ISSN : 0974-7419 Volume 12 Issue 11

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 12(11) 2013 [408-414]

Development and validation of a rapid HPLC method for the simultaneous estimation of nimesulide and tizanidine hydrochloride in pharmaceutical tablet dosage form

Nitin D.Rawool*, A.Venkatchalam

Department of Chemistry, Bhavan's College, Andheri (W), Mumbai-400058, (INDIA)

E-mail : nitin_rawool@yahoo.co.in

ABSTRACT

The objective of the present study was to develop a simple, precise and accurate reversed-phase HPLC method and subsequent validation of the same as per the ICH guidelines. The present study deals with the estimation by RP HPLC of two different drug components nimesulide (NMS) and tizanidine hydrochloride (TZN) present in a tablet formulation.

The chromatographic separation of NMS and TZN was done using phosphate buffer along with methanol as mobile phase, in the proportion of 50: 50. The separation is done on a C_{18} column and it is estimated at a λ max of 250 nm with a flow rate of 1ml/min. The retention times were about 3.7 and 9.3 min for TZN and NMS respectively. The specificity for interference of any peak with main peak of interest is checked. The repeatability was checked by system precision with relative standard deviation less than 1% in all instances, along with method reproducibility for TZN and NMS. The linearity ranges from a 10.02 to 30.07 mg/ml for TZN and 500.60 to 1501.80 mg/ml for NMS respectively. Correlation coefficients (*r*) of the regression equations were greater than 0.999 in all cases. The system suitability by precision is also checked to ensure that the analytical method is precise. The precision of the method was demonstrated using intra- and inter-day assay R.S.D. values which were less than 1%. The method was found to be accurate and precise for estimation of the two drugs simultaneously.

According to the validation results, the proposed method was found to be specific, accurate, precise and rapid. Hence the same can be applied to the quantitative analysis of tablets containing NMS–TZN binary mixtures.

KEYWORDS

ICH guidelines;
RP-HPLC;
Simultaneous;
Validation;
Nimesulide;
Tizanidine.

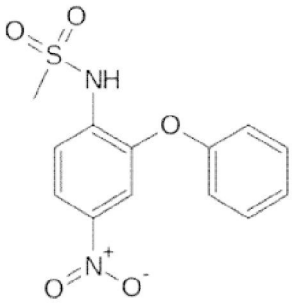
INTRODUCTION

Nimesulide is chemically N-(4-nitro-2-phenoxyphenyl) methane sulphonamide (Figure 1) and is official in the British Pharmacopoeia[1]. NMS is an antiinflammatory drug. NMS is yellow needle- like crystals, with a melting point of 143 °C to 144 °C. The molecular weight of NMS is 308.31 g/mol & the molecular formula is C13-H12-N2-O5-S. NMS is a selective COX-2 inhibitor used in a variety of inflammatory, pain and fever states. After oral administration the drug is rapidly and extensively absorbed. It is approved

for used in treatment of musculoskeletal disorder, dyemenorrhoea, thrombophlebitis and dental pain, inflammation. Some HPLC[2,3] and spectrophotometric[4,5] methods have been reported in the literature for its estimation.

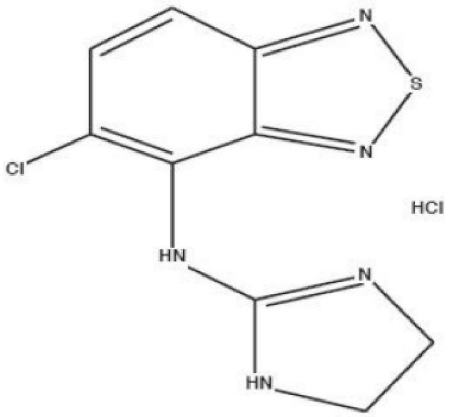
Tizanidine hydrochloride is chemically 5-chloro-N-(4, 5dihydro-1H-imidazol-2yl)-2, 1, 3-benzothiadiazol -4-amine hydrochloride (Figure 2). It is official in The United States Pharmacopoeia[6] and Indian Pharmacopoeia[7]. TZN is a white to off-white, fine crystalline powder, which is odorless or with a faint characteristic odor. TZN is slightly soluble in water and methanol; solubility in water decreases as the pH increases. TZN molecular formula is C9H8ClN5S- HCl and its molecular weight is 290.2 g/mol. TZN is a short-acting drug for the management of spasticity. TZN is an agonist at a2-adrenergic receptor sites and presumably reduces spasticity by increasing presynaptic inhibition of motor neurons. TZN is a skeletal muscle relaxant. Tizanidine is indicated for the treatment of spasticity due to multiple sclerosis and spinal cord injury.

