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NOVEL ANALYTICAL METHOD FOR THE SIMULTANEOUS ASSAY OF TIZANIDINE AND MEFENAMIC ACID BY RP-HPLC

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ABSTRACT

A simple, economic, rapid, accurate and precise analytical method for simultaneous determination of Tizanidine and Mefenamic acid in bulk and pharmaceutical dosage form was developed. Chromatographic separation was carried out using Symmetry C18 (250×4.6 mm×5 µm) with a mobile phase consisting of phosphate buffer (pH¬ 7.0) and acetonitrile in the ratio 55:45%v/v at a flow rate of 1 ml/min at a detection wavelength of 274 nm. The developed method resulted in the elution of Tizanidine and Mefenamic acid at 3.407 min and 4.750 min respectively. The calibration curves were linear (r^2=0.999) in the concentration range of 1-3 µg/ml for Tizanidine and 12.5-37.5 µg/ml for Mefenamic acid. The % RSD for Tizanidine and Mefenamic acid was found to be 1.55 and 0.64 respectively. The % mean recovery was found to be 99.65-100.25% for Tizanidine and 99.57-100.31% for Mefenamic acid. The LOD was found to be 0.08 µg/ml and 1.12 µg/ml and LOQ was found to be 0.25 µg/ml and 3.67 µg/ml for Tizanidine and Mefenamic acid respectively. The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Tizanidine and Mefenamic acid in drug substance and drug products.

INTRODUCTION:

Tizanidine is a central alpha-2-adrenergic receptor agonist and presumably reduces spasticity by increasing presynaptic inhibition of motor neurons. It is chemically 5-chloro-N-(4,5-dihydro-1H-imidazol-2-yl)-2,1,3-benzothiadiazol-4-amine (fig. no. 1). Mefenamic acid is an anthranilic acid derivative belongs to NSAIDs. It is chemically 2- [(2,3-dimethylphenyl)amino] benzoic acid (fig. no. 2). A combination of Tizanidine and Mefenamic acid is used to treat pain during periods, heavy bleeding during periods, fever and inflammation**.** A detailed literature survey revealed that there were analytical methods for the estimation of Tizanidine and Mefenamic acid alone and with other combinations[1-9]. Ganshyam et.al.,(2017) and Ashok et.al.,(2009) reported spectroscopic methods for the simultaneous estimation of Tizanidine and Mefenamic acid[10,11] but no HPLC methods reported for the simultaneous estimation of Tizanidine and Mefenamic acid. Hence an

attempt has been made to develop a rapid, sensitive, accurate and precise RPHPLC method for the simultaneous estimation of Tizanidine and Mefenamic acid in bulk drug and pharmaceutical formulation.

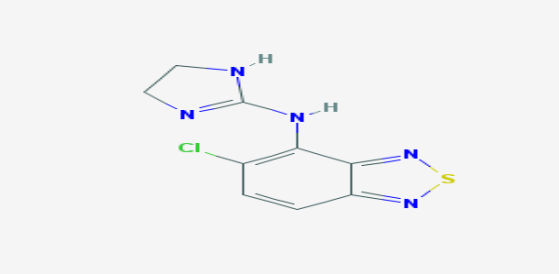

Figure 1. Structure of Tizanidine

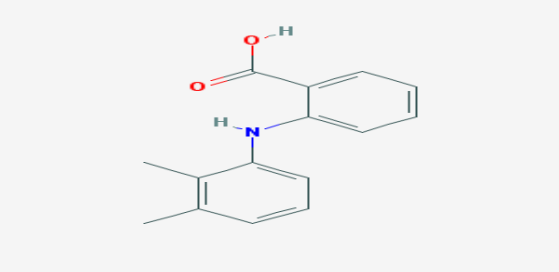

Figure 2. Structure of Mefenamic acid

MATERIALS AND METHODS

Materials

HPLC grade Mono potassium dihydrogen phosphate (KH_2PO_4), Dipotassium hydrogen phosphate (K_2HPO_4) and acetonitrile were purchased from Merck (Mumbai, India). Pharmaceutical grade Tizanidine and Mefenamic acid were supplied as a gift sample by Chandra labs (Hyderabad, India) and Mef T tablets (Tizanidine 2 mg & Mefenamic acid 25 mg) were purchased from local pharmacy.

