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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ASSAY FOR SIMULTANEOUS DETERMINATION OF PENTAPRAZOLE, DICLOFENAC, CHLOROXAZONE IN PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC

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Keywords:

Chlorzoxazone, Pantoprazole, Diclofenac, RP-HPLC

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ABSTRACT: Objective: A simple, accurate, precise method was developed for the simultaneous estimation of the Chlorzoxazone, Pantoprazole, and Diclofenac in solid dosage form. **Method:** Chromatogram was run through BDS C18 150 × 4.6 mm, 5 m. Mobile phase containing 0.01% KH_2PO_4 and Acetonitrile in the ratio of 52:48 v/v was pumped through column at a flow rate of 1.0 ml/min. The buffer used in this method was 0.01%. KH_2PO_4, Temperature was maintained at 30 °C. The optimized wavelength for Chlorzoxazone, Pantoprazole, and Diclofenac was 229.0 nm. **Results:** Retention time of Chlorzoxazone, Pantoprazole, and Diclofenac were found to be 2.229 min, 2.958 min, and 3.568 min. % RSD of system precision for Chlorzoxazone, Pantoprazole and Diclofenac were and found to be 0.9, 0.6 and 0.6, respectively. % RSD of method precision for Chlorzoxazone, Pantoprazole and Diclofenac were and found to be 0.6, 1.0 and 0.7 respectively. % recovery was obtained as 99.86%, 100.07% and 99.70% for Chlorzoxazone, Pantoprazole, and Diclofenac respectively. LOD values are obtained from regression equations of Chlorzoxazone, Pantoprazole and Diclofenac were 0.14 ppm, 0.24 ppm, 1.83 pm, and LOQ values are obtained from regression equations of Chlorzoxazone, Pantoprazole and Diclofenac were 0.42 ppm, 0.72 pm, 5.53 ppm respectively. The regression equation of Chlorzoxazone was y = 8321.9.x + 1397.8Pantoprazolee was y = 9806.1x + 6071.7 and of Diclofenac was y = 2575x + 4338.4. **Conclusion:** Retention times are decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

INTRODUCTION: Analytical chemistry is a branch of chemistry that deals with the identification of compounds and mixtures (qualitative analysis) or the determination of the proportions of the constituents (quantitative analysis).

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The techniques commonly used are titration, precipitation, spectroscopy, chromatography, etc. High-performance liquid chromatography (HPLC) is the fastest-growing analytical technique for analysis of drugs [1]. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids [2].

The reasons for the popularity of the method are its sensitivity, its ready adaptability to accurate quantitative determinations, its suitability for separating non-volatile species or thermally fragile ones and its widespread applicability to substances

that are of prime interest to the industry [3]. Sensitive detectors have transformed liquid column chromatography into high speed, efficient, accurate and highly resolved methods of separation [4]. The HPLC is the method of choice in the field of analytical chemistry since this method is specific, robust, linear, precise and accurate and the limit of detection is low. The present aim of the study was the simultaneous estimation of the drug Chlorzoxazone. Pantoprazole and Diclofenac sodium by using RP-HPLC method [5].

Chlorzoxazone: Chlorzoxazone is a centrally acting central muscle relaxant with a sedative drug. It is inhibiting the muscle spasm by exerting an effect primarily at the level of the spinal cord and subcortical areas of the brain. Chlorzoxazone is having molecular weight 169.57, Molecular Formula $C_7H_4C_lNO_2$, IUPAC Name: 5-chloro-2, 3-dihydro-1, 3- benzoxazol-2- one. The solubility of chlorzoxazone was in DMSO, ethanol, and methanol and pKa value were 9.396.

Structure:

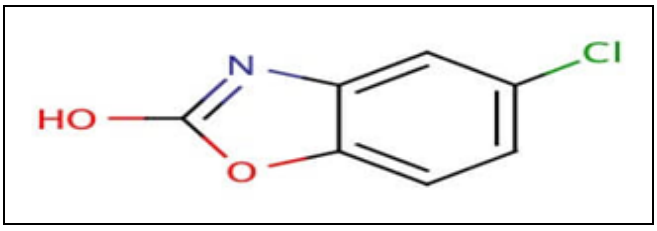

FIG. 1: STRUCTURE OF CHLORZOXAZONE

Pantoprazole: Pantoprazole is a proton pump inhibitor drug used for short-term treatment of erosion and ulceration of the esophagus caused by gastroesophageal reflux disease. The Pantoprazole is having molecular Weight 383.37, Molecular Formula $C_{16}H_{15}F_2N_3O_4S$, IUPAC Name 6-(Difluoromethoxy)-2- [(3, 4-dimethoxypyridin-2-yl) methanesulfinyl]-1H-1, 3-benzodiazole [7]. The solubility of pantoprazole was in methanol, pKa value was 6.43.

