Simultaneous Spectrophotometric Methods for the Estimation of Nimesulide and Tizanidine in a Tablet Dosage Form

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Accepted 21 August 2005
Revised 2 February 2005
Received 6 March 2004

Two new spectrophotometric methods (absorbance ratio method and multiwavelength spectroscopy) for the simultaneous estimation of nimesulide and tizanidine hydrochloride in their combined dosage form have been developed and validated for linearity, accuracy, precision, limit of detection, limit of quantitation and robustness. In the first method, the wavelength selected to develop equations were 297.8 nm and 318.8 nm in methanol for nimesulide and tizanidine respectively and the isoabsorptive point is 302.6 nm. The wavelengths 297.8 nm and 318 nm were selected for the multiwavelength spectroscopy method. Both drugs obeyed Beer–Lambert's law in the concentration range of up to 60 μg/ml for nimesulide and up to 30 μg/ml for tizanidine. The results of both methods have been statistically validated and were found to be satisfactory. These methods were found to be simple, rapid, accurate, precise and reproducible.

Nimesulide[1] (NMD) chemically N-(4-nitro-2-phenoxy phenyl) methane sulfonamide, is a new non-steroidal anti-inflammatory, analgesic and antipyretic agent. The various analytical methods such as spectrophotometric (UV/Vis), polarographic, HPLC and HPTLC methods have been reported for the estimation of nimesulide[1-4]. Tizanidine hydrochloride[5,6] (TZN) is 2,1,3- benzothiadiazol-4-amine, 5-chloro-N-(4,5-dihydro-1H-imidazole-2-yl) monohydrochloride, used as a central muscle relaxant. Tizanidine has been launched in the market recently, it is not official in IP, BP and USP. In analytical abstracts, differential colorimetry and differential-pulse polarography methods are reported for its estimation in tablet dosage form[7].

Literature survey reveals that no UV-spectrophotometric has yet been reported for the analysis of these two drugs in combination. Currently analytical methods for NMD and TZN are not found in any pharmacopoeia. No official Pharmacopoeial methods are available for their simultaneous estimation by absorbance ratio and multiwavelength methods in combined dosage form. A combination of 2 mg of TZN and 100 mg of NMD is commercially available as tablets. The objective of the investigation is to develop and validate a methodology for estimation of the combined dosage form by simultaneous UV spectroscopic methods[8,9].

The instruments and reagents used for this spectroscopic method were, Shimadzu UV/Vis spectrophotometer (Model-160A), analytical reagent grade methanol, reference standards of drugs used in this study obtained from authentic sources. Wavelength accuracy was ±0.5 nm with auto wavelength correction and 1 cm matched quartz cells.

Standard stock solution of NMD and TZN were prepared separately to the concentration of 100 μg/ml in methanol. NMD and TZN were further diluted to 50 μg/ml and 5 μg/ml, respectively. Both solutions were scanned over the range of 400 nm and 200 nm in the spectrum mode at scan speed (480/min) and the overlain spectra of two were recorded. Mixed standard for the pure drug was prepared from the stock solution of 60 μg/ml of NMD and 30 μg/ml of TZN. Seven mixed standards containing 0, 10, 20, 30, 40, 50 and 60 for NMD and 30, 25, 20, 15, 10, 5 and 0 for TZN were prepared. Beer-Lambert's law obeyed by NMD and TZN in the range of 0-60 μg/ml and 0-30 μg/ml, respectively.

Twenty tablets (brand name-Zulu and manufactured by

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Unichem Laboratories Ltd, Mumbai.) were weighed and crushed to a fine powder. An accurately weighed powder sample equivalent to 10 mg was transferred to a 100 ml volumetric flask and dissolved in 25 ml of methanol. After the immediate dissolution, the volume was made up to the mark with methanol. The solution was filtered through Whatmann filter paper No. 41 and was diluted to prepare the concentration range of 25 µg/ml and 5 µg/ml for TZN and NMD, respectively. The absorbance were recorded at 302.6 nm (isoabsorptive point) and 297.8 nm (λ_{max} of NMD) and the amount of drug present in the sample solution were obtained by using k and b value of calibration curve of absorbance ratio vs relative concentration of both drugs. The result of the tablet analysis, recovery studies and statistical validation data are given in Table 1 and 2.

