EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES

http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 02
540-553
Year: 2018

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ACECLOFENAC, PARACETAMOL AND CHLORZOXAZONE IN TABLET DOSAGE FORM

Tejal R. Patel*, Dr. Anuradha G. Patel, Dr. Shailesh V. Luhar and Dr. Sachin B. Narkhede

QA Department Smt. B.N.B Swaminarayan Pharmacy College, Salvav, Vapi, Gujarat, 396191.

***Corresponding Author: Tejal R. Patel**

QA Department Smt. B.N.B Swaminarayan Pharmacy College, Salvav, Vapi, Gujarat, 396191.

Article Received on 05/12/2017 **Article Revised on 26/12/2017** **Article Accepted on 16/01/2018**

ABSTRACT

To develop simple, accurate, precise, rapid and economical Stability Indicating RP-HPLC method for the Aceclofenac, Paracetamol and Chlorzoxazone in dosage form method included Shimadzu LC-2010, using Hypersil BDS- C_{18} (250* 4.6mm, 5µm) column and with mobile phase composition of Phosphate Buffer: Acetonitrile (80:20 % v/v)pH 5.5, at a flow rate of 1ml/min was used. Detection was carried out at 285 nm. Retention time of Aceclofenac, Paracetamol and Chlorzoxazone was found to be 10.093 min, 5.520 min and 7.023 min. For Stability Study Drug Were subjected to acid hydrolysis, alkaline hydrolysis, oxidative degradation and thermal degradation. The linear of the proposed method was investigated in the range of 5-15 µg/ml, 16.25-48.75 µg/ml and 12.5-37.5 µg/ml for Aceclofenac, Paracetamol and Chlorzoxazone. The limits of detection were 0.5473 µg/ml and 2.8318µg/ml and 1.8414µg/ml of Aceclofenac, Paracetamol and Chlorzoxazone and the limit of quantification were 1.6587µg/ml, 8.5813µg/ml and 5.5801 µg/ml of Aceclofenac, Paracetamol and Chlorzoxazone.

KEYWORDS: Aceclofenac, Paracetamol, Chlorzoxazone RP-HPLC method, forced degradation, validation.

MATERIALS AND METHODS

Aceclofenac 2-[(2',6- dichlorophenyl) amino] phenylacetoxyacectic Acid (ACE) inhibits synthesis of the inflammatory cytokines interleukins and tumor necrosis factor and inhibits prostaglandin E_2 production. It is official in British pharmacopeia 2009 and Indian pharmacopeia 2010. It is freely soluble in Methanol and water. Molecular weight of Aceclofenac 354.2 is gm/mol and formula is $C_{16}H_{13}Cl_2NO_4$.

Paracetamol N-(4-Hydroxyphenyl) acetamide it act as Analgesic, and anti-pyretic it is official in British pharmacopeia 2009, Indian pharmacopeia 2010 and United States Pharmacopoeia 29NF30. It is freely soluble in Methanol. Molecular weight of Paracetamol 151.163 is gm/mol and formula is $C_8H_9NO_2$.

Chlorzoxazone 5-chloro-2, 3-dihydro-1, 3 -benzoxazol-2-one it act as muscle relaxants, Benzoxazoles and Neuromuscular agent it is official in United States Pharmacopoeia 29NF30. It is freely soluble in Methanol. Molecular weight of Chlorzoxazone 169.565 is gm/mol and formula is $C_7H_4CINO_2$.

Aceclofenac is obtained from Amoli Organics Pvt. Ltd., Vapi, Gujarat, India.

Paracetamol is obtained from Granules India Limited, Telangana, India.

Chlorzoxazone is obtained from Vapi Care Pharma Pvt. Ltd., Vapi, Gujarat, India.

Instrumentation and Chromatographic method

The analysis of drug was carried on RP- HPLC, by using Shimadzu LC-2010, using Hypersil BDS- C_{18} (250 * 4.6 mm, 5µm) column and with mobile phase composition of Phosphate Buffer: Acetonitrile (80:20 % v/v) pH 5.5, at a flow rate of 1ml/min was used. Detection was carried out at 285 nm. Retention time of Aceclofenac, Paracetamol and Chlorzoxazone was found to be 10.093 min, 5.520 min and 7.023 min.

Determination of maximum absorbance

The standard solution of Aceclofenac, Paracetamol and Chlorzoxazone acid were scanned in range 200-400nm against mobile phase as blank Isobestic point of Aceclofenac, Paracetamol and Chlorzoxazone 285nm Thus the wavelength selected for the determination of Aceclofenac, Paracetamol and Chlorzoxazone 285nm.

Preparation of stock and standard solutions

Accurately weighed 10mg of Aceclofenac, 32.5mg of Paracetamol and 25mg of Chlorzoxazone were dissolved

in 100 ml volumetric flask containing 100 ml of Methanol which is considered as stock solution. Working standard solution of Aceclofenac, Paracetamol and Chlorzoxazone were prepared by making various dilutions of the drug solution from the stock solution. Five sets of the drug solution were prepared in the mobile phase containing 5-15 µg/ml of Aceclofenac, 16.25-48.75 µg/ml of Paracetamol and 12.5-37.5 µg/ml of Chlorzoxazone. Each of this drug solution was injected into the column and the peak area and retention time was recorded.

Assay of Marketed formulation (Branded tablet was taking)

Twenty tablets were weighed and average weight of a single tablet was calculated. Tablets were crushed and mixed using a mortar and pestle. Then drug sample equivalent to 10 mg of Aceclofenac, 32.5mg of Paracetamol and 25mg of Chlorzoxazone were accurately weighed and transferred into a 100ml volumetric flask and mixed with known amount of methanol and the active pharmaceutical ingredients were extracted into the methanol followed by ultra- sonication and then filtered through a whatman filter paper whatman filter paper no. 42. The drug sample was diluted by adding methanol to obtain a stock solution of 100µg/ml of Aceclofenac, 325 µg/ml of Paracetamol and 250 µg/ml of Chlorzoxazone.

Method validation

The Proposed method was validated according to ICH guidelines. The parameters assessed were linearity, precision, accuracy, LOD and LOQ.

System Suitability

System suitability tests are an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. System Suitability was performed on standard solution and system suitability parameters were calculated at the start of study for each parameter.

Linearity and Range

The linearity was determined at three levels over the range of 5-15 µg/ml of Aceclofenac, 16.25-48.75 µg/ml of Paracetamol and 12.5-37.5 µg/ml of Chlorzoxazone. Peak area of above linearity solution preparations were taken at each concentration three times.

Accuracy

Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and Results are calculated.

Precision Repeatability Study

Standard solutions of 5, 10, 15 µg/ml Aceclofenac and 16.25, 32.5, 48.75µg/ml Paracetamol and 12.5, 25, 37.5 µg/ml Chlorzoxazone were prepared and chromatograms were recorded. Area was measured of the same concentration solution three times and %RSD was calculated.

Intra-day precision

Mixed solutions containing 5, 10, 15 µg/ml Aceclofenac and 16.25, 32.5, 48.75 µg/ml Paracetamol and 12.5, 25, 37.5 µg/ml Chlorzoxazone acid were analysed three times on the same day % R.S.D was calculated.

Inter-day precision

Mixed solutions containing 5, 10, 15 µg/ml Aceclofenac and 16.25, 32.5, 48.75 µg/ml Paracetamol and 12.5, 25, 37.5 µg/ml Chlorzoxazone were analysed on three different days and % R.S.D was calculated.

Limit of Detection and Limits of Quantitation Limit of Detection (LOD)

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. The limit of detection (LOD) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline.

LOD = 3.3× Intercept / Slope

Limit of Quantitation (LOQ)

The limit of quantitation (LOQ) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline.

LOQ = 10 × Intercept / Slope

Robustness

The robustness of the method was established by making deliberate minor variations in the following method parameters.

a) pH of mobile phase: ± 0.2
b) Flow rate: ± 0.2 ml/min.
c) Change in the ratio of component in the mobile phase: ± 2%.

Stability studies

Stability Studies was carried out on the drug in order to check the stability of the drug by providing various stress conditions like acid, base, oxidation and thermal degradation compared with normal conditions. The purpose of force degradation method is to provide evidence that the analytical method is efficient in determination of drug substances in commercial drug product in the presence of its degradation products.

Acid hydrolysis: Take 1 ml solution of Aceclofenac 100 µg/ml and Paracetamol 325 µg/ml and Chlorzoxazone 250 µg/ml, 2 ml of 0.1N HCl was added. The solution was reflux for 6 hr at 60°C and transferred to a 10ml volumetric flask, cooled, neutralized by 0.1N NaOH and

diluted up to mark with methanol to get final concentration 10µg/ml of Aceclofenac, 32.5 µg/ml of Paracetamol and 25 µg/ml of Chlorzoxazone.

Alkaline hydrolysis:- Take 1 ml solution of Aceclofenac 100 µg/ml and Paracetamol 325 µg/ml and Chlorzoxazone 250 µg/ml, 2 ml of 0.1N NaOH was added. The solution was reflux for 6 hr at 60°C and transferred to a 10ml volumetric flask, cooled, neutralized by 0.1N HCl and diluted up to mark with methanol to get final concentration 10µg/ml of Aceclofenac, 32.5 µg/ml of Paracetamol and 25 µg/ml of Chlorzoxazone.

Oxidative degradation:- Take 1 ml solution of Aceclofenac 100 µg/ml and Paracetamol 325 µg/ml and Chlorzoxazone 250 µg/ml, 2 ml 10% H_2O_2 was added at room temperature for 6 hours and transferred to a 10ml volumetric flask, cooled diluted up to mark with methanol to get final concentration 10 µg/ml of Aceclofenac, 32.5 µg/ml of Paracetamol and 25 µg/ml of Chlorzoxazone.

