Derivative spectrophotometric for simultaneous estimation of propranolol hydrochloride and hydrochlorothiazide in synthetic mixture

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**ABSTRACT**
A simple, sensitive and economical spectrophotometric method for simultaneously estimation of PRO and HCTZ. The first derivative (D1) of the UV spectrum was used in the determination of both drugs in their synthetic mixtures. The Peak to baseline and Peak area at suitable wavelengths were used in the study. The linearity of both drugs was up to a concentration of (5-40 µg/ml). The analytical results of the estimation of PRO were, Rec% 97.179-102.424% and RSD% 0.001-4.996 %. While for estimation of HCTZ were, Rec% between 95.406-103.681% and RSD% 0.001-3.676%. The method was accurate, good repeatability and successfully applied in the estimation of both drugs in their synthetic mixtures.

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**INTRODUCTION**
The scientific name of Propranolol (1-isopropylamino-3-(1-naphthyloxy)-2 propranolol hydrochloride). The propranolol hydrochloride, as shown in Figure 1-a, has a molecular formula is C_{16}H_{22}CINO_{2} and the molecular mass is 298.807 g/mol. It is commonly used for Hypertension aMyocardial infarction, Anxiety tremor, Portal hypertension, Anxiety, as showed in Figure 1 (Mohammed et al., 2018). Also, it is useful in regulation Tachycardia (Tripathi, 2008) and considered as a non-selective inhibitor for beta receptors. Where it inhibits the receptor’s response of beta 1 and beta 2 competitively, so it leads to slowing in heart rate (Esteve-Romero et al., 2016).

Hydrochlorothiazide is (6-chloro-3,4-dihydro-2H-1,2,4-benzothiaphazine-7-,suiphonamide 1,1-dioxide). His molecular mass is 297.7 g/mol, as showed in Figure 2-b (Sivasubramanian and Lakshmi, 2015). It is a Diuretic anti-hypertension, for Edema treatment and swelling, which occur due to Heart failure, Cirrhosis, Kidney failure, Corticosteroids and Renal syndromes (Savaj et al., 2015) the Figure 1-a and Figure 1-b shows the chemical structure of Propranolol Hydrochloride and hydrochlorothiazide.

The binary mixture is used to treat heart-related diseases and hypertension (Savaj et al., 2015). There are many methods for the estimation of both drugs, individually or simultaneously. These methods were derivative spectrophotometric methods for the estimation of PRO and panadol Ruiz and . (1998) or PRO and Hydrolazine (Peña et al., 1991). HCTZ was estimated by the oxidative coupling method with phenylidiamine-O (Hasan and , 2019). HCTZ and Valsartan were estimated by first derivative method (Patel and ., 2012) The dual wavelengths and area under curve method were used for the simultaneous estimation of HCTZ and Olmesartan Medoxomil in their mixture (Ilango and Kumar, 2012). Both drugs were estimated by chro-
matographic methods individually or simultaneously (Kim et al., 2001; Hegazy et al., 2011; Umamaheshwari and , 2015). The Simultaneous Determination of Propranolol Hydrochloride and Isosorbide Mononitrate was done by Central Composite Rotatable Design (Khan et al., 2019). Some another methods were used, such as LC-MSIMS (Johannsen et al., 2019; Li and Hongbin, 2018). The present study aims to develop a new spectrophotometric method for simultaneously estimation of PRO and HCTZ by a first derivative method in pure form and in their synthetic mixture.

EXPERIMENTAL

Apparatus
A shimadzu UV-Vis 1650 spectrophotometer using a 1 cm quartz cell was used to the spectrophotometric measurements and ultrasound water bath produced by the lab tech. To dissolve the pure and samples.

Chemicals
Pure PRO 99.9% purched from Hubei-china and HCTZ 99.8% purched from Hetro-India.

