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Determination of Hydrochlorothiazide by Using its Quenching Effect on the Fluorescence of Bisoprolol Fumarate in Pharmaceutical Tablet Dosage Form by Indirect Spectrofluorimetry

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A rapid, precise, accurate and specific spectroflourimetric method was developed for the determination of hydrochlorothiazide from its pharmaceutical dosage forms. The method is based on quenching of the fluorescence of bisoprolol fumarate by hydrochlorothiazide. The fluorescence intensity of mixture of bisoprolol fumarate (in optimal concentration) and hydrochlorothiazide solution was measured at 298 nm using an excitation wavelength of 229 nm and the linearity range was found to be 2-20 µg/mL. The method was validated in terms of accuracy and precision. The result of study showed that the proposed spectroflourimetric method is useful for the routine determination of hydrochlorothiazide in pharmaceutical tablet dosage form.

Key Words: Hydrochlorothiazide, Spectrofluorimetry, Bisoprolol fumarate and Quenching effect.

INTRODUCTION

Hydrochlorothiazide (HCTZ), 6-chloro-3, 4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide-1, 1-dioxide, is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical preparations which decreases active sodium reabsorption and reduces pheripheral vascular resistance. The literature reveals many spectrophotometric[1-4], liquid chromatographic[3-7] and capillary electrophoresis[8] methods for the quantitative determination of hydrochlorothiazide alone or in combination with other antihypertensive drugs. However, to our best of knowledge, no method has been reported till date for the determination of hydrochlorothiazide by spectrofluorimetry. Hydrochlorothiazide itself is not a fluorescent compound but quenches the fluorescence of bisoprolol fumarate (BF). So, in this communication an attempt has been made to develop a spectrofluorimetric method for the determination of hydrochlorothiazide in bulk and in tablet dosage form by the use of its quenching effect on the fluorescence of bisoprolol fumarate.

EXPERIMENTAL

Shimadzu Spectrofluorimeter model RF-450 and a 1 cm × 1 cm fluorescent free quartz cell was used for all fluorimetric determinations.

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Methanol of analytical reagent grade (Allied Chemical Corporation, Vadodara, India) and double distilled water were used as solvent. Pure drug sample of hydrochlorothiazide and bisoprolol fumarate was kindly gifted by M/s. Sun Pharma Advanced Research Center, Vadodara, India.

About 50 mg each of hydrochlorothiazide and bisoprolol fumarate were accurately weighed and dissolved in 50 mL of methanol separately. Five mL of the above solutions were diluted separately to 50 mL with double distilled water to produce 100 µg/mL each of hydrochlorothiazide and bisoprolol fumarate in double distilled water.

Preparation of calibration curve: Into a series of eight 10 mL volumetric flasks 0.8 mL of standard stock solution of bisoprolol fumarate were added. The first flask was diluted up to the mark with double distilled water to give 8 mg/mL of bisoprolol fumarate. Into the remaining flasks, 0.2-2.0 mL of standard stock solutions of hydrochlorothiazide and 0.8 mL of bisoprolol fumarate were added and diluted with double distilled water to get 2, 4, 6, 8, 12, 16 and 20 µg/mL of hydrochlorothiazide in 8 µg/mL of aqueous bisoprolol fumarate (as background fluorescent indicator). Fluorescence was measured at 298 nm and calibration graph was obtained by plotting fluorescence intensity *versus* concentration of hydrochlorothiazide (excitation wavelength selected was 229 nm). The plot of fluorescence intensity against concentration of hydrochlorothiazide was found to decrease linearly in the range of 2-20 µg/mL of hydrochlorothiazide with correlation coefficient (R^2), slope (m) and intercept (c), 0.999, -2.3596 and 64.804, respectively.

Analytical method validation: Accuracy was determined by recovery study of hydrochlorothiazide, in which known amount of standard was added to pre-analyzed sample and subjected to the proposed spectrofluorimetric analysis. The study was performed at 3 different concentration levels. Intra-day precision and inter-day precision of the method were assessed from the results of triplicate analyses of the pure drug solution. The mean values and relative standard deviation values for replicate analysis at 10 different concentration levels were calculated. The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the ratio of 3.3 and 10.0 standard deviation of the blank (n = 7), respectively and the slope of the calibration line.

