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Research Article

Simultaneous determination of Propranolol hydrochloride and Hydrochlorothiazide in Tablets formulation using spectrophotometric technique (Simultaneous Equation Method)

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Abstract

A new spectrophotometric method was developed for simultaneous determination of compounds with interfering spectra in binary mixtures without previous separation, showing significant advantages over the conventional methods regarding minimal data manipulation and applicability. The proposed method was applied for the determination of Propranolol hydrochloride and Hydrochlorothiazide in Tablets formulation, for determination of sampling wavelength, 10 µg/ml of each of PRO and HCT were scanned in 200-400 nm range and sampling wavelengths were 289 nm for PRO and 270 nm for HCT are selected for development and validation of simultaneous equation method. For this method linearity observed in the range of 10-50 µg/ml for PRP and 5-25 µg/ml for HCT, and in their pharmaceutical formulation with mean percentage recoveries100.13± 0.86 and 100.07± 0.58, respectively. The method was validated according to ICH guidelines and can be applied for routine quality control testing.

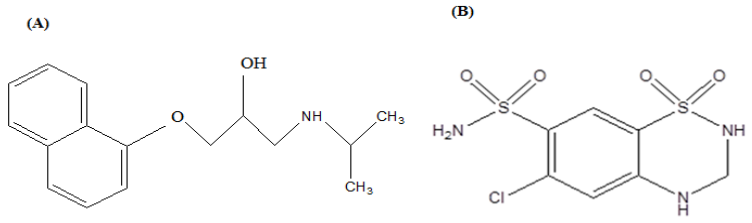
Keywords: Spectroscopic method, simultaneous equation method, propranolol hydrochloride and hydrochlorothiazide

1. Introduction

Many methods have been introduced for the analysis of binary mixtures among which the spectrophotometric based methods were the most simple, fast and applicable in almost all laboratories. Several manipulations were performed on the raw overlapping spectral data to enable mixture resolution for example, using different order derivatives[1-11].The aim of the present work was to develop a new simple, rapid, selective method for the simultaneous determination of components having overlapping spectra in binary mixtures, having the advantages of minimal data processing and a wider range of applications over the previously mentioned methods. To prove the ability of the newly described method in resolving the overlapping spectral data and simultaneous determination of each component, it was applied for the analysis of a mixture of propranolol hydrochloride (PRO) and hydrochlorothiazide (HCT) formulated together in the form of tablets widely used for the treatment of heart related problems accompanying several hypertension.

Propranolol hydrochloride is chemically (2 RS)-1-[(1- methyl ethyl) amino]-3-(naphthalene-l-yloxy)propan-2-ol hydrochloride[12]. Propranolol hydrochloride isa beta-adrenergic blocking agent that is used for treating high blood pressure, heart pain, abnormal rhythms of the heart, and some neurologic conditions, also used to Angina pectoris and coronary artery disease. Propranolol is useful in slowing and regulating Tachycardia[13]. Hydrochlorothiazide is chemically 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide[14].Hydrochlorothiazide is Diuretic and Antihypertensive used totreat excessive fluid accumulation and swelling (edema) of the body caused by heart failure, cirrhosis, chronic kidney failure, corticosteroid medications, and nephrotic syndrome. Combination of propranolol hydrochloride and hydrochlorothiazide is used to treat hypertension and heart related dieses[15].

Figure 1: (A) is Structure of propranolol hydrochloride and (B) is structure of Hydrochlorothiazide.