Thermal degradation:- Take 1 ml solution of Aceclofenac 100 µg/ml and Paracetamol 325 µg/ml and Chlorzoxazone 250 µg/ml, heat the solution for 6 hr at 80^0C and transferred to a 10ml volumetric flask, cooled diluted up to mark with methanol to get final concentration 10 µg/ml of Aceclofenac, 32.5 µg/ml of Paracetamol and 25 µg/ml of Chlorzoxazone.

RESULT AND DISCUSSION

Method development

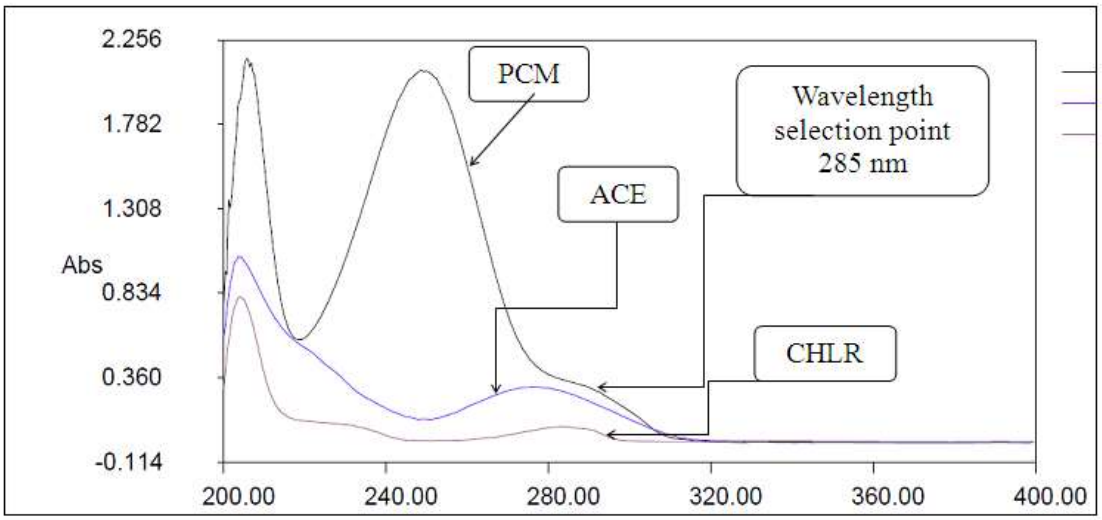

Figure no:-1 Determination of detection wavelength.

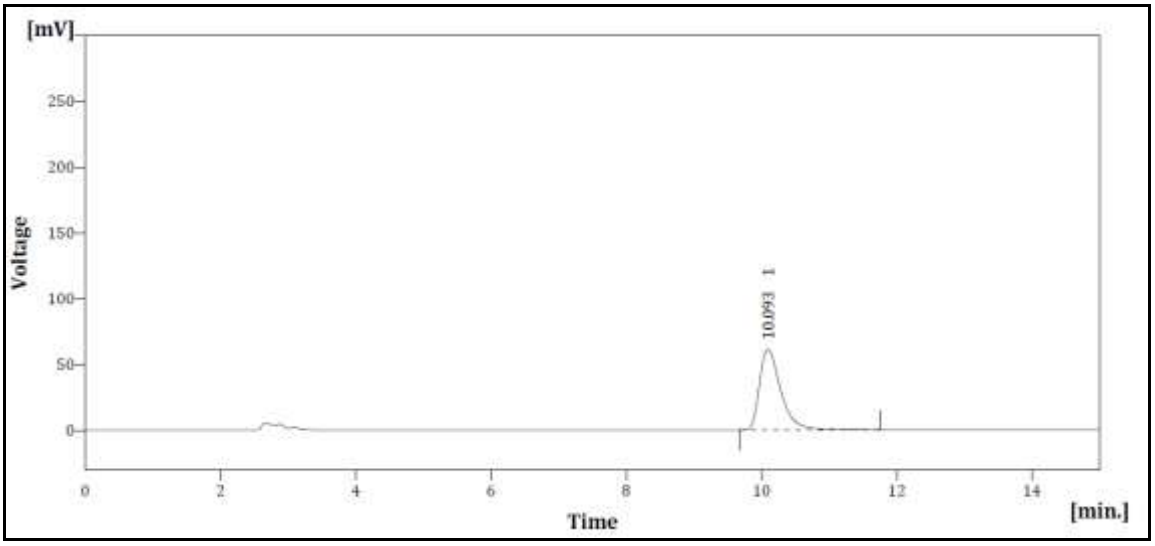

Figure no:-2 Chromatogram of Aceclofenac.

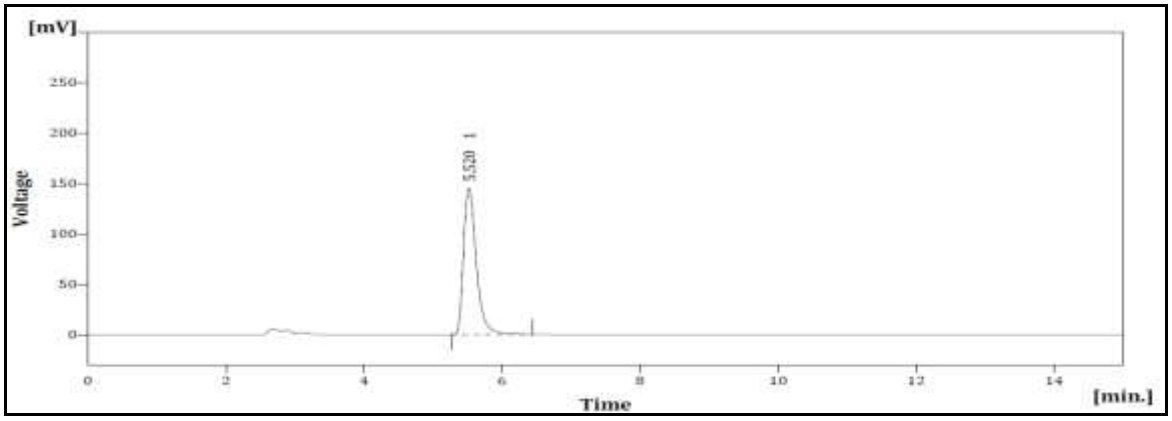

Figure no:-3 Chromatogram of Paracetamol.

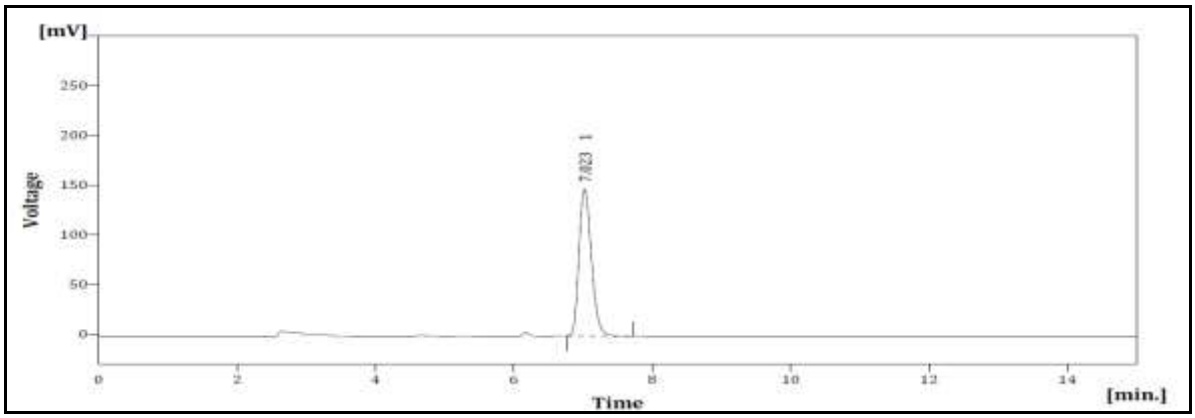

Figure no:-4 Chromatogram of Chlorzoxazone.

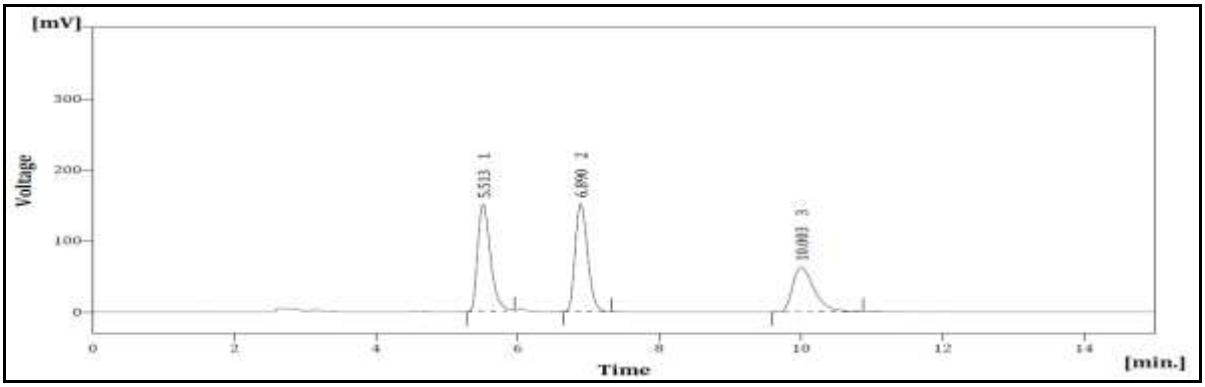

Figure no:-5 Chromatogram of formulation.