Structure:

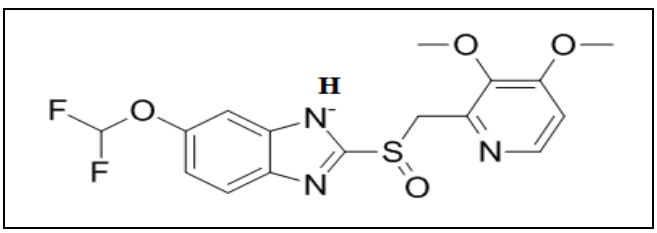

FIG. 2: STRUCTURE OF PANTOPRAZOLE

Diclofenac: Diclofenac is non-steroidal anti-inflammatory agent (NSAID) with antipyretic and analgesic actions. Diclofenac is having Molecular Weight 296.15, Molecular formula: $C_{14}H_{11}C_{l2}NO_2$ IUPAC Name: 2-{2-[(2, 6-dichlorophenyl) amino] phenyl} acetic acid. It is soluble in water and is having pKa value: 4.08.

Structure:

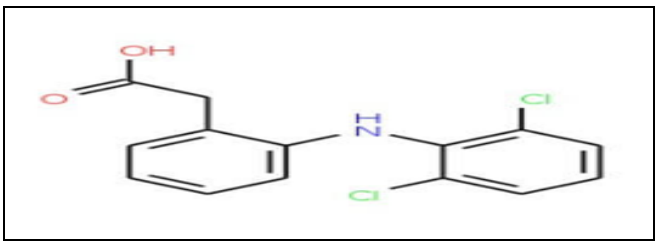

FIG. 3: STRUCTURE OF DICLOFENAC

MATERIALS AND METHOD:

Materials and Instruments: The materials used were AR grade or the best possible Pharma grade available and they are supplied by the manufacturer or supplier SD fine, Sigma Aldrich. The chemicals used are Water–HPLC grade, Acetonitrile, Triethylamine, Potassium dihydrogen orthophosphate, Orthophosphoric acid. Instruments used were Electronics Balance, pH meter, Waters HPLC2695 series with quaternary pumps, Photo Diode array detector, and autosampler integrated with empowering software, Ultra sonicator. UV double beam spectrophotometer with UV win [5].

General Procedure:

Sample Processing:

Diluents: Based upon the solubility of the drug diluents was selected0.03N KH_2PO_4: Acetonitrile (50:50 v/v)

0.03N KH_2PO_4 Buffer: Accurately weighed 4.08 gm of Potassium dihydrogen orthophosphate in a 1000 ml of volumetric flask add about 900 ml of milli-Q water added and degas to sonicate and finally make up the volume with water.

Preparation of Standard Stock Solutions: Accurately weighed 5 mg of Chlorzoxazone, 12.5 mg of Pantoprazole and 125 mg of Diclofenac and transferred to three 25 ml volumetric flasks separately. 10 ml of diluents were added to flasks and sonicated for 20 min. Flasks were made up with 0.03N KH_2PO_4: Acetonitrile (50:50 v/v) and labeled as Standard stock solutions 1, 2, and 3.

Preparation of Standard Working Solutions (100% Solution): 1 ml from each stock solution was pipette out and taken into a 10 ml volumetric flask and made up with 0.03 N KH_2PO_4: Acetonitrile (50:50 v/v)

Preparation of Sample Stock Solutions: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 25 mL of diluents was added and sonicated for 50 min, further the volume made up with diluent and filtered.

Preparation of Sample Working Solutions (100% Solution): From the filtered solution 1 ml was pipetted out into a 10 ml volumetric flask and made up to 10 ml with diluents (20 ppm, 50 ppm, and 500 ppm).

Preparation of Buffer:

0.1% OPA Buffer: 1 ml of orthophosphoric acid was diluted to 1000 ml with HPLC grade water.

RESULTS AND DISCUSSION: In the estimation of the three drugs four trails were conducted by using various solvent systems, columns, and experimental conditions. Finally in the optimized method the solvent system used was 0.01% KH_2PO_4: Acetonitrile (52:48 v/v), and other conditioned are represented in **Table 1**. Chromatograms of blank and placebo were showed in **Fig. 4** and **5**.