1.1. Theory

We can find out concentration of both the drug from combination mixture using the simultaneous equation method. In this method using the absorbance of both the drug and mixture at their wavelength and put this value in following equation and we can find out the concentration of drugs present in combination.

$$Cx = \frac{(A_2 \times Ay_1) - (A_1 \times Ay_2)}{(Ay_1 \times Ax_2) - (Ay_2 \times Ax_1)} \quad \text{---------- (1)}$$

$$Cy = \frac{(A_1 \times Ax_2) - (A_2 \times Ax_1)}{(Ax_2 \times Ay_1) - (Ax_1 \times Ay_2)} \quad \text{---------- (2)}$$

Where,

Cx = Concentration of drug X
Cy = Concentration of drug
A1 = Absorbanceof mixture at wavelength 1
A2 = Absorbance of mixture at wavelength 2
Ax1 = Absorptivity of drug A at wavelength 1
Ax2 = Absorptivity of drug A at wavelength 2
Ay1 = Absorptivity of drug B at wavelength 1
Ay2 =Absorptivity of drug B at wavelength 2

2. Material and method

2.1. Apparatus

A double beam UV/ Visible spectrophotometer (Shimadzu model2450, Japan) with spectral width of 2nm, 1 cm quartz cells was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software.

2.2. Reference samples

PRO and HCT reference standard are kindly supply by CIPLA LTD, Mumbai and CTX Life Science, Surat as a gift sample respectively.

2.3. Pharmaceutical formulation

Cipler-H tablet, labelled to contain 40 mg PRO and 20 mg HCT, manufactured by Cipla Ltd.

2.4. Materials and reagents: Methanol AR grade (RANKEM)

2.5. Standard solutions

2.5.1. Standard solution of Propranolol HCl (PRO)

Accurately weighed quantity of PRO10mg was transferred to 100ml volume tricflask, dissolved and diluted upto mark with Methanol to give a stock solution having strength 100µg/ml.

2.5.2. Standard solution of Hydrochlorothiazide (HCT)

Accurately weighed quantity of HCT 10mg was transferred into100ml volumetric flask, dissolved and diluted up to mark with Methanol to give a stock solution having strength 100µg/ml.

2.5.3. Preparation of standard mixture

Pipette out accurately 1 ml of PRO stock solution (100µg/ml), 0.5 ml of HCT stock solution (100µg/ml) in 10 ml volumetric flask and make up the volume up to the mark with Methanol. It gives solution containing PRO 10µg/ml, HCT 0.5µg/ml.

2.5.4. Test Sample Preparation

Dissolve tablet sample in 100 ml volumetric flask containing 100 ml methanol. Take 1 ml tablet sample solution in 10 ml volumetric flask and make up volume up to mark with methanol.

2.6. Procedures

2.6.1. Construction of calibration curves (linearity)

This series consisted of five concentrations of standard PRO solution ranging from10-50µg/ml. The solutions were prepared by pipetting out standard PRO stock solution (1ml, 2ml, 3ml, 4ml, 5ml) was transferred into a series of 10ml volumetric flasks and volume was adjusted upto mark with Methanol. A zero order spectra of the resulting solutions were recorded, measured the absorbance at 289.0 nm against a reagent blank solution (Methanol).Calibration curve was prepared by plotting absorbance versus respective concentration of PRO.

This series consisted of five concentrations of standard HCT solution ranging from 5-25 µg/ml. The solutions were prepared by pipetting out Standard HCT stock solution (0.5ml, 12ml, 1.5ml, 2ml and 2.5ml) was transferred into a series of 10 ml volumetric flasks and volume was adjusted upto mark with Methanol. A zero order spectra of the resulting solutions were recorded and measured the absorbance at 270 nm against a reagent blank solution (Methanol).Calibration curve was prepared by plotting absorbance versus respective concentration of HCT.

Figure 2: Overlain linear zero order spectra of PRO (Red) and HCT (Black) in2:1 ratio

