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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF PANTOPRAZOLE, DICLOFENAC & CHLORZOXAZONE BY RP-HPLC METHOD

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ABSTRACT

A simple, accurate, precise method was developed for the simultaneous estimation of the Chlorzoxazone (CLZ), Pantoprazole (PTP) and Diclofenac (DCF) in solid dosage form. Chromatogram was run through Discovery C18 150x4.6mm, 5µ. Mobile phase containing Buffer and Acetonitrile in the ratio of 42:58 v/v was pumped through column at a flow rate of 0.75ml/min. Buffer used in this method was 0.1% Orthophosphoric acid (OPA). Temperature was maintained at 30°C. Optimized wavelength for Chlorzoxazone. Pantoprazole and Diclofenac was 229.0 nm. Retention time of Chlorzoxazone, Pantoprazole and Diclofenac were found to be 2.233min, 2.763min and 3.759min %RSD of system precision for Chlorzoxazone, Pantoprazole and Diclofenac. were and found to be 1.0, 0.9and 0.6 respectively. %RSD of method precision for Chlorzoxazone, Pantoprazole and Diclofenac.were and found to be 0.6, 0.2 and 0.3 respectively. % recovery was Obtained as 99.88%, 99.81% and 99.75% for Chlorzoxazone, Pantoprazole and Diclofenac. respectively. LOD, LOQ values are obtained from regression equations of Chlorzoxazone, Pantoprazole and Diclofenac. were 0.14ppm, 0.22ppm, 2.88ppm and 0.42pm, 0.67ppm ,8.72 ppm respectively. Regression equation of Chlorzoxazone.wasy= 6226.1x + 745.53, Pantoprazole was y = 17987x + 3754.7. and of Diclofenac was y = 10072x + 32901.

KEYWORDS: Chlorzoxazone, Pantoprazole, Diclofenac, RP-HPLC.

INTRODUCTION

Chemically Chlorzoxazone(CLZ) was an 5-chloro-2,3-dihydro-1,3-benzoxazol-2-one.Molecular weight and Molecular formula of CLZ were 169.57 g/ mol $C_7H_4ClNO_2$ respectively. Chlorzoxazone is a centrally acting central muscle relaxant with sedative properties. It is claimed to inhibit muscle spasm by exerting an effect primarily at the level of the spinal cord and subcortical areas of the brain.Structure of the CLZ was shown in figure 1 (A).[1]

Chemically Pantoprazole (PTP) was an 6-(difluoro-ethoxy)-2-[(3,4-dimethoxypyridin-2-yl) methane-sulfinyl]-1H-1,3-benzodiazole.Molecular weight and Molecular formula of CLZ were 383.37g/ mol $C_{16}H_{15}F_2N_3O_4S$ respectively. Pantoprazole is a proton pump inhibitor drug used for short-term treatment of erosion and ulceration of the esophagus caused by gastroesophageal reflux disease. Structure of the PTP was shown in figure 1 (B).[2]

Chemically Diclofenac (DCF) was 2-{2-[(2,6-dichlorophenyl) amino] phenyl} acetic acid. Molecular weight and Molecular formula of CLZ were 296.15g/ mol and C14H11Cl2NO2 respectively. A non-steroidal anti-inflammatory agent (NSAID) with antipyretic and analgesic actions. It is primarily available as the sodium salt. Structure of the DCF was shown in figure 1 (C).[3]

Literature survey reveals there are several methods to estimated thee drugs in single or in combination of two drugs.[5-9] but there is only very few HPLC methods are available for simultaneous estimation of CLZ, PTP and DCF, so the scope of developing and alidating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes.

