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Spectrophotometric Methods for Simultaneous Estimation of Mefenamic Acid and Tizanidine in Tablets

ASHOK KUMAR*, SURENDER PANGHAL, SUDHA MALLAPUR,
RAMESH KUMAR† and AVANISH SINGH‡
Department of Pharmaceutical Chemistry, M.M.C.P., M.M. University, Mullana, Ambala-133 203, India
Fax: (91)(1731)304111; E-mail: ashokmmcp@rediffmail.com

Two spectrophotometric methods namely Vierordt's method and Q-analysis based on the derivation of the main spectra have been developed for the simultaneous analysis of mefenamic acid and tizanidine in a tablet formulation without involving any prior separation or masking. In Vierordt's method, the drugs were determined by using the absorptivity values of mefenamic acid and tizanidine at selected wavelengths, *viz.*, 216.8 and 234.3 nm, respectively. In Q-analysis method, isoabsorptive point was found to be at 224.6 nm. Both the drugs obey Beer's law in concentration range of 4-18 µg/mL (mefenamic acid) and 4-24 µg/mL (tizanidine). The results obtained were compared statistically by Student t-test and by the variance ratio F-test with those obtained by each method. Both the methods were found to be simple, rapid and accurate and can be adopted in routine analysis of drugs in formulations.

Key Words: Mefenamic acid, Tizanidine, Q-analysis.

INTRODUCTION

Mefenamic acid (MEF), N-(2,3- dimethylphenyl)-2-aminobenzoic acid is a non-steroidal antiinflammatory drug and a prostaglandin inhibitor useful in the treatment of patients with primary dysmenorrhea[1]. Mefenamic acid is official in BP[2], USP[3] and IP[4].

Tizanidine (TIZ), 5-chloro-4-{2-imidazolin-2-yl amino}-2,1,3-benzothiadiazole -4-amine is an α-adrenergic receptor agonist with myospasmolytic action. It has chemical structure unrelated to other muscle relaxants[5,6]. It is indicated for the treatment of back pain as monotherapy[7-9] or in combination with non steroidal antiinflammatory drugs (NSAIDs)[10]. The experimental and clinical studies suggest that tizanidine reduces the frequency of NSAID induced gastrointestinal (GI) adverse effects. In addition, experimental and clinical data suggests that tizanidine, when co-administered with an NSAID, has a synergistic effect in terms of efficacy and some protective effect against the GI adverse events caused by NSAIDs[10-19].

†Department of Chemistry, L.S. College, Muzaffarpur-842 001, India.
‡Department of Pharmaceutical Sciences, S.B.S.P.G.I., Dehradun-248 161, India.

There have been several reports on the determination of mefenamic acid individually or in its combination with other drugs, including the use of liquid chromatography[20-23], LC and GC-MS[24], thin layer chromatography[25], spectrophotometry[26,27], capillary electrophoresis[28,29], fluorescence spectrometry[30,31] and voltametry[32].

There are very few reports on analytical methods for estimation of tizanidine in bulk and its dosage form individually or in its combination with other drugs, including LC[33-38], GC-MS[39] and radioimmunoassay[40].

As per literature, no analytical method could be traced for the analysis of mefenamic acid and tizanidine combination in pharmaceutical dosage forms. Moreover the mefenamic acid and tizanidine mixture is not yet official in any pharmacopoeia. Therefore, simple, rapid and reliable methods for simultaneous estimation of these drugs in mixture seemed to be necessary.

Spectrophotometric methods of analysis are more economic and simpler, compared to methods such as chromatography and electrophoresis. The aim of this work was to develop reliable spectrophotometric methods namely Vierordt's method and Q-analysis based on the derivation of the main spectra for the simultaneous determination of mefenamic acid and tizanidine either in laboratory samples or in commercial dosage forms without any prior separation of individual drugs.

EXPERIMENTAL

A systronics UV-visible double beam spectrophotometer (Model 2101) with spectral bandwidth 2 nm and wavelength accuracy of ± 0.2 nm (with automatic wavelength correction) was used for all spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 10 mm matched quartz cells over the range of 400-200 nm. Single Pan Electrical Balance (DHONA 200D) was used for weighing throughout the experiment.

Pure sample of the drugs used were kindly donated by Lark Laboratories (India) Ltd. (mefenamic acid) and Mantena Drugs Pvt. Ltd, Hyderabad (tizanidine HCl). The gift samples were used as a standard without further purification. All the chemicals and solvents used in spectrophotometric analysis were of analytical reagent grade. Working solutions were prepared fresh daily by appropriate dilution using 0.1 N NaOH as a solvent. Commercial pharmaceutical preparations Meftal MR (Blue cross laboratories Ltd., Mumbai) are claimed to contain 500 mg of mefenamic acid and 2 mg of tizanidine were used in analysis.

