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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF HYDROCHLOROTHIAZIDE AND PROPRANOLOL IN BULK AND FORMULATION BY SIMULTANEOUS EQUATION METHOD

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ABSTRACT

The present study deals with UV spectrophotometric method development & validation for simultaneous estimation of Hydrochlorothiazide and Propranolol in dosage form by Simultaneous Equation Method. The wavelengths selected for the method were at the λmax 271nm and 289nm for Hydrochlorothiazide and Propranolol respectively. The linearity of Hydrochlorothiazide and Propranolol was found to be in the range of 4-24 µg/ml & 8-48µg/ml respectively. The linear correlation was obtained (r^2> 0.998) in the range of 4-24µg/ml for Hydrochlorothiazide at 271nm.The method was found to be linear (r^2> 0.998) in the range of 8-48 µg/ml for Propranolol at 289 nm. The limit of detection was 0.052 µg/ml and 0.461 µg/ml for Hydrochlorothiazide and Propranolol respectively. The limit of quantification was 0.158 µg/ml and 1.397 µg/ml for Hydrochlorothiazide and Propranolol. The accuracy of the method was found to be 97-98%.The simple and economical method was successfully applied for simultaneous determination of Propranolol and Hydrochlorothizide in combined dosage form. The proposed method was validated as per ICH guidelines.

Keywords: Propranolol, Hydrochlorothiazide, linearity, Simultaneous Equation Method.

INTRODUCTION

UV visible spectrophotometeric method[1-3] is very frequently employed in pharmaceutical analysis. It involves the measurement of the amount of ultraviolet (190-380nm) or visible (380-800nm) radiation absorbed by a substance in solution by an instrument which measures the ratio or a function of the ratio of the intensity of two beams of light in UV-Visible region. The basis of allspectrophotometric methods for multicomponent sample analysis is the property that the absorbance of a solution is the sum of absorbances of individual components or the measured absorbance is the difference between total absorbance of the solution in the sample cell and that of the solution in the reference (blank) cell. The various spectrophotometric methods which are used for estimation of drug in combine dosage form include simultaneous equation method[4-5], absorbance ratio method[6-8], derivative spectrophotometery[9-10] and dual wavelength quantition method.

Propranolol[11-12] is a non-selective beta blocker mainly used in the treatment of hypertension.

Propranolol is used in the treatment or prevention of many disorders including acute myocardial infarction, arrhythmias, angina pectoris, hypertension, hypertensive emergencies, hyperthyroidism and migraine. Chemically propranolol hydrochloride is 1-naphthalen-1-yloxy-3-(propan-2-ylamino) propan-2-ol hydrochloride.

Hydrochlorothiazide[13-14] is a first line diuretic drug of thiazide class that acts by inhibitingthe kidneys ability to retain water. This reduces the volume of the blood decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance. Chemically hydrochlorothiazide is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide1,1-dioxide.

Propranolol and hydrochlorothiazide[15-16] combination is used to treat high blood pressure (hypertension).

According to the literature available till today, all the UV-Visible methods are developed for Hydrochlorothiazide and Propranolol except Simultaneous Equation method. So the present communication reveals the Simultaneous estimation of Hydrochlorothiazide and Propranolol.

SIMULTANEOUS EQUATION METHOD OR VIERODT'S METHOD

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λmax different from the other, it may be possible to determine both drugs by the technique of simultaneous equations (Vierodt's method), provided certain criteria'sare applied. The information required is the aborptivities of X at andλ_1 and $\lambda_2$are ax_1 and ax_2 respectively. The aborptivities of Y at and λ_1 and $\lambda_2$are ay_1 and ay_2 respectively. The absorbances of the diluted sample at λ_1 and λ_2 are A_1 and A_2 respectively. Let Cx and Cy be the concentrations of X and Y respectively in the diluted sample. Two equations are constructed based upon the fact that at λ_1 and λ_2, the absorbance of the mixture is the sum of the individual absorbance of X and Y.