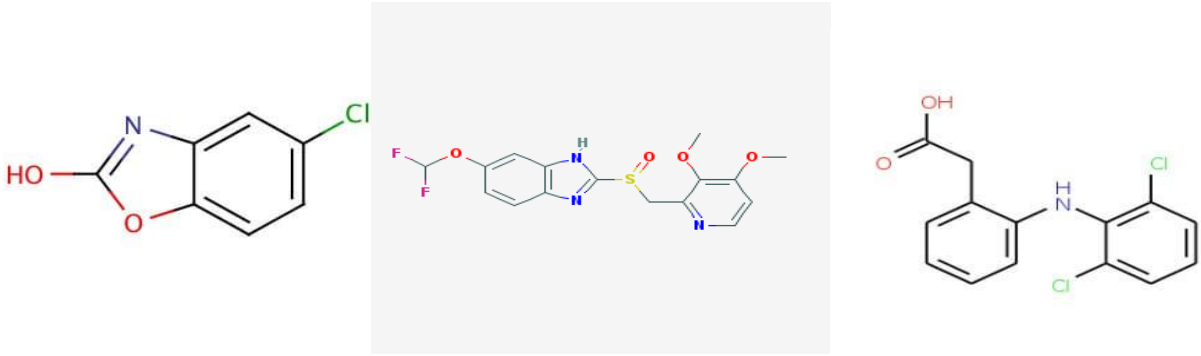

Figure 1: Structure of (A) Chlorzoxazone (B) Pantoprazole (C) Diclofenac.

MATERIALS AND METHODS

Reagents and Chemicals: The pharmaceutical drug samples of Chlorzoxazone. Pantoprazole and Diclofenac were obtained from Spectra Pharma Pvt. Ltd., Hyderabad. All the chemicals and solvents were used as HPLC grade. The pharmaceutical dosage form was purchased from local pharmacy.

Instrumentation: HPLC (waters 2695) system with Empower-2 software and 2996 module photo diode array detector equipped with a quaternary solvent delivery pump, automatic sampler unit, Discovery C18 150x4.6mm, 5m. As part of experimentation, additional equipment such as sonicator (ultrasonic cleaner power sonic 420), pH meter, vacuum oven (wadegati), water bath and other glassware were used for the present investigation.

Chromatographic conditions: The Discovery C18 150x4.6mm, 5m column was used for analytical separation. Potassium dihydrogen ortho phosphate and one drop of triethyl amine in every 100ml of buffer solution (pH3.0) and Acetonitrile was taken in the ratio of (58:42%v/v) mobile phase for the investigation with a flow rate of a 1.0ml/min. The temperature was maintained at 300C. The injection volume was 10µl and the UV detection was achieved at 229nm.

Preparation of potassium dihydrogen ortho phosphate buffer (pH:3.0): Accurately weighed 4.08 gms of potassium dihydrogen ortho phosphate in a 1000 ml of volumetric flask and add about 900 ml of milli-Q water and degas to sonicate and finally make up to the volume with water. Then added 1ml of triethyl amine and pH was adjusted to 3.5 with dilute orthophosphoric acid solution.

Preparation of mobile phase

Mixture of 600 ml of 0.01N KH2PO4 buffer (pH-3.5) and 400 ml of Acetonitrile in the ration of 58:42 v/v were mixed and degased in ultrasonic water bath for 15 minutes and filtered through 0.45 µ filter paper. Mobile phase was used as a diluent

***Preparation of mixture Standard stock solution*:** Weighed 5mg of Chlorzoxazone, 12.5 mg of Pantoprazole and 125mg of Diclofenac and transferred to three 25ml volumetric flasks separately. 10ml of Diluent was added to flasks and sonicated for 20mins. Flasks were made up with 0.03N KH2PO4: Acetonitrile (58:42 v/v) and labeled as Standard stock .Each flask was made up with diluent up to the mark. Pipette out 1ml from each stock solution taken into a 10ml volumetric flask and made up with diluent.

Preparation of Sample (Tablet) 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, 25ml of diluent added and sonicated for 50 min, further the volume made up with diluent and filtered.

Optimized chromatographic conditions

Column Used : Discovery C_{18} 150 x 4.6 mm, 5µ.
Mobile phase : buffer: Acetonitrile (58:42 v/v)
Flow rate : 0.75ml/min
Wavelength : 229.0 nm
Temperature : 30°C
Injection Volume : 10.0µl

