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# Simultaneous estimation of depoxetine hydrochloride and tadalafil as API in tablet dosage form by simultaneous equation method

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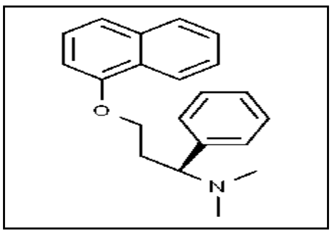
**Abstract**

A method for the simultaneous estimation of DPX Hydrochloride and TDX in a tablet dosage form using the simultaneous equation method was developed and validated. This method relies on the different maximum absorbance wavelengths of the two drugs: DPX Hydrochloride at 210 nm and TDX at 285 nm. Calibration curves were plotted for both drugs, and the concentrations in synthetic mixtures were determined using simultaneous equations derived from the absorbance measurements at these wavelengths. The method demonstrated high accuracy, precision, and specificity, adhering to ICH guidelines. In the linearity study at respective wavelengths, the linear regression equation for DPXHydrochloride, calibration curve at 285 nm was calculated by $y = 0.088x + 0.005$ ($r^2 = 0.999$), where y is absorbance and x is the value of various concentrations of standard solutions and the linear regression equation for Dapoxtine Hydrochloride, calibration curve at 210.0 nm was calculated by $y = 0.073x + 0.002$ ($r^2 = 0.999$) (Table – 3). Moreover, in the linearity study at of the other API at consecutive wavelengths, the linear regression equation for TDX, calibration curve at 285.0 nm was calculated by $y = 0.022x+0.035$ ($r^2 = 0.996$) and the linear regression equation for TDX, calibration curve at 210 m was calculated by $y = 0.034x - 0.013$ ($r^2=0.998$). The simultaneous equation method provided a reliable and accurate means of quantifying DPXHydrochloride and TDX in tablet formulations. The calibration curves were linear, and the method complied with ICH guidelines for specificity, accuracy, and precision. This method is advantageous for quality control in pharmaceutical formulations, offering simplicity and efficiency.

**Keywords:** TDX, Dapoxetine, simultaneous estimation method, beers law, wavelength

## Introduction

Dapoxetine, marketed as Priligy, among others, is a medication used for (S)-N, N-Dimethyl-3-(naphthalen-1-yloxy)-1-phenylpropan-1-amine. (fig.no - 1) The empirical formula is C21H23NO and molecular weight is 305.413 g/m. It is selective serotonin reuptake inhibitor, for the treatment of premature ejaculation [1]. TDX, sold under the brand name Cialis among others, is a medication used to treat erectile dysfunction, benign prostatic hyperplasia, and pulmonary arterial hypertension [2]. It is taken by mouth. Onset is typically within half an hour and the duration is up to 36 hours. The empirical formula is (2R,8R)-2-(1,3-benzodioxol-5-yl)-6-(trideuteriomethyl)-3, 6, 17-triazatetracyclo [8.7.0.03,8.011,16] heptadeca-1(10), 11, 13, 15-tetraene-4,7-dione Tadalafi-d3 and molecular weight is 392.4g/m.(fig. No. – 2) [3]. It works to treat erectile dysfunction by increasing blood flow to the penis during sexual stimulation. This increased blood flow can cause an erection. TDX treats PAH by relaxing the blood vessels in the lungs to allow blood to flow more easily.



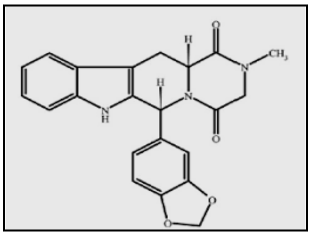


**Fig 1: (**Structure of DPX HCL) Fig. No. – 2 (Structure of TDX)

Literature survey reveals that the drug can be estimated only by LC/MS, Spectrophotometric method have been reported [4-6]. The aim of present work was to develop a simple, sensitive, precise and accurate UV/visible spectrophotometric method for the determination of DPXand TDX in its pure form and pharmaceutical formulation further to validate the developed method.

**Simultaneous Equation Method**

The Simultaneous Equation Method is a widely used analytical technique in spectroscopy for the simultaneous determination of two or more components in a mixture. This method is particularly useful when the components exhibit overlapping absorbance spectra at certain wavelengths, making their individual quantification challenging.

Principle

The principle behind the Simultaneous Equation Method involves utilizing the fact that each component in a mixture absorbs light at specific wavelengths. By measuring absorbance at two or more wavelengths where each component has different absorbance characteristics, it is possible to create a set of simultaneous equations. These equations can then be solved to determine the concentration of each component in the mixture.

