Chapter 3

A Validated Spectrofluorimetric Method for the Determination of Doxepin Hydrochloride in Commercial Dosage Forms
INTRODUCTION

Doxepin hydrochloride is chemically known as 1-Propanamine, 3-dibenz[b, e]oxepin-11(6H)-ylidene-N, N-dimethyl-, hydrochloride (CAS: 1229-29-4; M.W.: 315.84) [1,2]. It is a tricyclic antidepressant which is widely used for the treatment of depression and anxiety. It displays a potent central anticholinergic activity and can inhibit both norepinephrine and serotonin (5-HT) reuptake in synapses in brain. In general, lower dosages of doxepin hydrochloride are recommended. Where the presenting symptoms are mild in nature, it is advisable to initiate treatment at a dose of 10-50 mg daily. At high concentrations, severe adverse effects and toxicity can appear. Therefore, the analysis of doxepin hydrochloride is important for obtaining optimum therapeutic concentration and for quality assurance in pharmaceutical preparations.

The assay of doxepin hydrochloride in bulk and formulations is cited in The United States Pharmacopoeia [3] which is based on liquid chromatography. In view of the great importance in terms of its optimum oral dose and wide use of doxepin hydrochloride, various analytical methods have been reported which include high performance thin layer chromatography [4], high performance liquid chromatography [5,6], liquid chromatography coupled with mass spectrometry [7], capillary electrophoresis [8], electroanalytical method of analysis [9], spectrophotometry [10,11], and extractive spectrophotometry [12,13]. Spectrofluorimetry is attractive because of sensitivity, speed and simplicity. Most of the additives or excipients found in pharmaceutical preparations are not fluorescent in nature. Therefore, spectrofluorimetry is the good choice to analyze doxepin hydrochloride in pharmaceutical preparations. There are two methods available on spectrofluorimetry
which are based on dichloromethane extractable ion pair complexes of the drug with 9,10-dimethoxyanthracene-2-sulphonate [14] and tetraiodofluorescein [15].

This chapter describes an optimized and validated spectrofluorimetric method for the determination of doxepin hydrochloride in pharmaceutical formulations. The proposed spectrofluorimetric method is based on dichloromethane extractable ion pair complex formation of the doxepin hydrochloride with eosin at pH 4.52 (sodium acetate-acetic acid buffer solution). The extracted complex showed fluorescence intensity at 567 nm after excitation at 464 nm. The reaction conditions are optimized and validated as per International Conference on Harmonisation (USA) [16].
EXPERIMENTAL

Apparatus

Fluorescence spectra and measurements were made on a F-2500 Hitachi fluorescence spectrophotometer (Tokyo, Japan) equipped with xenon lamp and 1-cm quartz cells. Excitation and emission wavelengths were set at 464 and 567 nm with the excitation and emission slit widths of 10 nm, respectively. All measurements were performed at 25 ± 1 °C. An Elico LI 120 pH meter (Hyderabad, India) was used for pH measurement.

Reagents and standards

All reagents used were of analytical reagent grade. Eosin Y disodium salt (CAS: 17372-87-1, M.W.: 691.85, Fluka Chemie AG, Switzerland) solution, $2.89 \times 10^{-3}$ M was freshly prepared in distilled water. Buffer solutions ranging from 4.05 – 5.57 pH were prepared by mixing appropriate volumes of 0.2 M acetic acid and 0.2 M sodium acetate [17]. Doxepin hydrochloride reference standard drug was purchased from Sigma Chemical Company (St. Louis, USA). Spectra-25 capsule (Rexcin Pharmaceutical Private Limited, Himachal Pradesh, India) and Doxetar-25 capsule (Torrent Labs Pvt. Ltd., Ahmedabad, India) of doxepin hydrochloride were purchased from local market.

Doxepin hydrochloride test solution of 0.01 mg ml$^{-1}$ was prepared in distilled water.

Proposed procedure for the determination of doxepin hydrochloride

Aliquots of 0.05-0.4 ml standard doxepin hydrochloride (0.01 mg ml$^{-1}$) solution corresponding to 0.5-4.0 µg were pipetted into a series of 50 ml separating funnels. To each funnel, 3.2 ml of $2.89 \times 10^{-3}$ M eosin Y solution and 2.5 ml of
sodium acetate-acetic acid buffer solution of pH-4.52 were added and mixed well. The contents of the separating funnel were shaken vigourously with 5 ml of dichloromethane for 2.5 min and then allowed to separate the two layers. The fluorescence intensity of the organic layer was recorded at 567 nm after excitation at 464 nm. The amount of the drug was obtained either from the calibration graph or the regression equation.