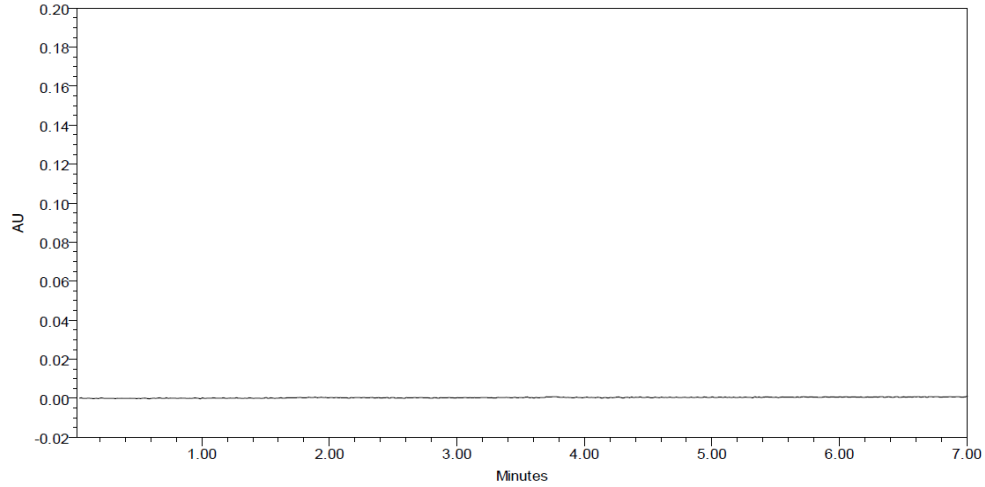

Figure 2: Blank chromatogram.

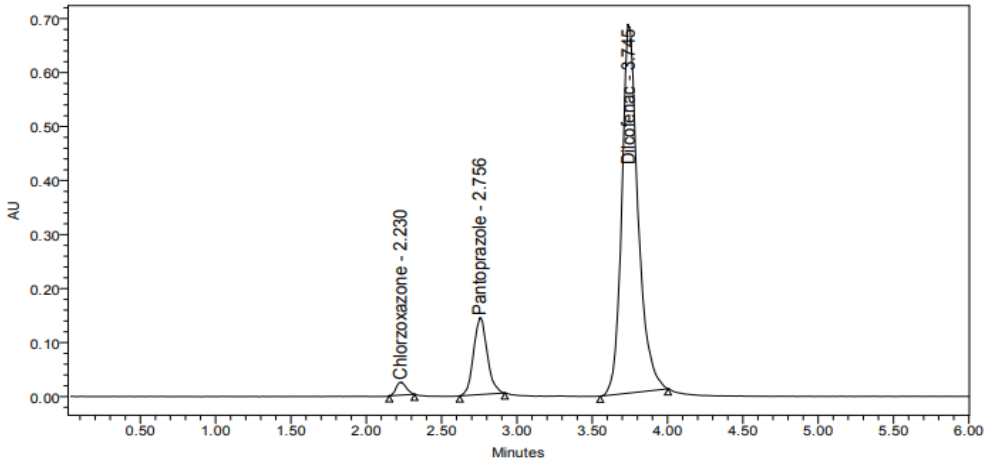

Figure 3: Chromatogram of standard mixture of CLZ, PTP & DCF.

	Peak Name	RT	Area	USP Tailing	USP Resolution	USP Plate Count
1	Chlorzoxazone	2.230	120465	1.1		5347.0
2	Pantoprazole	2.756	897292	1.1	3.6	4378.5
3	Dilcofenac	3.745	5131521	1.2	5.4	6203.7

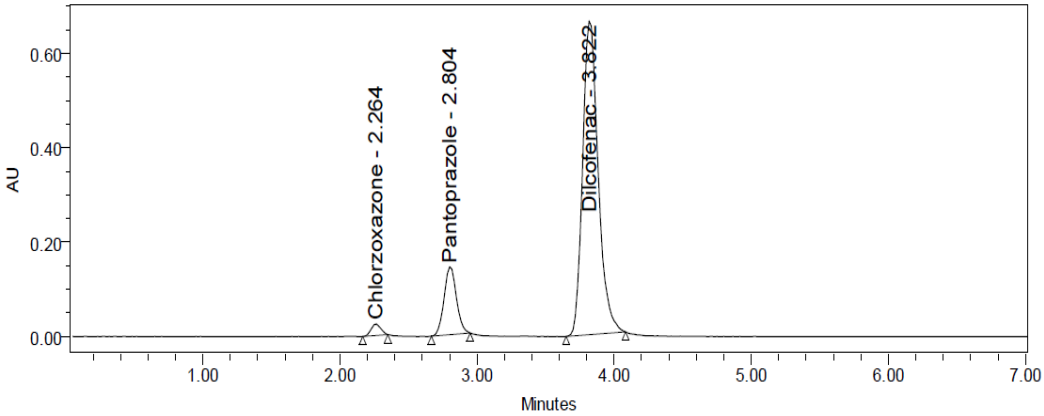

Figure 4: Chromatogram of sample mixture of CLZ, PTP & DCF.

