Shubham Borse et al /J. Pharm. Sci. & Res. Vol. 13(8), 2021, 502-507

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Method Development and Validation for the Simultaneous Estimation of Tizanidine and Aceclofenac by (UHPLC) RP-HPLC in Bulk and Tablet Dosage Forms

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Abstract

An accurate, precise and reproducible RP-HPLC method was developed for the simultaneous quantitative determination of Tizanidine (TZN) and Aceclofenac (AFN) in tablet dosage forms. Agilent (S.K.) Gradient System UV Detector and C_{18} column with 100mm x 4.6 mm i.d and 5µm particle size Acetonitrile: ph Buffer (75:25v/v) pH 3 was used as the mobile phase for the method. The detection wavelength was 301 nm and flow rate was 0.8 ml/min. In the developed method, the retention time of Tizanidine and Aceclofenac were found to be 2.80 min and 6.58 min. The LOD and LOQ of AZN were found to be 0.028 µg/ml and 0.087 µg/ml, AFN were found to be 0.1272µg/ml and 0.3875µg/ml, respectively. The proposed method is The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible. The developed method was validated according to the ICH guidelines. In this methods linearity, precision, range, robustness were observed. The method was found to be simple, accurate, precise, economic and reproducible. So the proposed methods can be used for the routine quality control analysis of TZN and AFN in bulk drug as well as in formulations.

Keywords: Tizanidine, Aceclofenac, method- development, validation, RP-HPLC

INTRODUCTION

Tizanidine is Skeletal muscle relaxant and official in IP-2007, USP 30-NF 25. Aceclofenac is an anti-inflammatory class of drug And official in European Pharmacopoeia. Tizanidine and Aceclofenac are available in combined tablet dosage form as an Muscle Relaxant and analgesic. Aceclofenac relieves pain by stimulating cartilage synthesis, Tizanidine is a short acting drug for the management of spasticity. It is an agonist at a 2-adrenergic receptor site & reduces the spasticity by increasing presynaptic inhibition of motor neurons [1-2].

Tizanidine Hcl chemically is 5-chloro-N-(2-imidazolin-2-yl) 2,1,3-benzothiadiazol-4-yl-amine (Figure 1). Tizanidine is a short acting drug for the management of spasticity. It is an agonist at a 2-adrenergic receptor sites & presumably reduces spasticity by increasing c inhibition of motor neurons. Aceclofenac chemically is 2-[2-[2-(2,6-Dichlorophenyl) amino phenyl] cetyl] oxyacetic acid (Figure 2). which is used as an effective NSAID having pronounced analgesic, antipyretic, anti-inflammatory property. It is belonging to developed NSAIDS of arylacetic acid type and structurally related to diclofenac. Aceclofenac-Tizanidine combination is more effective than aceclofenac alone and had a favourable safety profile in the treatment of acute low back pain and for rheumatic disorders. TZN & AFN is official in Indian Pharmacoepoeia 2007 respectively [3-6].

Literature review reveals that, Tizanidine also reported in combination with other drugs Similarly, Aceclofenac is reported for spectrophotometric, RP-HPLC and simultaneous estimation with other combinations [7-12]. Since no spectrophotometric method is reported for simultaneous estimation of Tizanidine and Aceclofenac in combination therefore, the present work, a successful attempt has been made to estimate both these drugs simultaneously by to simple RP-HPLC methods development. The present study aimed to develop a simple, sensitive, short retention time and accurate RP-HPLC method for the simultaneous determination of both Tizanidine and Aceclofenac together in pure and tablet dosage forms with high sensitivity, selectivity that can be used for the routine analysis of production samples. Validation of the developed method done in accordance with ICH guidelines. [13]

