International Journal of Farmacia

Journal Home page: www.ijfjournal.com

Analytical method development and validation for the estimation of Nimesulide and Tizanidine using RP-HPLC

Kaifi Azmath[1*], R. Vani[2*]

Deccan School of Pharmacy, Aghapura, Dar-Us-Salam, Hyderabad, Telangana

Corresponding Author: Kaifi azmath

Email: kaifiazmath@gmail.com

ABSTRACT

A simple and selective HPLC method is described for the determination of Tizanidine and Nimesulide in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 35 volumes of water and 65 volumes of Methanol with detection of 243nm. Linearity was observed in the range 20-100 µg/ml for Tizanidine (r^2 =0.999) and 20-100 µg /ml for Nimesulide (r^2 =0.999) for the amount of drugs estimated by the proposed methods were in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form**.**

Keywords: Nimesulide and Tizanidine **,** Reverse phase HPLC.

INTRODUCTION

Nimesulide is chemically named as N-(4-nitro-2-phenoxyphenyl) methane sulfonamide [1]. Tizanidne chemically named as 5-chloro-N-(4,5-dihydro-1H-imidazol-2-yl)-2,1,3-benzothiadiazol-4-amine [2]. Arthritis is a form of joint disorder that involves inflammation in one or more joints. The most common form of arthritis is osteoarthritis (degenerative joint disease), a result of trauma to the joint, infection of the joint, or age [3]. Nimesulide is a relatively COX-2 selective, non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties [4]. Its approved indications are the treatment of acute pain, the symptomatic treatment of osteoarthritis and primary dysmenorrhoea. Tizanidine is a drug that is used as a muscle relaxant [5]. It is a centrally acting $\alpha_2$adrenergic agonist [6]. It is used to treat the spasms, cramping, and tightness of muscles caused by medical problems such as multiple sclerosis, spastic diplegia [7], back pain. Zulu (Tizanidine/Nimesulide) exhibits muscle relaxant and pain relieving properties [8, 9]. Its mechanism of action is interfering with the pain sensations sent to the brain and inhibiting the synthesis of cyclooxygenase [10].

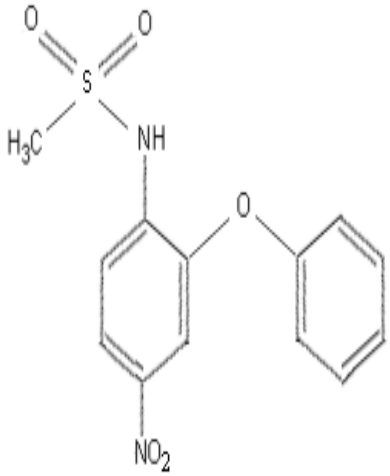

Fig 1: Nimesulide

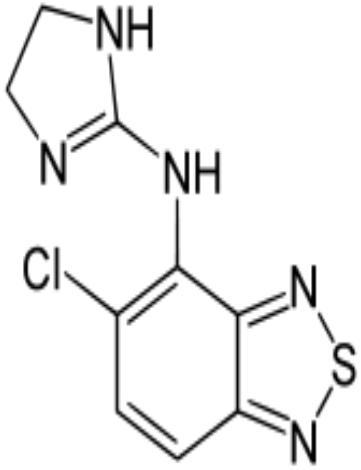

Fig 2: Tizanidine

EXPERIMENTAL WORK

Chemicals

Nimesulide and Tizanidine were obtained as gift sample from Chandra Pharma Research Laboratory in Hyderabad and marketed formulation was purchased from local market.

Instrument and chromatographic condition

All solvent used in this work are HPLC & AR grade. Instrument and chromatographic condition RP-HPLC Agilent 1220 infinity series separation model equipped with UV Detector was employed in this method. The EZchrom software was used for LC peak integration along data acquisition and data processing. The column used for separation of analyte is *Hypersil ODS (*150×4.6mm) 5µ. Mobile phase consisting of Methanol: water (65:35) v/v at flow rate 1 ml/min. it was filter through 0.45 µm nylon filter and sonicated for 5 min in ultrasonic bath. Sample was analyzed at 243 nm at an injection volume of 20µl.