The structure of both drugs is shown in Figure 1 & Figure 2.



Nimesulide (C13-H12-N2-O5-S)

Figure 1 : Structures of nimesulide.



Tizanidine hydrochloride (C9H8ClN5S- HCl)

Figure 2 : Structures of tizanidine hydrochloride.

Literature survey reveals that very few methods are reported for the simultaneous estimation of nimesulide and tizanidine in their combined dosage form[8,9].

There are some HPLC methods available for estimation of single component of drug of TZN in The United States Pharmacopoeia[10], and also in Indian Pharmacopoeia[11].

Analytical methods for simultaneous estimation of NMS and TZN by reverse phase chromatography have been reported[12]. In this method the mobile phase was a mixture of water & methanol with a pH adjustment of 4.15 with orthophosphoric acid. There are also some methods available for the estimation of NMS and TZN by a spectrophotometer using multiple wavelengths[13]. Also there are some spectrophotometric methods with various other combinations of NMS and TZN along with paracetamol[14] & TZN with different combinations[15].

The HPLC methods using the most commonly used mobile phase, shorter run time, easily available columns and simple detectors like the UV detectors are preferred. The present study describes the determination of NMS and TZN by using reverse phase chromatography, a C_{18} column, simpler mobile phase and a UV detector.

The use of HPLC is now a day's very much preferred in routine quality control analysis. It is important that well validated HPLC methods are to be developed for simultaneously estimating NMS and TZN. Hence, a new HPLC method for the simultaneous determination of NMS and TZN from the tablets was developed which is simple, rapid, selective and precise. The aim of this study is development of a simple, precise, rapid and accurate reverse phase HPLC method for the simultaneous estimation of NMS and TZN in pharmaceutical tablet dosage form. The method was validated in compliance with ICH guidelines[16-18].

EXPERIMENTAL

Chemicals and reagents

The pharmaceutical grade tizanidine hydrochloride and nimesulide were provided by the Bhavan's college and were certified to contain 100.16% (w/w) and 99.90% (w/w) respectively. HPLC grade methanol and water was procured from Merck, Mumbai, India. Diba-

sic potassium phosphate AR grade was procured from S. D. Fine Chemical, Mumbai, India. Formulation containing nimesulide 1000 mg and tizanidine 2 mg was procured from the market. All dilutions were performed in standard volumetric flasks.

Instrumentation

A liquid chromatography system consisting of a Shimadzu, VP LC-10AT equipped with a binary solvent delivery pump, column thermostat and UV detector was used for the study. A Rheodyne syringe loading manual injector with a 20 µl sample loop was used for the injection of analyte. The system was controlled, data was collected and processed by Class VP software. The separation was performed at ambient temperature, on reverse phase column used was Waters Bondapak C_{18}, 300 mm, 3.9mm ID, packed with 10 µ particle size.