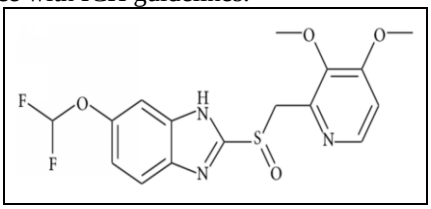

Figure 1: Structure of Tizanidine

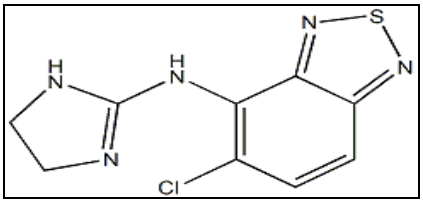

Figure 2: Structure of Aceclofenac

MATERIALS AND METHODS

Materials and Reagents

The analysis of the drug was carried out on Agilent (S.K.) gradient system UV detector. Equipped with reverse phase (Agilent) C_{18} column (4.6mm x 100mm; 2.5µm), a

SP930d pump, a 20µl injection loop and UV 730d (dad) absorbance detector and running Chemstation software.
Tizanidine and Aceclofenac were procured from R.S.I.T.C Jalgaon. Orthophopsphoric acid (OPA) (Avantor Performance material India Ltd. Thane, Maharashtra) and methanol, acetonitrile, (HPLC grade Merck Specialties Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai.), water, 0.45 µm filter (Millipore, Bangalore). A combination of Tizanidine 2 mg and 100 mg Aceclofenac in tablet formulation was procured from Intra Lab. India Pvt. Ltd. (Acent-TZ 100 : 2 mg brand).

Chromatographic Conditions

Column C_{18} (100 mm× 4.6 mm); particle size packing 2.5 µm ; detection wavelength of 301 nm; flow rate 0.7 ml/min; temperature ambient; sample size 20 µl; mobile phase Methanol : water (75 : 25) (pH 3 adjust with 0.1% OPA); run time of 15 mins.

Preparation of standard stock solution

Preparation of std. Tizanidine solution (Stock I)

An accurately weighed quantity, 2 mg of Tizanidine (TZN) was dissolved in methanol in a 10 ml volumetric flask and volume made up to 10.0 ml to produce a solution of 1000 µg/ml. From the freshly prepared standard stock solution (200 µg/ml), 0.1ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration of 10 µg/ml.

Preparation of std. Aceclofenac solution (Stock II)

An accurately weighed quantity, 100 mg of Aceclofenac (AFN) was dissolved in methanol in 10 ml volumetric flask and volume made up to 10.0 ml to produce a solution of 40 µg/ml From the freshly prepared standard stock solution (10000 µg/ml), 0.1 ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 0.4 µg/ml.

Preparation of std. Tizanidine and Aceclofenac solution (Stock III)

From the freshly prepared standard stock solution (200 & 10000 µg/ml), 0.1 ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 2-10 & 100-500 µg/ml. (Table 1 and Figure 3) respectively.

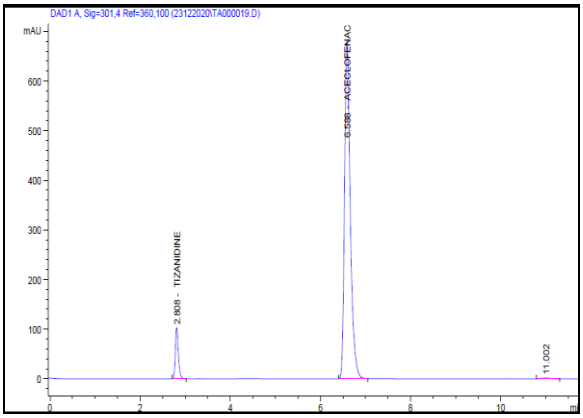

Figure 3: Chromatogram of standard combination of Tizanidine and Aceclofenac

Table 1:

Details of chromatogram of standard combination containi ng AZN and TMZ

Drug name	R.T	AREA	SYMM	TH.PLATES
TZN	2.80	474.71	0.71	9358
AFN	6.58	6038.10	0.69	14227

Method development and validation

Serial dilutions were done to prepared various concentration stock (Standard solution and diluted to get required concentration for calibration plot and which was injected [14-16].