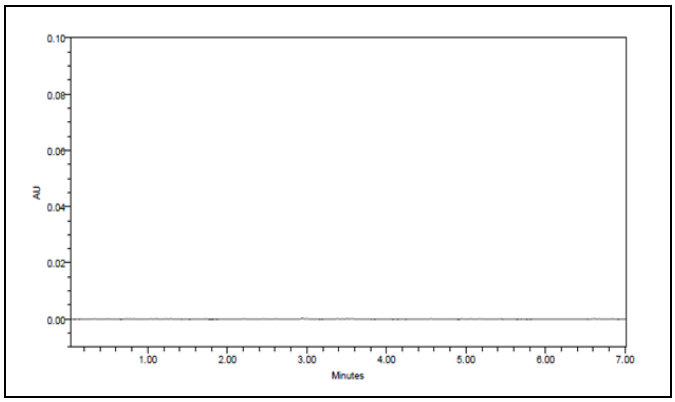

FIG. 4: BLANK CHROMATOGRAM

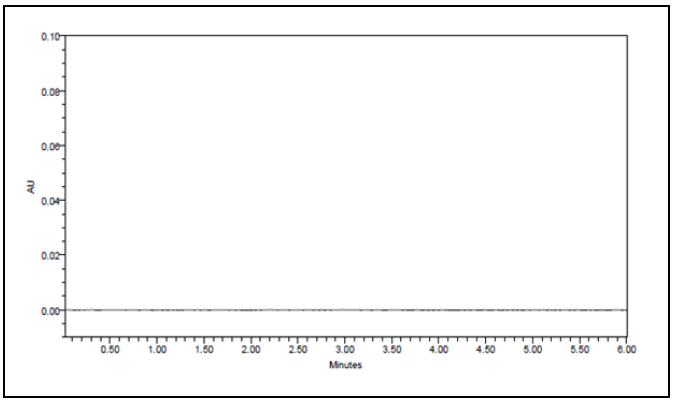

FIG. 5: PLACEBO CHROMATOGRAM

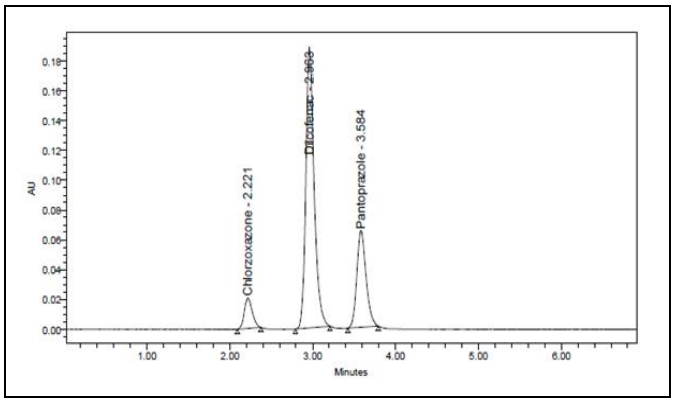

FIG. 6: OPTIMIZED CHROMATOGRAM

TABLE1: OPTIMIZED METHOD

Column Used	BDS C_{18} 150 × 4.6 mm, 5 µ
Mobile phase	0.01% KH_2PO_4: Acetonitrile (52:48 v/v)
Flow rate	1.0 ml/min
Diluent	0.01 N KH_2PO_4: Acetonitrile (50:50 v/v)
Wavelength	229.0 nm
Temperature	30 °C
Injection Volume	10.0 µl
Run time	7.0 Min

Chlorzoxazone, Pantoprazole, and Diclofenac were eluted at 2.221 min, 2.968 min, and 3.584 min, respectively with good resolution. The plate count and tailing factor were very satisfactory, so this method was optimized and to be validated. This method was optimized, and drugs were eluted with good retention time, resolution; all the system suitable parameters like Plate count and Tailing factor were within limits, it was shown in the **Fig. 6**.

Validation:

System Suitability Parameters: The system suitability parameters were determined by preparing standard solutions of Chlorzoxazone, Pantoprazole, and Diclofenac and the solutions were injected six times, and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections was not more than 2%. Plate count of the Chlorzoxazone Was 5439 ± 300, Pantoprazole was 4574 ± 300 and of Diclofenac was 6139 ± 300, tailing factor of Chlorzoxazone was 1.05 ± 0.2, Pantoprazole was 1.04 ± 0.2, and of Diclofenac was 1.220.1, resolution between Chlorzoxazone and Pantoprazole was 3.6 and resolution between Pantoprazole and Dilcofenac was 5.4. According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and they were within the limits. They were shown in **Table 2**, **Fig. 7**.

TABLE 2: SYSTEM SUITABILITY PARAMETERS

S. no.	Chlorzoxazone			Pantoprazole			Diclofenac		
Inj	RT (min)	TP	Tailing	RT (min)	TP	Tailing	RT (min)	TP	Tailing
1	2.220	2780	1.17	3.540	5241	1.22	2.937	4376	1.28
2	2.223	2817	1.18	3.546	5116	1.21	2.943	4610	1.27
3	2.223	3010	1.21	3.546	5214	1.23	2.944	4808	1.27
4	2.225	3023	1.13	3.548	5730	1.20	2.944	4383	1.28
5	2.225	2996	1.13	3.554	4891	1.20	2.949	4242	1.26
6	2.229	3083	1.15	3.568	5246	1.21	2.958	4540	1.26

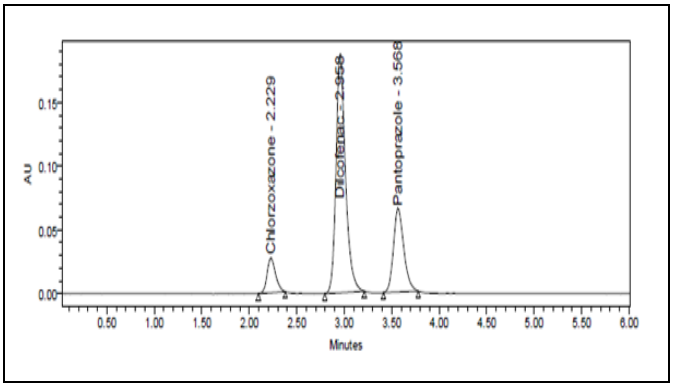

FIG. 7: SYSTEM SUITABILITY CHROMATOGRAM

Precision:

Preparation of Standard Stock Solutions: Accurately weighed 5 mg of Chlorzoxazone, 12.5 mg of Pantoprazole, and 125 mg of Diclofenac and transferred to three 25 ml volumetric flasks separately. 10 ml of diluent was added to flasks and sonicated for 15 min. Flasks were made up with 0.03 N KH_2PO_4: Acetonitrile (50:50 v/v) and labeled as Standard stock solutions 1, 2, and 3.