Chromatographic conditions

To develop a suitable LC method for the analysis of Nimesulide and tizanidine in their combined dosage form, different mobile phases were tried. The criteria employed for selecting the mobile phase for the analyses of the drugs were cost involve, time required for the analysis, better separation of drugs. Preparation of mobile phase: A mixture of monobasic potassium phosphate buffer and methanol was used as mobile phase. Buffer was prepared by accurately weighing 1.36 g of monobasic potassium phosphate in to a 1000 mL volumetric flask, dissolved by adding 500mL of distilled water to it and sonicated. After the salt was completely dissolved, diluted to volume with distilled water. The mobile phase with a mixture of monobasic potassium phosphate buffer, and methanol in the ratio of 50: 50 v/v was prepared. This mobile phase was filtered through 0.45 µ Nylon 6, 6 membrane filter and degassed in ultrasonic water bath. The mobile phase solvent was delivered through HPLC column Waters Bondapak C_{18}, 300 mm, 3.9mm, 10 µ, at a flow rate of 1.0 mL per min. All the experiments were performed in the isocratic mode. Detection of the analytes NMS and TZN was done at a wavelength of 250 nm. Injection volume of the analytes was set at a constant volume of 20 µl by using a fixed sample loop.

A typical HPLC chromatogram for simultaneous determination of NMS and TZN from blank and standard is shown in Figure 3 and Figure 4.

Preparation of standard stock solutions

In a 25ml volumetric flask 25.10 mg of TZN was added and mixed with 10 ml of mobile phase. This solution was sonicated to dissolve completely and diluted to 25 ml with mobile phase to form a solution of 1mg/mL. Similarly, in a 50ml volumetric flask 50.10 mg of NMS was added and mixed with 15 ml of mobile phase and the contents were sonicated to dissolve. Then using a graduated pipette 1 ml of previously prepared 1mg/mL of TZN solution was added to it. This solution was mixed and diluted to 50 ml with the same mobile phase. The final concentration obtained for TZN and NMS was 20.08 ppm and 1002.0 ppm respectively.

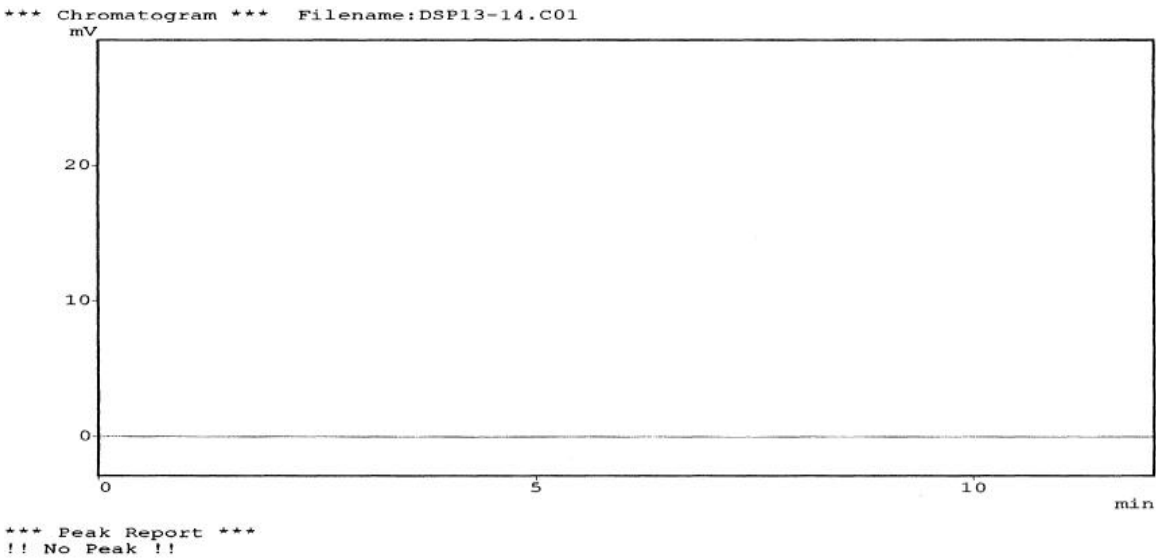
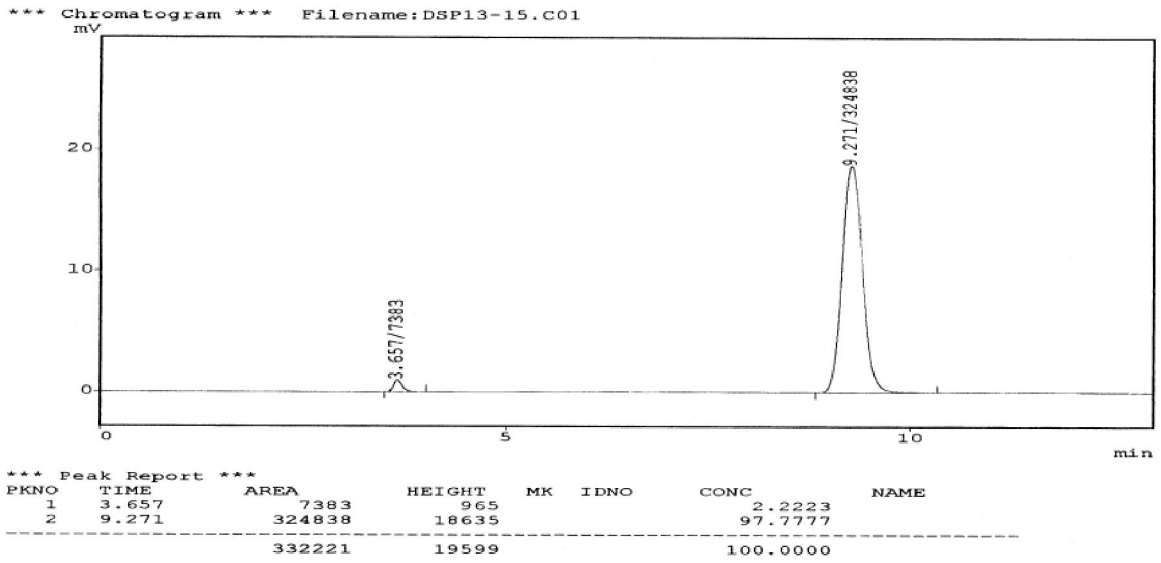