Assay preparation for commercial formulation

Weigh 20 Tizanidine and Aceclofenac combination tablets and calculated the average weight, accurately weigh and transfer the sample equivalent to 2 mg Tizanidine and 100 mg Aceclofenac into 10 ml volumetric flask .Add about 10ml ACN of diluent and sonicate to dissolve it completely and make volume up to the mark with diluents. Mix well and filter through 0.45 µm filter. Further pipette 0.1ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents. (10 µg/ml). The simple chromatogram of test Tizanidine and Aceclofenac Shown in (Figure 4) the amounts of Tizanidine and Aceclofenac per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Tablet Assay for %Label claim for % RSD Calculated, Result was shown in Table 2.

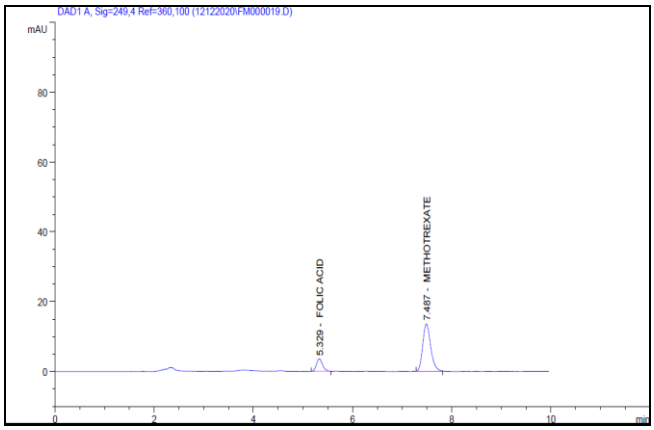

Figure 4: Chromatogram for marketed formulation

Table 2: Analysis of marketed formulation

Assay	Drug	Amt. Found	%Label Claim	SD	%RSD
Rp-HPLC Method	TZN	5.99	99.95	0.30	0.30
	ACF	301.09	100.36	0.94	0.94

RESULTS

Linearity and Range

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 2-10 µg/mL for Tizanidine and 100-500 µg/mL for

Aceclofenac Table 3 and 4 depict the calibration data of Tizanidine and Aceclofenac The respective linear equation for Tizanidine was y = 79.227 X – 0.36 and Aceclofenac equation y = 19.918 X + 43.378 where x is the concentration and y is area of peak. The correlation coefficient was 0.999 and 0.999. The calibration curve of Tizanidine and Aceclofenac is depicted in Figure 5 and 6.

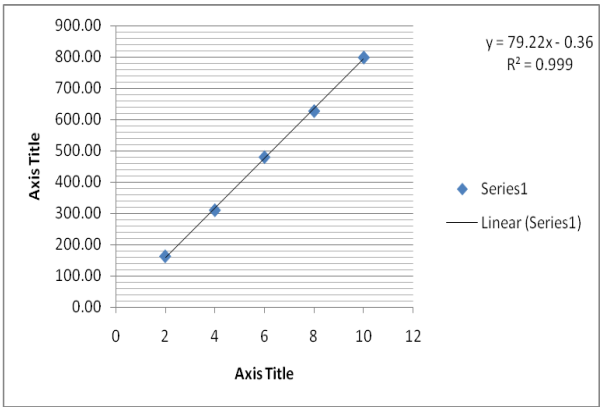

Figure 5: Calibration curve of Tizanidine

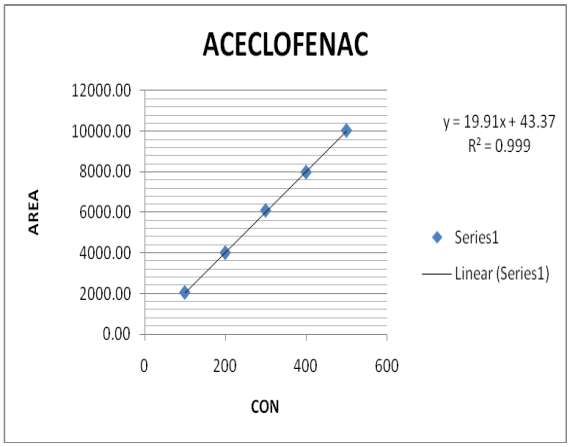

Figure 6: Calibration curve of Aceclofenac

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To a pre-analysed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed**.** The % recovery was found to be within 98-101%. Statistical validation of recovery studies are shown in Table 5, 6 and Fig. 7, 8 and 9.