Preparation of Standard Working Solutions (100% Solution): 1 ml from each standard stock solution was pipette out and taken into a 10 ml volumetric flask and made up with KH_2PO_4: Acetonitrile (20 ppm, 50 ppm, and 500 ppm).

Preparation of Sample Stock Solutions: 5 tablets were weighed and calculate the average weight of each tablet, then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 25 mL of diluent added and sonicated for 50 min; further the volume made up with diluent and filtered.

Preparation of Sample Working Solutions (100% Solution): 1 ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up with diluents (20 ppm, 50 ppm, and 500 ppm).

TABLE 3: PRECISION VALUES

S. no.	Area of Chlorzoxazone	Area of Pantoprazole	Area of Diclofenac
1	171126	500143	1290245
2.	172435	495628	1293549
3.	172284	500766	1301289
4.	169409	492683	1304374
5	169122	497427	1285299
6.	169432	496587	1289128
Mean	170536	497206	1293981
S.D	1512.6	2989.3	7409.4
% RSD	0.9	0.6	0.6

From a single volumetric flask of working standard solution, six injections were given, and the obtained areas were mentioned above **Table 3**. Average area, standard deviation, and % RSD were calculated for three drugs and obtained as 0.9%, 0.6%, and 0.6% respectively for Chlorzoxazone, Pantoprazole and Diclofenac. As the limit of Precision was less than "2" the system precision was passed in this method.

Linearity:

Preparation of Standard Stock Solutions: Accurately weighed 5mg of Chlorzoxazone, 12.5 mg of Pantoprazole, and 125 mg of Diclofenac and transferred to three 25 ml volumetric flasks separately. 10 ml of diluent was added to flasks and sonicated for 15 min. Flasks were made up with 0.03N KH_2PO_4: Acetonitrile (50:50 v/v) and labeled as Standard stock solutions 1, 2, and 3.

25% Standard Solution: 0.25 ml each from three standard stock solutions was pipette out and made up to 10 ml.

50% Standard Solution: 0.5 ml each from three standard stock solutions was pipette out and made up to 10 ml.

75% Standard Solution: 0.75 ml each from three standard stock solutions was pipette out and made up to 10 ml.

100% Standard Solution: 1.0 ml each from three standard stock solutions was pipette out and made up to 10 ml.

125% Standard Solution: 1.25 ml each from three standard stock solutions was pipette out and made up to 10 ml.

150% Standard Solution: 1.5 ml each from three standard stock solutions was pipette out and made up to 10 ml.

TABLE 4: LINEARITY FOR CHLORZOXAZONE, PANTOPRAZOLE AND DICLOFENAC

Chlorzoxazone.		Pantoprazole		Diclofenac	
Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Peak area
5	43034	12.5	131545	125	325141
10	86532	25	260353	250	654346
15	124455	37.5	370105	375	975309
20	171308	50	496077	500	1296108
25	208420	62.5	616050	625	1595660
30	249841	75	742462	750	1943275