Preparation of phosphate buffer

1.625 gm of Potassium Di Hydrogen ortho phosphate and 0.3 gms of Di Potassium Hydrogen ortho phosphate was weighed and dissolve in 100 ml of water and volume was made up to 550 ml with water. Adjust the P^H using ortho phosphoric acid .The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Preparation of Solutions

Preparation of mixed standard solution

Weigh accurately 4mg of Tizanidine and 100 mg of Nimesulide in 100 ml of diluents. From above stock solution 4µg/ml of Tizanidine and 100ug/ml Nimesulide is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram, and also as working standard concentration.

Preparation of Sample Solution

5 tablets (each tablet contains Nimesulide 100 mg and Tizanidine -4 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Nimesulide and Tizanidine (µg/ml) were prepared by dissolving weight equivalent to 10 mg of Nimesulide and 20 mg of Tizanidine and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of Nimesulide and Tizanidine was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Table 1: Summary of Chromatographic conditions

S.No.	Parameter	Description/value
1.	Stationary phase	Column ,Inertsil ODS 3V(250x4.6mm)5µm
2.	Mobile phase	Methanol: water(65:35)
3.	Detection wavelength	243nm
4.	pH	4.1
5.	Retention time	Tizanidine-2.513, Nimesulide -5.197
6.	Injection volume	20µl
7.	Run time	8 mins

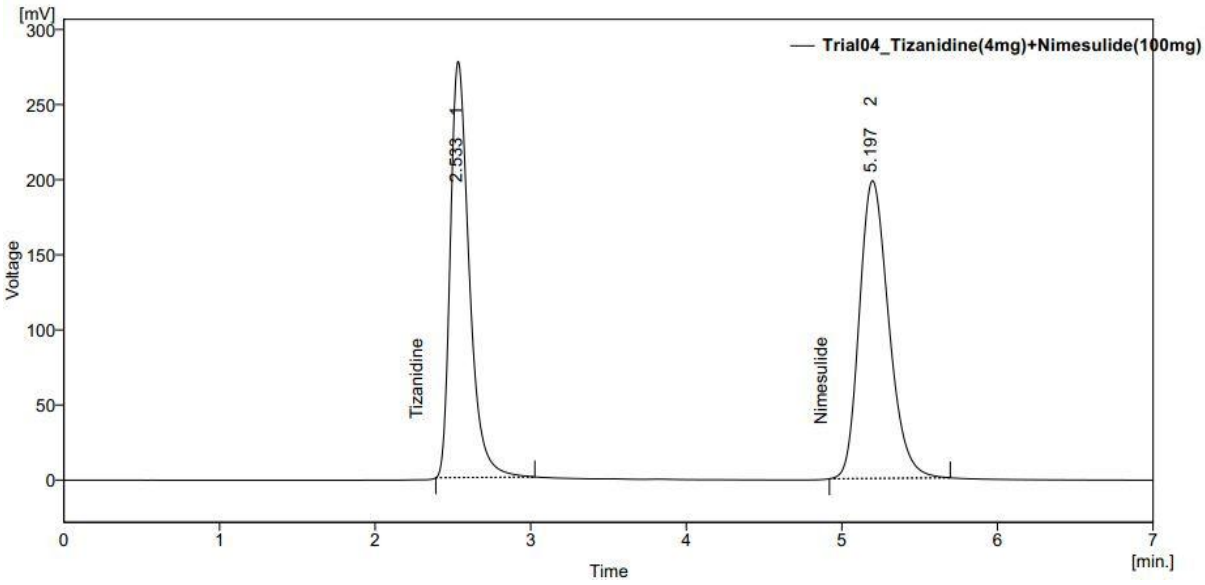

Fig 3: Typical chromatogram of Tizanidine and Nimesulide

Method validation

The validation of method was carried out as per ICH guideline. The parameters assessed were specificity, linearity, precision, accuracy, stability, LOD and LOQ. Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences including degradation product and related substances.

