EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 8
Issue: 6
379-385
Year: 2021

A STUDY OF DEGRADATIVE EFFECT USING VALIDATED LC-PDA METHOD FOR COMBINED DOSAGE FORM OF PARACETAMOL, TIZANDINE HCL AND ACECLOFENAC

Guganathan[1], Bharani Pandilla*[1], Vijayageetha R.[1], Sakthimaan Singh B.[1] and Sathiyasree G.[1]

[1]Department of Pharmaceutical Analysis, C.L. Baid Metha College of Pharmacy, the Tamilnadu Dr. M.G.R. Medical University, Chennai-600097, India.

***Corresponding Author: Bharani Pandilla**
Department of Pharmaceutical Analysis, C.L. Baid Metha College of Pharmacy, the Tamilnadu Dr. M.G.R. Medical University, Chennai-600097, India.

Article Received on 25/03/2021 **Article Revised on 15/04/2021** **Article Accepted on 05/05/2021**

ABSTRACT
To optimise a sensitive, robust and reliable HPLC technique for quantification of PAR, TZN and ACE in the presence of degradants and validate in accordance with ICH guidelines. Isocratic elution using mobile phase ratio of 0.1mM phosphate buffer pH 7.00:Acetonitrile (65:35) and phenomenex C_{18} (250 × 4.5 cm, 5µ) column used for chromatographic separation and the solvent delivery was 1.0mL/min and UV detection was programmed at 280 nm for 0-4 mins, 320nm for 4.01-10 mins and 280 nm for 10.01-45 mins. Retention time of PAR, TZN and ACE was 3.8 min, 5.6 min and 12.0 min respectively. The applied HPLC technique showed good linearity across the range of 250-750µg/ml concentration for PAR, 50-150µg/ml for ACE and 0.50-1.50µg/ml for TZN. The recoveries accomplished for PAR, TZN and ACE were 100.12, 99.78, and 100.78 respectively. The three drugs were subjected to acid, alkali, oxidation, sunlight and thermal degradation. The degradation products formed in all stress condition were separated from the principal peaks and the % RSD was found to be less than 2% which indicate that the method is highly specific and had excellent reproducibility. The proposed and validated technique can be efficiently applied for regular analysis of PAR, TZN and ACE in formulation.

KEYWORDS: RP-HPLC, PAR-Paracetamol, TZN-Tizanidine hydrochloride, ACE- Aceclofenac, stability indicating, Validation.

INTRODUCTION
PAR is chemically N-(4-hydroxyphenyl) acetamide "Fig. 1" and is an active metabolite of phenacetin. Generally it is safe at the recommended doses; on higher doses it may leads to toxicity, including liver toxicity.[1] TZN is an imadazoline derivative and chemically named as 5-chloro-N-(4, 5-dihydro-1H-imidazole-2-yl)-2, 1, 3-benzothiadiazol-4-amine hydrochloride "Fig. 2", a muscle relaxant and alpha-2-adrenergic agonist[2]. ACE is chemically [{2-(2, 6 dichlorophenyl) amino} phenylaceto-oxyacetic acid] "Fig. 3" and is a nonsteroidal anti-inflammatory drug (NSAID). It is weak acidic drug. ACE is highly soluble in alkaline solutions, less soluble in water and acidic solutions.[3]

Extensive review of literature revealed distinct analytical techniques were reported for the combination of PAR, TZN and ACE in pure form or in formulations either separately or in combined dosage forms with other drugs. Reported techniques include UV-visible spectroscopy[4-18] and chromatographic techniques like HPLC[19-33] and HPTLC.[34] Stability-indicating assay for concurrent determination of PAR, TZN and ACE in solid formulation has not been published. The current research work focused at establishing a simple, specific, accurate stability indicating RP-HPLC technique for the concurrent quantification of PAR, TZN and ACE in formulation. Validation of the proposed technique was in accordance with parameters of ICH recommendations such as accuracy, precision, linearity, range, robustness[35] and specificity (forced degradation).[36]

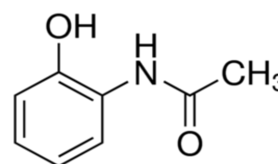

Fig. 1 N-(4-hydroxyphenyl) acetamide.