**Formation of Simultaneous Equations**

- Measure the absorbance of the mixture at the selected wavelengths.
- Using the absorbance data and the calibration curves, form a set of simultaneous equations based on Beer's Law:
- A1=ax1Cx+ay1CyA_1 = a_{x1}C_x+a_{y1}C_yA1 =ax1Cx+ay1Cy A2=ax2Cx+ay2CyA_2 = a_{x2}C_x + a_{y2}C_yA2=ax2Cx+ay2Cy

**Where**

- A1A_1A1 and A2A_2A2 are the absorbances at wavelengths 1 and 2.
- ax1a_{x1}ax1, ax2a_{x2}ax2 are the absorptivity coefficients of component X at wavelengths 1 and 2.
- ay1a_{y1}ay1, ay2a_{y2}ay2 are the absorptivity coefficients of component Y at wavelengths 1 and 2.
- CxC_xCx and CyC_yCy are the concentrations of components X and Y, respectively.

**Applications**

- Pharmaceutical analysis for quantifying drug mixtures.
- Environmental analysis for detecting multiple pollutants.
- Quality control in various industries where multiple components are present in a single sample.

**Materials and Methods**

TDX and DPX was estimated using UV Visible spectrophotometer at the wavelength of maximum absorbtion in methanol. The drug was characterized by solubility, melting point and other identification methods. The analysis of drug was carried out by UV visible method which was validated analytical parameters like linearity, precision and accuracy as per guidelines laid down by ICH.

**Physical Characteristics**

- **DPX Hydrochloride:** Off-white crystalline powder.
- **TDX:** White powder.

**Melting Point**

- DPX Hydrochloride**:** 182 °C (determined by capillary method using a melting point apparatus).
- TDX**:** 300 °C (determined by capillary method).

**Solubility Testing**

Excess amounts of DPX and TDX were dissolved in 100 mL distilled water.

- **Stirring:** Solutions were stirred for 48 hours on a magnetic stirrer at 100 rpm and room temperature (25±1 ºC).
- **Centrifugation:** Samples were centrifuged at 10,000 rpm for 10 minutes.
- **Filtration and Analysis:** Supernatants were filtered through a 0.22 µm syringe filter and analyzed using a UV spectrophotometer.

**Analytical Method**

**Spectrophotometric Analysis**:

- Range**:** 200 to 400 nm.
- λmax Determination**:** Maximum absorbance wavelengths (λmax) for DPX and TDX were identified and used for quantification.

**Estimation of DPX Hydrochloride and TDX in Synthetic Mixture**

The API mixture (with volumes of 0.1, 0.3, 0.2, 0.6, 0.3, 0.9 ml) was transferred to six different 10 ml volumetric flasks. Volume was made upto 10 ml. Absorbances of the solutions were measured at 285 nm and 210 nm. Absorptivity coefficients (ax1, ay1, ax2, ay2) were used to create two simultaneous equations for the analysis. Equations Used **–**

At 210 nm: A1=ax1·Cx+ay1·CyA1 = ax1 \cdot Cx + ay1 \cdot CyA1=ax1·Cx+ay1·Cy
At 285 nm: A2=ax2·Cx+ay2·CyA2 = ax2 \cdot Cx + ay2 \cdot CyA2=ax2·Cx+ay2·Cy

**Where**

- ax1=33.49ax1 = 33.49ax1=33.49 (DPXHydrochloride)
- ay1=88.69ay1 = 88.69ay1=88.69 (TDX)
- ax2=25.088ax2 = 25.088ax2=25.088 (DPXHydrochloride)
- ay2=73.87ay2 = 73.87ay2=73.87 (TDX)

**Rearranging and substituting into the equations**

Cy=A2−ax2·Cxay2Cy = \frac{A2 - ax2 \cdot Cx}{ay2}Cy=ay2A2−ax2·Cx

**Substituting Cy in the first equation gives**

Cx=A2·ay1−A1·ay2ax2·ay1−ax1·ay2Cx = \frac{A2 \cdot ay1 - A1 \cdot ay2}{ax2 \cdot ay1 - ax1 \cdot ay2}Cx=ax2·ay1−ax1·ay2A2·ay1−A1·ay2
Cy=A1·ax2−A2·ax1ax2·ay1−ax1·ay2Cy = \frac{A1 \cdot ax2 - A2 \cdot ax1}{ax2 \cdot ay1 - ax1 \cdot ay2}Cy=ax2·ay1−ax1·ay2A1·ax2−A2·ax1

Absorbances of the synthetic mixtures were measured at 285 nm and 210 nm. Concentrations of DPX hydrochloride and TDX were calculated using the derived equations. Absorbances for a 1:3 ratio of TDX to DPX hydrochloride are provided in Table 13, with values at both wavelengths.

