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Reverse Phase HPLC Method for Simultaneous Estimation of Tizanidine Hydrochloride and Nimesulide in Tablets

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A reverse phase high performance liquid chromatography method for the simultaneous estimation of tizanidine hydrochloride and nimesulide in tablets is presented. Cynopropyl column is used to retain tizanidine hydrochloride ($k'=1.52$) and also have reasonable retention for nimesulide ($k'=2.37$) with a good resolution and peak symmetry. Effect of change of chromatographic conditions such as pH, %organic modifier (acetonitrile) in mobile phase on retention of drugs were studied and optimized. Both the drugs showed linear response in the concentration range employed (tizanidine hydrochloride, 1.2-2.8 $\mu\text{g/ml}$ and nimesulide, 60-140 $\mu\text{g/ml}$) and was validated by least squares method at 95% confidence level. The results of analysis have been validated statistically and by recovery studies. The mean recoveries obtained for tizanidine hydrochloride and nimesulide were 99.6% and 100.1%, respectively.

Nimesulide (NIM), 4'-Nitro-2'-phenoxy methane-sulphonanilide, is widely used as an analgesic, anti-inflammatory and antipyretic drug¹. It acts as an inhibitor of prostaglandin synthetase and platelet aggregation. Tizanidine hydrochloride (TIZ), 5-chloro-N-(4,5-dihydro-1H-imidazole-2-yl)-2,1,3 benzothiadiazole, is a centrally acting muscle

relaxant². A combination of both these drugs, NIM (100 mg) and TIZ (2 mg) in each tablet, is marketed by Unichem Laboratories (Zulu).

Both these drugs are not official with United States Pharmacopoeia, Edn. 24 or European Pharmacopoeia 2000. A literature survey revealed no reported analytical methods for the simultaneous determination of TIZ and NIM either as

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active pharmaceutical ingredient or from dosage forms. Methods have been reported for estimation of NIM using techniques such as spectrophotometry³⁻¹⁶, flurometry¹⁷, electroanalytical^{18,19}, HPLC^{20,21}, HPTLC²²⁻²⁵ and some methods have also been reported for estimation of NIM with paracetamol²⁶, chloroxazone²⁷, meloxicam and flutamide²⁸. Methods reported for TIZ are based on electroanalytical techniques^{29,30} and SFC³¹. As no method is reported for simultaneous estimation of TIZ and NIM, an attempt has been made to develop a simple, accurate and rapid method for simultaneous estimation of these two drugs. Simultaneous estimation using UV spectrophotometry is difficult since the UV spectra of these two drugs overlap significantly and also there is large difference in labeled strength (TIZ- 2 mg and NIM-100 mg) in tablets. A simple reverse phase HPLC method for the simultaneous estimation of these two drugs from tablets is reported here.

A Thermo Separation Products HPLC consisting of pump P1000, autosampler AS 3000 and UV/Vis detector UV1000 and microcomputer with PC1000 software for data acquisition and processing was employed for entire chromatographic analysis. The column used was Zorbax CN (length: 25 cm; i.d. 4.6 mm; particle size 5 μ m) and was eluted with filtered and degassed mobile phase consisting 0.02 M sodium acetate buffer and acetonitrile in the proportion of 60:40 with apparent pH adjusted to 4.0 with acetic acid. The flow rate of mobile phase was 1.5 ml/ min. Detection was performed at 320 nm using range 0.2 auFS. TIZ and NIM were generous gifts from Novartis Ltd., Mumbai and Wockhardt Ltd., Aurangabad, respectively and both the drugs were used without further purification. Sodium acetate, acetonitrile, methanol (HPLC grade) and acetic acid (AR grade) were procured from Qualigens, Glaxo and Mumbai.

Stock standard solution of TIZ (100 μ g/ml) was prepared in distilled water and NIM (1000 μ g/ml) was prepared in acetonitrile. Stock solutions were suitably diluted using diluent, distilled water:acetonitrile (2:8), to obtain mixed standard solution of TIZ (2 μ g/ml) and NIM (100 μ g/ml). Test stock solution was prepared by grinding 10 tablets and powder equivalent to 1 tablet was transferred to 100 ml volumetric flask and to it 80 ml diluent was added. The solution was sonicated for 5 min in an ultra-sonic bath and mixed thoroughly by shaking. The volume was made up to the mark with diluent. This solution was filtered through Whatman filter paper No. 41 and 10 ml of filtrate was diluted to 100 ml with diluent to obtain test solution. Twenty microlitres of the test solution and standard solution were injected separately and chromatogram recorded upto 10 min.

A reverse phase HPLC method using C18 column, reported for analysis of NIM²¹ in formulation (mobile phase: 0.05 M phosphate buffer-methanol, pH 3.0) retained TIZ to a very less extent (k' =0.23), and the peak was not resolved sufficiently from diluent peak. It wasn't possible to increase the retention of TIZ without significantly increasing the retention of NIM using a C18 column and hence semi polar cynopropyl stationary phase was tried. Retention behavior of both TIZ and NIM at different pH, in the range of 3.0 to 7.5, of mobile phase consisting of 20 mM citrate-phosphate buffer and acetonitrile using CN column was studied. Reversal of elution orders of TIZ and NIM was observed between pH 3.0 and 4.0 and also between pH 6.0 and 7.5. At pH 4.0 there is good resolution between TIZ and NIM peaks and NIM is retained more than TIZ. Retention behavior of both drugs in mobile phases having pH 4.0, containing different proportions of acetonitrile (in the range of 30-60%) was studied. Retention of TIZ was relatively unaffected with change in proportion of organic modifier (acetonitrile) in mobile phase whereas retention of NIM decreases as proportion of organic modifier was increased. The elution order of TIZ and NIM were reversed at around 50% acetonitrile content in mobile phase. This observation can be correlated to lipophilic and nature of NIM³² and hydrophilic nature of TIZ. As the concentration of NIM (100 μ g/ml) is much