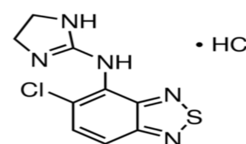

Fig. 2 5-chloro-N-(4, 5-dihydro-1H-imidazole-2-yl)-2, 1, 3-benzothiadiazol-4-amine hydrochloride.

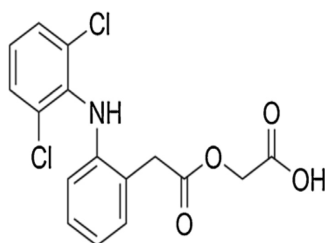

Fig. 3 [{2-(2, 6 dichlorophenyl) amino} phenylaceto-oxyacetic acid].

MATERIALS AND METHODS

Chemicals and reagents

PAR, TZN and ACE were procured from Sri Krishna Pharmaceuticals., Chennai. Potassium dihydrogen orthophosphate (A.R. grade) and Acetonitrile of HPLC grade was purchased from Rankem., India. MilliQ water from Millipore Ltd., Bangalore, India. Analytical grade reagents for stress studies purchased from Rankem., India. The tablet Gopayn MR PAR, TZN and ACE (500, 100 and 1 mg) was obtained from Sai Mirra Innoform Pvt Limited, Chennai, India.

Instrumentation

The HPLC system consists of a Waters 2695 Separation Module connected with photodiode array detector. The data was acquired by Empower software. Analytical balance (Shimadzu AUW220D), Ultra-sonicator (Spectrum Tek LMUC-12), Digital pH meter (Spectrum Tek), Hot air oven (In lab equipment Pvt. Ltd.) were used.

Chromatographic conditions

Chromatographic separation was done using Phenomenex C_{18} (250 x 4.6mm, 5µ,) and mobile phase was buffer:Acetonitrile (0.1mM phosphate pH 7.00) in the proportion of 65:35. The solvent delivery was 1.0ml/min. Sample volume at 20µL. UV detection was performed at 280 nm for 0-4 mins, 320nm for 4.01-10 mins and 280 nm for 10.01-45 mins at ambient temperature.

Working standard solution

Standard stock solution of ACE 1000µg/ml in methanol and TZN 10µg/ml in mobile phase were prepared. PAR 50 mg was accurately weighed, and 10 ml of ACE stock and 10 ml of TZN stock was added and diluted to obtain PAR, TZN and ACE concentration of 500, 1 and 100µg/ml respectively.

Sample stock preparation

Average weight of twenty tablets was estimated and tablets were pounded to powder and pulverised powder corresponding to average tablet weight was taken and dissolved with sufficient mobile phase, sonicated the solution for 20 minutes and made the volume with mobile phase to 100 ml.

Working sample preparation

The above sample stock solution was diluted with mobile phase to get desired concentration of PAR, TZN and ACE, 500, 1 and 100µg/ml respectively.

Forced Degradation studies

Forced degradation study involves exposure of drug product to thermal, light, oxidation, hydrolysis (acid, alkali). The forced degradation process is used to evaluate the interference of potential degradants during stability testing. The results help in investigating the mechanism of degradation of drug products on storage.

Stress degradation of formulation

Acid degradation

To 5 ml of the sample stock solution, 5 mL of 1M HCl was mixed and this was maintained at 60˚C for 2 hours and subsequently neutralised with base then made up with mobile phase to required concentration.

Alkali degradation

To 5 ml of the sample stock solution, 5 mL 1M NaOH was mixed and this was maintained at 60˚C for 2 hours and subsequently was neutralised with acid then made up with mobile phase to required concentration.

Oxidative degradation

To 5 ml of sample stock solution, 5ml of hydrogen peroxide (10% v/v) was mixed and this was maintained at 60˚C for 2 hours and made up with mobile phase to required concentration.

Photolytic degradation

The powdered tablets are placed in the UV light for 2 hours, sample of desired concentration of PAR, TZN and ACE (500, 1, 100 µg/mL) was prepared with mobile phase and analysed.

