

A Stability indicating Reverse-Phase liquid chromatographic method for the simultaneous estimation of DF and MP was developed. The chromatographic assay involves the use of Hi Q C18 W, 150 x 4.6mm, 5µm column with a simple mobile phase composition of Acetonitrile & HPLC Grade water in the ratio of 70:30%v/v at a flow rate of 1mL/min with U.V detection at wavelength of 220 nm. The method showed good linearity in the concentration range of 50-100 µg/mL for DF and 0.20-0.40 µg/mL for MP. The proposed method was also successfully applied to 20 tablets of marketed formulation (Arthotec). The developed method was successfully validated as per the ICH guidelines for following parameters. Accuracy, precision, repeatability, ruggedness, robustness, system suitability tests, etc. The RSD for Intra-day and Inter-day precision was found to be 0.96-1.85, 1.02-1.83 For DF and 0.55-0.59, 0.59-0.63 for MP. The average percentage recoveries for DF were found to be 90.83, 99.74, 100.21 and for MP it was found to be 100.83, 98.94, 99.72. which was in good agreement with labeled amount of Pharmaceutical formulation. The stability indicating capacity was tested by accelerated degradation of marketed formulation in acidic (0.1 N HCl), basic (0.1 N NaOH), Neutral (water), Oxidative (3% H_2O_2), Thermal (60^0C), Sunlight exposure.

Keywords: DF, MP, Stability Indicating, RP-HPLC, Assay Method, Force degradation, study

1. Introduction

The technique HPLC is so called because of its improved performance over the classical column chromatography. The technique basically involves the use of porous material as a stationary phase and the liquid mobile phase is pumped into the column under high pressure. The development of this technique is attributed to the small particle size of stationary phase. As the particle size is small the resistance to the flow of mobile phase is very high that is the reason why the high pressure is recommended.[1, 2] The stability indicating assays are defined as validated quantitative analytical methods that can detect the changes with time in the chemical, physical, or microbiological properties of the drug substance and drug product, and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference. Stress testing is the main tool that is use to predict stability problems, develop analytical methods, and identify degradation product and pathways. Stress testing is likely to be carried out on single batch of the drug substance. It should include the effect of temperature in 10ºC increments (Eg.50ºC, 60ºC etc). Above that for accelerated testing, humidity (Eg. 75% RH or greater) where appropriate oxidation and photolysis on the drug substance. The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension[13, 21]. Photostability testing should be an integral part of stress testing. The review of literature[9-17] has suggested that there are few methods reported for estimation of selected drugs singly and in combination however, no stability indicating assay method has been reported for estimation of these drugs in combined dosage form. So the present work was undertaken with following objective to developed economical, simple, accurate, precise and reproducible stability indicating assay method for estimation of these drugs in combined dosage form with the use of different modern instruments. Diclofenac sodium [Figure 1] chemically is 2-(2-(2, 6-dichlorophenylamino) phenyl) acetic acid and it is freely soluble in methanol and ethanol, sparingly soluble in water and acetic acid, practically insoluble in diethyl ether. It is used in Inflammatory disease (rheumatic arthritis, alkylosing spondylysis), Pain (postoperative orthopedic, gynecologic, Dysmenorrhoea). While misoprostol [Figure 2] chemically is Methyl 7-((1*R*, 2*R*, 3*R*)-3-hydroxy-2-((*S*, *E*)-4-hydroxy-4-methyloct-1-enyl)-5-oxocyclopentyl) heptanoate. And it is Soluble in ethanol, sparingly soluble in acetonitrile and practically insoluble in water. It is used in Antiulcer Agent, Oxytocics[18-19].

Figure 1: Chemical structure of Diclofenac sodium

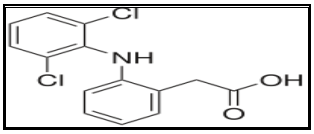
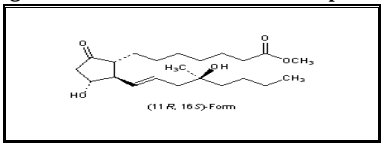


Figure 2: Chemical structure of Misoprostol



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2. Experimentals

2.1 Reagents and chemicals: DF supplied as a gift sample by Zim Labortaries Ltd, Nagpur and MP supplied as a gift sample by Wockheardt Pharamceutical Ltd, Aurangabad. All the chemicals used of HPLC Grade (Merk Ltd., Mumbai) and double distilled water was used for mobile phase preparation.