Preparation of Standard Solutions
Stock solution containing 100 μg/ml either PRO or HCTZ were prepared by dissolved 0.1g of each pure material in amount of distilled water for PRO the volume was completed to the mark with the same solvent in 100 ml volumetric flasks, while for HCTZ the ultrasound water bath was used for 12 min to dissolve the pure material completely before completing the volume in 100ml volumetric flask with the same solvent. Further dilutions were done using distilled water as described under the construction of calibration curves.

Analysis of Pharmaceutical Preparation
Twenty tablets of the pharmaceutical preparation (indicardin 10 mg) were weighted and were grinded by a ceramic mortar; they were mixed well and the weight equivalent to one tablet was taken from the mixture, which is 0.450g then it was dissolved in an amount of distilled water in 100 ml volumetric flack. The solution was filtered by using a filter paper (Whatman No.40) to get a clear solution. Then the volume was completed to the mark with the same solvent to prepare 100 μg/ml of PRO.

Ten tablets of the pharmaceutical preparation (HCTAIWA 25 mg) were weighted and were grinded by a ceramic mortar; they were mixed well and the weight equivalent to one tablet was taken from the mixture, which is 0.16048 g, then it was dissolved in amount of distilled water and by using the ultrasound water bath in 100 ml volumetric flask for 15 minute to dissolve the pure material completely. The solution was filtered by using a filter paper (Whatman No.40) to get a clear solution. Then the volume was completed to the mark with the same solvent to prepare 250 μg/ml of HCTZ.

Absorption Spectrum
A set of concentrations were prepared for both of PRO and HCTZ, with a range of concentrations of 5-40 μg/ml. Scanning for the wavelengths was done, which was around 190-400 nm to get the zero spectrum. The maximum absorption of HCTZ is at a wavelength of 272 nm, while for PRO at 290 nm.

The Simultaneous Determination of Propranolol and Hydrochlorothiazide
The procedure of The Determination of Propranolol Hydrochloride
Equal amounts of 1 ml HCTZ 100 μg/ml were transferred from the standard solution to a series of 10 ml volumetric flasks, then increasing quantities of PRO (50-400) μg/ml were added to these flasks and the volume was completed to the mark with distilled water. Zero spectrum of the mixtures was scanned between 190-400 nm and the values of the first derivative of these spectra were obtained depending on Peak to baseline at wavelength of 266 nm and Peak Area at 251-273 nm for the quantitative analysis of PRO under the optimum conditions namely, Medium scan speed, sampling interval 0.1nm, slit width 2nm, Δλ 20 and scaling factor 8.

The procedure of the Determination of Hydrochlorothiazide
Equal amounts of 1 ml PRO 50μg/ml were transferred from the standard solution to a series of 10 ml volumetric flasks, then increasing quantities of HCTZ (50-400) μg/ml were added to these flasks and the volume was completed to the mark with distilled water. The zero spectrum of the mixtures was scanned between 190-400 nm and the values of the first derivative of these spectra were obtained. The measurements of the first derivative depending on the Peak to baseline at wavelengths of 258,282 nm and Peak Area at 249-277 nm, 271-307.5 for quantitative analysis for HCTZ under the optimum conditions that were used with PRO.

RESULTS AND DISCUSSION
The Selection of the Optimum Conditions
A lot of solvents were used to dissolve both components such as distilled water, ethanol, methanol and their mixtures with or without HCl and NaOH. The results show that the distilled water was the best solvent for both components, safe, less expensive
Table 1: The effect of the Scaling factor values on the determination of Pro and Hctz

| Scaling Factor | PRO | HCTZ |
|----------------|-----|------|
|                | Slope | R²   | Slope | R²   |
| 1              | 0.0005*-0.0046* | 0.9985 - 0.9901 | 0.0023*-0.0042 | 0.9984 - 0.9974 |
| 2              | 0.0016-0.0140 | 0.9991 - 0.9919 | 0.0046 - 0.0085 | 0.998 - 0.9979 |
| 3              | 0.0021 - 0.0183 | 0.9993 - 0.9883 | 0.0093 - 0.0168 | 0.9979 - 0.9983 |
| 4              | 0.0031 - 0.0227 | 0.9996 - 0.9915 | 0.0139 - 0.0254 | 0.9979 - 0.9984 |
| 5              | 0.0036 - 0.0325 | 0.9995 - 0.9913 | 0.0162 - 0.0248 | 0.9979 - 0.9991 |
| 6              | 0.0040 - 0.0042 | 0.99960.9996 | 0.0163 - 0.0163 | 0.991 - 0.9979 |