**Analysis of doxepin hydrochloride in commercial dosage form**

The powder contents of two commercially available capsules of 25 mg strength of doxepin hydrochloride were taken in 50 ml of distilled water and kept for 10 min for complete dissolution of the drug. The mixture was filtered through Whatmann No. 42 filter paper (Whatmann International Limited, Kent, UK) in 100 ml standard volumetric flask. The residue was washed well with 3 × 10 ml portions of distilled water for complete recovery of the drug and diluted to volume with distilled water. This solution was further diluted to complete the analysis following the proposed procedure for the determination of doxepin hydrochloride.

**Procedure for reference method**

Aliquots of 0.2-3.0 ml of 0.001% standard doxepin hydrochloride solution corresponding to 0.2-3.0 µg ml\(^{-1}\) doxepin hydrochloride were pipetted into a series of 50 ml separating funnel. To each funnel, 2.90 \(\times\) \(10^{-3}\) M 9, 10-dimethoxyanthracene-2-sulphonate in 0.2 M HCl was added and mixed well. The contents of the funnel were shaken with 10 ml dichloromethane for 2.5 min and allowed to stand for clear separation of the two phases. The fluorescent intensity of the organic phase at \(\lambda_{em} = 448\) nm after excitation at 265 nm was measured against the reagent blank prepared
similarly except drug. The amount of the drug in a given sample can be estimated either from the calibration graph or the corresponding linear regression equation.

**Validation**

The proposed method has been validated for solution stability, specificity, linearity, precision, accuracy, robustness and bias.

**Solution stability**

The solution stability of aqueous doxepin hydrochloride was investigated by taking 200 µg ml⁻¹ doxepin hydrochloride. The drug solution was monitored spectrophotometrically by observing the UV spectra for 6 h. The spots on TLC plates for freshly prepared drug solution were monitored for 12 h at room temperature. The thin layer chromatography was performed using TLC plates coated with silica gel G (Merck Limited, Mumbai, India) and developed in the solvent system: benzene - dioxan - 7.75 M ammonia solution (30%) in the ratio of 2.5: 5.5: 2.0 v/v/v. Then the TLC plates were freed from mobile phase, dried and spots were detected in iodine chamber.

**Specificity and selectivity**

The specificity and selectivity of the proposed method was ascertained by the analysis of placebo solution of doxepin hydrochloride by taking 1 mg of pure doxepin hydrochloride with 50 mg glucose, 80 mg fructose, 150 mg lactose, 100 mg starch, and 150 mg sodium benzoate in 100 ml standard volumetric flask and diluted up to the mark with distilled water. This solution was analyzed following the proposed procedure for the determination of doxepin hydrochloride.
Linearity

The linearity of the proposed method was evaluated at nine concentration levels of 0.10, 0.16, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, and 0.80 $\mu\text{g ml}^{-1}$. Each concentration level was analyzed repeatedly for five times.

Precision

The intra-day (within day) and inter day (between days) precisions were evaluated using standard doxepin hydrochloride solution at three concentrations levels: 0.16, 0.40 and 0.80 $\mu\text{g ml}^{-1}$. The intra-day repeatability was assessed with five replicates for each of three working sample concentrations in a single day. The inter-day reproducibility was assessed with five replicates at each concentration over five days.

Accuracy

The accuracy of the proposed procedure was evaluated by the standard addition technique. In this technique, aliquot of 0.1 ml (or 0.2 mL) of active drug solution of spectra-25 capsule was spiked with 0, 0.05, 0.08, 0.1 and 0.15 ml of reference doxepin hydrochloride (0.01 mg ml$^{-1}$) into a 50 ml separating funnel. To each funnel, 3.2 ml of $2.89 \times 10^{-3}$ M eosin Y solution and 2.5 ml of sodium acetate-acetic acid buffer solution of pH 4.52 were added and mixed well. The funnel was shaken vigorously with 5 ml dichloromethane for 2.5 min, and then allowed to stand for clear separation of the organic layer from the aqueous phase. The nominal value was determined by measuring the fluorescence intensity at each concentration level.

Robustness

The robustness of the proposed method was evaluated by observing the influence of small variations of experimental variables i.e. concentration of eosin, volume of buffer solution of pH 4.52, shaking time and solvent. The exactness of each
operational parameter was checked by varying one experimental parameter at a time keeping the other parameters constant and the % recovery ± RSD of drug was calculated.