2.2 Instrument: HPLC system of JASCO JASCO Gradient Mode HPLC JASCO PU-2080 Plus Intelligent HPLC Pump. JASCO PU-2075 Plus Intelligent HPLC Detector. with column of Hi Q C18 W(150 mm x 4.6mm), 5µ is used. A gradient elution is performed using mixture of Acetonitrile & HPLC Grade water in the ratio of 70:30%v/v as a mobile phase at flow rated of 1 ml/min at detection wavelength of 220 nm.

2.3 Preparation of Mobile phase: The mobile phase was chosen based on literature survey and several trials with acetonitrile and water in various proportions. A mobile phase consisted of acetonitrile: water (70:30 v/v) was selected to achieve symmetrical peak and sensitivity.

2.3.1 Preparation of Stock Standard Solution (Solution A): Standard stock solution was prepared by dissolving 50.0 mg of DF and 0.2 mg of MP in 10.0 mL was water that give concentration 1000 and 4 µg/mL for DF and MP respectively.

2.3.2 Preparation of Working Standard Solution (Solution B): From the standard stock solution, the mixed standard solutions were prepared using acetonitrile to contain 50 µg/mL of DF and 0.2 µg/mL of MP.

2.4 Selection of detection wavelength: UV detector was selected, as it is reliable and easy to set at constant wavelength. A fix concentration of analyte were analysed at different wavelengths. As per the response of analyte, 220 nm was selected.

2.5 Linearity Study: From the standard stock solution of DF and MP 0.5 – 1.0 mL were taken in 10 mL volumetric flask diluted up to the with acetonitrile such that final concentration of DF and MP in the range 50-100 µg/mL of DF and 0.2-0.4 µg/mL of MP respectively. Volume of 20µl of each sample was injected with the help of Hamilton Syringe. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area versus the drug concentration.

2.6 Analysis of Marketed Formulation: Accurately weighed quantity equivalent to 50.0 mg of DF and 0.20 mg MP was transferred to 50 mL of volumetric flask containing water and volume was adjusted to mark with water, and filtered through Whatman filter paper. An appropriate volume, 0.5 mL was diluted to 10 mL with acetonitrile. The resulting solution (20 µl) was injected into the system and chromatogram was recorded. The concentration was determined by using linear regression equation. Amount of drug estimated in mg/tablet and percent label claim was calculated using following formula: Calculate the amount of DF/MP in mg / tablet using following formula:

$$\text{Mg/tablet} = \frac{AT1 \times WS1 \times Ds \times P1}{AS1 \times WT \times Dt} \times \text{Avg.wt}$$

Where,

AT1	=	Average area of DF/MP peak in test chromatograms
AS1	=	Average area of DF/MP peak in standard chromatograms
WS1	=	Weight of DF/MP working standard taken in mg
WT	=	Weight of sample taken in mg
P1	=	Potency of DF/MP working standard in % w/w on as such basis
Avg.wt.	=	Average weight of Tablet.
Ds	=	Dilution factor for standard.
Dt	=	Dilution factor for test.

Further calculate the amount of DF/MP present in % of Label claim using following formula

$$\% \text{ Label Claim} = \frac{\text{Assay (mg/tablet)} \times 100}{\text{Label claim of DF/MP}}$$

2.7 Method Validation: The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

2.7.1. Accuracy: It was done by recovery study using standard addition method at 80%, 100% and 120% level; known amount of DF and MP standard was added to preanalysed sample and subjected to the proposed HPLC method. The percent recovery was then calculated by using following formula

$$\% \text{ Recovery} = \frac{E_w - B}{C} \times 100$$

Where, E_w = Total drug estimated (mg)

B = Amount of drug contributed by preanalyzed capsule powder (mg)

C= Weight of pure drug added (mg).