The overlain spectra exhibit the absorbance maxima lies at 297.8 nm and 318.8 nm for NMD and TZN respectively, the point at 302.6 is isoabsorptive point of both the drugs. Based on the overlain spectra the sampling wavelength selected for method A was 302.6 nm (A1) and 318.8 nm (A2). The absorbance (A1/A2) is determined for the laboratory samples and tablet formulations in photometric mode and the concentration is obtained by plotting the graph of absorbance vs relative concentration. Standard deviation and co-efficient of variation are calculated. The wavelength selected to develop multiwavelength equations are 297.8 nm and 318 nm.

Tablet solutions of TZN and NMD were prepared at the concentration range of 25 µg/ml and 5 µg/ml, respectively. These tablet solutions were scanned over the range of 400

TABLE 1: RESULTS OF VALIDATION PARAMETERS

Validation Parameter	METHOD-A				METHOD-B			
	NMD		TZN		NMD		TZN	
Linearity	0.003	0.574	0.003	0.532	0.008	1.512	0.003	0.532
Accuracy	0.290	0.725	0.230	0.828	0.274	1.126	0.180	1.472
Precision	0.157	0.522	0.152	0.900	0.206	0.793	0.203	1.911
Repeatability	0.248	1.020	0.200	1.722	0.274	1.126	0.179	1.472
Intermediate Precision	0.157	0.522	0.152	0.900	0.206	0.792	0.202	1.910
Reproducibility	0.845	0.500	0.144	0.178	0.219	0.956	0.147	1.348
Robustness	0.310	1.367	0.045	0.309	1.367	0.644	0.045	0.309
LOD	0.043		0.083		0.046		0.026	
LOQ	0.127		0.250		0.013		0.078	

NMD is Nimesulide, TZN is Tizanidine Hydrochloride, LOD is limit of detection and LOQ is limit of quantitation, Method A is absorption ratio method, Method B is multiwavelength method. All values are µg/ml.

TABLE 2: RESULTS OF RECOVERY STUDIES OF TIZANIDINE AND NIMESULIDE

TZN tablet µg/ml	standard drug added µg/ml	TZN percentage found		NMD tablet µg/ml	standard drug added µg/m	NMD percentage found	
		Method A	Method B			Method A	Method B
5	5	96.5	98.9	25	5	98.4	99.7
5	10	98.9	99.2	25	10	98.6	99.7
5	15	98.4	98.9	25	15	99.5	99.9
5	20	99.5	99.3	25	20	99.2	99.7
5	25	98.6	99.0	25	25	99.2	99.4

NMD is Nimesulide, TZN is Tizanidine Hydrochloride, Method A is absorption ratio method, Method B is multiwavelength method.

nm to 200 nm and the absorbance was recorded at 302.6 nm and 297.8 nm for absorbance ratio method. The absorbance at wavelengths 297.8 nm and 318 nm were recorded for the multiwavelength spectroscopy method. The concentration of laboratory samples and tablet formulations is determined by using the mixed standards in multi-component mode of the spectrophotometer. Standard deviation and coefficient of variation are calculated and the results are given Table 3. Validation of the above two methods has been carried out as per ICH guidelines[10-16].

between 0.002 and 0.3.

Both the methods were validated according to ICH norms using accuracy/precision, repeatability/reproducibility, linearity/range, limit of detection (LOD)/limit of quantitation (LOQ), selectivity/specificity and robustness/ruggedness.

Based on the validation results, the proposed spectrophotometric methods for determination of NMD and TZN hydrochloride are found to be simple, rapid, precise and reproducible. The calibration curves were liner and obeyed Beer-Lambert's law in the given ranges. The validation parameters and recovery studies were statistically significant and were close to 100% reproducibility. Among the two methods the multiwavelength method have negligible standard deviation compared to absorption ratio method. So it can be concluded that the multiwavelength spectroscopy method can be applied for estimating combined dosage forms.