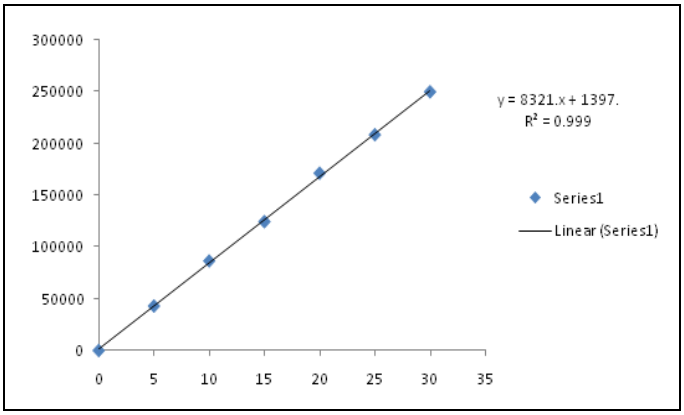

FIG. 8: CALIBRATION CURVE OF CHLORZOXAZONE

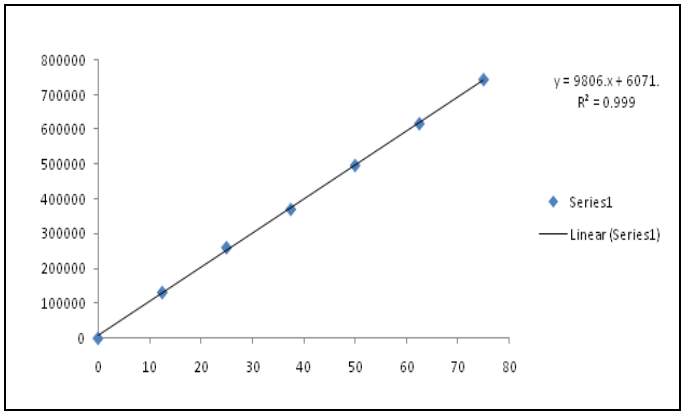

FIG. 9: CALIBRATION CURVE OF PANTOPRAZOLE

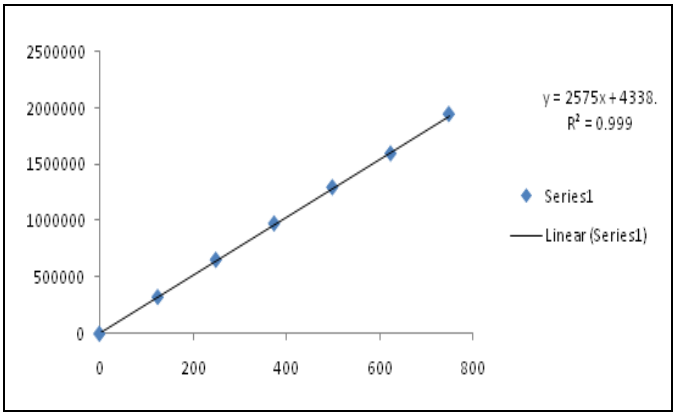

FIG. 10: CALIBRATION CURVE OF DICLOFENAC

Six linear concentrations of Chlorzoxazone. (5-30 µg/ml), Pantoprazole (12.5.75 µg/ml) and Diclofenac (125-750 µg/ml) were injected in a triplicate manner **Table 4**. Average areas were mentioned above, and linearity equations obtained for Chlorzoxazone were y = 8321.9.x + 1397.8 **Fig. 8**. Pantoprazole was y = 9806.1x + 6071 **Fig. 9** and of Diclofenac was y = 2575x + 4338.4 **Fig. 10.** The correlation coefficient obtained was 0.999 for all the three drugs.

Accuracy:

Preparation of Standard Stock Solutions: Accurately weighed 5 mg of Chlorzoxazone 12.5 mg of Pantoprazole and 125 mg of Diclofenac and transferred to three 25 ml volumetric flasks separately. 10 ml of diluent was added to flasks and sonicated for 15 min. Flasks were made up with 0.03 N KH_2PO_4: Acetonitrile (50:50 v/v) and labeled as Standard stock solutions 1, 2 and 3.

Preparation of 50% Spiked Solution: 0.5 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipette out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipette out, and made up to the mark with diluents.

Preparation of 150% Spiked Solution: 1.5 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipette out, and made up to the mark with diluents.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102

TABLE 5: ACCURACY OF CHLORZOXAZONE

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
	10	10.02	100.17	
50%	10	9.92	99.19	
	10	10.01	100.07	
	20	19.95	99.77	
100%	20	20.35	101.77	99.86%
	20	20.05	100.27	
	30	29.52	98.39	
150%	30	29.91	99.69	
	30	29.82	99.41	

TABLE 6: ACCURACY OF PANTOPRAZOLE

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
	25	25.07	100.28	
50%	25	25.50	101.98	
	25	24.93	99.74	
	50	49.68	99.37	
100%	50	49.89	99.77	100.07%
	50	50.45	100.90	
	75	75.06	100.08	
150%	75	74.38	99.17	
	75	74.54	99.38	

TABLE 7: ACCURACY OF DICLOFENAC

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
	250	251.09	100.44	
50%	250	248.23	99.29	
	250	248.68	99.47	
	500	502.58	100.52	
100%	500	496.16	99.23	99.70%
	500	501.92	100.38	
	750	746.77	99.57	
150%	750	740.70	98.76	
	750	747.37	99.65	

Three levels of Accuracy sample were prepared by the standard addition method. Triplicate injections were given for each level of accuracy and mean % Recovery was obtained as 99.86%, 100.07% and 99.70% for Chlorzoxazone, Pantoprazole, and Diclofenac respectively. The results were shown in **Table 5**, **6** and **7**. Robustness conditions like Flow minus (0/65 ml/min), Flow plus (0.85 ml/min), mobile phase minus, mobile phase plus, temperature minus (25 °C), and temperature plus (35 °C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected, and all the parameters were passed. % RSD was within the limit. The results were shown in **Table 8**.

TABLE 8: ROBUSTNESS DATA FOR CHLORZOXAZONE, PANTOPRAZOLE AND DILCOFENAC

S. no.	Condition	% RSD of Chlorzoxazone	% RSD of Pantoprazole	% RSD of Diclofenac
1	Flow rate (-) 0.65 ml/min	0.9	0.5	0.5
2	Flow rate (+) 0.85 ml/min	0.6	0.2	0.5
3	Mobile phase (-) 53B:47A	1.5	0.6	0.9
4	Mobile phase (+) 63B:37A	0.3	1.3	0.7
5	Temperature (-) 25°C	0.8	0.8	0.3
6	Temperature (+) 35°C	1.1	0.6	0.6

Robustness conditions like Flow minus (0.65 ml/min), Flow plus (0.85 ml/min), mobile phase minus (53B:47A), mobile phase plus (63B:37A), temperature minus (25 °C) and temperature plus (35 °C) was maintained and samples were injected in a duplicate manner. System suitability parameters were not much affected and all the parameters were passed. % RSD was within the limit.