Methods

Selection of wavelength

Standard solutions of Tizanidine and Mefenamic acid were prepared at the concentration of 10 µg/ml scanned by UV/Vis spectrophotometer at the range of 200-400 nm. UV spectrums of Tizanidine (fig. no. 3) and Mefenamic acid (fig. no. 4) were shown below. The isobestic point selected for simultaneous estimation was 274 nm (fig. no. 5).

Chromatographic conditions

The developed method used a reverse phase Symmetry C18 column (250x4.6 mm, 5µm), a mobile phase of phosphate buffer (p^H 7.0): Acetonitrile (55:45), flow rate of 1.0 ml/min and a detection wavelength of 274 nm using a UV detector.

Preparation of standard solution

Standard stock solution of Tizanidine and Mefenamic acid were prepared by dissolving 2 mg of Tizanidine and 25 mg of Mefenamic acid in sufficient mobile phase. After that, the solution was sonicated for 5 min and diluted to 100 ml with mobile phase. Further dilutions were prepared in 5 replicates of 2 µg/ml Tizanidine and 25 µg/ml Mefenamic acid which was made by adding 1 ml of stock solution to 10 ml of mobile phase.

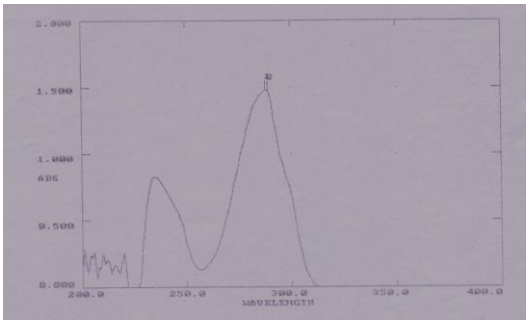

Figure 3. UV spectrum of Tizanidine

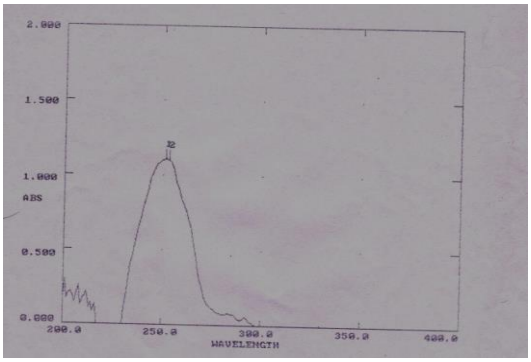

Figure 4. UV spectrum of Mefenamic Acid

Preparation of sample solution[12]

20 tablets each containing 2 mg of Tizanidine and 25 mg of Mefenamic acid were weighed and taken into a mortar and

crushed to fine powder and uniformly mixed. Sample solution of Tizanidine and Mefenamic acid were prepared by dissolving weight of tablet powder equivalent to 2 mg of Tizanidine and 25 mg of Mefenamic acid and dissolved in sufficient mobile phase. Then the solution was sonicated for 5 min, filtered and diluted to 100 ml with mobile phase. Further dilution of 2 µg/ml Tizanidine and 25 µg/ml Mefenamic acid was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Preparation of buffer solution

61.5 ml of 1M KH_2PO_4 and 38.5 ml of 1M K_2HPO_4 were taken into 200 ml of water, m9xed well and volume was made up to 1000 ml with water. The buffer was filtered through 0.45µ filter and sonicated for 20 min. The p^H of the resulted solution was found to be 7.0.