2.7.2. Precision: Precision of the method was studied as intra-day and inter- day variation and also repeatability of sample injections. Intra- day precision was determined by analyzing, the three different concentration 60 µg/mL, 80 µg/mL and 100µg/mL of DF and 0.24 µg/mL, 0.32 µg/mL and 0.40 µg/mL of MP respectively, for three times in the same day. Inter day variability was assessed using above mentioned three concentration analysed on three different days, over a period of one week.

2.7.3. Repeatability: It was performed by injecting sample 50 µg/mL of DF and 0.2 µg/mL of MP into the system and measuring the peak area. It was repeated for six times.

2.7.4. Ruggedness: Ruggedness of the method was studied by two different analyst using same operational and environmental condition. An appropriate concentration 50 µg/mL of DF and 0.2

µg/mL of MP was analysed and concentration were determined. The procedure was repeated for six times.

2.7.5. Robustness: Robustness of the method was studied by making deliberate variation in parameters such as flow rate (± 0.1mL), % of acetonitrile in the mobile phase composition (± 10%), and change in detection wavelength (±2 nm) and the effect on the results were examined. It was performed using 50 µg/mL and 0.2 µg/mL solution of DF and MP in triplicate.

2.7.6. System suitability test: According to USP, system suitability test are integral part of liquid chromatography methods. System suitability testing is essential for the assurance of the quality performance of the chromatographic condition were tested for system suitability testing.

2.8 Forced degradation studies

Forced degradation carried out by applying various stress conditions to study the effect over wide range of pH, heat, and oxidation and photo degradation using the following approach. Stress studies were conducted in aqueous solutions.

2.8.1. Acid Degradation: Accurately weight tablet equivalent to 50.0 mg of DF & 0.2mg of MP were dissolved in 5.0 mL of aqueous 0.1N hydrochloric acid in a separate volumetric flask and refluxed in round bottom flask on boiling water bath for 1 hr.

2.8.2. Alkali Degradation: Accurately weight tablet equivalent to 50.0 mg of DF & 0.2mg of MP were dissolved in 5.0 mL of aqueous 0.1N sodium hydroxide in a separate volumetric flask and refluxed in round bottom flask on boiling water bath for 1hr.

2.8.3. Neutral Degradation: Accurately weight tablet equivalent to 50.0 mg of DF & 0.2mg of MP were dissolved in 10.0 mL of water in a separate volumetric flask and kept at room temperature for 1hr.

2.8.4. Oxidative Degradation: Accurately weight tablet equivalent to 50.0 mg of DF & 0.2mg of MP were dissolved in 10.0 mL of 3% H_2O_2 in a separate volumetric flask and refluxed in round bottom flask on boiling water bath for 1hr.

2.8.5. Thermal Degradation: Accurately weight tablet equivalent to 50.0 mg of DF & 0.2mg of MP were uniformly spread as thin layer in a separate covered Petri-dish which were then kept in oven at 60°C for 24 hrs.

2.8.6. Photo Degradation: Accurately weight tablet equivalent to 50.0 mg of DP & 0.2mg of MP were uniformly spread as thin layer in a separate covered Petri-dish which were then kept in sunlight for 3 days.

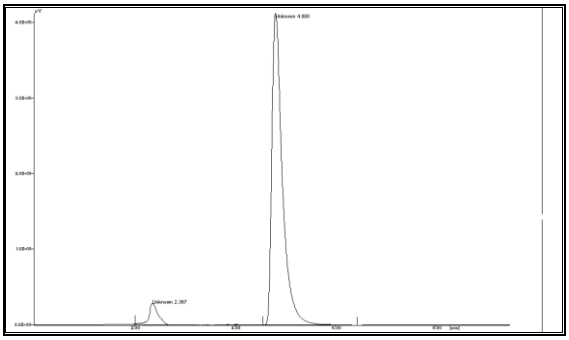
3. Results and Discussion

3.1HPLC Method Development and Optimization:

The finally optimized chromatographic conditions are.

Mobile phase	Mixture of Acetonitrile & HPLC Grade water in the ratio of 70:30%v/v
Column	Hi Q C18 W, 150 x 4.6mm, 5µm
Detection	220nm
Flow rate	1.0ml/min
Injection vol.	20µl
Column oven temp	Ambient

Figure 3: Optimized chromatogram of DF and MP.