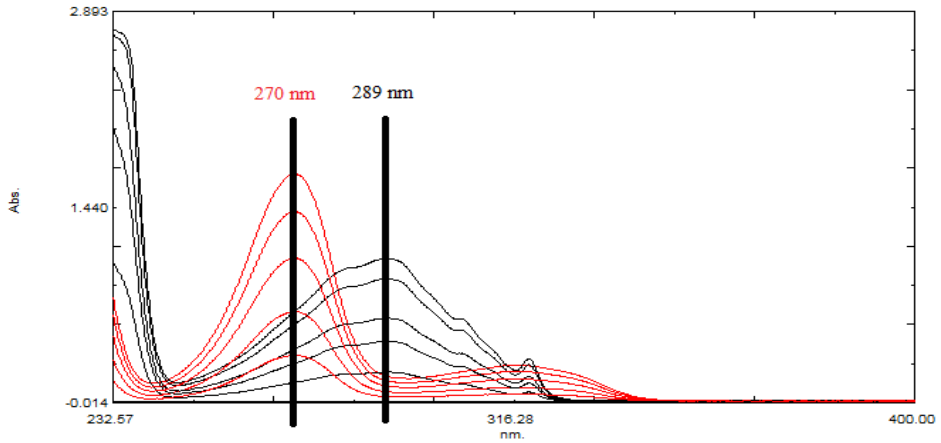


Figure 3: calibration curve of propranolol hydrochloride

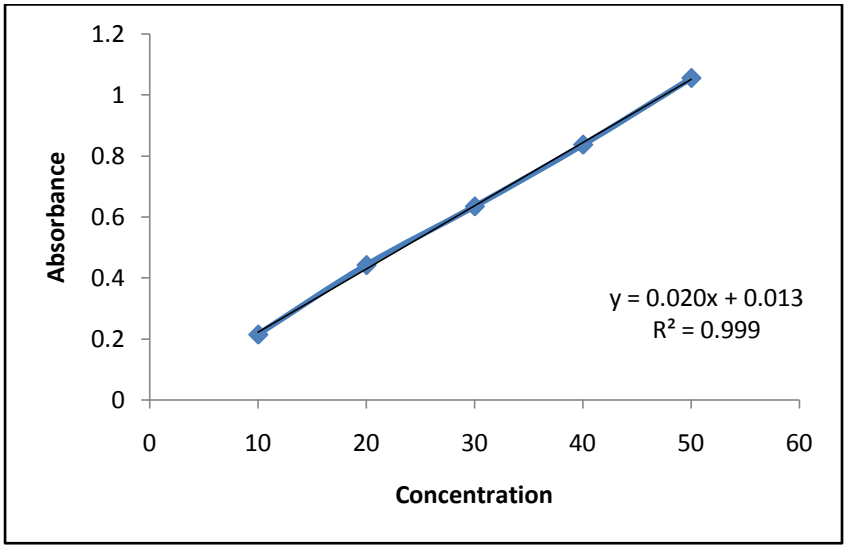
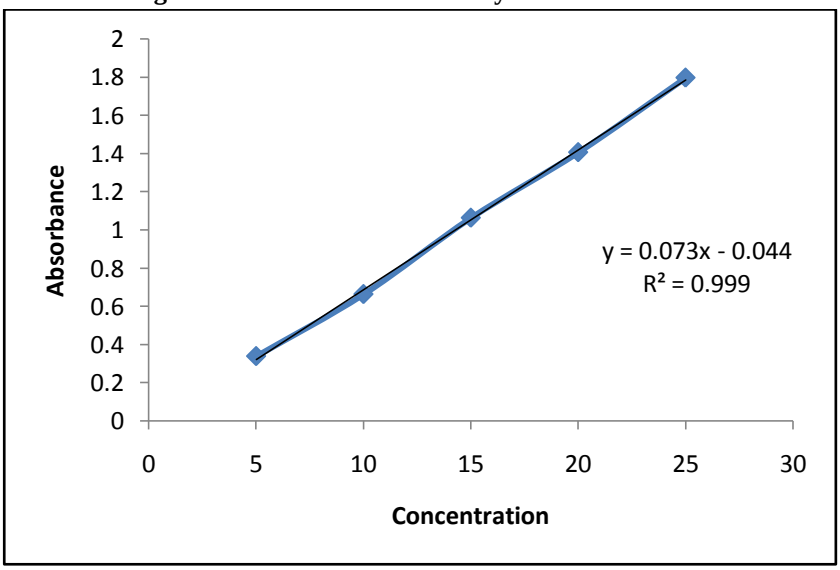