At $\lambda_1 A_1 = aX_1\ b\ Cx + aY_1 b\ Cy$---------- (1)

At $\lambda_2 A_2 = a\ X_2\ b\ Cx + aY_2\ b\ Cy$---------- (2)

For measurements in 1 cm cells b=1

Substituting for Cy in eq. (1) and rearranging

$$Cx = \frac{A_2\ a_{y1} - A_2\ a_{y2}}{A_{x2}\ a_{y1} - a_{x1}\ a_{y2}}$$

$$Cy = \frac{A_1\ a_{x2} - A_2\ a_{x1}}{A_{x2}\ a_{y1} - a_{x1}\ a_{y2}}$$

As an exercise one needs to drive modified equation containing a symbol b for path length for application in situations where A1 and A2 are measured in cells other than 1 cm path length. Criteria for obtaining maximum precision based upon absorbance ratios have been suggested that place limits on the relative concentration of the components of the mixture4. The criteria are that the ratios

$$A_2 / A_1 \text{ and } A_2 / A_1$$
$$aX_2 / a\ X_1 \text{ and} aY_2 / aY_1$$

Should lie outside the range 0.1-2.0 for the precise determination of X and Y respectively. These criteria are satisfied only when the λmaxof two component are reasonably dissimilar. An additional criterionis that the two components don't interact chemically thereby negating the initial assumption that the total absorbance is the sumof individual absorbances. The additiviy of the absorbance shouldalways be confirmed in the development of a new application of these techniques.

MATERIALS AND METHODS

Materials

Methanol was procured from Merck, India. Formulations of Hydrochlorothiazide and Propranolol are purchased from local market. Standard drugs of Hydrochlorothiazide and Propranololwere procured from Aurobindo laboratories.

Apparatus and conditions

A double beam UV/Visible spectrophotometer with data processing capacity was used. Absorption and overlain spectra of both test and standard solutions were recorded over the wavelength range of 200-400nm using 1cm quartz cell.

Preparation of standard stock solution

Stock solutions (100 µg/ml) of Hydrochlorothiazide and Propranolol were prepared by dissolving separately 10mg of drug in minimum quantity of methanol and finally diluted with water to make up the volume up to 100 ml. The maximum absorbance (λmax) of Hydrochlorothiazide and Propranolol were obtained at 271 nm and 289 nm, respectively for simultaneous estimation of Hydrochlorothiazide and Propranolol. A series of standard drug solutions in concentration range of 4-24 µg/ml for Hydrochlorothiazide and 8-48 µg/ml for Propranolol were prepared by diluting appropriate volumes of the standard stock solutions. The scanning for solution of Hydrochlorothiazide and Propranolol were carried out in the range of 200-400 nm against water as blank for obtaining the overlain spectra that was used in the analysis.

