Simultaneous RPHPLC Determination of Nimesulide and Tizanidine in Tablets

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A simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method has been developed for the simultaneous determination of nimesulide and tizanidine from tablets. The sample was analyzed using methanol: water in the ratio of 65:35, pH adjusted to 4.15 with orthophosphoric acid on an octadecylsilane C_{18} column. The effluent was monitored at 1.4 ml/min flow rate using 307 nm as detecting wavelength. The linear dynamic ranges for nimesulide and tizanidine were 0.2-1.0 μg/ml and 10-50 μg/ml, respectively. Coefficients of correlation obtained for nimesulide and tizanidine were 0.998 and 0.996 - respectively.

Nimesulide is an antiinflammatory drug. Chemically, nimesulide is N-(4-nitro-2-phenoxyphenyl)methane sulphonamide. It is approved for used in treatment of musculoskeletal disorder, dyemenorrhoea, thrombophlebitis and dental pain, inflammation. Some HPLC[1,2] and spectrophotometric[3,4] methods have been reported in the literature for its estimation. Tizanidine is a

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skeletal muscle relaxant, and is chemically 5-chloro-N-(4,5dihydro-1H-imidazol-2yl)-2,1,3-benzothiadiazol -4-amine hydrochloride. Tizanidine is indicated for the treatment of spasticity due to multiple sclerosis and spinal cord injury. Literature survey reveals that very few methods are reported for the simultaneous estimation of nimesulide and tizanidine in their combined dosage form[5,6]. Hence, a new HPLC method for the simultaneous determination of nimesulide and tizanidine from the tablets was developed which is simple, rapid, selective and precise.

Reference standards of nimesulide and tizanidine were obtained as gift sample from Indian Drugs ltd., Nagpur and Wallace Pharmaceuticals, Goa. Nimesulide and tizanidine tablets (Dicka Relax, Indian Drugs, Nagpur) were procured from the market. They had labeled content of 100 mg and 2 mg of nimesulide and tizanidine, respectively. Water and methanol HPLC grade, orthophosphoric acid reagents were used. High Pressure Liquid Chromatograph (Chemito), pump equipped with universal injector 20 µl loop size, UV/Vis detector (LC 6500) and computer-based data station were used. Column containing C_{18} (ODS) packing as the stationary phase was used. A water-methanol mixture was prepared by mixing 140 ml of water with 260 ml of methanol and its pH was adjusted to 4.15 with orthophosphoric acid.

About 500 mg nimesulide and 10 mg tizanidine were accurately weighed, transferred to 100 ml volumetric flask and dissolved in methanol. The volume was made up to the mark. An aliquot 0.02 ml of this solution was diluted to 10 ml with mobile phase to get the working standard solution (10 µg/ml for nimesulide and 0.2 µg/ml for tizanidine).

Twenty tablets were accurately weighed and finely powdered. Accurately weighed quantity of powder equivalent to 100 mg of nimesulide and 2 mg of tizanidine was transferred in 100 ml volumetric flask and dissolved in methanol and volume was adjusted to 100 ml with methanol and the solution was shaken for 10 min, filtered through grade-I filter paper and finally through 0.45 µm membrane filter paper. A 0.02 ml of aliquot filtrate solution was taken in 10 ml volumetric flask and diluted upto the mark with mobile phase. The mobile phase consisting of methanol: water (65:35), pH adjusted to 4.15 with orthophosphoric acid showed symmetric, sharp, reproducible peaks with good resolution. The flow rate was kept at 1.4 ml/min and 307 nm was selected as the wavelength for determination of eluted components. Several aliquots of standard nimesulide and tizanidine stock solution were taken in different 100 ml volumetric flasks and diluted upto the mark with mobile phase so that the final concentrations of nimesulide and tizanidine were in the range of 10-50 µg/ml and 0.2-10 µg/ml, respectively. The plots of peak area of each component against respective concentration of each corresponding drug were found to be linear in the range of 10-50 µg/ml and 0.2-10 µg/ml for nimesulide and tizanidine, respectively. Evaluation of two drugs was performed with UV detector at 307 nm. Peak areas were recorded for all the peaks. The coefficients of correlation were found to be 0.998 and 0.996 for nimesulide and tizanidine, respectively.

Twenty microlitres each of the working standard solution and sample solution were injected separately into the column. The detector responses were recorded for the same and the amounts of the drugs were calculated. The precision of the method was established by replicate analysis of the analyte (five times) using the proposed method. The low value of relative standared deviation (RSD) shows that the method is precise. The values are given in Table 1. To study the accuracy, reproducibility and precision of the proposed method, recovery experiments were carried out and results were obtained. The concentrations were found to be within 99-101% of the true concentrations of each drug, which indicates the accuracy of the method.

Analysis of tablets containing nimesulide and tizanidine was carried out by using optimized mobile phase

TABLE 1: RESULTS OF HPLC ASSAY

Expt. No.	Nimesulide (100 mg/tab)		Tizanidine (2 mg/tab)	
	Assay	Recovery	Assay	Recovery
1	100.75	100.33	1.9954	2.007
2	100.24	100.56	2.0106	2.0252
3	99.82	101.35	2.0248	2.0000
4	98.35	100.42	2.0116	2.0104
Mean	99.79	100.67	2.0106	2.0106
SD	±1.0325	±0.4664	±0.0120	±0.0106
RSD	0.0103	0.0047	0.0001	0.0001

TABLE 2: SUMMARY OF RESULTS OF SYSTEM SUITABITITY

Parameter		Nimesulide	Tizanidine
RSD		0.0045	0.037
Resolution		17.575	
Capacity factor		5.08	1.00
Asymmetry		1.5	2.4
Theoretical	Per column	6327	2546
Plates	Per meter	63265	25462

TABLE 3: DETERMINATION OF RUGGEDNESS PARAMETERS

Area under curve		% Label claim		Mean		S.D.		R.S.D.	
(Different analyst)									
NSD	TZN	NSD	TZN	NSD	TZN	NSD	TZN	NSD	TZN
180.6288	11.4562	99.83	98.26						
179.9668	11.7171	99.37	100.37	99.58	99.72	±0.231	±1.267	0.002	0.013
179.7792	11.7040	99.56	100.53						
(Different days)									
NSD	TZN	NSD	TZN	NSD	TZN	NSD	TZN	NSD	TZN
178.1429	11.7764	98.23	100.73						
179.6027	11.7052	99.37	100.57	99.12	100.54	±0.799	±0.207	0.008	0.002
181.3239	11.7526	99.77	100.32						

NSD stands for nimesulide and TZN stands for tizanidine

containing methanol and water (65:35), pH adjusted to 4.15 with orthophosphoric acid, and detection was done at 307 nm. The retention time for nimesulide was 6.84 min and for tizanidine was 2.09 min. The average content of nimesulide and tizanidine found were 99.79 mg/tab and 2.01 mg/tab, respectively. As per USP-XXIV, system suitability tests were carried out on freshly prepared solution of nimesulide and tizanidine and parameters obtained with 20 µl solution are shown in Table 2. The percentage recoveries of nimesulide (100.67%) and tizanidine (100.50%) reveal no interference of excipients. Ruggedness and robustness studies were carried out, as they are the measures to indicate the degree of reproducibility. It was observed that the method was rugged and robust as after carrying out the analysis by different analysts and on different days the results showed RSD well within permissible limits. The results are showed in Table 3.

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