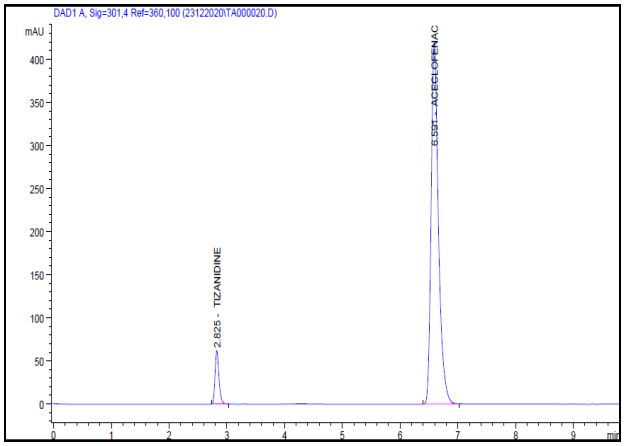

Figure 7: Chromatogram of Accuracy 80%

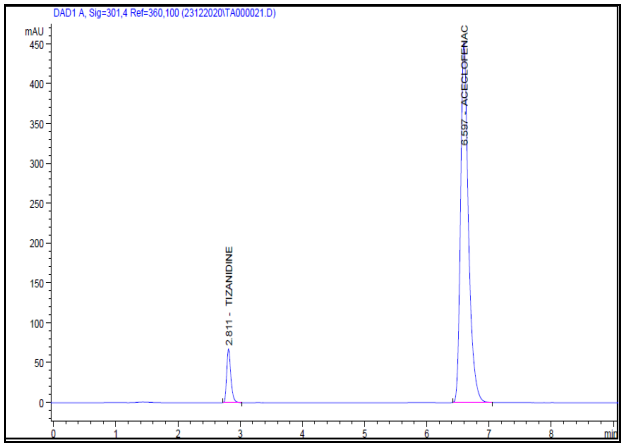

Figure 8: Chromatogram of Accuracy 100%

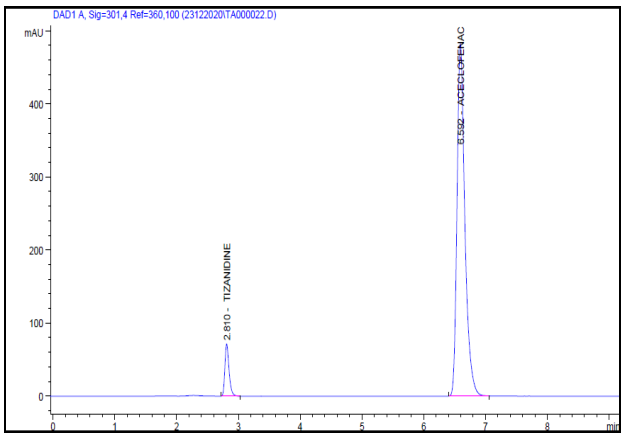

Figure 9: Chromatogram of Accuracy 120%

Table 3: Linearity data for Tizanidine

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
UHPLC Method	2	163.44	162.37	162.91	0.76	0.46
	4	310.1000	309.4900	309.80	0.43	0.14
	6	478.6900	479.6200	479.16	0.66	0.14
	8	627.0200	625.2200	626.12	1.27	0.20
	10	797.2400	796.7900	797.02	0.32	0.04
	Equation		y = 79.227 x – 0.36			
	R^2		0.999			