**Preparation of standard stock solution**
Accurately weighed DPX hydrochloride (10 mg) and TDX (10 mg) was separately transferred to 100 ml volumetric flask and dissolved in methanol. Both flask was shaken well and volume was made up to 100 ml with methanol as solvent. Thus, both final standard stock solution contained 100 µg/ml of DPX Hydrochloride and 100 ug/ml of TDX.

**Determination of wavelength of maximum absorbance**
The wavelength of DPX HCL and TDX were found to be 210 nm and 285 nm respectively. The intensity of peak increases with increase in concentration which was not interfering with wavelength of both drugs. So this method was found to be acceptable for simultaneous estimation of DPX HCL and TDX.

**Preparation of calibration curve**
Prepared a series of dilute solution from the above stock solution according to table in 10 ml volumetric flask. Measured the absorbance of both the solution at 210nm and 285nm using methanol as blank. Pipette out 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml of DPX hydrochloride stock solution (100 ppm) to a series of ten volumetric flask of 10 ml(fig. No. – 3,4) and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 ml of TDX stock solution (100 ppm) to a series of ten volumetric flasks of 10 ml. (fig. No. – 5,6)

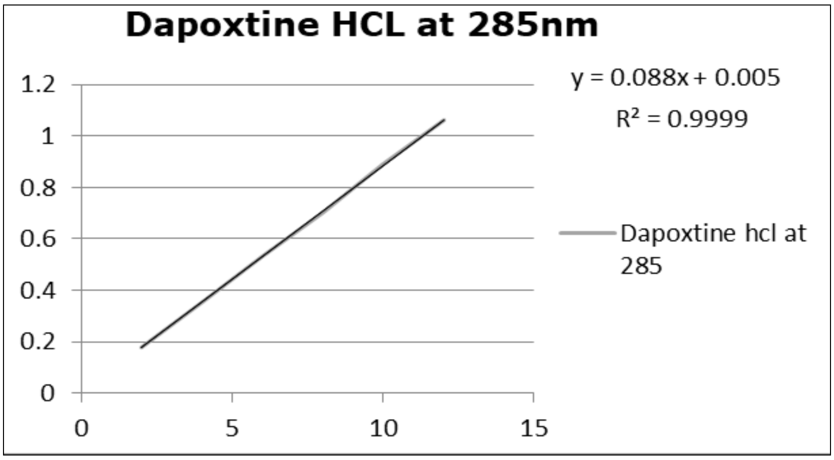



**Fig 1:** Calibration curve for DPXHydrochloride at 285 nm

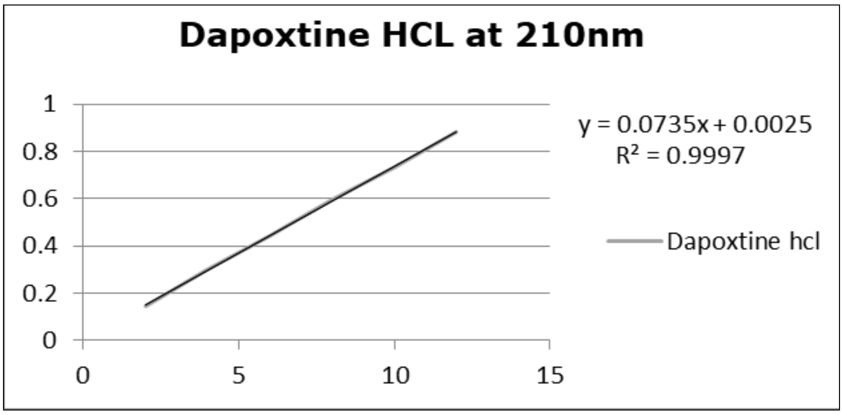



**Fig 2:** (Calibration curve for DPXHydrochloride at 210.0 nm)

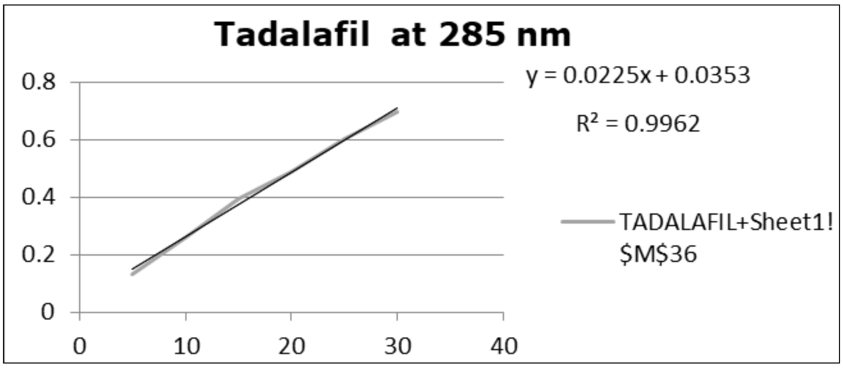



**Fig 3:** (Calibration curve for TDX at 285 nm)

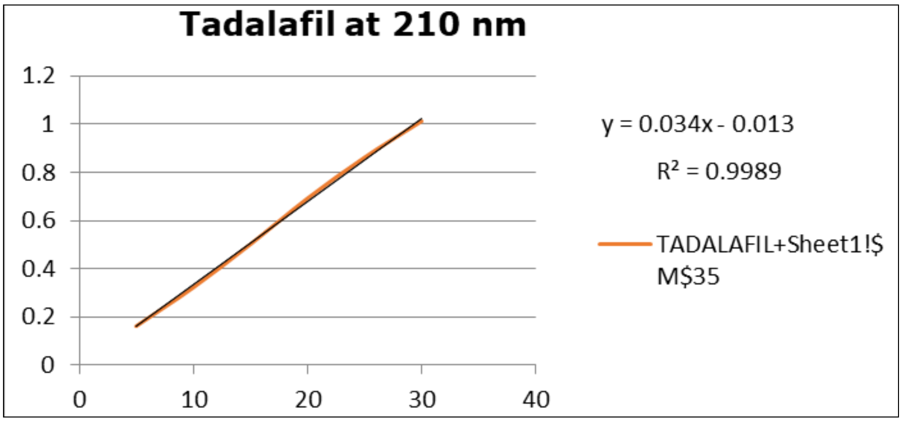



**Fig 4:** (Calibration curve for TDX at 210 nm)

**UV Method validation**

The ultra-spectrophotometric violet method was validated for accuracy, precision, linearity, detection of limit, Quantitation limit and robustness. Optical parameters like Beer's limit, slope, intercept and correlation coefficient were calculated from calibration curve.