Thermal degradation

The powdered tablets are placed in the Hot air oven for 2 hours at 60^0C sample solution of desired concentration of PAR, TZN and ACE (500, 1, 100 µg/mL) was prepared with mobile phase and analysed.

RESULTS AND DISCUSSION

Method development

During escalation of the proposed RP-HPLC method, various chromatographic conditions were scrutinized. Different trials were taken with different composition of water:acetonitrile, at ratio of 90:10 of water:acetonitrile resulted in poor peak shape of PAR and poor retention of TZN and ACE. During another trial, ratio of water:acetonitrile (70:30), resulted in defective peak shape of PAR, longer retention of ACE and no elution of TZN. Trials were taken with various buffers such as acetate and orthophosphate and ortho phosphoric acid with pH range of 3.5-7.5. The final mobile phase composition was fixed with phosphate buffer pH 7.00 (adjusted using orthophosphoric acid) at the concentration of 0.1mM phosphate buffer and

acetonitrile (65:35) which resulted in good peak of PAR, better retention of ACE. The detection wavelength of PAR and ACE was 280nm based on isobestic point of UV spectra. TZN was detected only at 320 nm, at this wavelength PAR and ACE were poorly detected. For this purpose, UV detection was programmed at 280 nm for 0-4 mins, 320 nm for 4.01-10 mins and 280 nm for 10.01-45 mins.

The last trial condition was optimised, the retention time of PAR, TZN and ACE was at 3.8 min, 5.6 min and 12.0 min. The optimised parameter of the HPLC technique was validated in accordance with ICH and regulatory recommendations.

Method validation

System suitability

System suitability was estimated by six replicated injection of working standard solution at 100% concentration. The result obtained for the system suitability parameters are summarised in the "Table 1" and the standard chromatogram in "Fig. 4".

Table.1 Parameters of system suitability for PAR, TZN and ACE.

PARAMETER	RESPONSE		
	PAR	TZN	ACE
RT(min)	*3.81*	*5.62*	*12.07*
Relative standard deviation(% RSD)	*1.23*	*1.30*	*1.69*
Theoretical plates	*3257.8*	*7911.1*	*10811.2*
Tailing factor	*0.95*	*1.5*	*1.6*