Table 4: Linearity data for Aceclofenac

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
UHPLC Method	100	2040.92	2037.61	2039.27	2.3405	0.1148
	200	3996.3900	3997.7700	3997.08	0.9758	0.0244
	300	6076.3500	6081.7100	6079.03	3.7901	0.0623
	400	7960.9000	7963.9700	7962.44	2.1708	0.0273
	500	10007.9000	10022.8000	10015.35	10.5359	0.1052
	Equation		Y= 19.918X + 43.378			
	R^2		0.999			

Table 5: Result of Recovery data for Tizanidine and Aceclofenac

Drug	Level (%)	Amt. taken (µg/ml)	Amt. Added (µg/ml)	Absorbance Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
TZN	80%	2	1.6	3.67±0.006	1.67±0.006	104.39±0.42
	100%	2	2	4.00±0.009	2.58±0.003	99.15±1.27
	120%	2	2.4	4.37±0.002	2.58±0.003	100.03±1.46
AFN	80%	100	80	180.48±0.08	80.48±0.08	100.59±0.10
	100%	100	100	199.33±0.07	99.33±0.07	99.33±0.07
	120%	100	120	219.42±0.02	119.42±0.02	99.51±0.02

***mean of each 3 reading for RP-HPLC method**

Table 6: Statistical Validation of Recovery Studies Tizanidine and Aceclofenac

Method	Level of Recovery (%)	Drug	% RSD	Standard Deviation*	Mean % Recovery
RP-HPLC Method	80%	TZN	0.006	0.006	104.39
		AFN	0.009	0.003	99.15
	100%	TZN	0.002	0.003	100.03
		AFN	0.08	0.08	100.59
	120%	TZN	0.07	0.07	99.33
		AFN	0.02	0.02	99.51

***Denotes average of three determinations for RP-HPLC method**

System suitability parameters

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of TZN and AFN system suitability parameters were studied. The result shown Figure 10 and Table 7.

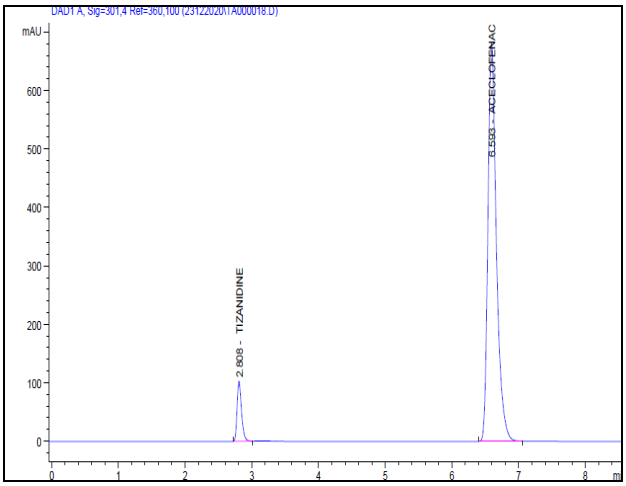

Figure 10: Chromatogram of system suitability

Table 7: Repeatability studies on RP-HPLC for

Method	Conc. of TNZ & AFN (mg/ml)	Peak area	Amount found (mg)	% Amount found
HPLC TZN Method	5	472.66	5.98	99.69
	5	474.28		
	Mean	473.47		
	SD	0.14		
	%RSD	0.24		
HPLC AFN Method	300	6043.7	301.38	100.46
	300	6044.23		
	Mean	6043.96		
	SD	0.96		
	%RSD	0.02		

Tizanidine and Aceclofenac

Precision

The method was established by analyzing various standards of AZN and AFN. All the solution were analyzed thrice in order to record any intra-day & inter-day variation in the result. The result obtained for interday and intraday variation are shown in the Table 8 and Figure 11.