VALIDATION

The above optimized chromatographic method has been validated for the assay of CLZ, PTP & DCF using the following parameters [International Conference on Harmonization (ICH) 1995]. Linearity was studied to find out the relationship of concentration with Peak area. Six different concentrations of Chlorzoxazone, Pantoprazole and Diclofenac (CLZ, PTP & DCF)drug mixtures respectively. Each concentration of solution was injected into the HPLC and chromatogram was recorded. The calibration graph was constructed by plotting the peak versus the final concentration of the each drug (µg/ml) and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of mixtures of Chlorzoxazone(5mg) +Pantoprazole(60mh) +Dilcofenac (5mg) respectively. The precision of each method was ascertained separately from the peak area by actual determination of five replicates of a fixed amount of drug (Chlorzoxazone(5mg) +Pantoprazole(60mh) +Dilcofenac (5mg) respectively). The %RSD (percentage relative standard deviation) was calculated for precision and ruggedness. The accuracy of the method was shown by analyzing the model mixtures containing 80,100 and 120% of Chlorzoxazone, Pantoprazole and Diclofenac. After the measurement, the Amount found and individual recoveries were calculated. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the linearity data using the formulae LOD = 3.3×standard deviation /slope; LOQ = 10×standard deviation /slope. Robustness was performed by following the same method with different flow rate.

RESULTS AND DISCUSSION

The regression equation for CLZ was found to be y = 6242x + 399.4 (slope, intercept and correlation coefficient were found to be 6226, 745.5 and 0.999 respectively) and linear over beer's range of 5-30 µg/ml. The regression equation for PTP was found to be y = 18019x + 2011 (slope, intercept and correlation coefficient were found to be 17986, 3754.7 and 0.999 respectively) and linear over beer's range of 12.5-75 µg/ml. The regression equation for DCF was found to be y = 10100x + 17625 (slope, intercept and correlation coefficient were found to be 10072, 32900 and 0.999 respectively) and linear over beer's range of 125-750 µg/ml. Linearity graph of CLZ,PTP & DCF were shown in Figure 5, 6 & 7 respectively. Linearity data was shown in table 1. The precision and ruggedness were determined using the % RSD of the peak area for six replicate preparations of the drug. %RSD of system precision for Chlorzoxazone, Pantoprazole and Diclofenac were and found to be 1.0, 0.9 and 0.6 respectively. %RSD of method precision for Chlorzoxazone, Pantoprazole and Diclofenac were and found to be 0.9, 0.7 and 0.9 respectively. % recovery was obtained as 99.88%, 99.81% and 99.75% for Chlorzoxazone, Pantoprazole and Diclofenac respectively. The calculated RSD values were less than 2. Precision and ruggedness data are presented in Table 2. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 50%, 100% and 150% of standard solution of drug CLZ, PTP & DCF and along with 5 µg/mL of placebo solution within the linearity ranges. The mean percentage recoveries were found to be 99.88%, 99.81% and 99.75%w/w for 50%, 100% and 150% respectively. The mean percentage recoveries were found to be 100.05±0.32, 99.20±0.17 and 99.82±1.02%w/w for 50%, 100% and 150% respectively for MET. The mean percentage recoveries were found to be 99.82±0.51, 100.33±0.76%w/w and 99.89±0.63 for 50%, 100% and 150% respectively for DIA. The results of accuracy were shown that the developed method have a good percentage recovery at different concentrations of drugs. LOD for GLU, MET and DIA was found to be 2.23 µg, 0.51µg and 0.04 µg respectively. LOQ for GLU, MET and DIA was found to be 6.77 µg, 1.54 µg and 0.13 µg respectively. Summary of all the validation parameter shown in table 4.

Degradation

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Table 1: Linearity table for CLZ, PTP and DCF.