Figure 3 : Chromatogram of blank preparation.



*** Peak Report ***

PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	3.657	7383	965			2.2223	
2	9.271	324838	18635			97.7777	
		332221	19599			100.0000	

Figure 4 : Chromatogram of tizanidine hcl and nimesulide in standard preparation.

Sample preparation

A commercial brand, Nicip T tablet was procured for testing suitability of the proposed analytical method to estimate tizanidine and nimesulide in tablet formulation. The label claim was 2 mg and 100 mg for tizanidine and nimesulide respectively. Twenty tablets were weighed and average weight was determined. These tablets were crushed to a fine powder, and weighed quantity of powder equal to the average weight was transferred in the 100 ml volumetric flasks. Then 50ml of mobile phase is added in this volumetric flask. The contents of the flask were allowed to dissolve with intermittent sonication to ensure complete solubility of the drug. The mixture was diluted to 100ml with mobile phase, thoroughly mixed and then filtered through 0.45 µ nylon filter. The final concentration of the solution was 20 ppm for TZN and 1000 ppm for NMS. A 20 µl of each of this solution was injected into the HPLC system. The drug content in the test preparation was quantified by comparing with the known amount of standard injected. The amounts of TZN– NMS in binary mixtures or dosage forms were individually calculated using the related linear regression equations.

RESULTS AND DISCUSSION

System suitability

System suitability tests are used to verify that the reproducibility of the equipment is adequate for the analysis to be carried out. System suitability tests were performed as per the USP to confirm the suitability and reproducibility of the system.

Twenty microlitres of the above standard solution of TZN and NMS was injected each time in to the stream of mobile system at a flow rate of 1 ml/min. The solution was injected six times into the column and the corresponding chromatograms were obtained. From these chromatograms the area under the peaks and respective retention time of the drug were noted. The retention time of tizanidine and nimesulide observed was 3.675 and 9.271 min respectively. A model chromatogram is shown in the Figure 3 and 4. Using these values of the two drug substances the precision was checked for the area and retention time of both the drugs. The system suitability was carried out by injecting standard solution of tizanidine (20 ppm) and nimesulide (1000 ppm) into the chromatographic system to check the reproducibility of peak areas. The % RSD observed was 0.51 and 0.20 for tizanidine and nimesulide respectively. The results of system precision are as shown in (TABLE 1).