Figure 4: Calibration curve of hydrochlorothiazide



2.6.2. Analysis of laboratory-prepared mixtures

Laboratory-prepared mixtures containing different ratios of PRO and HCT were prepared. By applying the procedure under linearity, absorbance at 289.0 nm was recorded for PRO and 270.0 nm were recorded for HCT. The concentration of each drug in each mixture was calculated from its corresponding Cx and Cy equation. Validity of the method was assessed by spiking the pharmaceutical formulation by known amounts of standard drug powders (standard addition technique). The recovery of the added standards was then calculated after applying the proposed method.

2.6.3. Application of the proposed method for the simultaneous determination of PRO and HCT in Cipler – H tablet

Take one tablet and dissolved into a 100-ml beaker and sonicated in 100 ml methanol for 15 min, filtered into 100- ml volumetric flask. The residue was washed three times each using 10 ml methanol and completed to the mark with the same solvent. Transfer accurately 1 ml of the extracted solution into a 10-ml measuring flask. One millilitres of PRO working solution (20µg/ml) equivalent to 40 µg PRO was added and completed to the mark with methanol. The general procedure under linearity was followed.

3. Results and discussion

The absorbance wavelength for PRO and HCT found to be 289 and 270 nm, respectively, which is different and hence non-overlapping. Thus simultaneous determination of PRO and HCT in bulk mixture-I and tablet solution-I was found to be successful by simultaneous equation.

3.1. Specificity

The specificity of the method was investigated by observing any interference of one drug with other two drugs in bulk mixture and tablet solution. Similarly the interference of excipients of tablet with drugs was investigated.

3.2. Linearity and range

The linearity of method is its ability within a given range to obtain test results which are directly or through a mathematical transformation, proportional to the concentration of analyte. Linearity of the method was determined at five concentration levels for PRO and HCT independently.

3.3. Accuracy

The accuracy of an analytical method is the closeness of the test results to the true value. It was tested by spiking standard PRO solution in different concentration 80, 100 and 120% to a tablet solution. The tablet solution was analyzed at 289 nm for estimation of PRO. Similarly, the accuracy for HCT was determined at 270 nm, respectively.

3.4. Precision

The intra-day precision (repeatability) of method was determined by measuring the absorbance of tablet solution-I at 289 and 270 nm for PRO and HCT, respectively; within a laboratory over a short period of time. The inter-day precision (intermediate precision) was determined by measuring the absorbance of tablet solution-I at 289 and 270 nm for PRO and HCT, respectively, within a laboratory on three consecutive days, by different analysts. The %RSD was calculated for intra and inter-day precision.

3.5. LOD and LOQ

The LOD of an analytical method is the lowest amount of analyte in a sample which can be detected but not necessarily quantified. The detection limit (DL) of method was determined by equation, DL = (3.3 σ)/S, where, σ– standard deviation of blank response, S– slope of the calibration curve. The quantitation limit (QL) of analyte was determined by equation DL = (10 σ)/S, where, σ– standard deviation of blank response, S– slope of the calibration curve

3.6. Robustness and ruggedness

Robustness and ruggedness of the method has been evaluated at two different levels i.e. change in stock solution and changing the instrument.

4. Analysis of commercial tablets

The proposed method was successfully applied to the analysis of both mixtures in their pharmaceutical preparations. Results obtained were precise and in good agreement with the labelled claim as concluded from the satisfactory values of % recovery and RSD (%) gathered in table 2 and 3. Proposed method is precise and accurate, and give same result in same day and between the day and this data is gathered in table 1. When we change in standard stock concentration or changed the instrument that time also this proposed method give good result this data also gathered in table 4 and 5.

Table 1: Inter day and intraday precision data for propranolol and hydrochlorothiazide in three different concentration ranges.