2. Linearity:

Table 1: Linearity studies of DF

Concentration of DF [µg/mL]	Peak Area	± SD (n=5)	% RSD
50	6325229	55804.33	0.88
60	7590270	76336.61	1.02
70	8855325	46660.01	0.52
80	10120363	48156.14	0.47
90	11385410	213570.03	1.87
100	12650458	231509.12	1.83

Figure 4: Linearity studies of DF.

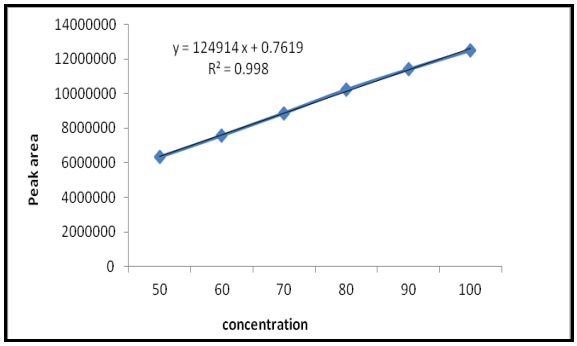
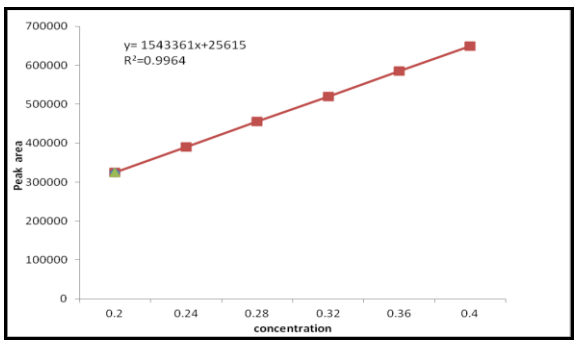


Table 2: Linearity studies of MP

Concentration of MP [µg/mL]	Peak Area	± SD (n=5)	% RSD
0.20	354975	6704.38	1.88
0.24	399970	2194.38	0.55
0.28	454965	2599.80	0.57
0.32	519963	2670.91	0.52
0.36	581875	2965.73	0.50
0.40	650120	3509.13	0.59

Figure 5: Linearity studies of MP



3.2 Analysis of Marketed formulation:

Table 3: results of marketed formulation analysis

Brand Name: Arthotec **Avg. Wt: 2034 mg**

Sr. No.	Weight of std.(mg)		Weight of sample	Peak area of std		Peak area of sample		% Label claim	
	DF	MP	(mg)	DF	MP	DF		DF	MP
1.	50	0.2	2034	6325229	354975	6314028	351870	99.82	99.12
2.			2032			6305156	351269	99.68	98.95
3.			2036			6312439	351109	99.72	98.89
							Mean	99.74	98.94
							±S.D. n=3	0.1475	0.1193
							%RSD	0.14	0.12

3.3 Method Validation[6]:

3.3.1. Accuracy: It was ascertained by recovery studies based on standard addition method at level of 80%, 100%, 120%. The average percentage recoveries for DF was found to be 90.83, 99.74, 100.21 and for MP it was found to be 100.83, 98.94, 99.72. which was in good agreement with labeled amount of Pharmaceutical formulation.

Table 4: Recovery studies of DF

Label claim (mg/Tab)	Amount Added (mg)	Total Amount	Amount Recovered (mg)	% RSD	% Recovery
50	40 (80%)	90	89.78	0.96	90.83
50	50(100%)	100	99.74	0.14	99.74
50	60(120%)	110	107.26	0.88	100.21

Table 5: Recovery studies of MP

Label claim (mg/Tab)	Amount Added(mg)	Total Amount	Amount Recovered (mg)	% RSD	% Recovery
0.2	0.16 (80%)	0.36	0.363	0.89	100.83
0.2	0.20 (100%)	0.40	0.390	0.12	98.94
0.2	0.24 (120%)	0.44	0.437	0.92	99.72

3.3.2. Precision:

Precision of the method was studied as intra-day and inter- day variation and also repeatability of sample injections. Intra- day precision was determined by analyzing, the three different concentration 60 µg/mL, 80 µg/mL and 100µg/mL of DF and 0.24 µg/mL, 0.32 µg/mL and 0.40 µg/mL of MP respectively, for three times in the same day. Inter day variability was assessed using above mentioned three concentration analysed on three different days, over a period of one week.