*for + ve Peak, + for – ve Peak

Table 2: The results of the simultaneous estimation of PRO and HCTZ at a range of concentrations 5-40 μg/ml

| Compound | Order of derivative | λ nm | R² | Slope | LOD μg/ml | LOQ μg/ml |
|----------|---------------------|------|----|-------|----------|----------|
| PRO in presence of HCTZ | Peak to baseline | 266 | 0.9988 | 0.0033 | 0.1804 | 0.6016 |
| | Peak Area | 273-251 | 0.9979 | 0.01316 | 0.5766 | 1.9222 |
| | Peak to baseline | 258 | 0.9973 | 0.0163 | 1.0900 | 3.6335 |
| HCTZ in presence of PRO | Peak Area | 282 | 0.9991 | 0.0170 | 0.0371 | 0.0553 |
| | Peak to baseline | 272-249 | 0.9994 | 0.3325 | 0.4711 | 1.5705 |
| | Peak Area | 307.5-271 | 0.9982 | 0.3355 | 0.6339 | 0.3068 |

Table 3: A comparison between the analyzing characteristics for the proposed method and other analyzing methods.

| Parameters | Present Method PRO in presence 10 μg/ml HCTZ | Other Method (Shanmugasundaram and Kamarapu, 2018) |
|------------|-----------------------------------------------|--------------------------------------------------|
| λ max(nm)  | 266                                           | 289                                              |
| Linear range (μg/mL) | 5-40                                        | 8-48                                             |
| Correlation coefficient | 0.9979-0.9988                                | 0.998                                           |
| The detection limit (μg/mL) | 0.1804-0.5766                                | 0.461                                           |
| Quantitative limit (μg/mL) | 0.6016-1.9222                                 | 1.397                                           |
| RSD%       | 4.926-0.001                                   | —                                                |
| Rec%       | 102.667-96.666                                | 98-97                                            |
| Parameters | Present Method HCTZ in presence 5 μg/ml PRO | Other Method (Shanmugasundaram and Kamarapu, 2018) |
| λ max(nm)  | 258,282                                       | —                                                |
| Linear range (μg/mL) | 5-40                                        | 20-60                                           |
| Correlation coefficient | 0.9973-0.9994                                | —                                                |
| Detection limit (μg/mL) | 0.0371-1.0900                                | 0.95                                            |
| Quantitative limit (μg/mL) | 0.0553-3.6335                                | 3.01                                            |
| RSD%       | 0.002-3.565                                   | —                                                |
| Rec%       | 95.406-104.132                                | —                                                |
and available. Regarding to the other conditions, different values of $\Delta \lambda$ were used, which was around 20-160nm to choose an appropriate value of $\Delta \lambda$ and the best value was 20nm. It's notice that when there is an increasing in $\Delta \lambda$ value, the spectrum becomes distorted. A changing of the scaling factor was done from 1-10, whereas the method was more sensitive at the value 8 of the scaling factor and this is shown through the value of the slope, the value of $R^2$ was high so the value 8 was chosen as in Table 1. The best concentration of HCTZ was chosen, which is $10 \mu g/ml$ as a constant concentration to determine the PRO. Also, the concentration of PRO was chosen, which is $5 \mu g/ml$ as a constant concentration to determine HCTZ.

**The Simultaneous Determination of Propranolol Hydrochloride and Hydrochlorothiazide**

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Figure 1: (a)-a structure of PRO (b)-a structure of HCTZ

![Figure 1](image1)

Figure 2: The absorption spectrum A for HCTZ, B for PRO and C for their mixture

![Figure 2](image2)
Absorption Spectrum

A set of concentrations were prepared for PRO and HCTZ, with a range of concentrations up to 5-40 μg/ml. Scanning for the wavelengths was done, which was around 190-400 nm and its zero spectrum was obtained. The maximum absorption of HCTZ is at a wavelength of 272 nm, while for PRO at 290 nm.