Precision	Conc.		Propranolol*	Hydrochlorothiazide*
	PRO	HCTZ	289 nm	270 nm
Intraday (n=3) Abs. ±% RSD	10	5	0.247±0.8097	0.464±0.3287
	20	10	0.465±0.1240	0.909±0.2784
	30	15	0.773±0.2587	1.482±0.1401
Interday (n=3) Abs. ± % RSD	10	5	0.214±0.2693	0.338 ±0.2958
	20	10	0.442±0.1305	0.666±0.1501
	30	15	0.635±0.2409	1.056±0.5463

Table 2: Accuracy data for propranolol hydrochloride with % recovery and % RSD

Concentration of PRO from formulation (µg/ml)	Amount of PRO spiked (µg/ml)	Total amount (µg/ml)	Amount found (µg/ml)	% recovery	S.D	% RSD
20	16	36	36.337	101.709	0.001	0.299
20	20	40	40.880	100.464	0.00577	0.5245
20	44	44	44.282	101.369	0.00095	0.9885

Table 3: Accuracy data for hydrochlorothiazide with % recovery and % RSD

Concentration of HCT from formulation (µg/ml)	Amount of HCT spiked (µg/ml)	Total amount (µg/ml)	Amount found (µg/ml)	% recovery	S.D	% RSD
10	8	18	18.307	100.465	0.005292	0.359
10	10	20	20.112	101.886	0.00500	0.9950
10	12	22	22.301	100.436	0.00095	0.9470

Table 4: Robustness and ruggedness data into that change in stock concentration in that concentration of stock – 1 is 100 µg/ml and concentration of stock – 2 is 500 µg/ml.

Condition	Conc.	Different Stock Solution Preparation		Change in λ_{max}	
		Stock-1*	Stock-2*	λ_{max}= 289 nm	λ_{max}= 289 + 0.2 nm
Propranolol Mean (n=3) ± % RSD	10	0.256±0.225	0.266±0.375	0.256±0.225	0.251±0.395
	20	0.486±0.118	0.496±0.116	0.486±0.118	0.48±0.208
	30	0.749±0.133	0.735±0.549	0.749±0.133	0.753±0.202
		Stock-1*	Stock-2*	λ_{max}= 270 nm	λ_{max}= 270 + 0.2 nm
Hydrochlorothiazide Mean (n=3) ± %RSD	5	0.455±0.126	0.495±0.202	0.455±0.126	0.451±0.639
	10	0.879±0.114	0.906±0.127	0.879±0.114	0.872±0.114
	15	1.384±0.150	1.414±0.107	1.384±0.150	1.387±0.110

Table 5: Result of all validation and development parameters for this proposed method for propranolol and hydrochlorothiazide.

Sr.No	Parameter	Propranolol	Hydrochlorothiazide
1	Range (µg/ml)	289 nm	270 nm
2	Correlation coefficient (r^2)	10 - 50	5 - 25
3	Regression equation	y = 0.0208x + 0.0138	y = 0.0732x - 0.0449
4	Inter day precision	0.9993	0.9991
5	Intraday precision	0.1057	0.0315
6	Accuracy (%recovery ± SD)	0.3205	0.0955
7	LOD (µg/ml)	0.13 – 0.26 0.12 – 0.80	0.15-0.54 0.14-0.32
8	LOQ (µg/ml)	99.74 ± 0.299	100.48 ± 0.359
9	Robustness and ruggedness	0.118-0.225	0.114-0.150

5. Conclusion

A novel, simple, rapid and sensitive method is proposed for the analysis of two binary mixtures with overlapping spectra. The method involves the generation of absorbance spectra followed by measurement of the absorbance. The proposed method does not require any sophisticated mathematical treatment for the absorption data, and it exhibits several advantages over other spectrophotometric methods for resolution of binary mixtures. The applicability of the developed method was evaluated through the determination of both drug combinations in several laboratory-prepared mixtures and in pharmaceutical tablets with good accuracy and precision. Therefore, the presented methodology is adequate for the routine quality control analysis of these fixed-dose combinations.

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