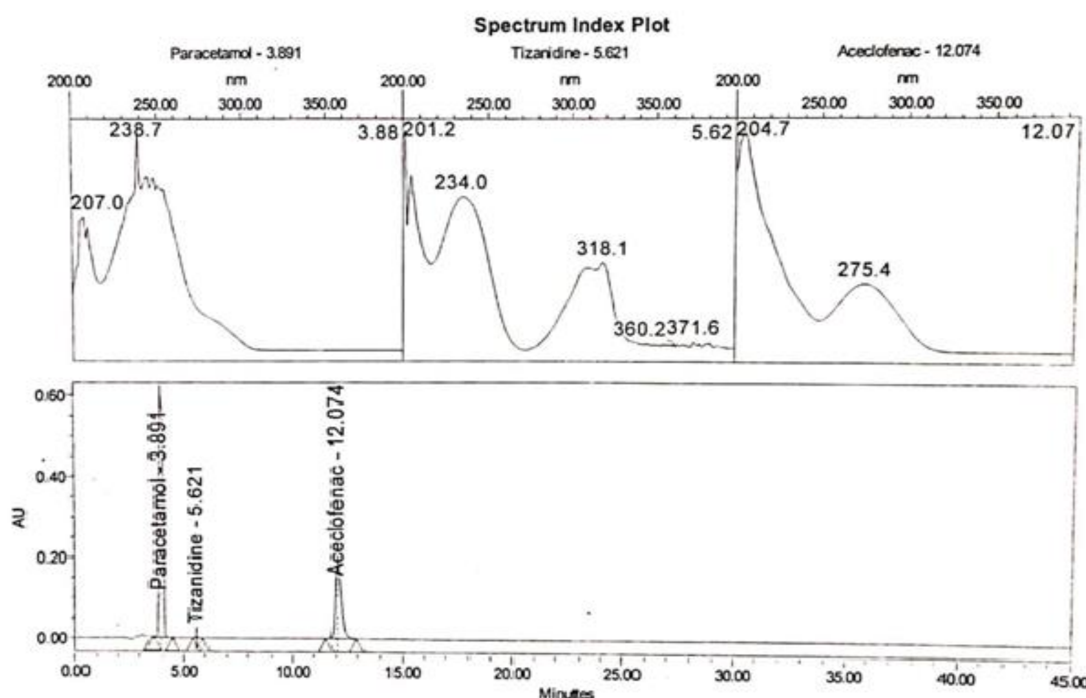

Fig. 4 Chromatogram of standard.

Linearity & Range

Linearity was found in the range of 250-750µg/mL for PAR, 0.5-1.5µg/mL for TZN and 50-150µg/ml for ACE. The peak area and concentration is subjected to statistical analysis to calculate the regression parameters and correlation coefficients. Results were presented in the "Table 2".

Table.2 Regression values for PAR, TZN and ACE.

PARAMETERS	RESPONSE		
	PAR	TZN	ACE
LINEARITY			
Range	*250.0-750.0µg/Ml*	*50.0-150.0µg/mL*	*0.50-1.50µg/mL*
Slope	*186071.1*	*38893.3*	*108559.1*
Intercept	*352331.5793*	*126492.1931*	*5332.062069*
r^2	*0.9998*	*0.9998*	*0.9998*

Precision
Repeatability was performed by injecting six replicate injections of PAR, TZN and ACE standard and sample (500µg/mL, 1µg/mL and 100µg/mL) on the same day as intraday precision study and on a different day for inter day precision. The results are reported in the "Table 3" which reflects that the % RSD is less than 2%.

Table.3 Precision for PAR, TZN and ACE.

DRUG		MEAN (ASSAY) ± SD	% RSD
Intra –day Precision (n=6)	***PAR***	*99.74±0.23*	*0.235*
	TZN	*99.10±0.57*	*0.580*
	ACE	*99.13±0.59*	*0.591*
Inter-day precision (n=6)	***PAR***	*98.08±0.87*	*0.886*
	TZN	*99.02±0.59*	*0.591*
	ACE	*98.04±0.88*	*0.893*

Accuracy (%Recovery)
The accuracy was demonstrated by calculating % recovery of PAR, ACE and TZN by standard addition method. The known concentration of standard solutions consisting 250, 0.5 & 50µg/ml, 500, 1 & 100µg/ml and 750, 1.5 & 150µg/ml of PAR, TZN and ACE denoting 50%, 100% and 150% as working solution. Above prepared solution were injected in triplicate manner and the results were reported in the "Table 4".

Table.4 Accuracy for PAR, TZN and ACE.

DRUG	RECOVERY LEVEL	AMOUNT ADDED (MCG)	AMOUNT FOUND(MCG)	ABSOLUTE MEAN	%RECOVERY ± SD (N=3)
PAR	*50%*	*250.45*	*249.12*	*250.04±0.44*	*101.57±0.94*
	100%	*499.14*	*488.45*	*499.77±0.55*	*100.13±0.78*
	150%	*749.45*	*749.01*	*749.45±0.22*	*100.12±0.17*
TZN	*50%*	*0.49*	*0.46*	*0.48±0.01*	*99.52±1.07*
	100%	*0.98*	*0.95*	*0.96±0.02*	*100.71±1.23*
	150%	*1.49*	*1.43*	*1.49±0.03*	*99.10±0.41*
ACE	*50%*	*49.65*	*48.45*	*49.55±0.10*	*101.57±0.94*
	100%	*99.56*	*98.67*	*99.41±0.16*	*100.13±0.78*
	150%	*149.33*	*149.11*	*149.40±0.32*	*100.12±0.17*

Robustness
The optimised HPLC technique showed robustness when purposeful changes in the solvent delivery (± 0.2 ml/min) and pH from the optimised chromatographic condition (± 0.2 unit) of the buffer did not show any change in elution time of the analyte peaks. The results are discussed in the "Table 5".

Table. 5 Robustness for PAR, TZN and ACE.