The absorption spectra of both drugs are severely overlapped as in Figure 2, so the simultaneous estimation of these components by the direct spectrophotometric method was impossible. The first derivative method was used in quantitative estimation without separation.

Figure 3 and Figure 4 represent the first derivative spectrum for a mixture of PRO with concentrations up to 5-40 μg/ml in the presence of 10 μg/ml HCTZ and the first derivative spectrum for a mixture of HCTZ with concentrations up to 5-40 μg/ml in the presence of 5μg/ml PRO.

Construction Of Calibration Curves

Calibration curves were constructed in according to optimum conditions of the suggested procedure, the linearity for all of the calibration curves was around 5-40 μg/ml for each PRO and HCTZ. The values of the slope for all of the calibration curves were around 0.0033-0.3355 and the values of LOD and LOQ were around 0.0371-1.0900 μg/ml and 0.0553-3.6335 μg/ml respectively, it was calculated based on ICH(21). The values of $R^2$ was around 0.9994-0.9979 for both drugs.

The results of the simultaneous estimation of PRO and HCTZ at a range of concentrations 5-40 μg/ml for each of them in the presence of the another, shown in Table 2.

Accuracy and Precision

The accuracy and precision for the proposed method were tested through the calculation of recovery percentage Rec% and relative standard deviation RSD% for the concentrations of calibration curves and by doing seven repetitions for each measurement process (n=7). The results show that the first derivative method has good accuracy and precision in the estimation of both drugs. The values of Rec% were between (96.666-102.667%) and (95.406-104.132%) and the values of RSD% were around (0.001-4.926%) and (0.002-3.565%) for PRO in the presence of HCTZ and for HCTZ in the presence of PRO respectively.

Interferences Effect

The effect of the additives (lactose, Mg stearate, Aerasil, Maize starch, TALC, PHB, MHB) that was
Figure 4: The first derivative for a mixture of HCTZ with a concentration up 5-40 to μg/ml in the presence of 5μg/ml PRO added to the drug were studied. Each additive was added separately to the pure material according to its percentage in a pharmaceutical formula. The results show that these additives have no significant effect when analyzing 30 μg/ml of PRO in the presence of 10 μg/ml of HCTZ and when analyzing 30 μg/ml of HCTZ in the presence of 5 μg/ml of PRO according to the proposed method. The Rec% value was around 98.37709-103.939% and 95.50102-104.817%. The value of the RSD% was around 0.1417528-0.810316 and 0.090489-0.482102 for PRO and HCTZ, respectively.

Application of Suggested Method

The proposed method was used in the quantitative estimation of the pharmaceutical form HCTAIWA 25 μg/ml at concentrations of 30,35 in the presence of 5 μg/ml PRO and the pharmaceutical form Indicardin 10 μg/ml at concentration 20,25 in the presence of 10 μg/ml HCTZ, each measurement was done seven times (n=7) for both drugs. The modes of the derivative which were used are Peak Area and Peak to Baseline. The results showed the success of the application of this method, the Rec% value was around 95.2370-104.2420% and 98.3333-104.0354%. The RSD% value was around 0.1885-1.5230% and 0.327-4.7334% for PRO in the presence of HCTZ and for HCTZ in the presence of PRO, respectively.

The Methods Comparison

A comparison between the analyzing characteristics of the suggested method and other analyzing method was done. Table 3 shows the results of this comparison (Ny et al., 2015).

CONCLUSIONS

The first derivative method which is used in the determination of PRO and HCTZ simultaneously, consider as a useful method due to its analyzing properties, its a simple, accurate, sensitive, economical method, useful in increasing the speed of work, low economical cost, does not need an expensive solvents and without losing the accuracy in the determination of drugs in the pharmaceutical formula.
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