Linearity

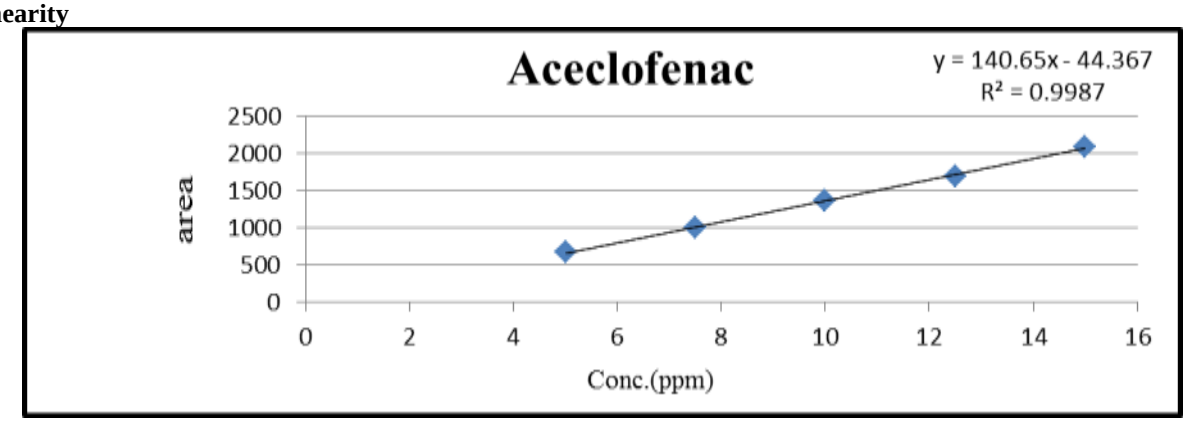

Figure no:-6 Calibration curve of Aceclofenac.

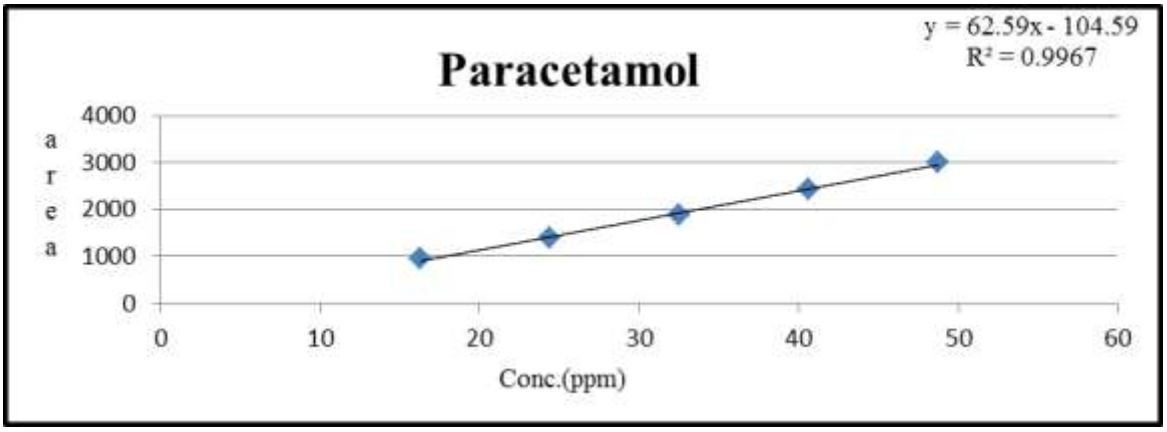

Figure no:-7 Calibration curve of Paracetamol.

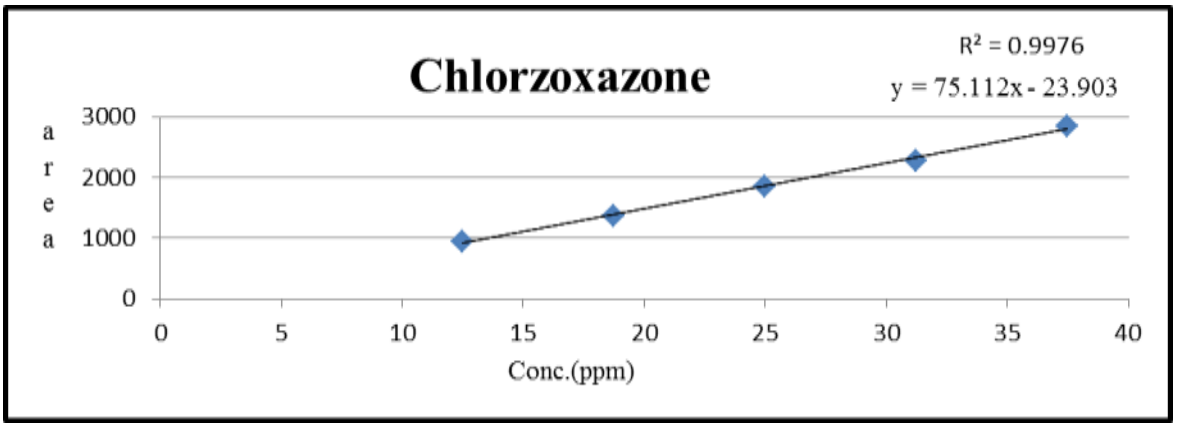

Figure no:-8 Calibration curve of Chlorzoxazone.

Table 1: System suitability parameters.

Parameters	MEAN ± S.D. (n=6) Paracetamol	MEAN ± S.D. (n=6) Chlorzoxazone	MEAN ± S.D. (n=6) Aceclofenac	Standard value as per IP
Retention time	5.56±0.020	6.96±0.017	10.09± 0.030	_
Tailing factor	1.64±0.020	1.43±0.011	1.86±0.016	Not greater than 2.0
Theoretical plates	4433±5.019	6952±3.741	5289±5.329	Not less than 2000
Resolution	_	4.19	7.03	More than 1.5

Accuracy

Table 2: Recovery study of Aceclofenac.

Level	Actual Conc. (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Peak Area of Sample	% Recovery	Mean % Recovery ± S.D (n=3)	%RSD
80%	5	4	9	1134.04	101.71	100.54 ±1.26	1.254
	5	4	9	1128.57	100.70		
	5	4	9	1120.49	99.21		
100%	5	5	10	1267.63	101.15	100.63 ±0.057	0.575
	5	5	10	1259.92	100.00		
	5	5	10	1264.90	100.74		
120%	5	6	11	1407.42	101.53	100.94 ±0.055	0.548
	5	6	11	1401.96	100.86		
	5	6	11	1398.51	100.43		

Table 3: Recovery study of Paracetamol.

Level	Actual Conc. (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Peak Area of Sample	% Recovery	Mean % Recovery ± S.D (n=3)	%RSD
80%	16.25	13	29.2	1690.34	99.21	99.16±0.58	0.59
	16.25	13	29.25	1694.29	99.72		
	16.25	13	29.25	1685.22	98.55		
100%	16.25	16.25	29.25	1873.63	98.34	99.12±0.88	0.89
	16.25	16.25	32.5	1879.48	98.94		
	16.25	16.25	32.5	1890.44	100.08		
120%	16.25	19.5	35.75	2067.42	98.66	99.80±0.84	0.85
	16.25	19.5	35.75	2060.07	98.03		
	16.25	19.5	35.75	2079.49	99.70		

Table 4: Recovery study of Chlorzoxazone.

Level	Actual Conc. (µg/ml)	Amount of standard spiked (µg/ml)	Total amount (µg/ml)	Peak Area of Sample	% Recovery	Mean % Recovery ± S.D (n=3)	%RSD
80%	12.5	10	22.5	1660.91	100.54	99.40±1.22	1.232
	12.5	10	22.5	1653.56	99.57		
	12.5	10	22.5	1642.49	98.10		
100%	12.5	12.5	25	1834.22	98.76	98.54±0.20	0.204
	12.5	12.5	25	1831.95	98.52		
	12.5	12.5	25	1830.44	98.36		
120%	12.5	15	27.5	2023.68	98.99	98.73±0.32	0.333
	12.5	15	27.5	2016.51	98.36		
	12.5	15	27.5	2021.89	98.83		

Precision

Table 5: Repeatability study of the drugs.

Drug	Conc(µg/ml)	Intraday precision		Intraday precision	
		Mean ± S.D (n=3)	%R.S.D	Mean± S.D (n=3)	%R.S.D
ACE	5	686.509 ±12.13	1.76	703.24 ± 11.48	1.63
	10	1388.42 ± 19.05	1.37	1388.88 ± 18.24	1.35
	15	2094.35 ± 12.63	0.60	2105.90 ± 9.61	0.45
PCM	16.25	969.97 ± 16.74	1.66	1001.99 ± 8.14	0.81
	32.5	1956.78 ± 34.92	1.78	1936.32 ± 10.66	0.05
	48.75	2925.94 ± 40.81	1.39	2926.61 ± 37.04	1.26
CHLR	12.5	945.64 ± 9.93	1.05	984.59 ± 17.66	1.79
	25	1901.47 ± 3.94	0.20	1898.10 ± 16.15	0.85
	37.5	2860.59 ± 11.59	0.40	2865.90 ± 21.68	0.75

Limit of Detection and Limits of Quantitation Limit of Detection (LOD)

Table 6: LOD and LOQ of the drugs.

Drugs	LOD (µg/ml)	LOQ (µg/ml)
ACE	0.5473	1.6587
PCM	2.8318	8.5813
CHLR	1.8414	5.5801

Force Degradation studies

Table no 7: Force Degradation studies of drugs.