Sensitivity:

LOD Sample Preparation: 0.25 ml each from three standard stock solutions was pipette out and transferred to 3 separate 10 ml volumetric flask and made up with diluents from the above solutions 0.1 ml, 0.1 ml and 0.1 ml of Chlorzoxazone, Pantoprazole and Dilcofenac solutions respectively were transferred to 10 ml volumetric flasks and made up with the same diluents. LOD values are obtained from regression equations of Chlorzoxazone, Pantoprazole, and Diclofenac were 0.14 ppm, 0.24 ppm, 1.83 pm. The result was depicted in **Fig. 11**.

LOQ Sample Preparation: 0.25 ml each from three standard stock solutions was pipette out and transferred to 3 separate 10 ml volumetric flask and made up with diluents from the above solutions 0.3 ml, 0.3 ml and 0.3 ml of Chlorzoxazone, Pantoprazole and Dilcofenac solutions respectively were transferred to 10 ml volumetric flasks and made up with the same diluents.

LOQ values are obtained from regression equations of Chlorzoxazone, Pantoprazole and Diclofenac were 0.42 ppm, 0.72 pm, 5.53 ppm respectively. The chromatogram was shown in **Fig. 12**. The result of sensitivity was depicted in **Table 9**.

TABLE 9: SENSITIVITY TABLE OF CHLORZOXAZONE, PANTOPRAZOLE AND DILCOFENAC

Molecule	LOD (µg/ml)	LOQ (µg/ml)
Chlorzoxazone	0.14 µg/ml	0.42 µg/ml
Pantoprazole	0.24 µg/ml	0.72 µg/ml
Dilcofenac	1.83 µg/ml	5.53 µg/ml

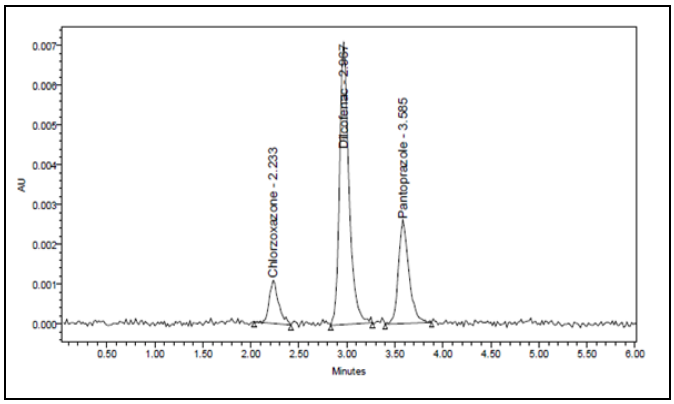

FIG. 11: LOD CHROMATOGRAM OF STANDARD

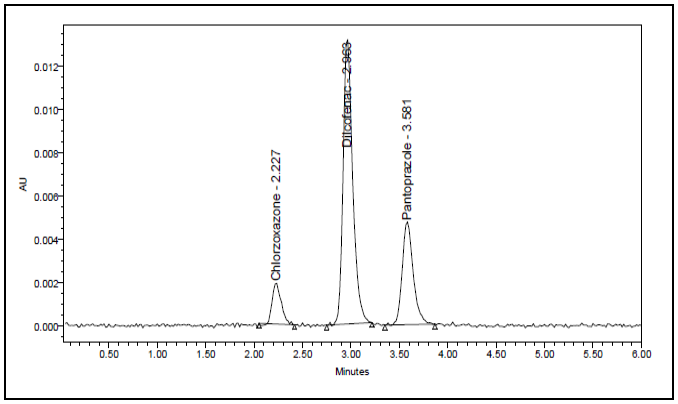

FIG. 12: LOD CHROMATOGRAM OF STANDARD

Assay: Alldex DT plus (50 + 20 + 500) the label claim Pantoprazole 50 mg Chlorzoxazone20mg Diclofenac 500 mg per unit formulation Assay was performed with the above formulation. Average % Assay for Chlorzoxazone, Pantoprazole, and Diclofenac. Obtained was 99.28%, 99.24% and 100.12% respectively. The results were shown in **Tables 10**, **11** and **12**.

TABLE 10: ASSAY DATA OF CHLORZOXAZONE

S. no.	Standard Area	Sample area	% Assay
1	171126	168913	98.85
2	172435	170840	99.98
3	172284	171067	100.11
4	169409	170128	99.56
5	169122	169928	99.44
6	169432	171996	100.65
Avg	170536	170479	99.77
Stdev	1512.6	1063.6	0.622
%RSD	0.9	0.6	0.6

TABLE 11: ASSAY DATA OF PANTOPRAZOLE

S. no.	Standard Area	Sample area	% Assay
1	500143	494402	99.24
2	495628	508518	102.07
3	500766	501974	100.76
4	492683	506325	101.63
5	497427	500386	100.44
6	496587	504538	101.27
Avg	497206	502691	100.90
Stdev	2989.3	5002.1	1.00
% RSD	0.6	1.0	1.0

TABLE 12: ASSAY DATA OF DICLOFENAC

S. no.	Standard Area	Sample area	% Assay
1	1290245	1288257	99.46
2	1293549	1302223	100.54
3	1301289	1302368	100.55
4	1304374	1311342	101.24
5	1285299	1290713	99.65
6	1289128	1290961	99.67
Avg	1293981	1297644	100.18
Stdev	7409.4	9074.5	0.701
% RSD	0.6	0.7	0.7

Degradation Studies: Degradation studies were performed at different conditioned such as degradation by hydrogen peroxide, acid, alkali, neural, dry heat, photo radiations the results were shown in **Table 13**, **14,** and **15**.