VARIATION PARAMETERS	ACCURACY %^{a}± SD			RELATIVE STANDARD DEVIATION (%RSD)		
	PAR	TZN	ACE	PAR	TZN	ACE
Solvent delivery rate (±0.2 ml/min)						
0.9mL/min	*99.86±1.2*	*98.12±0.4*	*97.32±0.6*	*0.15*	*1.35*	*0.84*
1.1mL/min	*100.04±0.7*	*99.43±0.8*	*99.23±1.3*	*0.92*	*1.48*	*0.20*
Changes in pH (± 2.0%)						
pH6.95	*96.93±1.1*	*95.67±1.4*	*97.13±0.9*	*0.06*	*1.14*	*0.46*
PH7.05	*97.45±0.9*	*98.34±0.5*	*99.56±0.4*	*0.30*	*1.04*	*0.27*

$^{a=}$*shows the accuracy of 3 times of injection*

Solution stability
The stability in analytical solution was determined by analyzing both the control and the sample solution over a period of 24 hrs kept over a table top. The retention time and peak area of PAR, TZN and ACE did not show any significant changes. (% RSD less than 2.0). This indicates the solutions were stable for 24 hrs, which was sufficient to complete whole analytical process without any stability issues.

Specificity (forced degradation studies)
A stability-indicating method evaluates the active pharmaceutical ingredients, without the intrusion from excipients, potential impurities and degradation products. And also to determine the inherent stability nature of the

raw material and dosage form. Stress study confesses the mechanistic approach of degradation products and its pathway.[36, 37]

"Table 6" provides the percentage degradation of analyte molecule and its recovery during stress condition. There was no interference of placebo and blank during stress studies. Among the various stress conditions, PAR degraded in acidic environment about 3.32% with a recovery of 96.68% and stable in all other conditions. TZN degraded highly in oxidative condition about 10.05% with a recovery of 89.95%. ACE degraded highly in acidic environment about 13.42% with the recovery of 86.58% and moderate degradation in alkaline condition about 7.12% with the recovery of 93.88%. The degradants were well separated from the three main peaks. The chromatogram of degraded peaks was fetched in the "Fig. 5". The peak purity data conveys that the degraded peaks does not disturb the purity of significant main peaks of the three analytes.

Table.6 Specificity (Forced degradation study).

DEGRADATION CONDITION	DEGRADATION %[a]			PEAK PURITY			PEAK THRESHOLD		
	PAR	TZN	ACE	PAR	TZN	ACE	PAR	TZN	ACE
Acid hydrolysis	*3.32*	*4.58*	*13.42*	*2.191*	*0.501*	*0.087*	*37.057*	*0.572*	*0.289*
Alkali hydrolysis	*2.33*	*2.09*	*7.12*	*1.802*	*0.456*	*0.624*	*9.602*	*0.612*	*0.765*
Oxidative degradation	*3.19*	*10.97*	*2.99*	*2.004*	*0.608*	*0.077*	*21.431*	*0.900*	*0.282*
Thermal degradation	*2.99*	*1.99*	*1.99*	*2.224*	*0.19*	*0.082*	*14.983*	*0.609*	*0.288*
Photolysis degradation	*1.65*	*3.46*	*0.90*	*2.491*	*0.368*	*0.082*	*31.302*	*0.567*	*0.282*