Stress Condition	% Degradation of API			% Degradation of pharmaceutical dosage form (Tablet)		
	ACE	PCM	CHLR	ACE	PCM	CHLR
Acid Hydrolysis	8.12	5.52	6.06	8.58	5.15	5.82
Alkaline Hydrolysis	4.16	8.39	4.06	4.99	9.47	4.43
Oxidative	6.65	6.60	8.30	8.38	6.79	7.25
Thermal	5.23	3.60	5.06	5.29	4.29	6.72

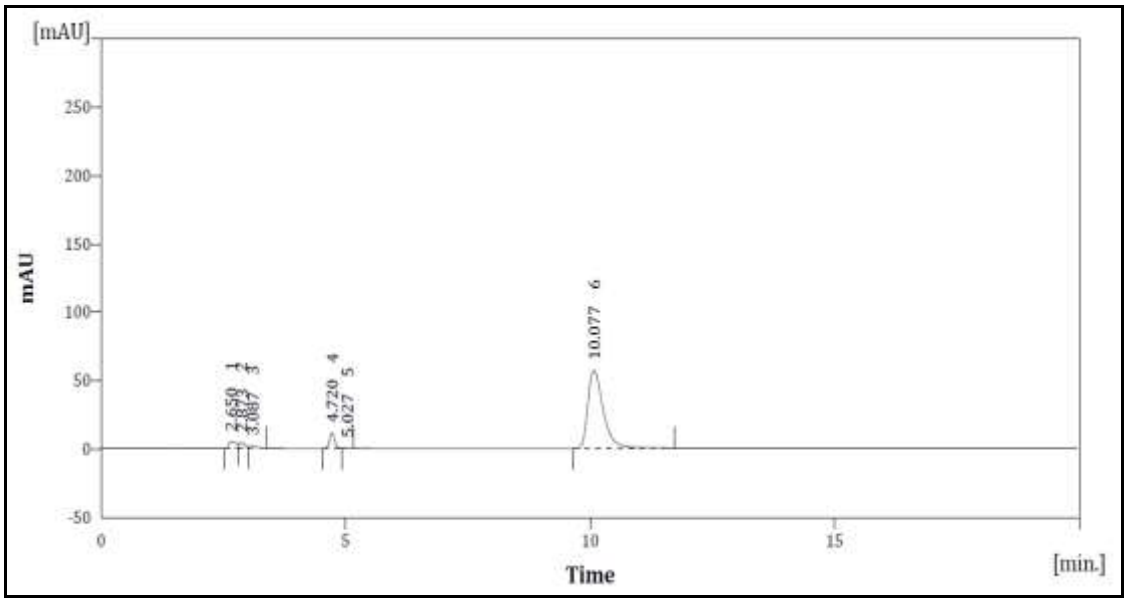

Figure no 9: Acid Hydrolysis of Aceclofenac.

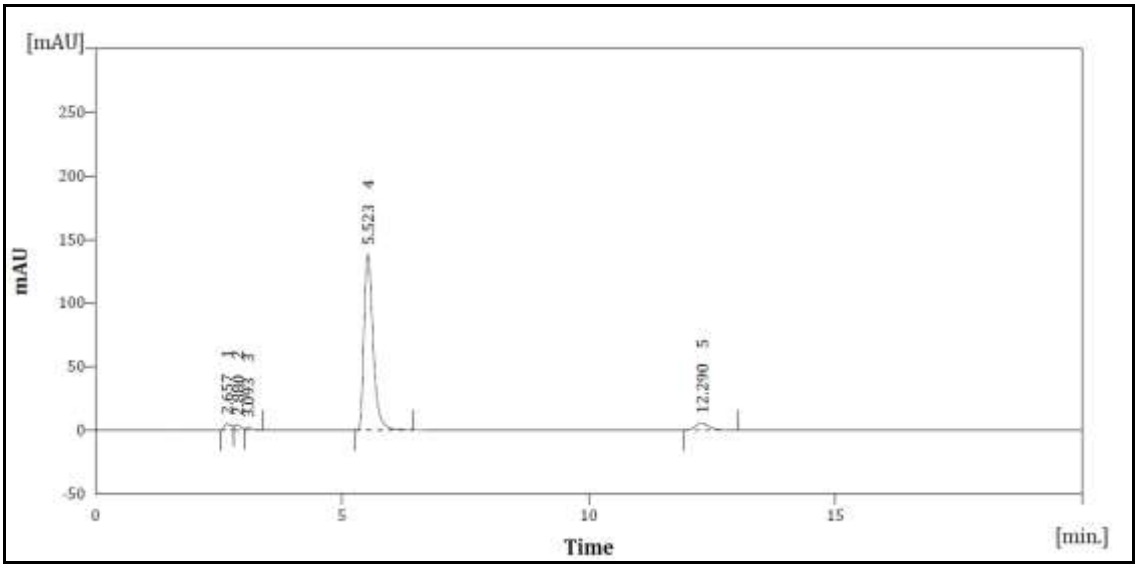

Figure no:-10 Acid Hydrolysis of Paracetamol.

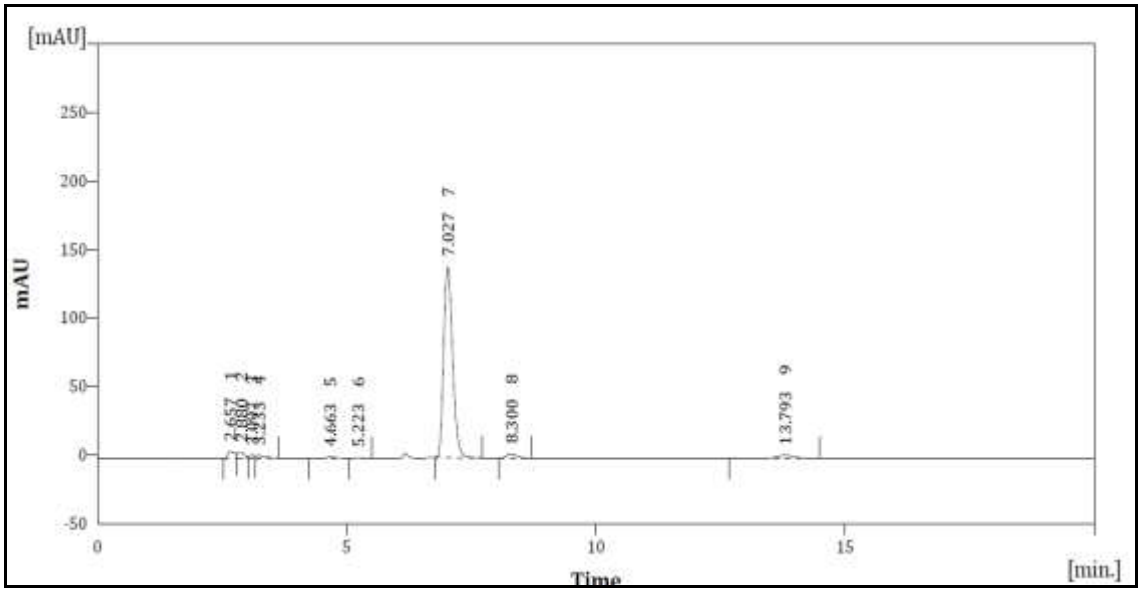

Figure no:-11 Acid Hydrolysis of Chlorzoxazone.

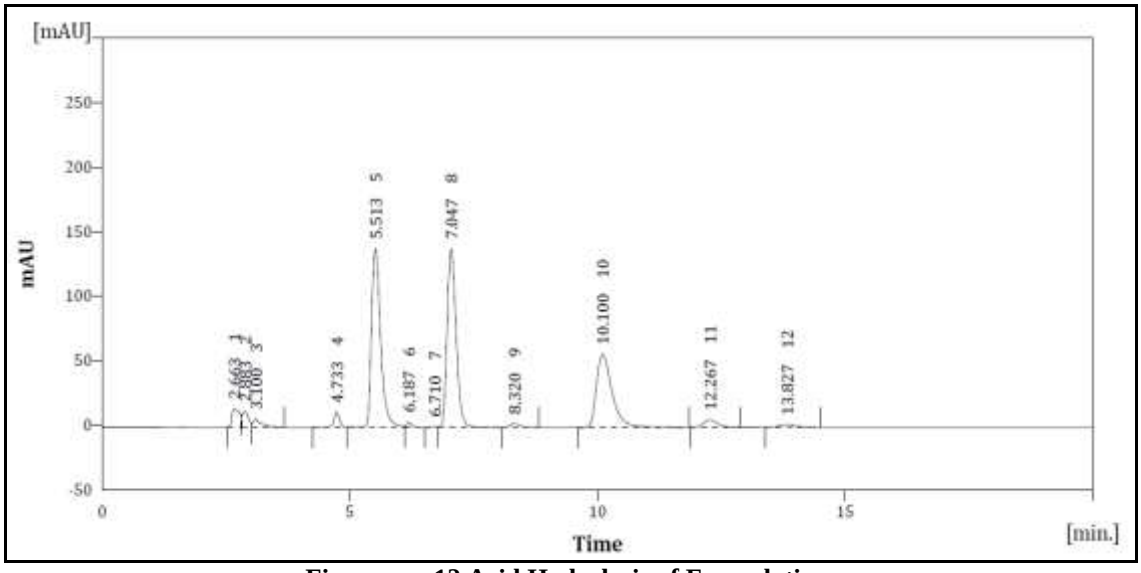

Figure no:-12 Acid Hydrolysis of Formulation.