Acid Degradation Studies: To 1 ml of stock solution Chlorzoxazone, Pantoprazole, and Diclofenac, 1 ml of 2 N Hydrochloric acid was added and refluxed for 30 min at 60 °C. The resultant solution was diluted to obtain 5 µg/ml, 60 µg/ml and 5 µg/ml of all components and 10 µl solutions were injected into the system and the chromate grams were recorded to assess the stability of the sample. The result was showed in **Fig. 13.**

Alkali Degradation Studies: To 1 ml of stock solution Chlorzoxazone, Pantoprazole, and Diclofenac, 1 ml of 2 N sodium hydroxide was added and refluxed for 30 min at 60 °C. The resultant solution was diluted to obtain 5 µg/ml, 60 µg/ml and 5 µg/ml of all components and 10 µl were injected into the system, and the chromatograms were recorded to assess the stability of sample. The result was showed in **Fig. 14.**

Oxidation: To 1 ml of stock solutions of Chlorzoxazone, Pantoprazole and Dilcofenac. 1 ml of 20% hydrogen peroxide (H_2O_2) were added separately. The solutions were kept for 30 min at 60 °C. For HPLC study, the resultant solution was diluted to obtain 5 µg/ml, 60 µg/ml and 5 µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The result was showed in **Fig. 15.**

Dry Heat Degradation Studies: The standard drug solution was placed in an oven at 105 °C for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted obtain 5 µg/ml, 60 µg/ml and 5 µg/ml of all components and 10 µl were injected into the system and the chromate grams were recorded to assess the stability of the sample. The result was showed in **Fig. 16.**

Photo Stability Studies: The photochemical stability of the drug was also studied by exposing the 50 µg/ml, 600 µg/ml, and 50 µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1 day or 200 Watt h/m^2 in photostability chamber. For HPLC study, the resultant solution was diluted to obtain 5 µg/ml, 60 µg/ml and 5 µg/ml of all components and 10 µl were injected into the system, and the chromatograms were recorded to assess the stability of sample. The result was showed in **Fig. 16.**

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 6 h at a temperature of 60 °C. For HPLC study, the resultant solution was diluted to obtain 5 µg/ml, 60 µg/ml and 5 µg/ml of all

components and 10 µl were injected into the system, and the chromate grams were recorded to assess the stability of the sample. The result was showed in **Fig. 17.**

TABLE 13: DEGRADATION DATA OF CHLORZOXAZONE

S. no.	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	2.45	0.204	0.273
2	Alkali	2.14	0.247	0.267
3	Oxidation	5.14	0.231	0.293
4	Thermal	3.94	0.108	0.268
5	UV	0.72	0.120	0.268
6	Water	0.02	0.115	0.270

TABLE 14: DEGRADATION DATA OF PANTOPRAZOLE

S. no.	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	3.84	0.303	0.424
2	Alkali	5.64	0.276	0.435
3	Oxidation	4.88	0.307	0.393
4	Thermal	1.24	0.247	0.398
5	UV	0.95	0.231	0.390
6	Water	0.95	0.238	0.386

TABLE 15: DEGRADATION DATA OF DICLOFENAC

S. no.	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	5.70	0.305	0.334
2	Alkali	7.87	0.143	0.327
3	Oxidation	4.57	0.234	0.397
4	Thermal	2.18	0.136	0.307
5	UV	0.93	0.132	0.302
6	Water	0.54	0.140	0.303