**Linearity**

Appropriate aliquots of TDX and DPX hydrochloride working standard solution were taken in different 10 ml volumetric flasks and dilute up to the mark with distilled water to obtain final concentration of 1-10 ug/ml for and 5-50 ug/ml.

**Range**

The range of the analytical method was decide from the interval between the upper and lower level of the calibration curve by plotting curve. It includes linearity range, working range, target range.

**Precision**

The precision of analytical method express the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample. Intraday precision was determined by analysing the drugs at concentrations 3.5 ug/ml for TDX and 17.5 ug/ml for DPX and each concentration for three times, on the same day. Inter day precision was determined similarly, but the analysis is carried out daily, for two consecutive days. Repeatability (intraday) of the method was determined by analysing six samples of the same concentration of the drug. The absorbance of each was measured and reported in terms of relative standard deviation to obtain the variation.

**Accuracy**

The stock solution, 2 ml of the pre analyzed tablet (1:3::TDX:DPX) solution (10ug/ml TDX and 30ug/ml of DPX) was taken and transferred to 10 ml volumetric flask, volume was made up to the mark with methanol to get desired stock solution. Further 0.3 ml and 0.6 ml of pre analyzed stock solution was taken and 0.2 amd 0.6, 0.8ml of standard solution of API mixture was added and volume make up to the mark with methanol. This method meant for recovery of mixture of TDX and DPX hydrochloride by spiking with pure standard of API mixture solution.