Chlorzoxazone		Pantoprazole		Diclofenac	
Conc. (µg/mL)	**Mean Peak area**	**Conc. (µg/mL)**	**Mean Peak area**	**Conc. (µg/mL)**	**Mean Peak area**
5	32211	12.5	228334	125	1280316
10	62590	25	455696	250	2529876
15	93471	37.5	673596	375	3788627
20	126300	50	908071	500	5201587
25	156328	62.5	1124158	625	6268434
30	187317	75	1354206	750	7567601
Slope	6226.131		17986.79		10072.01
Intercept	745.5333		3754.733		32900.93
Correlation	0.999		0.999		0.999

Table 2: System precision table of CLZ, PTP & DCF.

S. No	Peak Area of Chlorzoxazone.	Peak Area of Pantoprazole	Peak Area of Diclofenac
1.	124507	901653	5159724
2.	125548	917895	5244013
3.	122826	913053	5197235
4.	125659	904277	5204493
5.	126635	901515	5236821
6.	124864	895692	5183841
Mean	125106	905681	5204355
S.D	1295.8	8232.4	31906.9
%RSD	1.0	0.9	0.6

Table 3: Degradation data of CLZ, PTP & DCF.

S.NO	Degradation Condition	Chlorzoxazone.			Pantoprazole			Diclofenac		
		% Drug Degraded	Purity Angle	Purity Threshold	% Drug Degraded	Purity Angle	Purity Threshold	% Drug Degraded	Purity Angle	Purity Threshold
1	**Acid**	10.00	0.301	0.522	6.26	0.547	0.730	4.64	0.109	0.288
2	**Alkali**	5.16	2.128	2.628	5.07	0.478	0.537	3.01	0.086	0.292
3	**Oxidation**	3.54	0.113	0.605	4.81	2.128	2.628	6.23	0.124	0.289
4	**Thermal**	2.28	2.030	2.402	3.78	0.332	0.538	2.50	0.086	0.287
5	**UV**	1.25	1.074	1.101	1.88	0.464	0.588	1.08	0.350	0.599
6	**Water**	0.33	1.840	2.370	0.94	0.385	0.522	0.87	0.088	0.288

Table 4: Summary of validation data of CLZ, PTP & DCF.

Validation	Parameters	Chlorzoxazone	Pantoprazole	Diclofenac
	Range (µg/ml)	5-30µg/ml	12.5-75µg/ml	125-750µg/ml
	Regression coefficient	0.999	0.999	0.999
Linearity	**Slope(m)**	6226	17986	10072
	Intercept(c)	745.5333	3754.733	32900.93
Assay	**Mean % content**	99.23%	99.24%	100.12%
Specificity		Specific	Specific	Specific
System precision	**%RSD**	1.0	0.9	0.6
Method precision	**%RSD**	0.6	0.2	0.3
Accuracy % recovery	**% recovery**	99.88%	99.81%	99.75%
LOD		0.14µg/ml	0.22µg/ml	2.88µg/ml
LOQ		0.42µg/ml	0.67µg/ml	8.72µg/ml

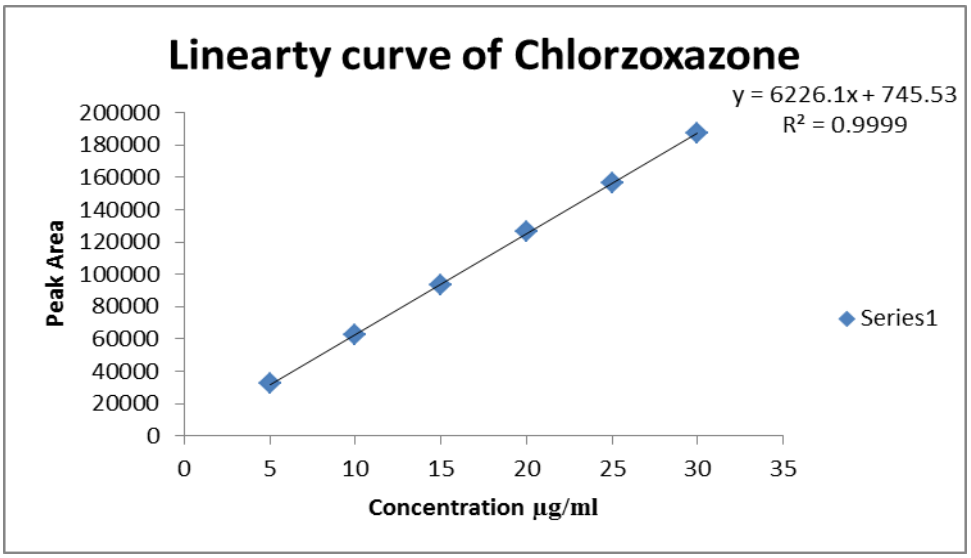

Figure 7: Linearity curve of chlorzoxazone.