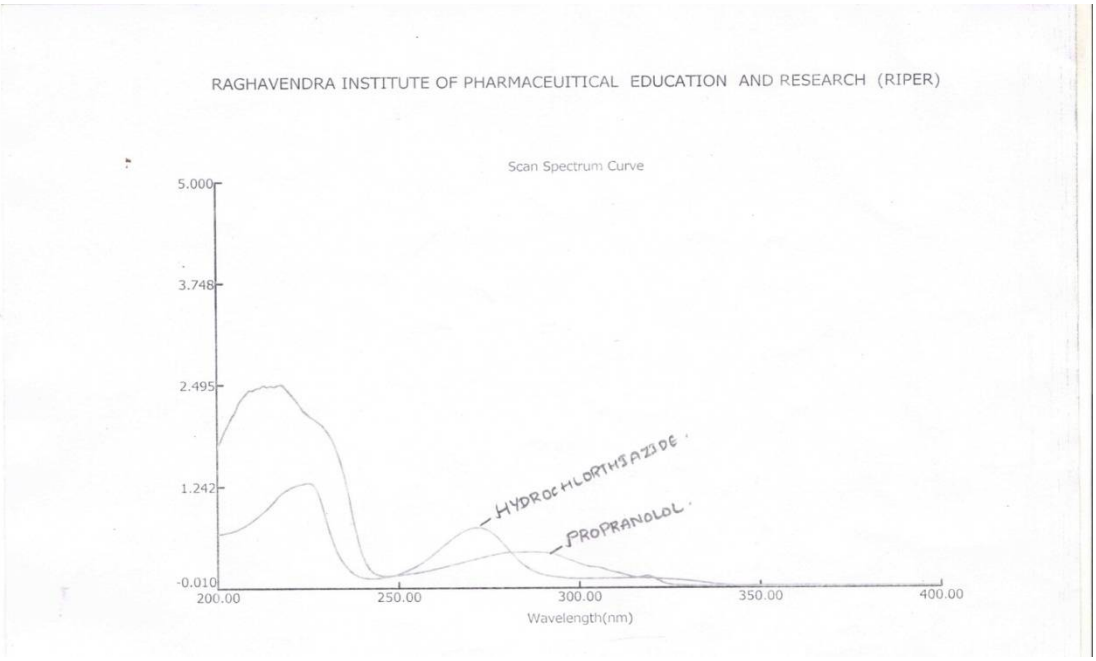

Fig. 1: Overlain spectra of Hydrochlorothiazide and Propranolol

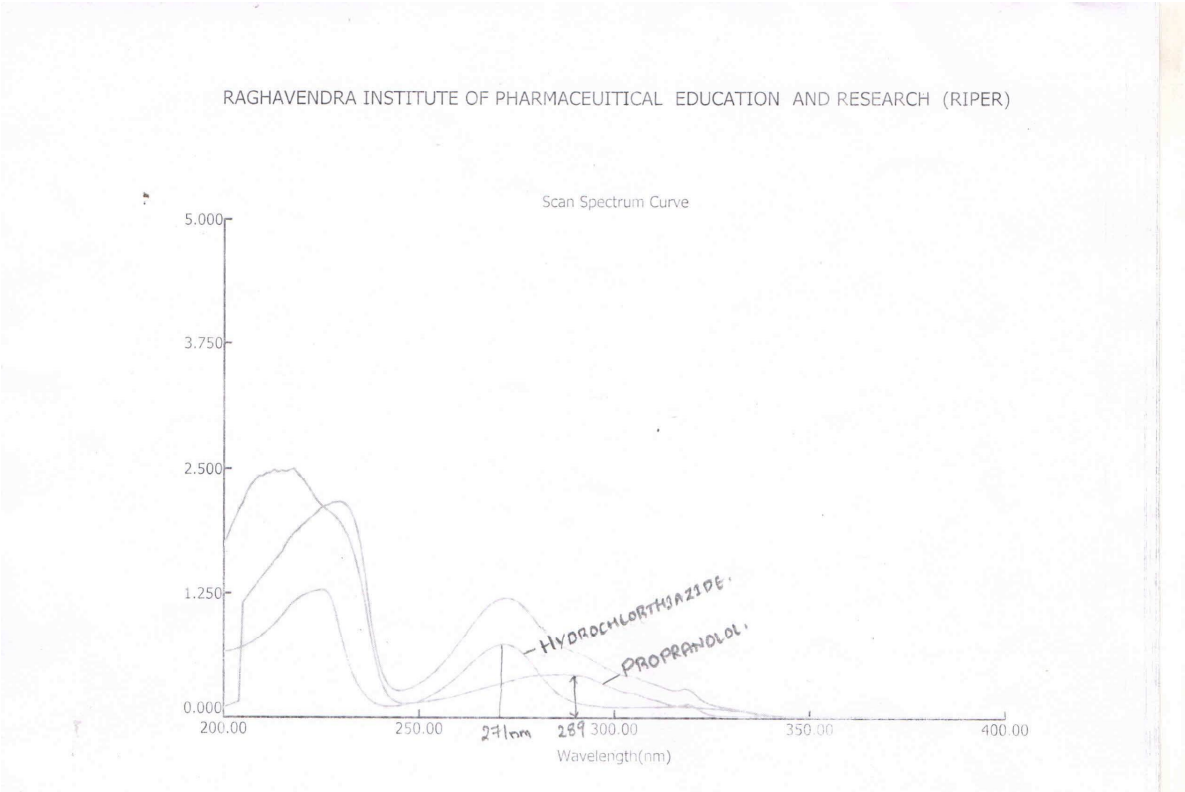

Fig. 2: Simultaneous Over lain spectra of Hydrochlorothiazide and Propranolol

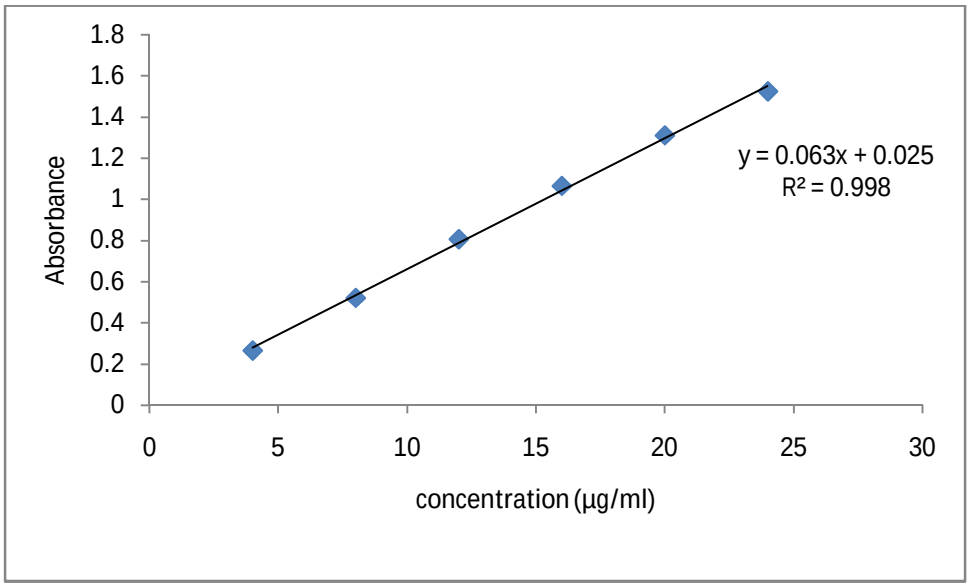