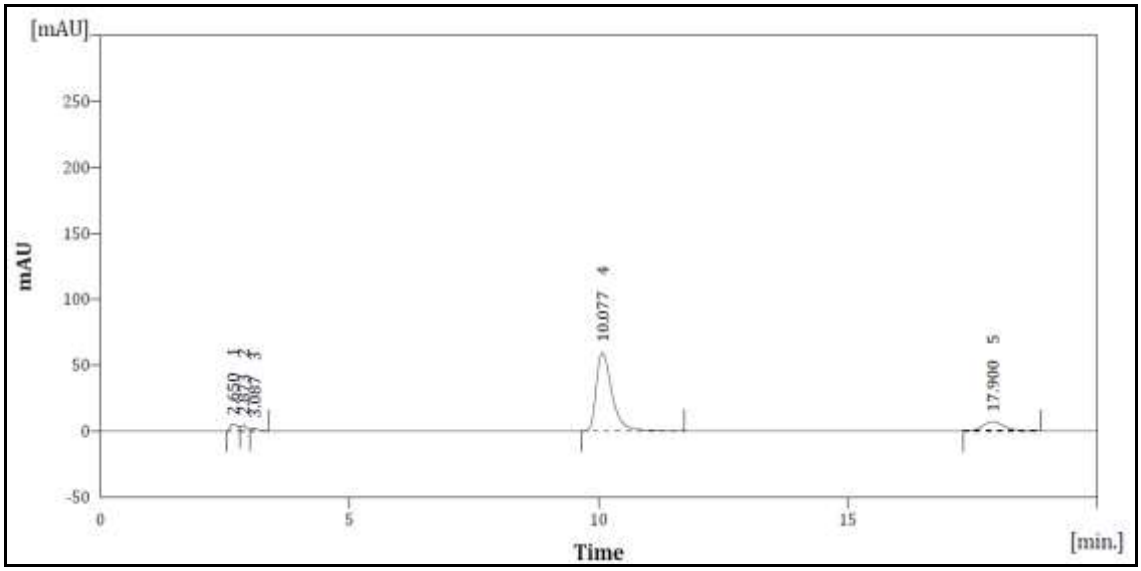

Figure no:-13 Alkali Hydrolysis of Aceclofenac.

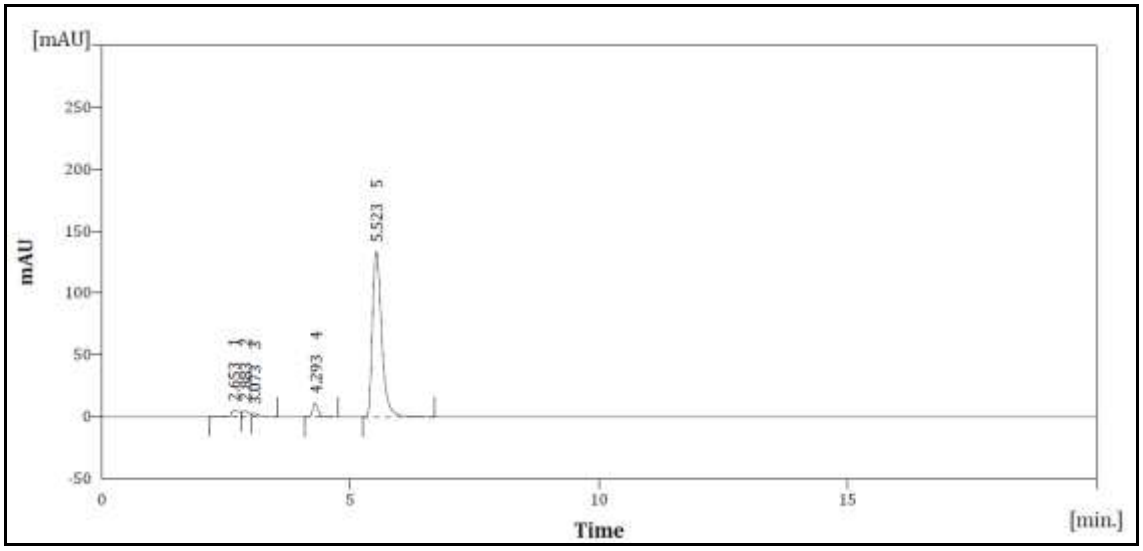

Figure no:-14 Alkali Hydrolysis of Paracetamol.

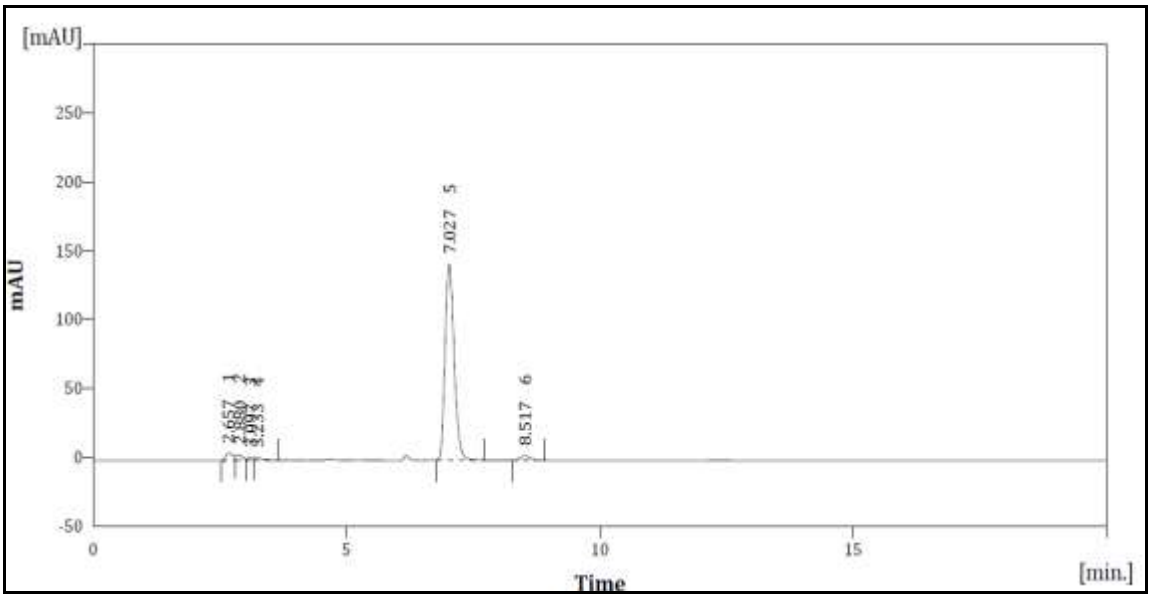

Figure no:-15 Alkali Hydrolysis of Chlorzoxazone.

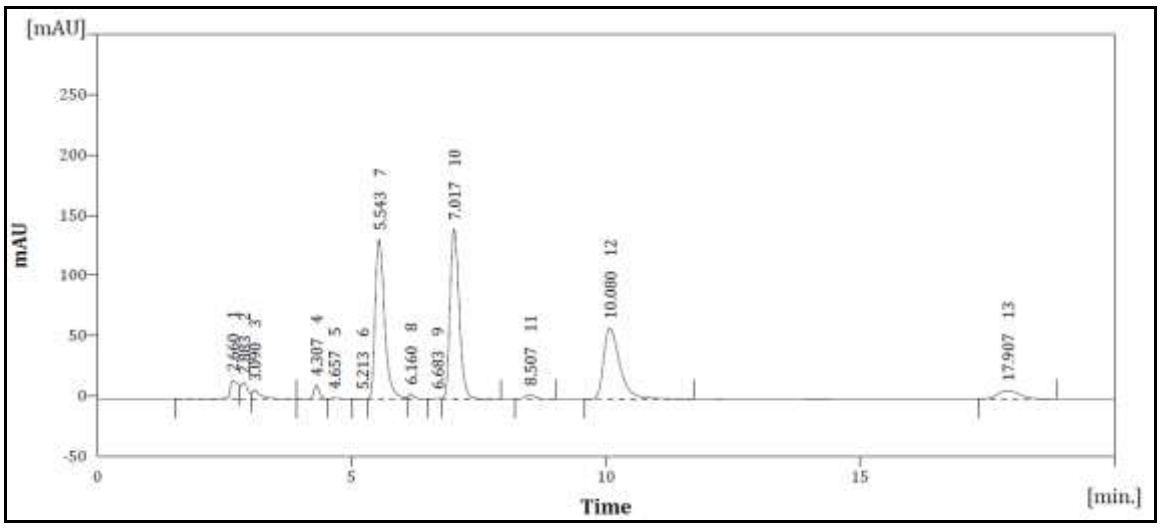

Figure no:-16 Alkali Hydrolysis of Formulation.