Accuracy

The accuracy was determined by calculating % recoveries of Tizanidine and Nimesulide. It was carried out by adding known amount of each analyte corresponding to three conc. Levels (50, 100, and 150) of the label claims to the excipients. At each level, six determinations were performed and the accuracy results were expressed as percent analyte recovered by proposed method.

Precision

Method precision

Precision of an analytical method is usually expressed as the standard deviation. Method precision was demonstrated by preparing six samples as per test method representing single batch and were chromatographed. The precision of the method was evaluated by computing the %RSD of the results. The individual values and the low % RSD observed on the values are within acceptance criteria and indicates that method is precise.

Linearity

The purpose of the test for linearity is to demonstrate that the entire analytical system (including detector and data acquisition) exhibits linear responses and directly proportional over the relevant conc. Range for the target conc. of the analyte. The linear regression data for the calibration plot is the indicative of a good linear relationship between peak and concentration over wide range. The correlation coefficient was indicative of high significance.

Robustness

Robustness of method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase, flow rate and temperature. This deliberate change in the method has no effect on the peak tailing, peak area and theoretical plates finally the method was found to be robust.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts. % RSD Assay values between two analysts not greater than 2.0%, hence the method was rugged.

Limit of Detection and Limit of Quantitation

The LOD can be defined as the smallest level of analyte that gives a measurable responses and LOQ was determined as the lowest amount of the analyte that was reproducibly quantified. These two parameters were calculated using formula based on standard deviation of the response and slope. LOD and LOQ were calculated by equation, LOD=3.3 x σ/s and LOQ= 10 x σ/s, where s = standard deviation, S = slope of calibration curve

Assay of Nimesulide and Tizandine in pharmaceutical dosage form

Assay of marketed product was carried out by using the developed method. Sample solutions were prepared and injected into RP –HPLC system. The sample solution was scanned at 243 nm. The percentage drug estimated was found to be 100.22% and 99.97% respectively as Tizanidine and Nimesulide. The chromatogram showed two single peaks of Tizanidine and Nimesulide was observed with retention time 2.519 and 5.19 min respectively.

Forced degradation studies

Stress studies are performed according to ICH guidelines under following conditions.

Acid degradation

To 5 ml of sample solution add 1 ml of 0.1 N HCL and sonicate place it aside for 3 hrs, then neutralize the solution with 1ml of base and then transfer above solution into 10 ml volumetric flask dilute with mobile phase and record the chromatogram.

Alkaline degradation

To 5 ml of sample solution add 1 ml of 0.1 N NaOH place it aside for 3 hrs, then neutralize the solution with 1ml of acid and then transfer above solution into 10 ml volumetric flask dilute with mobile phase and record the chromatogram.

Peroxide degradation

To 5 ml of sample solution add 1 ml of 3% H_20_2 and sonicate place it aside for 3 hrs, then transfer above solution into 10 ml volumetric flask dilute with mobile phase and record the chromatogram.

Photolytic degradation

Expose about 100 mg of sample in UV light chamber at 243 nm for 3 hrs. weigh accurately this power equivalent to 40 mg of Nimesulide and 10 mg of Tizanidine into a 100 ml volumetric flask and make up the volume and sonicate for 30 minutes with intermittent shaking and volume is made up to the mark with mobile phase and record the chromatogram.

Thermal degradation

Expose about 100 mg of sample in to dry heat at 80^0C for 3 hrs. weigh accurately this power equivalent to 40 mg of Nimesulide and 10 mg of Tizanidine into a 100 ml volumetric flask and make up the volume and sonicate for 30 minutes with intermittent shaking and volume is made up to the mark with mobile phase and record the chromatogram.