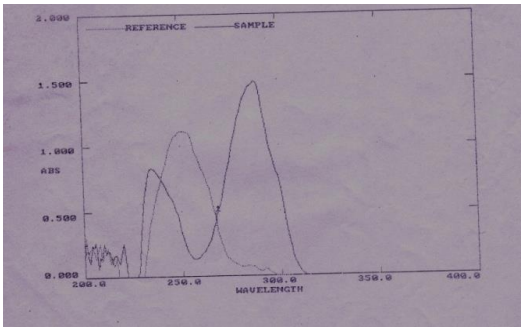

Figure 5. UV overlap spectrum of Tizanidine and Mefenamic acid hydrochloride

RESULTS AND DISCUSSION

Method development

Different chromatographic conditions were tried for better separation and resolution. Symmetry C18 (250×4.6mm, 5µm) column was found satisfactory. Peak purity of Tizanidine and Mefenamic acid was checked using UV detector and 274 nm was considered satisfactory for detecting both the drugs with adequate sensitivity. A number of solvents in the different ratio over a wide range of p^H were tried, but either peak shape was broad or resolution was not good. Repeated trials to obtain good, sharp peak with an efficient resolution between two peaks of Tizanidine and Mefenamic acid done on a C18 column in isocratic HPLC mode with a mobile phase consisting of phosphate buffer (p^H 7.0): Acetonitrile (55:45). A typical RP-HPLC chromatogram for simultaneous determination of Tizanidine and Mefenamic acid was shown in (fig. no. 6).

Method validation

The developed RP-HPLC method was validated for parameters like system suitability, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ) and robustness according to ICH guidelines[13].

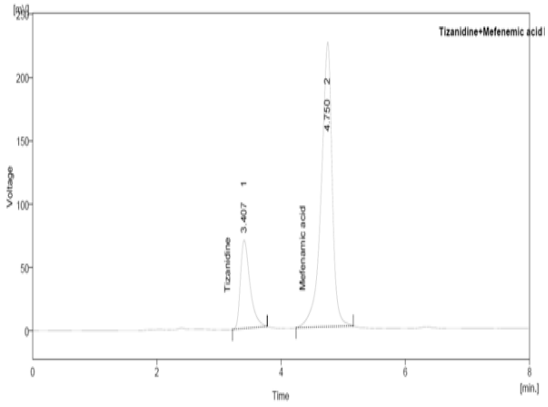

Figure 6. Chromatogram of Tizanidine and Mefenamic acid

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated. The system suitability parameters were tabulated in (Table no. 1). All the parameters were found to be within the limits.

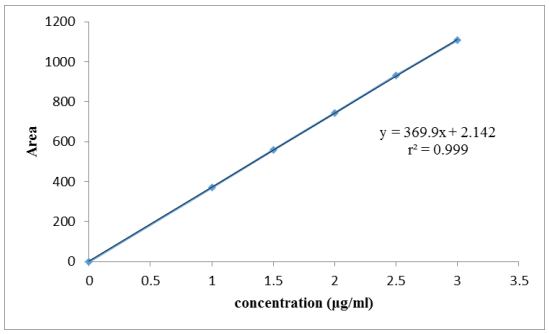

Figure 7. Calibration curve of Tizanidine

Table 1. System suitability data for Tizanidine and Mefenamic acid

Analytes	Retention time	Resolution	Tailing factor	Theoretical plates
Tizanidine	3.407 min	-	1.822	4250
Mefenamic acid	4.750 min	12.854	1.134	7695

Table 2. Linearity data of Tizanidine and Mefenamic acid

Level (%)	Tizanidine		Mefenamic acid	
	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
50	1.0	372.27	12.50	1379.78
75	1.5	558.40	18.75	2069.67
100	2.0	744.54	25.00	2759.56
125	2.5	930.67	31.25	3449.45
150	3.0	1106.81	37.50	4099.35
Correlation coefficient		0.999	0.999	

Table 3. Method precision data of Tizanidine and Mefenamic acid

n	Tizanidine Area	Mefenamic acid Area
Injection 1	743.60	2762.23
Injection 2	754.48	2716.63
Injection 3	744.86	2759.56
Injection 4	725.80	2737.84
Injection 5	756.01	2741.33
Injection 6	755.47	2759.76
Average	746.70	2746.23
SD	11.60	17.81
%RSD	1.55	0.64