Specificity

Specificity can be described as the capability of the method to accurately measure the response of the analysed compound with no interferences originating from sample matrix. High percentage recovery observed with assay samples of pharmaceutical dosage forms, including standard addition experiments, indicates that

Full Paper

the proposed method was not affected by interferences from excipients used in formulations. The peaks obtained from recovery experiments of dosage forms, were checked for uniformity using UV spectra taken from different points of the peak of interest. These spectra were super-imposable when overlaid; showing that there was no other co-eluting peaks, at the peak of analytes, TZN and NMS.

TABLE 1 : Result of system suitability.

TZN - 20 ppm		NMS - 1000 ppm	
Injection No.	**Area count**	**Injection No.**	**Area count**
1	7383	1	324838
2	7389	2	325485
3	7434	3	324987
4	7438	4	324517
5	7488	5	324368
6	7425	6	323580
Average	7426	Average	324629
Std. Dev.	38.13	Std. Dev.	645.86
% RSD	0.51	% RSD	0.20

Linearity

The calibration curves for TZN and NMS in binary mixtures were constructed by plotting the peak area of TZN and NMS against the respective concentration. The Linearity curve was made using standard solutions containing TZN and NMS at six different concentrations ranging from 50 to 150% of nominal concentrations such as 10 ppm to 30 ppm for TZN and 500 ppm to 1500 ppm for NMS. Twenty microlitres were injected each time in the stream of mobile phase, at a flow rate of 1 ml/min. Each of these dilutions of different concentration was injected in duplicate into the column and the corresponding chromatograms were obtained. From these chromatograms the area under the peak of the drug were noted. Using these values, the mean area of the drug was calculated. The regression of the drug concentration was completed. The linear concentrations range of 10 ppm to 30 ppm for TZN and 500 ppm to 1500 ppm for NMS was selected for routine analysis purpose. Six levels were prepared and each level was injected in duplicate in to the chromatographic system. Mean peak area of each level was calculated. Graph of mean peak area vs. concentration was plotted and the best-fit line was determined by regression. % Intercept and Correlation coefficient was obtained. The % co-relation co-efficient was 0.9999 TZN and 0.9999 for NMS. The plot of peak area vs. concentration of each analyte was found to be linear within the concentration ranges stated above. The results of Linearity are shown in (TABLE 2).

TABLE 2 : Results of linearity.

Level	TZN conc. In ppm.	TZN average area count	NMS conc. In ppm.	NMS average area count
Level 1	10.02	3906	500.60	181097
Level 2	14.03	5439	700.84	229640
Level 3	20.05	7607	1001.20	309259
Level 4	22.05	8315	1101.32	330419
Level 5	26.06	9860	1301.56	382812
Level 6	30.07	11210	1501.80	435326
Slope		365.0111		253.8824
Intercept		283.9077		53001.9540
Correlation coefficient (r^2) (n=6)		0.99975		0.9997

Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively. ICH guideline Q2B[17] describes several approaches for determination of detection and quantitation limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. The LOD and LOQ values obtained for these compounds of the developed method are presented in (TABLES 3).

TABLE 3 : Results of LOD and LOQ.

	TZN			NMS		
	Ppm	**Avg. area**	**Avg.S/N ratio**	**ppm**	**Avg. area**	**Avg. S/N ratio**
LOD	2.005	788	4.23	3.004	1173	3.61
LOQ	6.014	2185	12.38	8.010	3093	11.49

Precision

The precision of the proposed method were assessed as repeatability and intermediate precision performing seven replicate injections of standard and three different sample solutions, which were freshly prepared and analyzed (TABLES 1 and 4). These experiments were repeated over a period of 24 hrs to evaluate day-to-day variability (intermediate precision). As can be seen in (TABLES 4), the % R.S.D. values of the mea-

surements ranged between 0.99% and 0.42% for TZN and NMS respectively. The % R.S.D. of assay results obtained in intermediate precision study were not greater than 2%, confirming good precision of the proposed method between days.

TABLE 4 : Results of method precision and intermediate precision.