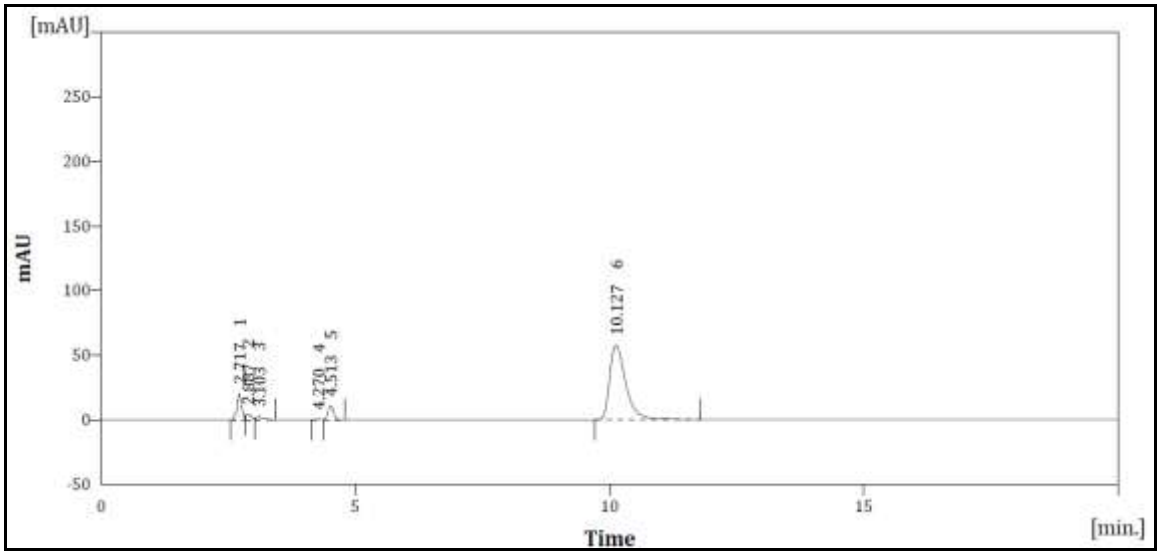

Figure no:-17 Oxidative Hydrolysis of Aceclofenac.

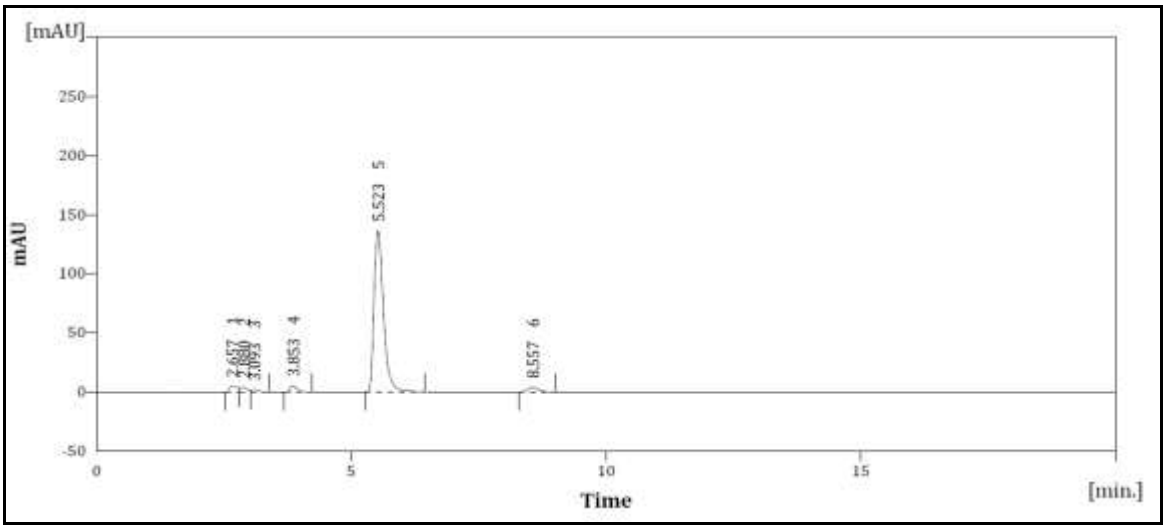

Figure no:-18 Oxidative Hydrolysis of Paracetamol.

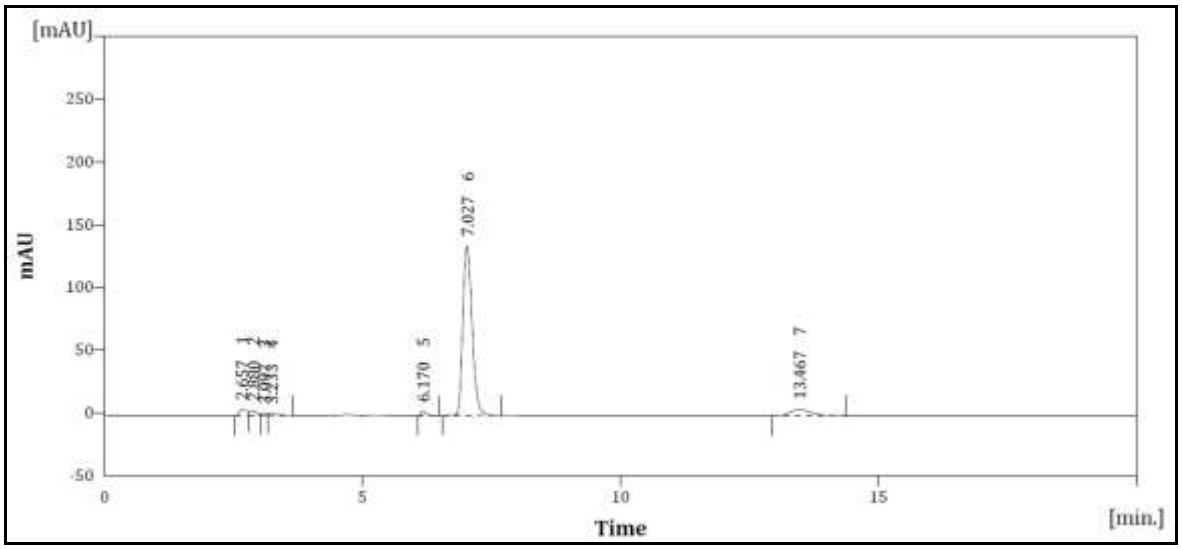

Figure no:-19 Oxidative Hydrolysis of Chlorzoxazone.

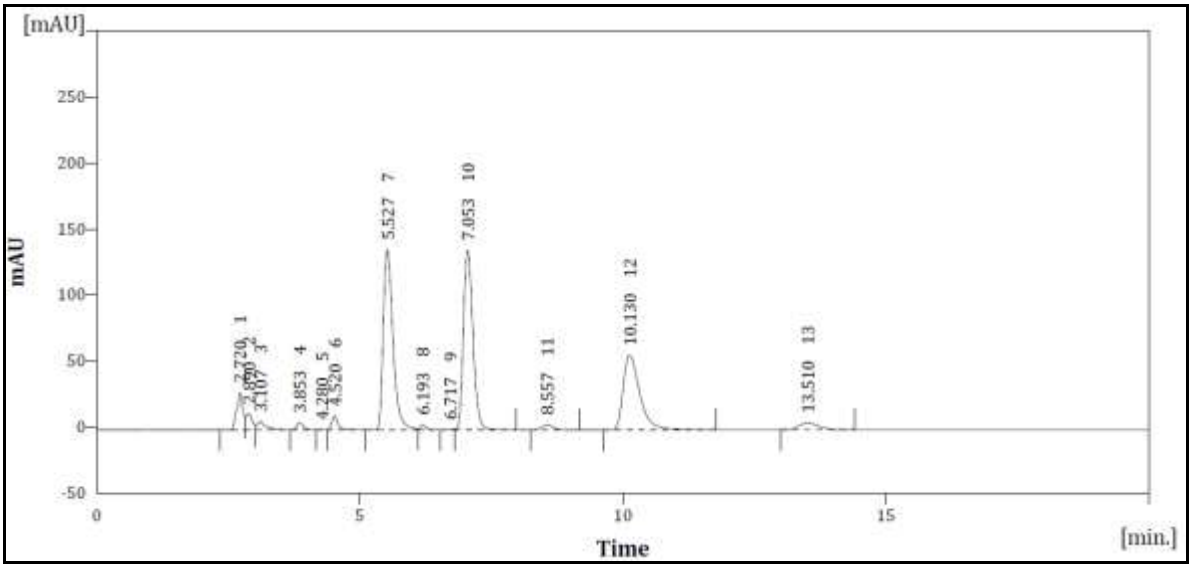

Figure no:-20 Oxidative Hydrolysis of Formulation.

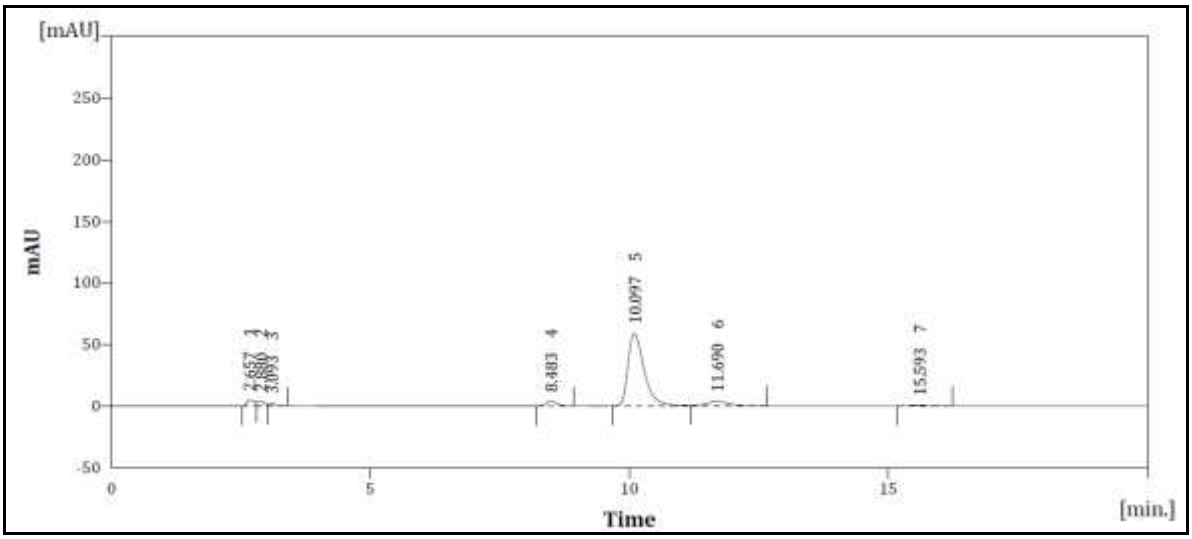

Figure no:-21 Thermal Hydrolyisis of Aceclofenac.