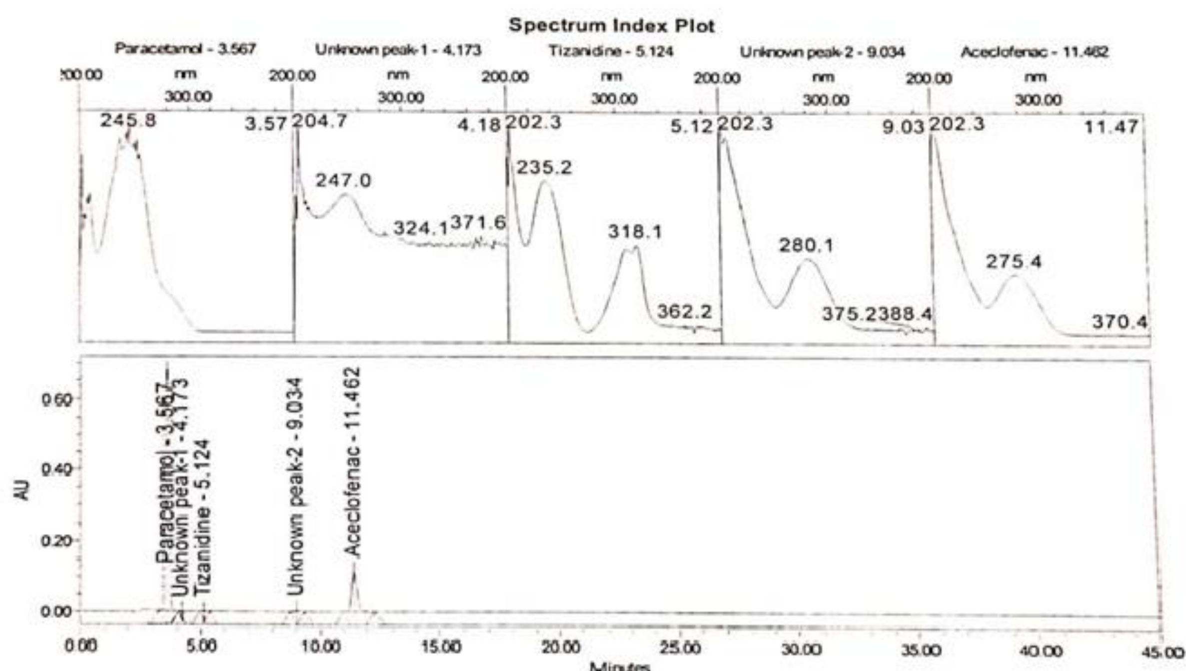

Fig. 5 Chromatogram of sample.

Filter integrity

Filter integrity testing was used to persuade the filters are integral and fulfil their intended properties. Filters such as Polyvinylidene fluoride, nylon membrane filter, Teflon filter and Polytetrafluoroethylene membrane filters were used for sample preparation. Percentage deviation where within 2% and it shows that there no interference was observed.

CONCLUSION

The developed stability indicating assay technique has been validated in accordance with regulatory and ICH recommendations. The optimised technique is linear, specific, robust and stability indicating to quantify PAR, TZN and ACE in formulation. Stress studies accomplished for the three analytes in different condition such as thermal, photolytic, oxidation, hydrolysis (acid and alkali). The stress studies showed that degradation was within the limits and degraded peaks were well resolved from the principal peaks. The significant main peaks were attaining good peak purity based on the PDA detection. The developed assay method is assessment for quantitative estimation of PAR, TZN and ACE in formulations.

ACKNOWLEDGEMENT

The authors are grateful to Sai Mirra Innopharm Pvt Ltd Chennai and C. L. Baid Metha College of Pharmacy for the grant of necessary equipment, facility & chemicals to accomplish the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERNCES

1. Martindale, The Extra Pharmacopoeia. 31st ed., London: Royal Pharmaceutical Society of Great Britain, 1996; 36–37: 81–83.
2. British National Formulary, Pharmaceutical Press, 2018; 1094: 178
3. Arslan SA, Tirnaksiz FA. A Nonsteroidal anti-inflammatory drug: Aceclofenac. J.Pharm. Sci, 2010; 35(2): 105-18.
4. Gondane SJ, Deshpande MM, Mahajan MP, Sawant SD. Spectrophotometric method development and validation for estimation of Tizanidine and Aceclofenac in bulk drug & tablet formulation. Int. J. ChemTech. Res, 2011; 3(2): 620-624.
5. Rohit Shah, Chandrakant Magdum, Shital Kumar Patil, Dhanya Kumar Chougule, Nilofar Naikwade. Validated spectroscopic method for estimation of Aceclofenac from tablet formulation. Res. J. Pharm. and Tech, 2008; 1(4): 123-129.
6. Madhukar Badgujara A, Kiran Mangaonkarb V. Spectrophotometric simultaneous determination of Paracetamol and Aceclofeanc in tablet dosage form. Res. J. Pharm. Bio. Chem Sci, 2014; 5(4): 110.
7. Chandrashekhar Narajji, Hardik Patel R, Manohar Karvekar D, Suresh Babu AR. Simultaneous estimation of Aceclofenac, Paracetamol and Tizanidine in their combined dosage forms by spectrophotometric and RP- HPLC method. J. Anal Bio anal. Techniques, 2011; 2(4): 2155-9872.
8. Ashraful Islam SM, Sharif Abuzar, Pijush Kumar Paul. Validation of UV spectrophotometric and RP-HPLC methods for the simultaneous analysis of Paracetamol and Aceclofenac in marketed tablets. Int. J. of Pharm. & Life Sci, 2011; 2(12): 1267-1275.
9. Sujata Gondane J, Mrunalini Deshpande M, Mahajan MP. Simultaneous Estimation of Tizanidine and Aceclofenac in bulk drug and tablet formulation by Q-analysis and area under curve. Int. J. Pharm. Sci. Review & Res, 2011; 8(1): 121-128.
10. Permender Rathee, Kamal Dua, Sushila Rathee, Vikash Kumar. Statistical design for optimization and determination of Tizanidine Hcl using Folin-Ciocalteu (Fc) as chromogenic reagent. Pharm. Anal. Acta, 2014; 5(7): 2153-2435.
11. Sumit Saparia, Padmavathi P, Prabhu, Subrahmanyam EVS, Jignesh Kaneria, Sagar Bechara, et al. Development of new analytical methods and their validation for the determination of Tizanidine hydrochloride in bulk and marketed formulations. Asian J. Biomed. Pharm. Sci, 2013; 3(23): 57-60.
12. Giri A, Saritha B, Sreenivasulu Reddy T. Quantitative estimation of Tizanidine in pharmaceutical formulations by visible spectrophotometry using gold (III). Int. J. Pharm. Sci. Rev. Res, 2014; 27(2): 97-101.
13. Nagwa Ahimda HS, Mariam S, Abu- Naja, Yasmin Doghman SA. Determination of Paracetamol in tablet by difference spectrophotometric method. Asian. J. Chem, 2009; 21(3): 2233-2240.
14. Ogunneye Adeyemi Lawrence, Adewuyi Gregory Olufemi, Omoboyowa Damilola Alex, Saraye Taiwo Kayode. Spectrophotometric determination of Acetaminophen content of different brands of Paracetamol tablets from South-West Nigeria. J. Res. Enviroment. Sci. Toxicol, 2012; 1(5): 251-257.
15. Pawar VT, Pishwakar SA, More HN. Spectrometric estimation of Aceclofenac and Paracetamol from tablet dosage form. Int. Res. J. Pharm, 2011; 2(1): 93-98.
16. Pratiksha V, Rathi. A comparative estimation of content of Paracetamol in different formulations using UV-Visible spectrophotometer. World J. Pharm. & Pharm. Sci, 2012; 7(2): 1473-1481.
17. Dixit RB, Patel JA. Spectrophotometric determination of Paracetamol drug using 8-hydroxyquinoline. Int. J. Pharm. Sci. Res, 2014; 5(6): 2393-2397.
18. Rajan Rele V. Simultaneous spectrophotometric estimation of Paracetamol and Aceclofenac by second order derivative method in combined dosage form. J. Chem. Pharm. Res, 2015; 7(6): 512-517.
19. Poonam Patil P, Ware AL, Priyanka A. Development and validation of RP-HPLC method for simultaneous determination of Paracetamol, Aceclofenac and Tizanidine in bulk and tablet formulation. Inventi, 2012; 1(1): 1-4.
20. Dudhe PB, Jadhav S, Sawarkar V, Nagras MA. Method development and validation for simultaneous determination of Aceclofenac and Tizanidine in bulk and marketed formulation. Int. J. PharmTech. Res, 2013; 5(3): 1212-1216.
21. Siva Kumar R, Senthilnathan P, Kumar Nallasivan P, Sam Solomon WD, Venkat Narayanan R. Validated reverse phase HPLC method for the determination of Aceclofenac and Tizanidine in tablets. Asian. J. Pharm. Res & Health care, 2010; 2(1): 84- 235
22. Momin MY, Yeole PG, Puranik MP, Wadher SJ. Reverse Phase HPLC Method for Determination of Aceclofenac and Paracetamol in tablet dosage form. Indian J. Pharm. Sci, 2006; 68(3): 154-161.
23. Kekare MB, Choukekar MP, Vaidya VV, Singh GR. Simultaneous RP-HPLC determination of Aceclofenac and Tizanidine in pharmaceutical preparations. Anal. Chem. An Indian Journal, 2008; 7(6): 776-779.
24. Godse VP, Deodhar MN, Bhosale AV, Sonawane RA, Sakpal PS, Borkar DD, et al. Reverse phase HPLC method for determination of Aceclofenac and Paracetamol in tablet dosage form. Asian J. Res. Chem, 2009; 2(1): 214-221.
25. Amit Samel, Bhanu Raman. Simultaneous estimation of related impurities of Tizanidine hydrochloride in its active pharmaceutical ingradient