Method precision	TZN			NMS		
	Area	mg/tab	% Assay	Area	mg/tab	% Assay
Sample1.	7549	1.99	99.25	323744	99.86	99.86
Sample2.	7460	2.09	100.26	324038	99.97	99.76
Sample3.	7448	2.00	100.09	323744	99.96	99.96
Intermediate precision						
Sample1.	7689	1.99	99.56	338544	99.76	99.76
Sample2.	7668	1.96	98.09	339630	100.74	100.74
Sample3.	7640	2.02	100.95	338944	100.65	100.65
		Avg.	99.70		Avg.	100.16
		Std. Dev.	0.9841		Std. Dev.	0.4249
		% RSD	0.99		% RSD	0.42

Accuracy

Accuracy of the proposed method was established by recovery experiments using standard addition method. This study was employed by addition of known amounts of TZN and NMS onto known concentration of commercial tablets sample. The pure standards at these three levels were added to the sample. The resulting mixtures were analyzed as described in Section. The experiment was carried out at three different levels i.e. 110 %, 120 %, and 130 % of the working concentration of TZN (20 ppm) and NMS (1000 ppm). From the amount estimated, the percentage recovery was calculated. The recovery experiments, using Nicip T tablets containing TZN and NMS, showed recovery from 99.87 to 101.49% with mean recovery of 100.64 and from 98.35 to 102.50% with mean recovery of 100.24% with R.S.D. values of 0.63 and 1.48% for TZN and NMS, respectively. The percent recovery is in between 98 % to 102 % which indicates specificity and accuracy of the method. Results obtained from recovery studies are as shown in (TABLE 5).

TABLE 5 : Results of accuracy by recovery.

TZN			NMS		
Level-%	Average% Result	Average% Recovery	Level-%	Average% Result	Average% Recovery
110	109.58	100.44	110	112.10	99.73
110	111.05	99.87	110	112.32	100.47
110	111.64	99.77	110	112.34	102.50
120	122.01	100.75	120	118.20	101.37
120	122.52	100.34	120	118.23	100.20
120	124.04	101.17	120	118.30	101.98
130	130.81	100.08	130	124.51	98.66
130	132.89	101.29	130	124.77	98.86
130	133.77	101.49	130	124.91	98.35
	Average	100.64		Average	100.24
	Std. dev	0.6331		Std. dev	1.4862
	% RSD	0.63		% RSD	1.48

DISCUSSION AND CONCLUSION

This validated HPLC method has been proved to be simple, precise, rapid and reliable. To achieve sharp peaks with good resolution under isocratic conditions, mixture of monobasic potassium phosphate salt solution and acetonitrile in different proportion were tested as mobile phase on a C_{18} stationary phase. The mixture of monobasic potassium phosphate salt solution and methanol in the proportion (50: 50 v/v) proportions was proved to be the most suitable for estimation. The UV overlay spectra showed that both the drugs absorbed appreciably at 250nm & hence this wavelength was selected for detection of both the compounds. Since the chromatographic peaks were better defined, resolved with this system, under the above mentioned chromatographic conditions, the retention time obtained for TZN and NS were 3.7 and 9.0 min respectively. The calibration curve for TZN and NMS was found to be linear over the range of 10.02 to 30.07 ppm of TZN and 500.60 to 1501.80 ppm of NMS, respectively. The data of regression analysis of the calibration curves is shown in (TABLE 2). The proposed method provides a good resolution between TZN and NMS. Using this single procedure, it is possible to perform quantitative analysis of two different analytes from a tablet dosage forms within a short analysis time. The developed method reported herein was validated by evaluation of the validation parameters as described in ICH-

Q2B guideline. System suitability, specificity, linearity, LOD, LOQ values, precision and accuracy of the proposed technique were obtained during the validation studies. The results of the validation and the precision test parameters are summarised in (TABLE 1). The developed method was found to be simple, sensitive and selective for analysis of TZN and NMS in combination without any interference from excipients. Thus the method was successfully used for determination of tizanidine and nimesulide in pharmaceutical formulation. The results of assay for combined dosage form using proposed method are summarised in (TABLE 4).