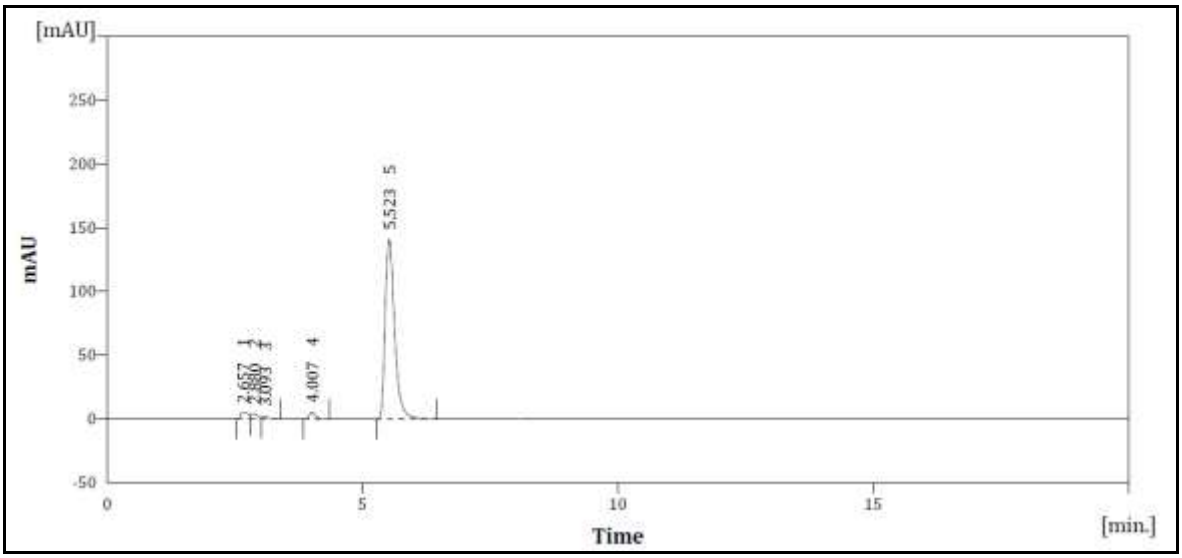

Figure no:- 22 Thermal Hydrolysis of Paracetamol.

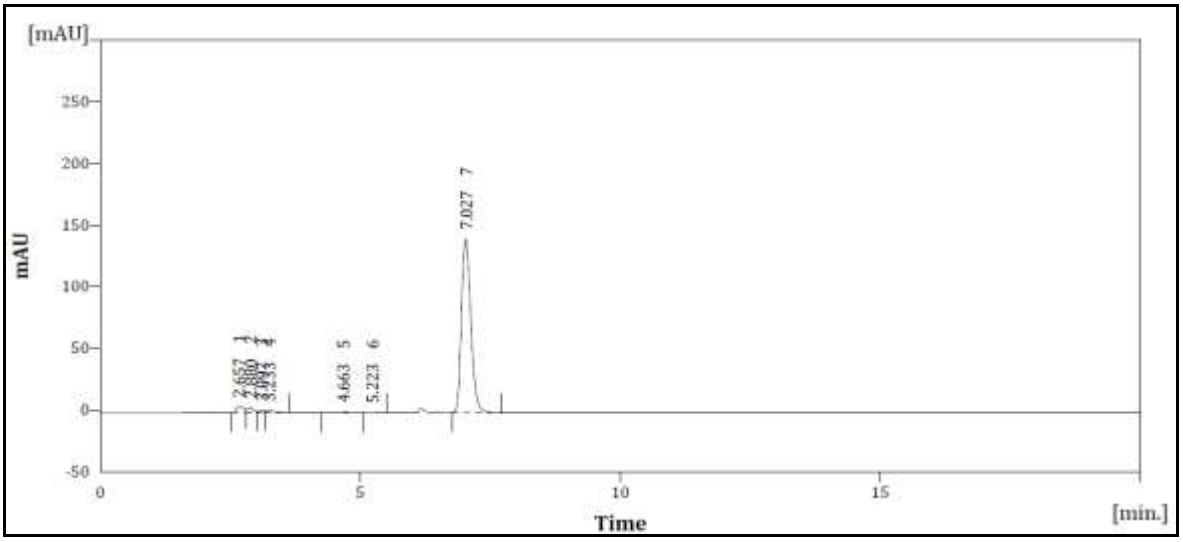

Figure no:-23 Thermal Hydrolysis of Chlorzoxazone.

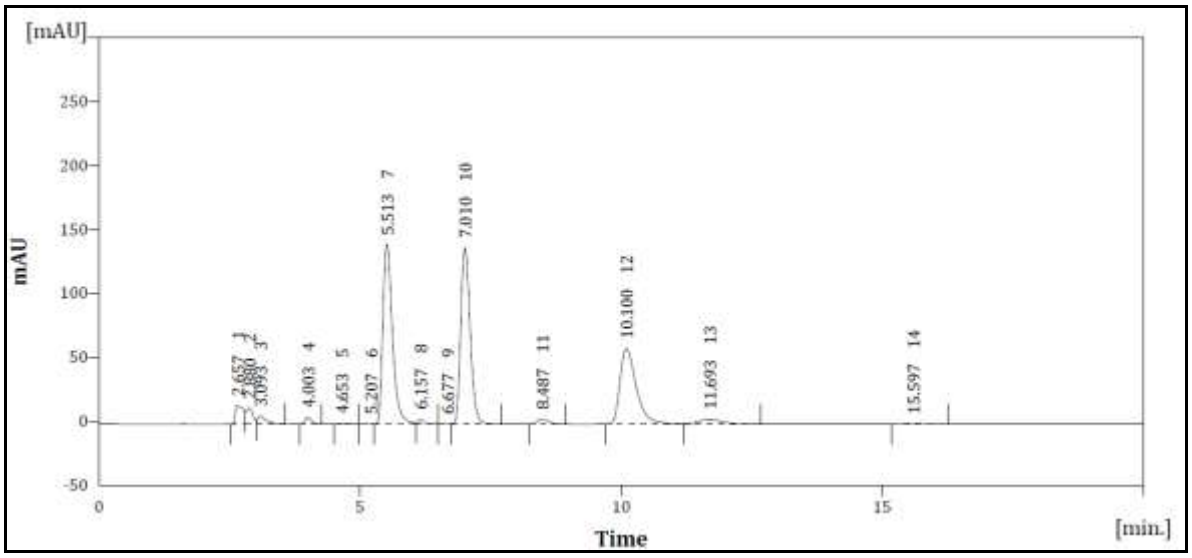

Figure no:-24 Thermal Hydrolysis of Formulation.

CONCLUSION

- Development HPLC Method can resolve all Degradants peak of drug. No chromatographic interference from tablet excipients was found.
- It is concluded that the developed method is specific. The test parameters were also performed and were found to be within acceptable criteria. The method can be successfully employed for the simultaneous determination of Aceclofenac, Paracetamol and Chlorzoxazone in pharmaceutical formulation.

REFERENCES

1. Skoog Da et al., Fundamental of Analytical Chmistry. Homson Rooks/ Cole Publication; 8th Edn, 2005; 973-979.
2. Analytical Chemistry; 5th Edn; Thomson Brooks/Cole Publication, Singapore, 1994; 566-568.
3. Sharma BK. In Instrumental Methods of Chemical Analysis. Goel Publication House; 23rd Edn; New Delhi, 2002; 1-16.
4. Jeffery G., Bassett J., Mehdham J. and Denney R., In Text of quantitative Chemical Analysis; 5th Edn, 1989; 34.
5. Swarbrick J. and Boylan J. In Encyclopedia of pharmaceutical technology, Marcel Dekker Inc., Vol. I, 2002; 363-416.
6. Meyer VR, Practical high Performance liquid chromatography, 2th Edn; John Wiley and sons, London, 1993; 26-258.
7. Mendham J., Denny R., Barnes J., Thomas M. In Vogels Text Book of Quantitative Chemical Analysis; 6th Edn; Pearson education, 2002; 123-136.
8. Sethi P. In HPLC quantitative analysis of Pharmaceutical formulations, CBS publication and distributors, New delhi, 2001.
9. Snyder L. and Kirkland J. In Introduction to Modern Liquid Chromatography; 2th Edn; John Wiley & Sons Inc, New York, 1997; 267-400.