Record the peak area of stressed samples then compares it with peak area of unstressed sample to determine the % degradation.

$$\% \text{ degradation} = \frac{\text{Response of unstressed sample} - \text{response of stressed sample}}{\text{Response of unstressed sample}} \times 100$$

RESULTS AND DISCUSSION

Nimesulide and Tizanidine combination is used in the treatment of muscle relaxant and pain relieving properties, cramping, and tightness of muscles.

Literature survey reveals that various methods for the estimation of Nimesulide and Tizandine individually and in combination with other drugs is also reported, So here an attempt has been made to develop simple, accurate, sensitive, rapid and economic method for estimation of Nimesulide and Tizanidine from combine dosage form using HPLC. Specific objectives includes with less flow rate.

The conditions in HPLC were optimized in order to obtain the drugs separation of eluted compounds. Initially various composition of mobile phase & PH range were tried in order to have a good separation of the titled ingredients. The composition of Mobile phase & PH selections were based on peak parameters like height, tailing, capacity factor, symmetric factor, theoretical plates, run plates, run time & resolution. The system with Methanol: water(65:35) was found to be robust with PH 4.1. The optimum wavelength for detection was 243 nm at which both drugs have good response. System suitability test were carried out on a freshly prepared stock solution % RSD of peak area of five replicated injections of standards were taken & was found to be less than 2%. Specificity of the method developed was performed & it was found that there is no interference of excipients with the analyte which indicate that the method is specific for the analysis of the analytes in their dosage form. Assay was determined for both Standard & sample solution & the % assay was found to be 100.22 and 99.97%.and respectively. Calibration curves was found to be linear in the concentration range of 40-140 µg/ml for NIM and TIZ . Accuracy studies of the method developed wad determined by spiking the known amount of analyte to sample solution and the percentage mean recovery was found to be 100.22% and 99.97% for TIZ and NIM respectively. Precision of the method & system were found to be within limits. Limit of detection was found to be 3.63µg/ml and 2.34µg/ml for NIM & TIZ respectively and Limit of Quantification was found is to be 7.09µg/ml &9.11µg/ml for NIM & TIZ respectively. Robustness of the method was determined by varying flow rate and wavelength & Ruggedness of the method was determined by carrying out the determination by two different analyst.

Samples containing Nimesulide and Tizanidine were subjected to various stress conditions and it was found that overall net degradation was within the limits without any significant degradation products.

A RP-HPLC method developed for Nimesulide and Tizanidine shows that the results obtained for RP-HPLC are promising with better resolution in set chromatographic conditions. The developed methods were statistically validated which suggested that the methods were within the acceptable limits hence these methods can be used for the routine determination of Ceftazidime and Avibactam in bulk drug and pharmaceutical formulation.