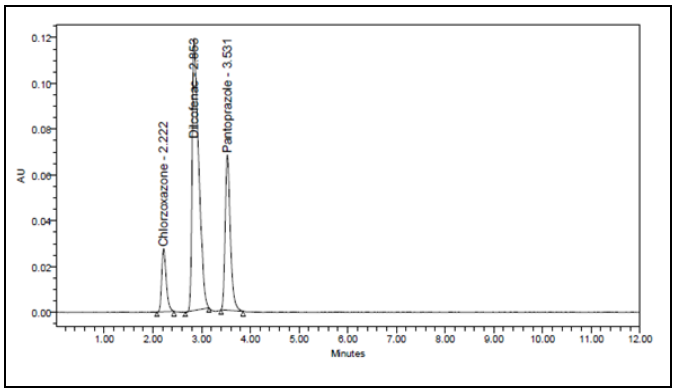

FIG. 13: ACID DEGRADATION CHROMATOGRAM OF CHLORZOXAZONE PANTOPRAZOLE AND DICLOFENAC

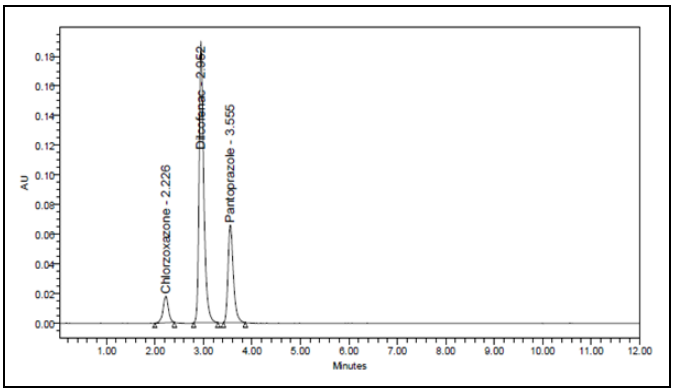

FIG. 14: BASE DEGRADATION CHROMATOGRAM OF CHLORZOXAZONE, PANTOPRAZOLE AND DICLOFENAC

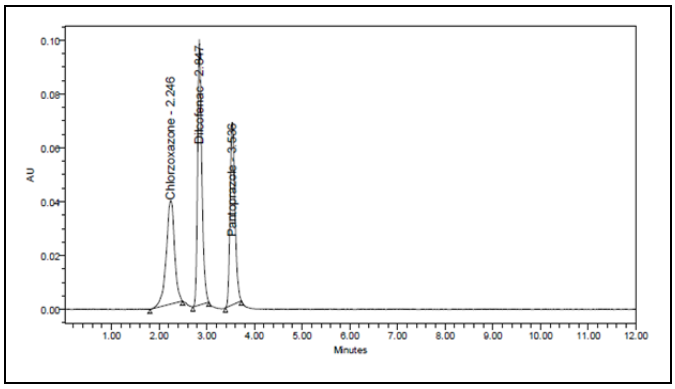

FIG. 15: PEROXIDE DEGRADATION CHROMATOGRAM OF CHLORZOXAZONE, PANTOPRAZOLE AND DICLOFENAC

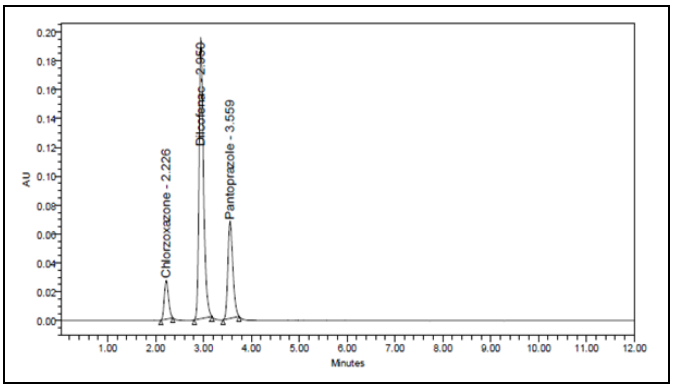

FIG. 16: THERMAL DEGRADATION CHROMATOGRAM OF CHLORZOXAZONE, PANTOPRAZOLE AND DICLOFENAC

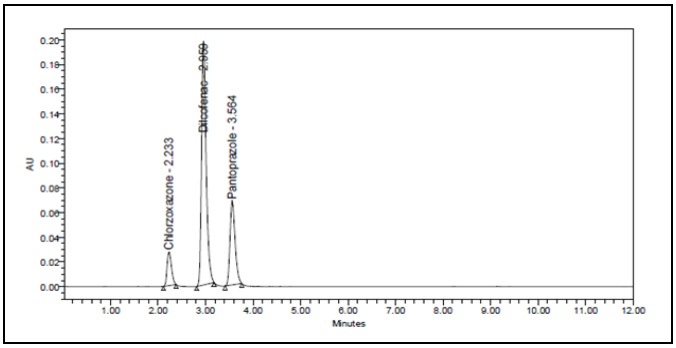

FIG. 17: UV DEGRADATION CHROMATOGRAM OF CHLORZOXAZONE, PANTOPRAZOLE AND DICLOFENAC

CONCLUSION: A simple, accurate, precise method was developed for the simultaneous estimation of the Chlorzoxazone, Pantoprazole, and Diclofenac in tablet dosage form. The retention time of Chlorzoxazone, Pantoprazole and Diclofenac were found to be 2.229 min, 2.958 min, and 3.568 min. % RSD of system precision for Chlorzoxa-zone, Pantoprazole and Diclofenac were and found to be 0.9, 0.6 and 0.6 respectively. % RSD of method precision for Chlorzoxazone, Pantoprazole and Diclofenac were and found to be 0.6, 1.0 and 0.7 respectively. % recovery was obtained as 99.86%, 100.07% and 99.70% for Chlorzoxazone, Pantoprazole, and Diclofenac respectively.

LOD values are obtained from regression equations of Chlorzoxazone, Pantoprazole and Diclofenac were 0.14 ppm, 0.24 ppm and 1.83 ppm and LOQ values are obtained from regression equations of Chlorzoxazone, Pantoprazole and Diclofenac were 0.42 ppm, 0.72 ppm, and 5.53 ppm respectively. The regression equation of Chlorzoxazone was y = 8321.9.x + 1397.8 Pantoprazolee was y = 9806.1x + 6071.7 and of Diclofenac was y = 2575x + 4338.4. Retention times are decreased so the method developed was simple and economical that can be adopted in regular Quality control tests in Industries.

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All are given moral support to put my views in writing the paper. During my research work and the writing of the research paper, every author played key role and they are encouraged me in every aspect of this tenure.

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