**Results**

The concentration of the API mixture of TDX and DPX hydrochloride was accurately determined using the simultaneous equations, demonstrating the method's efficacy for this ratio (Table -2). It was concluded that the method was linear and obeyed the Lambert-Beer's law in the concentration range of 1 to 10µg/ml at 210 nm and 285 nm wavelength for TDX. Similarly, the Lambert-Beer's Law was obeyed in the concentration range of 5 to 50 µg/ml at 285.0 nm and 210 nm wavelength for DPX hydrochloride. In the linearity study at respective wavelengths, the linear regression equation for DPX hydrochloride, calibration curve at 285 nm was calculated by y = 0.088x +0.005 ($r^2$ = 0.999), where y is absorbance and x is the value of various concentrations of standard solutions and the linear regression equation for Dapoxtine Hydrochloride, calibration curve at 210.0 nm was calculated by y =0.073x + 0.002 ($r^2$ = 0.999) (Table – 3). Moreover, in the linearity study at of the other API at consecutive wavelengths, the linear regression equation for TDX, calibration curve at 285.0 nm was calculated by y = 0.022x+0.035 ($r^2$ = 0.996) and the linear regression equation for TDX, calibration curve at 210 m was calculated by y = 0.034x - 0.013 ($r^2$=0.998).

**Table 1:** Optical parameters & regression characteristic for DPX Hydrochloride and TDX

| Parameters | DPXHydrochloride | | TDX | |
|---|---|---|---|---|
| | 285 nm | 210 nm | 285 nm | 210 nm |
| Beers's law limit | 5-35 | 5-35 | 2-14 | 2-14 |
| Molar absorptivity ($mole^{-1}$ $cm^{-1}$) | $0.886\times10^3$ | $3.377\times10^3$ | $0.245\times10^3$ | $0.338\times10^3$ |
| Sandell's sensitivity | 0.0344 | 0.0904 | 0.1589 | 0.1152 |
| Regression equation (y= mx ± c) slope(m) intercept (c) | 0.088 | 0.073 | 0.022 | 0.035 |
| | 0.005 | 0.002 | 0.035 | -0.013 |
| Correlation coefficient($r^2$) | 0.999 | 0.999 | 0.996 | 0.998 |

**Table 2:** (Estimation of TDX and DPX Hydrochloride in marketed tablet dosage form by proposed UV spectroscopic method)

| Brand Name | Label Claim (mg) | Drug Found (mg) | | Mean ± SD | % Drug Found | | Mean % ±SD | % RSD |
|---|---|---|---|---|---|---|---|---|
| | | TDL | DPX | | TDL | DPX | | |
| Tadapox | 10 mg TDL + 30 Mg DPX | 10.21 | 74.42 | TDL 10.20±0.412 | 102.1 | 99.22 | TDL 100.2±0.387 | 0.388 |
| | | 9.98 | 74.18 | | 99.81 | 98.90 | | |
| | | 10.18 | 75.09 | DPX 74.34±0.322 | 101.8 | 100.1 | DPX 99.50±0.529 | 0.532 |
| | | 10.17 | 74.31 | | 101.7 | 99.08 | | |
| | | 9.89 | 75.14 | | 98.94 | 100.2 | | |

**Table 3:** Summary of Validation Parameters

| Validation Parameters | | DPX HCL | TDL |
|---|---|---|---|
| Specificity | | 0.406 | 0.206 |
| Liniarity | | 5-50 | 1-10 |
| Accuracy | | 96.0 | 101.4 |
| Range | Working range | 5-30 | 1 -12 |
| | Target Conc. | 17.5 | 3.5 |
| | Target range | 14<br>17.5<br>21 | 2.8<br>3.5<br>4.2 |
| Precision | Repeatability | 0.454 | 0.470 |
| | Intraday | 0.157 | 0.106 |
| | Interday | 0.287 | 0.20 |
| Robustness | Change in speed | 0.23 | 0.40 |
| | Change in Wavelength | 1.2 | 0.42 |
| Limit of detection | | 0.299 | 0.207 |
| Limit of quantitation | | 0.629 | 0.905 |

## Discussion

The method involves using spectrophotometric measurements at two distinct wavelengths and applying simultaneous equations to determine the concentrations of both components in a synthetic mixture. This approach is effective for accurate quantification of DPX hydrochloride and TDX. The simultaneous equation method provided a reliable and accurate means of quantifying DPX hydrochloride and TDX in tablet formulations. The calibration curves were linear, and the method complied with ICH guidelines for specificity, accuracy, and precision. This method is advantageous for quality control in pharmaceutical formulations, offering simplicity and efficiency.

## Conclusion

The simultaneous equation method for the estimation of DPX hydrochloride and TDX in tablet dosage forms was validated successfully. The method is linear, precise, accurate, and specific, making it suitable for routine analysis in pharmaceutical quality control.

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