Analysis of tablet: A total of 20 tablets were accurately weighed and powdered in a mortar. An amount equivalent to 5 mg was taken and dissolved in 10 mL of methanol by magnetically stirring for 5 min. About 10 mL of water was added and stirred for further 5 min. The mixture was transferred to two centrifuge tubes and centrifuged at 1000 rpm for 5 min. The supernatant was transferred to a 100 mL volumetric flask through a Whatman No. 42 filter paper. The paper was washed thrice with double distilled water and the combined filtrate was made up to the mark with double distilled water. Suitable aliquot of above sample solution and standard solution of bisoprolol fumarate was diluted to get concentration of hydrochlorothiazide and bisoprolol fumarate in the range specified in calibration curve.

Fluorescence was measured and the concentration of hydrochlorothiazide was found from the calibration curve.

RESULTS AND DISCUSSION

During the investigation it was observed that hydrochlorothiazide quenches fluorescence intensity of bisoprolol fumarate at 298 nm. This property has been successfully exploited to develop a spectrofluorimetric method for determination of hydrochlorothiazide. Thus, the proposed method involves interaction of hydrochlorothiazide and bisoprolol fumarate followed by determination of hydrochlorothiazide.

Different solvents *viz*., methanol, 0.1 N HCl, 0.1 N H_2SO_4 and methanolic water were used for preparation of standard and sample solutions. Methanolic water was selected as solvent since it gave maximum fluorescence intensity of bisoprolol fumarate at 298 nm.

The fluorescence quenching interaction of hydrochlorothiazide on bisoprolol fumarate was studied as a function of hydrochlorothiazide and bisoprolol fumarate concentration. The result revealed that quenched fluorescence intensity was found to be proportional to the concentration of hydrochlorothiazide in the range of 2-20 μg/mL when concentration of bisoprolol fumarate was 8 μg/mL. Therefore, 8 μg/mL of bisoprolol fumarate was chosen for further investigation.

The method showed good linearity in the concentration range of 2-20 μg/mL with the correlation coefficient of 0.9999. The optimized method parameters, regression characteristics and the data obtained from the measurement are shown in Table-1. Also, the standard and sample preparation required less time and no tedious extraction was involved. From the recovery study shown in Table-2, it was found that 100.91 % of hydrochlorothiazide was recovered which indicated high accuracy of the method. The precision of the method was checked in terms of interday and intraday, where method was repeated on 3 different days and also repeated for 3 different time periods in the same day. The percentage relative standard deviation was (RSD %) was 0.51 and 0.93 % for interday and intraday study, respectively, which is well within the acceptable limit of 2 %. The limit of detection (LOD) and limit of quantification (LOQ) for hydrochlorothiazide were found to be 0.495 and 1.649 μg/mL, respectively. The signal to noise ratio was 3 for LOD and 10 for LOQ. The selectivity of the method was checked by analyzing hydrochlorothiazide along with common excipients. It was found that the excipients did not interfere with the estimation of hydrochlorothiazide. The assay value of hydrochlorothiazide tablets was found to be 99.57 ± 0.831 % (Table-3).

This demonstrates that the developed spectrofluorimetric method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be successfully applied for the routine quality control of bulk and tablet dosage form of hydrochlorothiazide within a short analysis time.

TABLE-1
OPTIMIZED METHOD PARAMETERS AND LINEAR REGRESSION DATA FOR CALIBRATION CURVE

Method parameters	Optimized values	Method parameters	Optimized values
Solvent*	Methanol and double distilled water	Scan speed	Fast (1)
Excitation wavelength (nm)	229	Concentration range (µg/mL)	2-20
Emission wavelength (nm)	298	Slope (m)	-2.3596
Ordinate scale	3	Intercept (b)	64.804
Abscissa scale	2	Correlation coefficient	0.999
Scanning range (nm)	200-600	RSD (%)	0.692

*Methanol was used to dissolve the drug and further dilution was made with double distilled water.

TABLE-2
RECOVERY STUDY DATA

Sample	Amount claim per tablet (mg)	Amount added (%)	Total amount added (mg)	Amount recovered* (mg)	Recovery ± SD** (%)	RSD** (%)
1	50	80	40	40.02	100.05 ± 0.155	0.155
2	50	100	50	49.97	99.89 ± 0.345	0.345
3	50	120	60	60.07	100.12 ± 0.225	0.224

*Mean of three determinations; **SD: Standard deviation of three determinations; RSD: Relative standard deviation of three determinations.

TABLE-3
ANALYSIS OF COMMERCIAL TABLET FORMULATION

Label claim (mg/tablet)	Amount found.* (mg)	Amount estimated* ± SD** (%)	RSD** (%)
50	49.83	99.67 ± 0.845	0.276

*Mean for three determinations. **SD: Standard deviation of three determinations; RSD: Relative standard deviation of three determinations.

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