Development and Validation of Spectrophotometric Methods for Simultaneous Estimation of Tramadol Hydrochloride and Chlorzoxazone in Tablet Dosage Form

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Two simple, accurate, and precise methods for simultaneous estimation of tramadol hydrochloride and chlorzoxazone in combined dosage form have been described. The first method employs formation and solving of simultaneous equations using 272.20 and 248.30 nm as two analytical wavelengths. The second method is absorption ratio method, which uses 272.20 and 257.50 nm as two analytical wavelengths. Both the methods allow the simultaneous determination of tramadol hydrochloride and chlorzoxazone in concentration ranges employed for this purpose with the standard deviation of <1.0% in the assay of tablet.

A new fixed dose combination containing tramadol hydrochloride (TRM) and chlorzoxazone (CLZ) is available in the market in tablet dosage form for relieving low back pain and cervical spondylosis. USP monograph describes high performance liquid chromatography (HPLC)[1] method for assay of CLZ tablet and European pharmacopoeia describes potentiometric[2] method for assay of TRM hydrochloride. Literature survey reveals that reports are available for estimation of TRM hydrochloride by GC[3] in plasma and brain tissue of mice and rats, using HPLC[4-6] in plasma and urine, and spectrohotometry[7,8] in pharmaceutical formulations. Several spectrophotometric[9,10] and HPLC[11,12] methods are reported for estimation of CLZ in combination with other drugs from pharmaceutical formulations and biological fluid. However, no method is yet reported for simultaneous estimation of tramadol hydrochloride and chlorzoxazone in combined dosage form. Hence, two spectrophotometric methods have been developed to estimate these two drugs from tablet dosage form.

MATERIALS AND METHODS

UV/Vis. double beam spectrophotometer, model-Shimadzu UV 2401 PC with 1 cm quartz cells was used.

Preparation of solutions:
TRM hydrochloride standard stock solution (0.6 mg/ml) was prepared by transferring accurately weighed 60 mg portion of TRM in 100 ml volumetric flask and volume was made up with 0.1 N NaOH in methanol to give concentration of 600 µg/ml. CLZ standard stock solution (0.1 mg/ml) was prepared by transferring accurately 10 mg portion of CLZ in 100 ml volumetric flask and volume was made up with 0.1 N NaOH solution in methanol to give concentration of 100 µg/ml.

Simultaneous equations method (Method 1):
Selection of analytical wavelengths was done by taking pure samples of TRM and CLZ which were separately dissolved in 0.1 N NaOH in methanol to give two solutions of 60 and 10 µg/ml, respectively. They were scanned in the wavelength range of 200-400 nm. From the overlain spectra (fig. 1), wavelengths 272.20 and 248.30 nm were selected for the formation of simultaneous equations. For constructing a calibration curves, two series of different concentrations in range of 30-300 µg/ml for TRM and 5-50 µg/ml for CLZ were prepared from stock solutions. The calibration curves were plotted at 272.20 and 248.30 nm. The absorptivities (A1%, 1 cm) of both the drugs at both the wavelengths were determined. These

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TABLE 1: REGESSION ANALYSIS OF THE CALIBRATION CURVES

Method	Drug	Wavelength (nm)	Concentration range (µg/ml)	Intercept (RSD)	Slope	r^2
1	TRM	272.20	30-300	0.0082	0.0059	0.999
		248.30	30-300	0.006	0.006	0.9959
	CLZ	272.20	5-50	0.0326	0.0234	0.9952
		248.30	5-50	0.0295	0.049	0.998
2	TRM	272.20	30-300	0.0087	0.0059	0.999
		257.50	30-300	0.016	0.0019	0.997
	CLZ	272.20	5-50	0.00331	0.0234	0.9955
		257.50	5-50	0.0265	0.0422	0.9972

Method 1 is the simultaneous equation method while Method 2 is absorption ratio method, RSD is relative standard deviation and r^2 is correlation coefficient

calculated values were the mean of five independent determinations.

The absorbance and absorptivities values at the particular wavelengths were calculated and substituted in the following equations to obtain the concentrations: $A_1 = 0.56 \times 10^2$ Cx + 2.77×10^2 Cy- (1) and $A_2 = 0.04 \times 10^2$ Cx + 4.01×10^2 Cy- (2), where A_1 and A_2 are absorbance of sample solution at 248.30 and 272.2 nm, respectively. Cx and Cy are concentration of TRM hydrochloride and CLZ, respectively (in mole/l) in sample solution. By substituting the value of Cx from Equation (1) into Equation (2), the value of Cy can be obtained. Now substituting this value of Cy in one of the equations, the value of Cx can be obtained. The validity of formed equations was checked by preparing five mixed standards, measuring their absorbances at respective wavelengths and comparing these with the absorbances calculated using above formed equations.

Absorption ratio method (Method 2):

From the overlain spectra of TRM and CLZ 272.20 nm was taken as λ_{max} for TRM and 257.50 nm as isobestic point for estimation of TRM and CLZ, respectively.