Fig. 3: Regressed standard curve of Hydrochlorothiazide at λmax 271nm

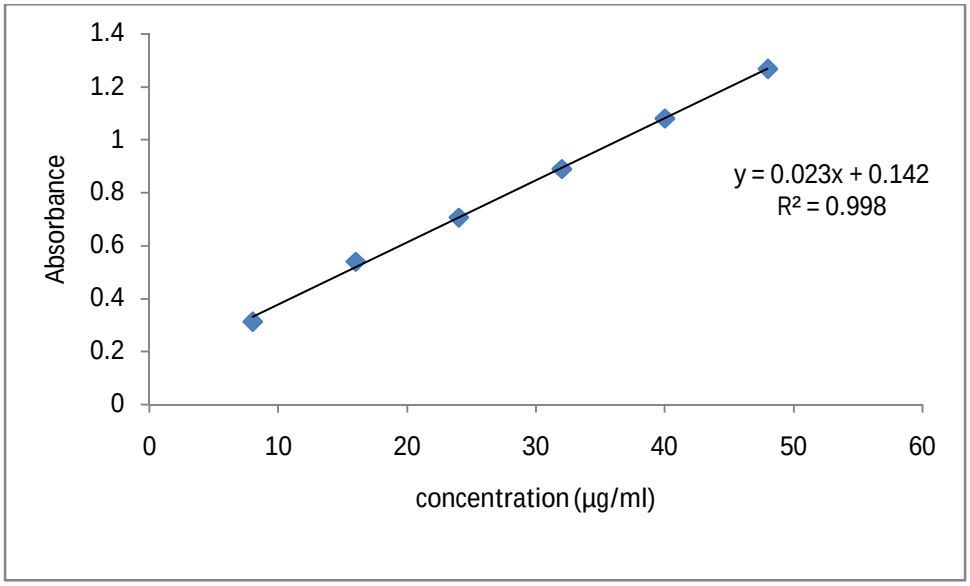

Fig. 4: Regressed standard curve of Propranolol at λmax 289nm

Table 1: Estimation of Hydrochlorothiazide and Propranololby simultaneous equation method