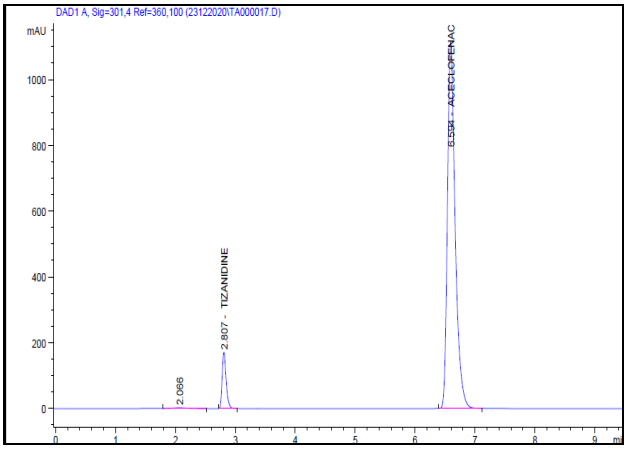

Figure 11: Chromatogram of Precision

Robustness

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate on retention time and tailing factor of drug peak was studied. The results indicate that less variability in retention time and tailing factor were observed Table 9.

DISCUSSION

The proposed methods for simultaneous estimation of AZN and AFN in tablet dosage forms were found to be simple, accurate, economical and rapid. The method was validated as per the ICH Q2 (R1) guidelines. Standard calibration yielded correlation coefficient (r^2) 0.999 for both AZN and TMN at all the selected wavelengths. The values of % RSD are within the prescribed limit of 2 %, showing high precision of methods and recovery was close to 98%-101% for both drugs. Results of the analysis of pharmaceutical formulations reveal that the proposed method is suitable for their simultaneous determination with virtually no interference of any additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully for simultaneous estimation of AZN and AFN in formulations.

CONCLUSION

The developed HPLC methods in that linearity, precision, range, robustness were found to be more accurate, precise and reproducible. The methods were found to be simple & time saving. All proposed methods could be applied for routine analysis in quality control laboratories.

Acknowledgements

The authors are thankful to the Principal, Gangamai College of Pharmacy, Nagaon, Dist. Dhule for providing necessary facilities for research work. They are also grateful to R.S.I.T.C Jalgaon for giving gift samples of pure drugs.

Table 8: Intraday and Inter day Precision studies on RP-HPLC method for TZN and AFN

Method	Drug	Conc. (µg/ml)	Interday Precision		Intraday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
RP-HPLC Method	TZN	2	160.62±0.96	101.61	160.62±0.96	101.61
		6	475.16±1.39	100.04	475.16±1.39	100.04
		10	794.49±0.25	100.34	794.49±0.25	100.34
	ACF	100	2025.47±0.96	99.55	2021.47±0.96	99.35
		300	6046.96±0.96	100.51	6044.04±096	100.46
		500	9997.33±0.82	99.99	9995.78±0.63	99.97

***Mean of each 3 reading for RP-HPLC method**

Table 9: Result of Robustness Study of Tizanidine and Aceclofenac

Parameters	Conc. (µg/ml)	Amount of detected (mean ±SD)	% RSD	Amount of detected (mean ±SD)	% RSD
		For TZN		For AFN	
Chromatogram of flow change 0.7 ml	4+200	359.78±2.06	0.57	4573.28±0.25	0.07
Chromatogram of flow change 0.9 ml	4+200	1423.35±0.84	0.65	3452.93±0.26	0.09
Chromatogram of comp change wavelength change 300 nm	4+200	283.9±1.49	0.53	4271.6±0.19	0.07
Chromatogram of comp change wavelength change 302 nm	4+200	338.97±2.69	0.79	3628.11±0.98	0.03
Chromatogram of mobile phase change 74+26 ml	4+200	311.0±2.20	0.71	3933.0±0.4	0.10
Chromatogram of mobile phase change 76+24 ml	4+200	313.35±2.34	0.75	1710.49±0.23	0.07

Conflict of Interest

Authors have no conflicts of interest to declare.

Abbreviation used: HPLC: High performance liquid chromatography; **UV:** Ultraviolet; **ICH:** International Conference on Harmonization; **LOQ:** Limit of quantitation; **LOD:** Limit of detection; **RSD:** Relative standard deviation; **RT:** Retention time; **OPA:** Orthophosphoric acid; **AZN:** Tizanidine; **AFN:** Aceclofenac; **FDA:** Food and Drug Administration; **SD:** Standard deviation.

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