Table 6: Precision studies on DF

Conc. [µg/mL]	Intra-day Amount found [µg/mL]			Inter-day Amount found [µg/mL]		
	Mean	±SD n=3	% RSD	Mean	±SD n=3	% RSD
60	59.72	76336.61	1.02	58.91	74229.31	0.96
80	78.90	48156.14	0.47	79.43	49170	0.48
100	98.54	231509.12	1.83	99.20	241609.12	1.85

Table 7: Precision studies on MP

Conc. [µg/mL]	Intra-day Amount found [µg/mL]			Inter-day Amount found [µg/mL]		
	Mean	±SD n=3	% RSD	Mean	±SD n=3	% RSD
0.24	0.241	2144.38	0.55	0.236	2294.38	0.59
0.32	0.325	2670.91	0.52	0.331	2610.73	0.48
0.40	0.412	3509.13	0.59	0.409	3459.13	0.63

3.3.3 Repeatability:

Table 8: Repeatability studies on DF and MP

Concentration of DF [µg/mL]	Peak Area	Concentration of MP [µg/mL]	Peak Area
50	6325229	0.20	351870
50	6339270	0.20	351201
50	6314059	0.20	351269
50	6295780	0.20	351109
Mean	6314018		351362
± SD	18371.75		820.79
%RSD	0.29		0.20

3.3.4. Ruggedness: Ruggedness of the method was studied by two different analyst using same operational and environmental condition. An appropriate concentration 50 µg/mL of DF and 0.2 µg/mL of MP was analysed and concentration were determined.

Table 9: Ruggedness studies on DF

Condition	Mean	± SD n=3	%RSD
Analyst I	6325229	38371.75	0.59
Analyst II	6339270	40715.50	0.64

Table 10: Ruggedness studies on MP

Condition	Mean	± SD n=3	%RSD
Analyst I	350132	1210.05	0.34
Analyst II	347690	1597.98	0.45

3.3.5. Robustness:

Table 11: Robustness studies on DF

Condition	Mean	± SD n=3	%RSD
Change in flow rate (± 0.1 ml)	6286519	36492.14	0.58
Change in detection wavelength (± 2 nm)	6289541	35715.50	0.60

Table 12: Robustness studies on Mp

Condition	Mean	± SD n=3	%RSD
Change in flow rate (± 0.1 ml)	342890	1574.49	0.35
Change in detection wavelength (± 2 nm)	349803	2598.56	0.57

3.3.6. System suitability test:

Table 13: System suitability studies for DF and MP.

System Suitability Parameter	Standard	Proposed Method of MP	Proposed Method of DF
Retention time (t_R) [min]	5-10 min	2.3	4.8
Resolution	Should be > 2	-	2.98
Theoretical plate (N)	More than 2000	2078	3510

Force degradation studies[4]:

Table 14: Summary of force degradation studies.

Condition	%Assay DF	% Degradation DF	%Assay MP	% Degradation MP
Initial sample	99.74	-	98.95	-
0.1N HCL	97.24	2.53	98.50	-
0.1N NaOH	97.74	-	98.50	0.45
3% H_2O_2	98.44	1.30	97.90	1.05
Thermal	98.96	0.78	97.37	0.89
Neutral	99.72	-	-	-
Sun	96.45	3.29	98.50	0.45