TABLE 3: RESULTS OF ANALYSIS OF TABLET FORMULATION

Amount Present (µg/ml)		TZN percentage found		NMD percentage found	
TZN	NMD	Method A	Method B	Method A	Method B
5	50	97.8	100.0	98.7	99.7
5	50	99.2	100.0	99.2	99.8
5	50	99.6	99.4	97.8	99.7
5	50	98.8	99.6	98.5	99.6
5	50	98.2	97.9	99.2	97.9
5	50	99.2	99.5	97.8	100.0

NMD is Nimesulide, TZN is Tizanidine Hydrochloride, Method A is absorption ratio method, Method B is multiwavelength method.

In the present work, two methods namely multi wavelength spectroscopy and graphical absorbance ratio method (Q-analysis) were developed for the simultaneous spectroscopic estimation of NMD and TZN in commercially available tablet dosage form. With methanol solvent, the overlain spectra of the two drugs at 298.8 nm and 318.8 nm were selected as the sampling wavelength for NMD at a concentration of 50 µg/ml and TZN at a concentration of 5 µg/ml, respectively.

The two wavelengths selected for absorbance ratio method were 297.8 nm (max of NMD) and 302.6 nm. The absorbance ratio (A1/A2) at 297.8 nm (A1) and 302.6 nm (A2) was plotted against relative concentration of drugs. The k and b values were obtained from those plots were used to determine the unknown concentration of these two drugs. In multiwavelength method, the maximum absorbance of NMD at 297.8 nm had least absorbance in TZN. Similarly at 318.8 nm TZN had maximum absorbance but least absorbance for NMD. The recovery studies of the drug added were found to be 96.5 to 99.5%. The standard deviation for validation parameters of drugs in tablet formulation was found

ACKNOWLEDGEMENTS

Author wishes to thank the Director, S. G. S. I. T. S., Indore for providing necessary facilities for this work. One of the authors, MSK is grateful to Unichem Laboratories, Mumbai for the gift sample of pure drugs.

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HPLC Method for Simultaneous Estimation of Rofecoxib and Tizanidine hydrochloride in Tablets

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Accepted 21 August 2005
Revised 2 February 2005
Received 8 September 2004

A reverse phase high performance liquid chromatography method for the simultaneous estimation of rofecoxib and tizanidine hydrochloride in tablets is developed. The determination was carried out on a Wakosil C18 (250x4.6 mm, 5 µm) column using a mobile phase of acetonitrile and phosphate buffer pH 5.0 (50:50%v/v). The flow rate was 0.5 ml/min with detection at 240 nm. The retention time for tizanidine hydrochloride was 4.9 min and rofecoxib 12.2 min. Rofecoxib showed a linear response in the concentration range of 50-200 µg/ml and tizanidine hydrochloride 10-80 µg /ml. The results of analysis have been validated statistically and by recovery studies. The recoveries obtained for standard rofecoxib and tizanidine hydrochloride from the formulation ranged from were 98.20 to 101.6%.

Rofecoxib is widely used as an analgesic and antiinflammatory drug. Tizanidine hydrochloride is a centrally acting muscle relaxant. A tablet formulation containing rofecoxib (50 mg) and tizanidine hydrochloride (6 mg) in each tablet is marketed in India. Literature survey revealed that rofecoxib and tizanidine Hydrochloride can be estimated independently by a few spectrophotometric[1-3] and HPLC[4-6] methods. There is no official method published for simultaneous estimation of rofecoxib and tizanidine hydrochloride. Hence attempts were made to develop a simple, accurate and rapid HPLC method for simultaneous estimation of these two drugs in marketed tablet dosage forms.

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A Shimadzu HPLC SPD 10-AT Chromatograph equipped with UV/Vis detector and a Rheodyne injector with 100 ml external loop was used. A Wakosil C18 (250x4.6 mm, 5 µm), was the column employed. Elution was carried out using a mobile phase of composition potassium dihydrogen phosphate (0.02 M), pH 5.0 and acetonitrile in ratio of 50:50 v/v and a flow rate of 0.5 ml/min. The detector was set at 240 nm. Response of the peak areas were recorded and integrated using Winchrom oracle software. Standard samples of rofecoxib and tizanidine hydrochloride were gift sampled by Sun Pharmaceutical Industries Ltd., Mumbai. Acetonitrile of HPLC grade (Ranchem) and potassium dihydrogen phosphate of AR grade (Qualigens) were used.

Standard stock solutions of rofecoxib (1000 µg/ml) and tizanidine hydrochloride (120 µg/ml) were prepared in the