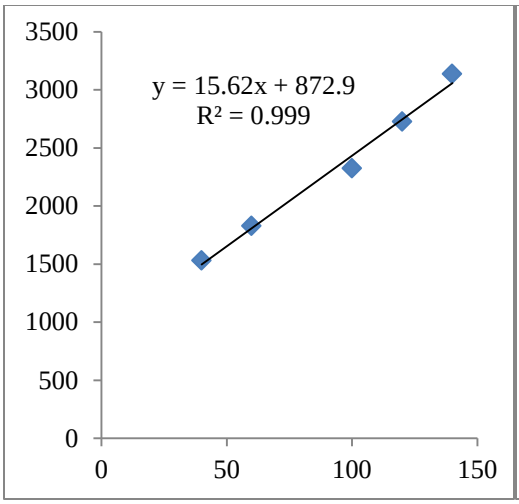

Fig 4: Linearity graph of Tizanidine

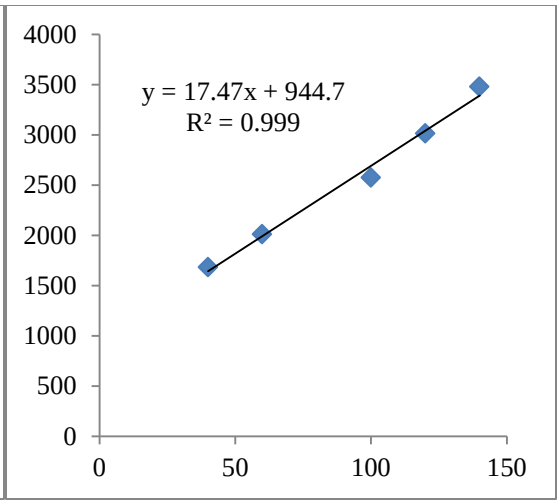

Fig 5: Linearity graph of Nimesulide

Table 2: Results of Tizanidine

S. No.	Conc. (µg/ml)	Area
1	40	1530.419
2	60	1829.682
3	100	2325.506
4	120	2728.038
5	140	3138.275

Table 3: Results of Nimesulide

S. No.	Conc. (µg/ml)	Area
1	40	1681.599
2	60	2011.338
3	100	2575.330
4	120	3014.365
5	140	3479.875

Table 4: Results for Method precision of Tizanidine and Nimesulide

Tizanidine			**Nimesulide**		
S. No	**Retention time**	**Area**	**S. No**	**Retention time**	**Area**
1	2.520	2307.992	1	5.190	2567.676
2	2.517	2310.290	2	5.203	2538.698
3	2.517	2323.689	3	5.203	2551.448
4	2.503	2310.647	4	5.177	2556.486
5	2.533	2334.444	5	5.197	2555.680
6	2.527	2330.088	6	5.190	2577.911
Average	2.519	2319.525	Average	5.193333	2557.983
SD	0.010232	-	SD	0.009893	-
% RSD	0.405393	-	% RSD	0.190109	-

Recovery results of Tizanidine

Table 5: Recovery results for Tizanidine

Recovery level	Accuracy Tizanidine					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered	%Recovery	
50%	2	2319.455	2321.82	2	100.00	
	2	2338.467				
	2	2307.566				
100%	4	2728.038	2733.146	4.5	105.94	
	4	2728.038				104.74
	4	2743.362				
150%	6	3138.275	3142.61	7.89	108.28	
	6	3140.528				
	6	3149.047				

Table 6: Recovery results for Nimesulide

Recovery level	Accuracy Nimesulide					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered	%Recovery	
50%	80	2563.423	2569.571	80.56	100.33	
	80	2577.055				
	80	2568.237				
100%	100	3014.365	3016.010	103.78	117.76	
	100	3014.365				109.08
	100	3019.302				
150%	120	3479.875	3494.181	151.96	109.15	
	120	3488.541				
	120	3495.127				

Observation

The percentage mean recovery of Tizanidine and Nimesulide is 104.74% and 109.08 % respectively.

Table 7: Results for precision of Tizanidine and Nimesulide

Tizanidine			Nimesulide		
S. No	Retention time	Area	S. No	Retention time	Area
1	2.520	2307.992	1	5.190	2567.676
2	2.517	2310.290	2	5.203	2538.698
3	2.517	2323.689	3	5.203	2551.448
4	2.503	2310.647	4	5.177	2556.486
5	2.533	2334.444	5	5.197	2555.680
6	2.527	2330.088	6	5.190	2577.911
Average	2.519	2319.525	Average	5.193333	2557.983

SD	0.010232	-	SD	0.009893	-
% RSD	0.405393	-	% RSD	0.190109	-

Table 8: Result of Robustness study

	Tizanidine		Nimesulide	
Parameter	**Retention time (min)**	**Tailing factor**	**Retention time (min)**	**Tailing factor**
Flow Rate				
0.8 ml/min	3.073	1.647	6.277	1.647
1.2 ml/min	2.147	1.640	4.377	1.381
Wavelength				
241nm	2.523	1.643	5.163	1.404
243nm	2.523	1.643	5.163	1.404

Table 9: Results for Ruggedness

Tizanidine	**% Assay**	**Nimesulide**	**% Assay**
Analyst 01	99.09	**Analyst 01**	101.56
Analyst 02	99.09	**Analyst 02**	99.22