Figure 6: Chromatogram of acid degradation

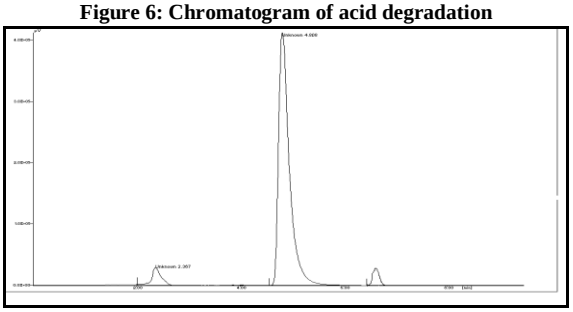


Figure 7: Chromatogram of Alkali degradation

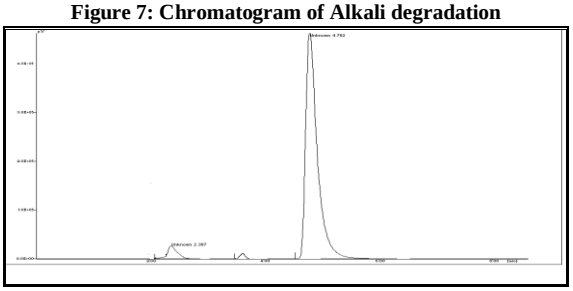


Figure 8: Chromatogram of Neutral degradation

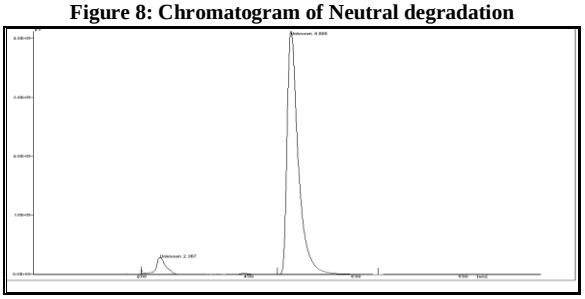


Figure 9: Chromatogram of oxidative degradation

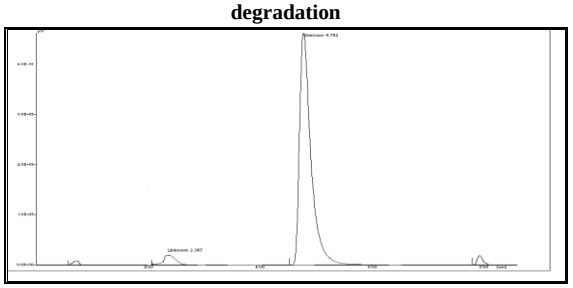


Figure 10: Chromatogram of sunlight degradation

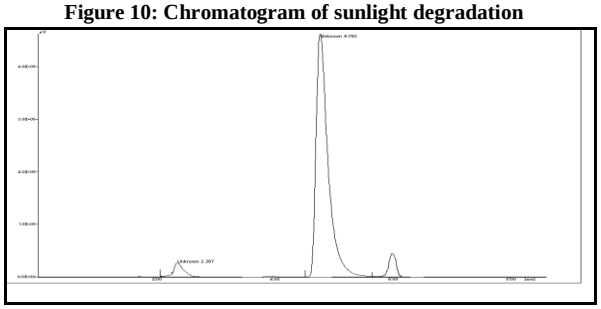
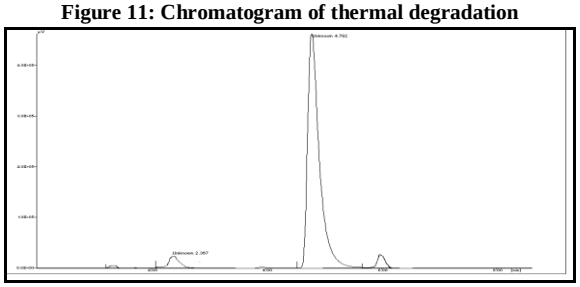


Figure 11: Chromatogram of thermal degradation



The parent drug peak was well resolved from all the degradants generated under various stress conditions. However it could not be ascertain that peaks of degradants with similar retention times under different stress condition were same chemical entity or different. In this regard further studied may be pursued in order to isolate and characterize degradants of different stress conditions. The DF was susceptible to acid, photolytic, thermal and oxidative degradation and MP was susceptible to alkali, oxidative, thermal and photolytic degradation in the marketed formulation.

4. Conclusion

The proposed method was validated as Per the ICH Guidelines. The proposed method also showed the good resolution between DF and MP with run time of 15 min. The method is very simple and rapid and no where involves complicated sample preparation and mobile phase preparation. Also the proposed method showed good specificity and selectivity in order to determine DF and MP in the presence of their degradation products. The linearity and reproducibility data of the drugs carried out by this method showed that no major interference is caused in the estimation of the drugs. Therefore the method can be use for routine quality control of these drugs.

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