10. Ahuja S. and Scypinski S. In Handbook of Modern Phamaceutical Analysis, Academic Press, United States of America, 2001; 3: 349.
11. ICH QIA (R2). 2005 Stability Testing of New Drug Substances and Products, November 2012.
12. Saranjit Singh and Monika Bakshi, "Guidance on Conduct of Strees Tests to Determine Inherent Stability of Drugs." *Journal of Pharmatical Technology*, 2000; 1-14.
13. ICH Q2A. 2005 Validation Of Analytical Method, November 2012.
14. The Merck Index, An Encyclopedia of Chemicals, Drugs And Biologicals; Fourteenth Edition; Published by Merck Research Laboratories, 2006; 5.
15. Tripathi KD., Essential of Medical Pharmacology; Sixth Edition; Jaypee Brothers, Medical Publishers [P] Ltd, New Delhi, 2008; 184-201.
16. www.chemspider.com/Chemical-Structure.64809.html.
17. The Merck Index, An Encyclopedia of Chemicals, Drugs And Biologicals; Fourteenth Edition; Published by Merck Research Laboratories, 2006; 9.
18. "Drug bank: Paracetamol". https://www.drugbank.ca/drugs/DB00316.
19. The Merck Index, An Encyclopedia of Chemicals, Drugs And Biologicals; Fourteenth Edition; Published by Merck Research Laboratories, 2006; 364.
20. "Drug bank: Chlorzoxazone". https://www.drugbank.ca/drugs/DB00356.
21. Health and Family Indian Pharmacopoeia–2010, The Indian Pharmacopoeia Commission, Ghaziabad, Govt. of India Ministry of Welfare, 2: 770.
22. British Pharmacopoeia (BP); The Stationery Office. London, U.K, Vol I, 2009; 44.
23. Gharge D. and Dhabale P., "Simultaneous Estimation of Aceclofenac and Paracetamol in Solid Dosage Form by RP-HPLC Method", *International Journal of Chem Tech Research*, 2010; 2: 942-946.
24. Azhlwar S. and, Kochupappy TR., "Stability indicating HPLC method for simultaneous determination of Drotaverine and Aceclofenac", *International Journal of Pharmacy and Pharmaceutical Sciences*, 2011; 3: 245-250.
25. Rath SK., Sarangi RR. and Panda SK, "UV-Spectrophotometric method for simultaneous estimation of Drotaverine hydrochloride and Aceclofenac in bulk and their formulation", *International Journal of Biological & Pharmaceutical Research*, 2011; 2: 55-59.
26. Srujani Ch,, Sravanthi B. and Madhuri D., "Validated UV Spectrophotometric Methods for the Estimation of Aceclofenac in Bulk and Pharmaceutical Formulation", *Scholars Academic Journal of Pharmacy*, 2014; 3: 471-476.
27. Hossain Md. F. and Bhadra SU., "The ICH guidance in practice: Stress degradation studies on Aceclofenac and development of a validated stability-indicating reversed-phase HPLC assay in tablet dosage form," *Scholars Research Library Der Pharma Chemica*, 2013; 5: 131-146.
28. Kesharwani S. and Kohali D., "Development and Validation of analytical methods for Simultaneous Estimation of Diacerein and Aceclofenac in Bulk and Tablets using UV-visible spectroscopy", *International Journal of ChemTech Research*, 2010; 2: 1816-1822.
29. Gandhi S., Deshpande P. and Rajmane V., "Method development and validation for simultaneous estimation of Drotaverine hydrochloride and Aceclofenac in tablet dosage Form by RP- HPLC", *International Journal of Pharmaceutical Sciences Review and Research*, 2010; 4: 49-52.
30. Health and Family Indian Pharmacopoeia–2010, The Indian Pharmacopoeia Commission, Ghaziabad, Govt. of India Ministry of Welfare, 3: 1861.
31. British Pharmacopoeia (BP); The Stationery Office. London, U.K, 2009; 3: 2968.
32. The United States Pharmacopeia (USP 30), The National Formulary (NF 25), United State Pharmacopoeial Convention Inc. Rockville, U. S. A, Vol I, 2007; 21.
33. Bhimavarapu R., Kanikanti D. and Gowthami N., "Forced Degradation Study of Paracetamol in tablet formulation using RP- HPLC", *Bulletin of Pharmaceutical Research*, 2011; 1: 13-17.
34. Golla M., Reddy R. and Aliekya A., "RP-HPLC Method Development and Validation for the Simultaneous Estimation of Paracetamol and Flupiritine Maleate in Pharmaceutical Dosage", *Journal of Scientific and Innovative Research*, 2013; 2: 634-641.
35. Kamble RM. and Singh SG., "Stability-Indicating RP-HPLC Method for Analysis of Paracetamol and Tramadol in a Pharmaceutical Dosage Form", *E-Journal of Chemistry*, 2012; 9: 1347-1356.
36. Nayak S., Sarangi RR. and Panda SK., "UV-Spectrophotometric method for simultaneous estimation of Paracetamol and Ondancetron in bulk and their formulation", *International Journal of Biological & Pharmaceutical Research*, 2011; 2: 45-49.
37. Boyka GT., Bistra DK. and Dimitar RR., "HPLC Assay and Stability Studies of Tablets Containing Paracetamol and Caffeine", *International Journal of Pharmaceutical Sciences Review and Research*, 2013; 18: 138-142.
38. Nimila C., Balan P. and Chiranjeevi N., "Development and validation of a reverse phase HPLC method of simultaneous estimation of Tolperisone hydrochloride and Paracetamol in tablet dosage form", *International Journal of Pharmacy and Phamaceutical Sciences*, 2012; 4: 84-88.
39. Maslarska V. and Tencheva J., "Simultaneous determination and validation of Paracetamol and Codeine phosphate in pharmaceutical preparation by RP-HPLC", *International Journal of Pharmacy and Phamaceutical Sciences*, 2013; 5: 417-419.

40. Vaidya VV., Singh GR. and Choukekar MP., "Simultaneous RP HPLC Determination of Aceclofenac, Paracetamol and Tzanidine in Pharmaceutical Preparations", *E-Journal of Chemistry*, 2010; 7: 260-264.
41. Shah D., Patel B. and Bhavsar A., "Stability indicating RP-HPLC method for simultaneous estimation of Paracetamol and Pamabrom in bulk and combined pharmaceutical dosage form", *World Journal of Pharmacy and Pharmaceutical Sciences*, 2014; 3: 1009-1020.
42. The United States Pharmacopeia (USP 30), The National Formulary (NF 25), United State Pharmacopoeial Convention Inc. Rockville, U. S. A, Vol-I, 2007; 501.
43. Rathod D., Chaudhari B. and Vasava A., "Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Ibuprofen and Chlorzoxazone", *PHARMAGENE*, 2013; 1: 65-68.
44. Prajapati KM. and Patel SA., "Spectrophotometric Estimation of Chlorzoxazone and Diclofenac Sodium in Synthetic Mixture by First Order Derivative Spectrophotometry", *International Journal of Pharmamedix India*, 2013; 1: 258-269.
45. Yadav SS., Jagtap AS. and Rao JR., "Simultaneous Determination of Paracetamol, Lornoxicam and Chlorzoxazone in Tablets by High Performance Thin Layer Chromatography", *Scholars Research Library Der Pharma Chemica*, 2012; 4: 1798-1802.
46. Kulkarni MB., Dange PB. and Walode SG., "Stability indicating thin-layer chromatographic determination of chlorzoxazone, diclofenac sodium and paracetamol as bulk drug: Application to forced degradation study", *Pelagia Research Library Der Pharmacia Sinica*, 2012; 3: 643-652.
47. Babu SA. and Roosewelt C., "Simultaneous estimation of Aceclofenac, Paracetamol and Chlorzoxazone in tablets", *Indian Journal of Pharmaceutical Sciences*, 2007; 69: 692-694.
48. Patel AV., Bhavsar G. and Kapadiya N., "Validation and Development of RP- HPLC Assay Method for Estimation of Paracetamol, Aceclofenac and Chlorzoxazone in Combined Tablet Dosage Form", *The Pharmaceutical and Chemical Journal*, 2015; 2: 25-30.
49. Meher VK., Babu SA. and Roosewelt C., "Simultaneous estimation of Paracetamol, Aceclofenac and Chlorzoxazone in oral dosage form using spectrophotometric method", *Analytical Chemistry an Indian Journal*, 2007; 5: 46-50.
50. Rathinavel G., Thakur D. and Premanand DC., "Validated RP-HPLC Method for Estimation of Aceclofenac, Paracetamol and Chlorzoxazone in Dosage Form", *Scholars Research Library Der Pharma Chemica*, 2010; 2: 286-296.
51. Devadasu Ch., Rao DG. and Rao NM., "Development and validation of RP-HPLC method for simultaneous determination of Paracetamol, Aceclofenac and Chlorzoxazone in combined dosage form", *World Journal of Pharmacy and Pharmaceutical Sciences*, 2013; 3: 667-681.
52. Kumar RM., Venkatesh K. and Vaidhyalingam., "Simultaneous estimation of Paracetamol, Chlorzoxazone and Aceclofenac in Pharmaceutical Formulation by HPLC Method", *International Journal of Chem Tech Research*, 2009; 1: 457-460.
53. Pawar UD., Naik AV. and Sulebhavikar AV., "Simultaneous Determination of Aceclofenac, Paracetamol and Chlorzoxazone by HPLC in Tablet Dose Form", *E-Journal of Chemistry*, 2009; 6: 289-294.
54. Shaikh KA. And Devkhile AB., "Simultaneous Determination of Aceclofenac, Paracetamol, and Chlorzoxazone by RP- HPLC in Pharmaceutical Dosage Form", *Journal of Chromatographic* Science, 2008; 46: 649-652.
55. Dieter Neuser., Analgesic Combination World Intellectual Property Organization WO9846235, 1998.
56. Francois Dietlin., Stable liquid paracetamol compositions and method for preparing same USPTO US6028222A, 1997.
57. Krishna Devarakonda., Gastric retentive extended release pharmaceutical compositions USPTO US8741885B1, 2012.
58. Laura Corradini., Method of treating inflammatory pain USPTO US8298536, 2009.
59. Andrew Xian Chen., Sustained Release Pharmaceutical Compositions for Highly Water Soluble Drugs USPTO US8221792, 2006.