Table 4. Accuracy data of Tizanidine and Mefenamic acid

Level (%)	Tizanidine		Mefenamic acid	
	% Recovery	% Mean*	% Recovery	% Mean*
50	99.75		99.12	
50	99.25	99.82	99.98	99.57
50	100.48		99.62	
100	99.75		100.50	
100	99.83	99.65	99.07	100.04
100	99.37		100.56	
150	100.16		100.11	
150	100.08	100.25	100.39	100.31
150	100.51		100.44	

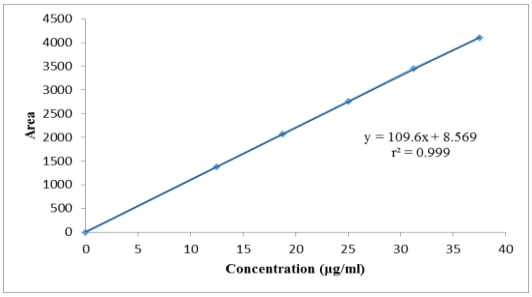

Figure 8. Calibration curve of Mefenamic acid

Linearity

Linearity was evaluated by analysis of standard solutions of Tizanidine and Mefenamic acid of five different concentrations from 50-150 % of target concentration. The range of linearity was found 1-3 µg/ml for Tizanidine and 12.5-37.5 µg/ml for Mefenamic acid. The peak area ratio and concentration of each drug was subjected to regression analysis to calculate the calibration equations and

Table 5. Robustness data for Tizanidine and Mefenamic acid

Parameter	Tizanidine			Mefenamic acid		
	Retension time*	Tailing*	Plate count*	Retension time*	Tailing*	Plate count*
Less flow rate (0.8 ml/min)	4.377	1.951	4255	5.960	1.136	7698
More flow rate (1.2 ml/min)	2.930	1.922	4245	3.997	1.133	7692
Less wavelength (272nm)	3.507	1.971	4253	4.790	1.133	7693
More wavelength (276 nm)	3.530	1.974	4254	4.773	1.138	7692

* mean of 6 observations

Correlation coefficients (fig. no. 7 and 8). The regression data obtained for Tizanidine and Mefenamic acid was represented in (Table no. 2). The result shows that within the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD was calculated using the formula 3.3 times σ/s where σ is the standard deviation of the intercept obtained for calibration curve and s is the slope of the calibration curve. The LOD of Tizanidine and Mefenamic acid was found to be 0.08 µg/ml and 1.12 µg/ml respectively. Similarly LOQ is calculated using the formula 10 times σ/s. The LOQ of Tizanidine and Mefenamic acid was found to be 0.25 µg/ml and 3.67 µg/ml respectively.

Precision: The method precision was demonstrated by injecting standard solutions of Tizanidine and Mefenamic acid as per the test procedure and the chromatograms of six standard solutions were recorded. The results of precision were tabulated in (Table 3). The % RSD of Tizanidine and Mefenamic acid was found to be 1.55 and 0.64 respectively. % RSD values were within the limits and the method was found to be precise.

Accuracy: Accuracy of the developed method has been carried out by recovery studies by applying the standard addition method. A known quantity of standard drug concentration corresponding to 50%, 100%, and 150% were added to preanalysed sample solutions. Each set of addition were repeated three times. The accuracy was expressed as the percentage of analytes recovered by the assay. The (Table no. 4) lists the recoveries of the drug from a series of spiked concentrations. The results indicate the method was found to be accurate.

Robustness:

To determine the robustness of the developed method, experimental conditions were deliberately altered, and the system suitability parameters were evaluated. The solutions were prepared as per the test method and injected at different variable conditions like flow rate (± 0.2 ml/min.) and wavelength (± 2 nm), system suitability parameters were compared. The results were tabulated in (Table no. 5). At the flow rate of 1.0 ml/min and wave length of 274 nm, shows a sharp peak with good resolution and rest of the flow rates and wave lengths were found to be not satisfactory but passed all system suitability parameters indicating that the method was robust.

CONCLUSION

The proposed RP-HPLC method was found to be simple, rapid, sensitive, accurate and precise. Hence the developed method can be useful for routine analysis of Tizanidine and Mefenamic acid in bulk and pharmaceutical formulation.

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CONFLICT OF INTERESTS

Declare none

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