Table 10: Results of Forced Degradation studies

Stress condition	**Peak Area**		**% Degradation**	
	TIZ	**NIM**	**TIZ**	**NIM**
Unstressed sample	2192.417	267.545	0	0
Acid	3981.699	391.641	0.81	0.46
Alkaline	3934.512	396.009	0.79	0.48
Oxidation	3943.709	391.379	0.79	0.46
Photolytic	3951.770	397.622	0.80	0.49
Thermal	3971.642	385.227	0.81	0.43

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Tizanidine and Nimesulide was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department and in industries, approved testing laboratories studies in near future. Forced degradation studies carried out are helpful to determine stability of this drug in combination. Forced degradation studies were carried under various stress conditions. It was found to be stable and the net degradation was within the limits without any significant degradation products.

REFERENCES

[1]. **Chatwal G.R., Anand S.K.,** Instrumental methods of chemical analysis, Himalaya Publishing House, Mumbai, 5, 2.566, 2.626- 2.636.

[2]. **Kaushal C, Srivastava B,** A Process of Method Development: A Chromatographic Approach. J Chem Pharm Res, 2(2), **2010,** 519-545.

[3]. **Willard,** "Instrumental Methods of Analysis "CBS Publisher and Distributors, Delhi. 7, 514

[4]. **Patel RM, Patel PM, Patel NM,** Stability Indicating HPLC Method Development- A Review, Int Res J Pharmacy 2(5), **2011,** 79-87.
[5]. **Loyd R. Synder, Joseph j. krikland ans Joseph L.Glajch**, Practical HPLC Method Development; 2, 1997, 1-14, 14-16, 40, 202, 206, 253.
[6]. **Douglas, A.; Skoog, F.; James, H.; Stanley**, R. C. Liquid Chromatography. Instrumental Analysis, Cengage Learning India Pvt. Ltd.: New Delhi, 9, 2007, 893 - 934.
[7]. **Skoog; Holler; Crouch;** Liquid Chromatography**.** Instrumental Analysis**,** Cengage Learning India**.** New Delhi**.** 2011, 893**.**
[8]. **Chung Chow Chean, Herman Lam, Y.C. Lee, and Xue-Ming Zhang**, Analytical Method Validation and Instrumental Performance Verification, John Wiley & Sons, 2004
[9]. **P.P. Sharma,** Validation in Pharmaceutical Industry, Concepts, Approches & guidelines, Vandana Publications, Delhi, 1, 365-376
[10]. ICH, Q2A, Text on Validation of Analytical Procedures, International Conference on Harmonization, **1994**, Geneva.
[11]. Sillén H**,** Mitchell R**,** Sleigh R**,** Mainwaring G**,** Catton K**,** Houghton R**,** Glendining K, International Journal of Pharma and Bioanalysis 7(12), 2015, 1423-34
[12]. Beaudoin ME**,** Gangl ET**.** US National Liberary of Medicine National Institutes of Health; Bioanalysis 8(2), 2016, 111-22.
[13]. Moreno Ade H**,** Salgado HR. Journal of AOAC 91(4), 2008, 739-43.
[14]. **Andréia de Haro Moreno, Hérida Regina Nunes Salgado**. Scientific And Academic Publishing, Physical Chemistry, 2(1), 2012, 6-11.
[15]. **B. Hiiremath and B. H. M. Mruthyun jayaswam,** International Research Journal of Pharmacy, 4(11), 2013
[16]. http://www.drugbank.ca/drugs/DB00438 Drug Bank: Nimesulide (DB00438)
[17]. https://www.drugbank.ca/drugs/DB09060 Drug Bank: Tizanidine(DB09060)
[18]. Huynh-Ba K., (2009), Handbook of Stability Testing in Pharmaceutical Development, Springer Science, LLC 233 Spring street, Newyork, NY 100313, USA Pages 17-35
[19]. Yoshioka S., Stella J.V. Stability of Drugs And Dosage Forms, Kluwer Academic Publishers, Newyork, 2002, 3-15, 61-105.