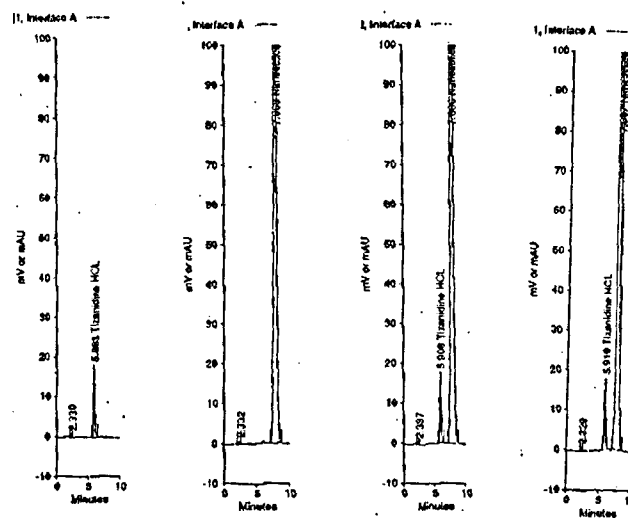


Fig. 1a. **Fig. 1b.** **Fig. 1c.** **Fig. 1d.**
Fig. 1: Chromatograms of standard and test solutions. Chromatograms showing (a) Standard solution TIZ, (b) Standard solution of NIM (c) Standard solution containing both TIZ and NIM and (d) Test solution of commercially marketed tablets containing both TIZ and NIM.

TABLE 1: RECOVERY EXPERIMENT.

Name of drug	Amount added ($\mu\text{g/ml}$)	Amount Recovered ($\mu\text{g/ml}$) (n=3)	Recovery (%)	Average Recovery (%)
TIZ	0.200	0.198	99.20	99.56
	0.400	0.393	98.23	
	0.600	0.607	101.25	
NIM	10.00	10.10	100.97	100.11
	20.00	19.95	99.77	
	30.00	29.88	99.60	

Recovery experiment data for TIZ and NIM showing amounts of drug added and recovered from sample solution at each level (n=3), percentage recovery and the average percentage recovery of three levels. TIZ stands for tizanidine hydrochloride and NIM for nimesulide.

greater than that of TIZ ($2 \mu\text{g/ml}$) in both test and standard solutions, in cases of lower column efficiency a tailing in NIM peak will reduce the resolution between two peaks if it elutes prior to TIZ. So the apparent pH 4.0 and 40% organic modifier was selected for mobile phase that retains the NIM more than TIZ. With the above mentioned composition of mobile phase a good resolution between TIZ and NIM peaks was achieved with a reasonably short runtime of 10 min.

The mobile phase was optimized with sodium acetate buffer (20 mM) and acetonitrile in the proportion of 60:40 and apparent pH adjusted to 4.0 with acetic acid. The typical chromatograms obtained with standard and test solutions are shown in fig. 1. The retention factors k' obtained for TIZ and NIM were 1.52 and 2.37 (retention time 5.9 and 7.9 min), respectively. The resolution, R_f , was 4.2 and tailing factors for TIZ and NIM peaks were 1.3 and 1.01, respectively. The UV detection was carried out at 320 nm as TIZ and NIM both show λ_{max} around that wavelength. The UV absorption of TIZ is much more than NIM at this wavelength and because of which it was possible to have appreciable response for TIZ ($2 \mu\text{g/ml}$) and also detector response was not saturated for NIM ($100 \mu\text{g/ml}$) peak. Thus it was possible to analyse both the drugs in single chromatogram.

The proposed method was validated as per standard analytical procedures³³. System precision and assay precision experiments yielded results that were precise with percentage relative standard deviation less than 2.0%. Linearity experiment was performed thrice for both the components and response was found to be linear in the range of 1.2 to $2.8 \mu\text{g/ml}$ for TIZ and 60 to $140 \mu\text{g/ml}$ for NIM. Regression lines obtained at 95% confidence interval using least square

method. Correlation coefficient 'r' values (n=3) for both TIZ and NIM were ≥ 0.999 , and % relative standard deviations of slope values were 1.42 and 0.34 for TIZ and NIM, respectively. The responses of both the drugs were linear in the said range. Recovery study was carried out at three levels. Standard drug solutions containing drugs in the range 10–30% of nominal concentration ($2 \mu\text{g/ml}$ for TIZ and $100 \mu\text{g/ml}$ for NIM) was added to previously analyzed tablet sample solution (test solution). Amount of drug recovered at each level (n=3) was determined. Percentage recovery at each level was calculated. Table 1 shows recovery study data for TIZ and NIM. The average recovery obtained for TIZ and NIM were 99.56% and 100.11%, respectively.

Based on the validation study data it can be concluded that the proposed method is accurate and precise for the analysis of both drugs. No interference was found from excipients used in tablet formulation and hence the method is suitable for analysis of tablet formulation. The method is simple and has a runtime of 10 min, which makes it especially suitable for routine quality control analysis work.

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