S.No.	Drug	λ_1 (271nm)	λ_2 (289nm)
1.	Hydrochlorothiazide	0.06058 (ax_1)	0.01358 (ax_2)
2.	Propranolol	0.01408 (ay_1)	0.01775 (ay_2)
3.	Mixture	1.056	0.565

Table 2: Linearity of Hydrochlorothiazide and Propranolol

S.No.	Hydrochlorothiazide		Propranolol	
	Concentration(µg/ml)	Absorbance at 271nm	Concentration(µg/ml)	Absorbance at 289 nm
1	4	0.267	8	0.314
2	8	0.522	16	0.540
3	12	0.807	24	0.707
4	16	1.065	32	0.890
5	20	1.310	40	1.081
6	24	1.523	48	1.268
Regression Valuer²	0.998		0.998	
Regression equation	Y = 0.063x + 0.025		Y = 0.0235x + 0.1424	

Table3: Assay

S.No.	Drug	Amount taken(µg/ml)	Amount Found± S.D (n=3)	Assay	%RSD
1.	Hydrochlorothiazide	12	12.2± 0.1414	101.66%	0.168
2.	Propranolol	24	22.5± 1.0606	93.75%	0.650

Table 4: Recovery studies

S.No.	Name of the drug	Amount of sample (µg/ml)	Recovery level	Amount added (µg/ml)	Amount recovered (µg/ml)	%Recovery
1.	Hydrochlorothiazide	12	80%	9.6	20.55	97.57
			100%	12.0	23.57	98.21
			120%	14.4	24.82	97.80
2.	Propranolol	24	80%	19.8	43.13	98.47
			100%	24.0	47.25	98.45
			120%	28.8	48.89	98.22

Table 5: Optical characteristics of the present method

S.No.	Parameter	Hydrochlorothiazide	Propranolol
1.	λ_{max}	271nm	289nm
2.	Beer's limit (µg/ml)	4-24	8-48
3.	Regression equation	Y = 0.063x + 0.025	Y = 0.0235x + 0.1424
	a) Slope	0.063	0.0235
	b) Intercept	0.025	0.1424
	c) Co-relation coefficient	0.998	0.998
4.	LOD (µg/ml)	0.052	0.461
5.	LOQ (µg/ml)	0.158	1.397
6.	Precision	<2%	<2%
7.	% Recovery	98.25-99.83	98.66-99.4

RESULTS AND DISCUSSION

The selected drugs Hydrochlorothiazide and Propranolol in Bulk and Formulation were estimated by using simultaneous equation UV spectrophotometric method as per ICH guidelines. The method was validated for all validation parameters as per ICH guidelines. The linearity range for Hydrochlorothiazide and Propranolol was 4-24 µg/ml and 8-48 µg/ml respectively, with r^2 value of 0.998 and 0.998 respectively. The % RSD for intraday and inter-day precision was <2%. The method has been validated in assay of active pharmaceutical ingredients. The accuracy of the method was validated by recovery studies and was found to be significant and under specification limits, with % recovery 98-99 (i.e., within the acceptable range 97-102%). The assay results were found to be 101.6% and 93.75% (within the acceptable limits).

CONCLUSION

An accurate and precise simultaneous equation spectrophotometric method has been developed and validated for the analysis of Hydrochlorothiazide and Propranolol in formulation. The percentage recovery and found concentrations of active ingredients were within the acceptable limits. As the LOD and LOQ values were very low. Hence this method can be used for the estimation of Hydrochlorothiazide and Propranolol in bulk&formulation for quality control studies.

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