The developed method can be used for the simultaneous estimation of tizanidine and nimesulide. It can serve as an easy, cost effective and efficient HPLC method for routine quality control analysis of tablet dosage forms containing tizanidine and nimesulide.

ACKNOWLEDGEMENTS

The authors are grateful to Bhavan's college, Andheri, for providing the necessary facilities for the research work.

REFERENCES

[1] British Pharmacopoeia, British pharmacopoeia commission office, MHRA, London, **2**, 1552-1553 **(2012)**.

[2] S.S.Zarapkar, N.P.Bhandari, U.P.Halkar; Simultaneous determination of nimesulide and chlorzoxazone in pharmaceutical dosage by RP-HPLC. Indian Drugs, **37**, 467 **(2000)**.

[3] K.E.Nagoji, S.Vijaysrinivas, K.M.Kumar, N.Mathivanan, S.M.Kumar, M.E.Rao; Simultaneous RP-HPLC estimation of nimesulide and diclofenac sodium. Indian J.Pharm.Sci., **65**, 407 **(2003)**.

[4] S.J.Rajput, G.Randive; Assay of nimesulide in pharmaceutical dosage formulation. Eastern Pharmacist, **475**, 113 **(1997)**.

[5] K.E.Nagoji, S.S.Rao, M.E.Rao, K.V.Rao; New spectrophotometric method for the estimation of nimesulide in pharmaceutical dosage formulation. Eastern Pharmacist, **496**, 117 **(1999)**.

[6] The united states pharmacopoeia, The united states pharmacopeia convention, Rockville, **3**, 4877-4878 **(2012)**.

[7] Indian pharmacopoeia, The Indian pharmacopoeia commission, Ghaziabad, **3**, 2230-2231 **(2010)**.

[8] M.N.Reddy, T.K.Murthy, D.G.Sankar; Simultaneous estimation of nimesulide and tizanidine hydrochloride in their combined dosage form. Eastern Pharmacist, **521**, 121 **(2001)**.

[9] M.S.Shingare, K.R.Naidu, U.N.Kale; RP HPLC method for simultaneous estimation of tizanidine HCl and nimesulide in tablets. Indian J.Pharm.Sci., **65**, 315 **(2003)**.

[10] The united states pharmacopoeia, The united states pharmacopeial convention, Rockville, **3**, 4878-4879 **(2012)**.

[11] Indian pharmacopoeia, The Indian pharmacopoeia commission, Ghaziabad, **3**, 2230-2231 **(2010)**.

[12] H.Nimje, S.P.Wate, D.P.Dharkar, R.Razdan; Simultaneous RPHPLC determination of nimesulide and tizanidine in tablets. Indian J.Pharm.Sci., **69**, 281-3 **(2007)**.

[13] S.K.Mantra, N.S.H.N.Moorth, C.Kartthikeyan, P. Trivedi; Simultaneous spectrophotometric methods for the estimation of nimesulide and tizanidine in tablet dosage form. Indian J.Pharm.Sci., **67**, 501-4 **(2005)**.

[14] A.Chandratrey, R.Sharma; Simultaneous spectrophotometric estimation and validation of three component tablet formulation containing paracetamol, nimesulide and tizanidine. Indian Journal of Chemical Technology, **17**, 229-32 **(2010)**.

[15] R.Siva Kumar, P.Kumar Nallasivan, P.R.Vijai Anand, T.Akelesh, R.Venkatnarayanan; Spectrophotometric methods for simultaneous estimation of aceclofenac and tizanidine. International Journal of Pharm.Tech.Research, **2**, 945-49 **(2010)**.

[16] ICH-Q2A, Guidelines for industry: Text on validation of analytical methods, March, **(1995)**.

[17] ICH, Q2B, Validation of analytical procedures: Methodology, International Conference on Harmonization, Nov., **(1996)**.

[18] I.S.Krull, M.E.Swartz; LC/GC N, Am.Magazine, **17(3)**, 244-46 **(1999)**.