Vierordt's method (method 1): This method is based on the solving of equations with two unknowns using $A_{1\%}^{1\,cm}$ (absorbance value of the 1 % solution in 10 mm cell) values calculated from absorbances measured at two suitable wavelengths for two compounds in the mixture have an absorption minimum and maximum inversely. The quantification analyses of mefenamic acid and tizanidine in a binary mixture were performed with the following equations:

$$Cx = (A_2\, ay_1 - A_1\, ay_2)/ax_2ay_1 - ax_1ay_2 \quad (1)$$

$$Cy = (A_1\, ax_2 - A_2ax_1)/ax_2ay_1 - ax_1ay_2 \quad (2)$$

where Cx and Cy are the concentrations of X and Y respectively in the diluted sample, ax_1 and ax_2 are absorptivities of X at λ_1 and λ_2, ay_1 and ay_2 are absorptivities of Y at λ_1 and λ_2. The absorbance of the diluted samples at λ_1 and λ_2 are A_1 ($A_1 = ax_1bcx + ay_1bcy$) and A_2 ($A_2 = ax_2bcx + ay_2bcy$), respectively.

Q-Analysis method (method 2): The drugs that obey Beer's law at all wavelengths and the ratio of absorbances at any two wavelengths are a constant value independent of concentration or pathlength. The concentration of individual components may be calculated by mathematical treatment of the simultaneous equations:

$$Cx = Qm\text{-}Qy/Qx\text{-}Qy)\times A_1 /ax_1 \quad (3)$$

$$Cy = Qm\text{-}Qy/Qy\text{-}Qx)\times A_1/ax_1 \quad (4)$$

where $Qm = A_2/A_1$, A_1 is absorbance of sample at isoabsorptive point, A_2 is absorbance of sample at λ_{max} of one of the two components, $Qx = ax_2/ax_1$, $Qy = ay_2/ay_1$, ax_1 and ax_2 represent absorptivities of X at λ_1 (isoabsorptive point) and λ_2 (λ_{max} of one of the two components) and ay_1 and ay_2 denote absorptivities of Y at λ_1 (isoabsorptive point) and λ_2, respectively; Cx and Cy be the concentration of X [should lie outside the range of (0.1-0.2)] and Y, respectively.

Procedure

Preparation of standard stock solution: Standard stock solution of mefenamic acid and tizanidine of strength 100 µg/mL each was prepared separately by dissolving 25 mg of mefenamic acid and tizanidine in 20 mL of methanol in a separate 250 mL volumetric flask and volume was made up to the mark with the 0.1 N NaOH (in distilled water).

Preparation of working standard solutions: Suitable aliquots of the stock solution of mefenamic acid and tizanidine were diluted separately with 0.1 N NaOH to obtain 4-18 µg/mL of mefenamic acid and 4-24 µg/mL of tizanidine.

Determination of λ_{max} and isoabsorptive point: The above stock solution was further diluted to get the concentration of 12 µg/mL (each of mefenamic acid and tizanidine) and scanned over the range of 400-200 nm using the solvent as blank. The overlain spectrum of both the drugs was recorded (Fig. 1). λ_{max} of mefenamic acid and tizanidine was found to be 216.8 and 234.8 nm, respectively and isoabsorptive point was found to be at 224.6 nm.

Analysis of tablet: Twenty tablets were accurately weighed and powdered in a mortar. An accurately weighed amount equivalent to 25 mg of mefenamic acid and 0.1 mg of tizanidine was taken. Furthermore, it was demonstrated that ratio of both drugs present in some formulations were not in working range. Hence standard addition of 24.9 mg of tizanidine is made[41]. The mixture was then dissolved in 20 mL of methanol in 100 mL calibrated flask. After 0.5 h of mechanical shaking, the solution was filtered in a 250 mL calibrated flask through Whatmann filter paper No. 41. The residue was washed three times with 10 mL of solvent and then the volume was completed to 250 mL with the same solvent. The sample solution thus prepared was further diluted to get the solutions containing mefenamic acid and tizanidine in 12:12 µg mL^{-1} proportion, respectively.

RESULTS AND DISCUSSION

The absorption spectra of mefenamic acid and tizanidine overlapped closely shown in Fig. 1. For this reason, the determination of the above compounds was not possible by direct measurement of absorbances in zero-order spectra. By using Vierordt's method, the determination of the two compounds is possible for direct absorbance measurements in their zero-order spectra. For this procedure, the absorbance values were measured at 216.8 and 234.3 nm, selecting the maximum and the minimum wavelengths of the two compounds. In Q-analysis method, isoabsorptive point was found to be at 224.6 nm. Both the drugs obey Beer's law in concentration ranges of 4-18 µg/mL of mefenamic acid and 4-24 µg/mL of tizanidine. The parameters used in both methods were shown in Table-1 and the equations used have been explained in the method's section. The developed methods were validated accurately and summary of results of various validation parameters are listed in Table-2. The proposed methods were successfully applied to the analysis of mefenamic acid and tizanidine in combined pharmaceutical formulations without any interference of excipients and pretreatments and results obtained are listed in Table-3.