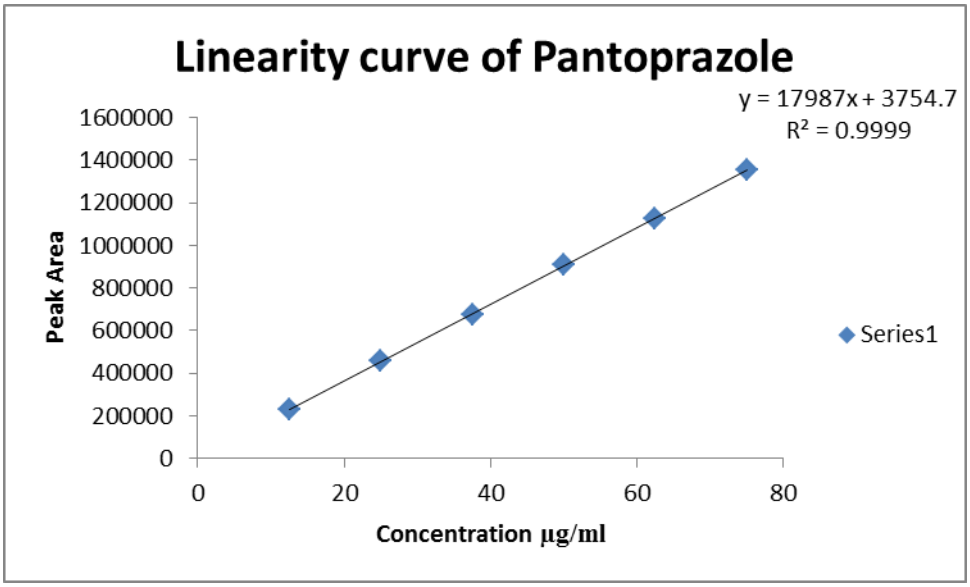

Figure 5: Linearity curve of Pantoprazole.

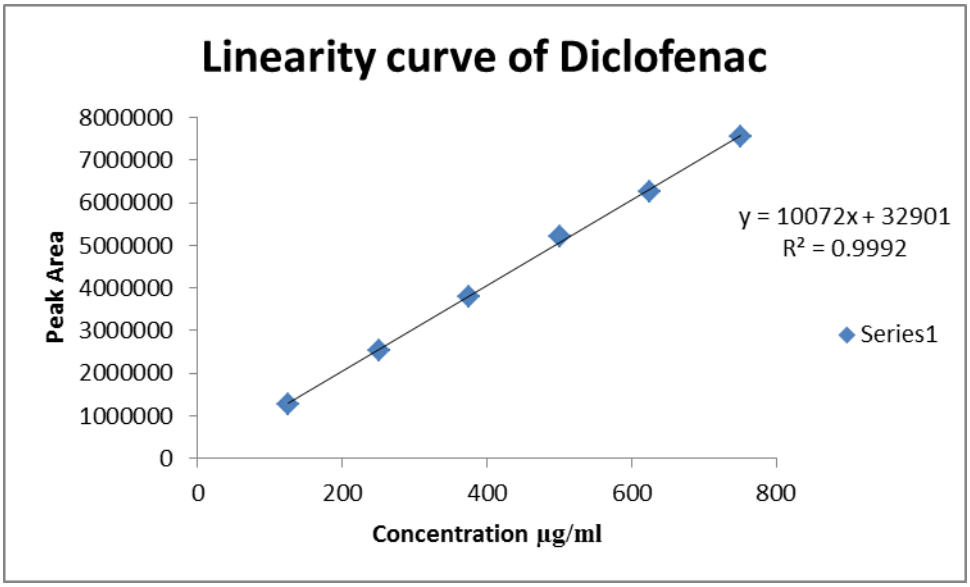

Figure 6: Linearity curve of Diclofenac.

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the Chlorzoxazone, Pantoprazole and Diclofenac in Tablet dosage form was developed and the proposed method as suitable for routine analysis of CLZ, PTP & DCF.

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