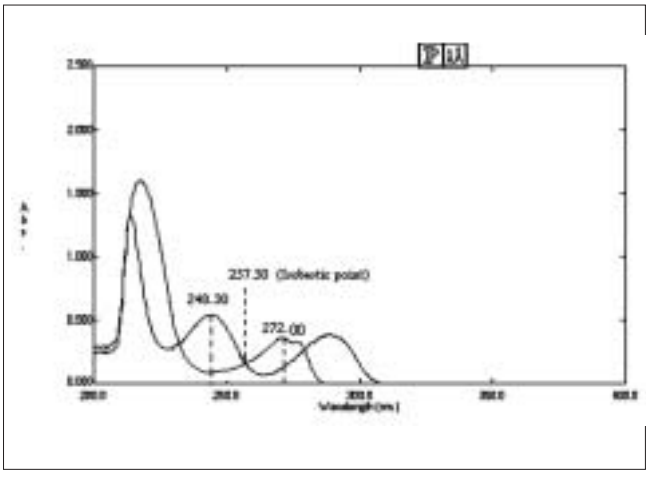

Fig. 1: Overlain spectra of TRM and CLZ

Series of different concentrations in range of 30-300 µg/ml for TRM and 5-50 µg/ml for CLZ were prepared from stock solutions. The calibration curves were plotted at 272.20 and 257.50 nm. The absorptivities (A1%, 1 cm) of both the drugs at both the wavelengths were determined. These calculated values were the mean of five independent determinations.

Estimation from tablets:

Twenty tablets of brand Muzox (Stedman Pharmaceuticals Ltd., Label claim CLZ 250 mg and 50 mg TRM) were weighed and finely powdered. Accurately weighed tablet powder equivalent to 100 mg was taken in 100 ml volumetric flask. To it 580 mg of pure TRM was added and sonicated for 5 min with 50 ml 0.1 N methanolic NaOH. The volume was made to mark. Aliquot portion of this solution was further diluted to achieve final concentration of 60 µg/ml for TRM and 10 µg/ml for CLZ. The absorbances were noted at respective wavelengths. The concentration of each drug in tablet formulation was determined using above methods.

RESULTS AND DISCUSSION

The overlain spectra of TRM and CLZ in the concentration ratio of 6:1 showed that the peaks are well resolved thus satisfactory criteria for obtaining maximum precision based on absorbance ratios The criteria being the ratios (A_2A_1/aX_2aX_1) for drug Y and (aY_2aY_1/A_1A_2) for drug X should lie outside the range of 0.1-2.0 where A_1 and A_2 represent absorbance of tablet solution at λ_1

TABLE 2: ASSAY RESULTS OF TRAMADOL HYDROCHLORIDE AND CHLORZOXAZONE IN MARKETED FORMULATION

Tablet	Method 1		Method 2	
	% TRM	% CLZ	% TRM	% CLZ
Muzox	100.11% ±0.75	101.29% ±0.80	100.86% ±0.85	99.93% ±0.87

Method 1 is the simultaneous equations method while Method 2 is absorption ratio method

TABLE 3: SUMMARY OF VALIDATION PARAMETERS

Parameters	Method 1		Method 2	
	TRM	CLZ	TRM	CLZ
Linearity range (µg/ml)	30-300	5-50	30-300	5-50
Correlation coefficient (r^2)	at 248.30		at 257.50	
	0.9959	0.9980	0.9970	0.9972
	at 272.20		at 272.20	
	0.9990	0.9952	0.9990	0.9955
Precision (R.S.D.)	0.0310	0.0090	0.00104	0.0166
Ruggedness				
Intraday (n=3)	0.7609	0.4336	0.5244	0.5819
Interday (n=3)	0.5461	0.5918	0.8543	0.9314
Accuracy (%)	100.27	99.78	100.19	99.79
Reproducibility	Reproducible	Reproducible	Reproducible	Reproducible
Specificity	Specific	Specific	Specific	Specific

Method 1 is the simultaneous equation method while Method 2 is absorption ratio method and r^2 is correlation coefficient

and λ_2, aX_1 and aX_2 represents absorptivities of X at λ_1 and λ_2 and aY_1 and aY_2 denote absorptivities of Y at λ_1 and λ_2, respectively. In the present contest, the above criteria found to be satisfied for TRM (X) and CLZ (Y) where λ_1 is 272.20 nm and λ_2 is 248.30 nm. In overlain spectra, CLZ shows two distinct peaks, one at around 248.30 and other at 292.00 nm. The peak at 248.30 nm was found to be prominent hence for simultaneous equations method; the peak was used for determination of CLZ. Since only one prominent peak exists for TRM at 272.20 nm, the same was used for its determination. Absorbance was determined at both the wavelengths. Calibration curves were plotted and regression analysis was carried out (Table 1). The absorbitivity was then calculated and substituted in Equations 1 and 2 along with absorbance values to obtain concentration of drugs.

In absorption ratio method, two wavelengths are selected from overlain spectra out of which one is isobestic point and another is λ_{max} of one of the drugs. The spectra of TRM and CLZ when overlaid indicated that the isobestic point was at 257.50 nm at which estimation of CLZ was done and estimation of TRM was done at its λ_{max}, 272.20 nm.

Both the methods were successfully used to estimate the amounts tramadol hydrochloride and chlorzoxazone in marketed tablet formulation containing tramadol hydrochloride 50 mg and chlorzoxazone 250 mg. The results obtained were comparable with the corresponding labeled amounts (Table 2).

By observing the validation parameters (Table 3), both the methods were found to be specific, accurate, precise, repeatable, and reproducible. However, Absorption ratio method has an advantage of simpler calculations over the simultaneous equations method. Hence, both methods can be employed for routine analysis of tablet for assay as well as dissolution testing.

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