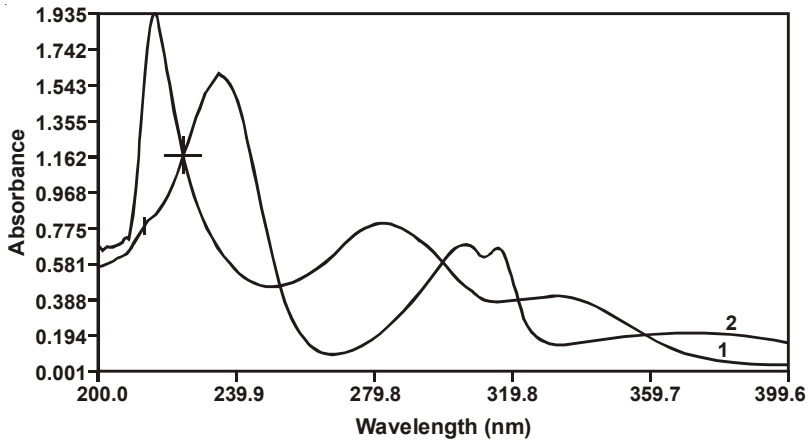

Fig. 1. Zero-order spectra of 12 µg/mL mefenamic acid and 12 µg/mL tizanidine HCl
1 = Mefenamic acid, 2 = Tizanidine hydrochloride

TABLE-1
EXPERIMENTAL PARAMETERS USED FOR THE SIMULTANEOUS DETERMINATION OF MEFENAMIC ACID AND TIZANIDINE

Parameters	Absorptivity values	
	Mefenamic acid [a]	Tizanidine [b]
λ_{MEF} (216.8 nm)	106.501190	42.265923
λ_{TIZ} (234.8 nm)	37.014881	84.023735
λ_{iso} (256.4 nm)	65.859524	59.670536

[a,b]Average of three readings.

TABLE-2
RESULTS OF VALIDATION PARAMETERS OBTAINED BY PROPOSED METHODS

Parameters	Method 1		Method 2	
	Mefenamic acid	Tizanidine	Mefenamic acid	Tizanidine
Range (μg mL^{-1})	4-24	4-28	4-24	4-28
Slope	0.1064	0.08215	0.1064	0.0597
Intercept	0.0005	0.02146	0.0005	0.0009
Correlation-coefficient (R^2)	0.9999	0.9997	0.9999	0.9999
Accuracy	99.778±0.918	99.588±0.95	99.268±1.334	100.106±1.062
Precision (% RSD)	1.788	1.624	2.422	1.8436
Reproducibility (% RSD)	1.669	1.148	1.501	1.2280

Method 1 = Vierordt's method; Method 2: Q-Analysis method.

TABLE-3
RESULTS OBTAINED FOR THE ANALYSIS OF
TABLETS BY DEVELOPED METHODS

Formulation	% Labeled claim obtained		Coefficient of variation		Standard error	
	Mefenamic acid[a]	Tizanidine[a]	Mefenamic acid[a]	Tizanidine[a]	Mefenamic acid[a]	Tizanidine[a]
Method 1						
Tablet-I	98.74 ± 0.1706	(100.42) ± 0.5122	0.03456	0.22307	0.12066	0.00319
Tablet-II	98.96 ± 0.0421	(98.82) ± 0.4261	0.03223	0.08554	0.11924	0.00125
Method 2						
Tablet-I	99.94 ± 0.3682	(100.42) ± 0.342	0.12614	0.15437	0.45777	0.002168
Tablet-II	98.72 ± 0.1525	(98.82) ± 0.2461	0.26949	0.10884	0.98167	0.001541

[a]Mean value of three determinations for each formulation ± Standard deviation.
Values in parentheses correspond to the parameters calculated after accounting for the actual values for tizanidine, that is, values without standard addition.
Method 1: Vierordt's method; Method 2: Q-Analysis method.

TABLE-4
STATISTICAL COMPARISON OF THE RESULTS
OBTAINED BY THE DEVELOPED METHODS

Drugs	[a]Method 1	[b]Method 2
Mefenamic acid (mean ± SD)	99.778 ± 0.918	99.268 ± 1.334
$t_{calculated}$	0.864	1.248
$t_{theoretical}$	2.260	2.260
$F_{calculated}$	0.425	1.045
$F_{theoretical}$	3.180	3.180
Tizanidine (mean ± SD)	99.588 ± 0.95	100.106 ± 1.062
$t_{calculated}$	0.768	0.954
$t_{theoretical}$	2.260	2.260
$F_{calculated}$	0.245	0.478
$F_{theoretical}$	3.180	3.180

Results obtained are average of 10 experiments for each; SD = Standard deviation.
[a]Method 1: Vierordt's method; [b]Method 2: Q-Analysis method.

Statistical comparison of the results of the developed methods: The results obtained from the analysis of commercial formulations were compared statistically by Student t-test and by the variance ratio F-test with those obtained by each method. The calculated values of the Student t-values at 95 % confidence level and the variance ratio F-values did not exceed the theoretical values indicating that there were no significant differences among the results of the developed methods represented in Table-4.

Conclusion

For routine analytical purpose, it is desirable to establish methods capable of analyzing large number of samples in a short time period with good accuracy and precision without any prior separation step. The proposed spectrophotometric methods described in this paper meet these requirements. It can be inferred that the methods described here are simple, accurate and could be used for rapid and reliable determination of mefenamic acid and tizanidine hydrochloride in quality control laboratories.

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