by Reversed-Phase Liquid Chromatography. Asian J. Chem, 2009; 21(2): 176-182.
26. Deepali Gharge, Pandurang Dhabale. Simultaneous estimation of Aceclofenac and Paracetamol in solid dosage form by RP-HPLC method. Int. J. ChemTech. Res, 2010; 2(2): 942-946.
27. Gopinath R, Rajan S, Meyyanathan SN, Krishnaveni N, Suresh B. A RP-HPLC method for simultaneous estimation of Paracetamol and Aceclofenac in tablet. Indian J. Pharm. Sci, 2007; 69(1): 137-140.
28. Niti Bhardwaj, Ahuja P, Farah Iram, Asif Husain. Synthesis and analytical method development of a new pro-drug of Aceclofenac. Int. J. Pharm. Sci. Res, 2015; 6(9): 3872- 3876.
29. Karthika Paul, Nagarajan JSK, Chandan RS. Standardization on RP-HPLC method for the estimation of Aceclofenac in dosage form. Int. J. Res. Pharm. Chem, 2011; 1(4): 212- 219.
30. Khalid A, Attia M, Mohamed Nassar WI, Ashraf Abdel-Fattah. A comparative study of different approaches for stability indicating determination of Tizanidine in presence of its oxidative degradation product. Int. Pharm & Pharm. Res, 2016; 5(3): 166-195.
31. Mariana Brandalise, Pamela CL, Ferreira Leonardo Meneghini, Andrea Pereira G, Pedro Fröelich E, Ana Bergold M. Stability-indicating method for determination of Tizanidine hydrochloride by LC-DAD method using chemometric approach. Drug Anal. Res, 2017; 1(2): 61-66.
32. Vaidya VV, Singh GR, Choukekhar MP, Houkekar Kekare MB. Simultaneous RP-HPLC Determination of Aceclofenac, Paracetamol and Tizanidine in pharmaceutical preparations. E-J. of Chem, 2010; 7(2): 260-264.
33. Raghunadha Gupta C, Vijaya Ratna, J, Younoos Mohammad. Development and validation of new RP-HPLC method with UV detection for the determination of aceclofenac in plasma. J. Pharm. Res, 2011; 4(2): 338-339.
34. Mahadik KR, Partaker AR, Himani Agrawal, Neeraj Kaul. Stability-indicating HPTLC determination of Tizanidine hydrochloride in bulk drug and pharmaceutical formulations. J. Pharm. Biomed. Anal, 2003; 33(2): 545-552.
35. Validation of Analytical Procedures: Text and Methodology (Q2 (R1)). International Conference on harmonization, Geneva, Switzerland on Nov, 2005.
36. Stability testing of new drug substances and Products. (Q1A). International Conference on Harmonization, Geneva, Switzerland on Nov, 2003.
37. Reynolds DW, Facchine KL, Mullaney JF, Alsante KM, Hatajik TD, Motto MG. Available guidance and best practices for conducting forced degradation studies. Pharm. Tech, 2002; 48-56.