**Evaluation of bias**

The bias has been evaluated by means of point and interval hypothesis tests [17]. In interval hypothesis test, the test method is compared with the reference method and considered to be acceptable if the mean recovery is within ± 2.0 % of that of the reference method [18] i.e.

\[ 0.98 < \frac{\mu_2}{\mu_1} < 1.02 \]

which can be generalized to

\[ \theta_L < \frac{\mu_2}{\mu_1} < \theta_U \]

where \( \theta_L \) and \( \theta_U \) are lower and upper acceptance limits, respectively. The limits of this confidence interval can be calculated using the following quadratic equation:

\[ \theta^2 \left( \frac{\bar{x}_1^2 - S_p^2 t_{\text{tab}}^2}{n_1} \right) + \theta \left(-2\bar{x}_1\bar{x}_2 \right) + \left( \frac{\bar{x}_1^2 - S_p^2 t_{\text{tab}}^2}{n_2} \right) = 0 \]

where \( \bar{x}_1 \) and \( \bar{x}_2 \) are mean values based on \( n_1 \) and \( n_2 \) measurements, respectively. \( S_p \) is the pooled standard deviation and \( t_{\text{tab}} \) is the tabulated one-sided t-value, with \( n_1 + n_2 - 2 \) degrees of freedom at 95% confidence level.
RESULTS AND DISCUSSION

Spectral studies

The literature citation revealed that doxepin hydrochloride is not fluorescent in nature but when reacted with 9, 10-dimethoxyanthracene-2-sulphonate [14] and tetraiodofluorescein [15], the fluorescent ion pair complexes are formed which are extractable in dichloromethane and thus, can be utilized for the estimation of doxepin hydrochloride in pharmaceutical formulations. In the similar manner, aqueous doxepin hydrochloride solution when treated with aqueous eosin Y disodium salt solution at pH 4.52, a fluorescent light pink ion pair complex is formed which is quantitatively extracted in dichloromethane. The fluorescent intensity was measured at 567 nm after excitation at 464 nm. The reaction is confirmed as eosin Y in blank solution did not form ion pair complex in the absence of drug and can not be extracted in dichloromethane. Fig. 3.1 shows the excitation and emission spectra of ion-pair complex.

Stoichiometry

The stoichiometric ratio between doxepin hydrochloride and eosin Y at pH 4.52 was evaluated by Job’s method of continuous variations [19]. For this, different volumes that is 0, 0.4, 1.2, 1.8, 2.0, 2.2, 2.6, 2.65, 2.67, 2.7, 3.2, 3.8, and 4.0 ml of 1.0 × 10⁻⁴ M doxepin hydrochloride were added with 4.0, 3.6, 2.8, 2.2, 2.0, 1.8, 1.4, 1.35, 1.33, 1.3, 0.8, 0.2 and 0 ml of 1.0 × 10⁻⁴ M eosin Y into a 50 ml separating funnel and analysis was completed by following the recommended procedure. The fluorescence intensity was measured at 567 nm after excitation at 464 nm and plotted against the mole fraction of drug (Fig. 3.2).
Fig. 3.1: Excitation (a) and emission (b) spectra of fluorescent ion-pair complex:
0.1 μg ml⁻¹ doxepin hydrochloride + 3.2 ml of 2.89 × 10⁻³ M eosin Y + 2.5 ml of buffer solution of pH 4.52. The complex was extracted in 5 ml dichloromethane.
Fig. 3.2: Job’s plot to establish the stoichiometry of the reaction.
It is apparent from the figure that the combining molar ratio between doxepin hydrochloride and eosin Y is 2:1. The apparent formation constant and standard Gibbs free energy \( (\Delta G^\circ) \) were calculated and found to be \( 5.65 \times 10^{13} \) and \(-78.467\ \text{kJ mol}^{-1}\), respectively.

**Mechanism of the reaction**

Doxepin hydrochloride as a derivative of dibenzoxepine possessing nitrogen atom of tertiary amine group in the aliphatic chain offers a basic characteristic to the drug. The drug is protonated in the presence of sodium acetate- acetic acid buffer solution of pH 4.52. Eosin Y is tetrabromofluorescein disodium salt, loses its two sodium ions in distilled water, thus one mole of eosin provides two negative sites and forms a fluorescent ion pair complex with 2 moles of protonated doxepin which is extractable in dichloromethane. The extracted dichloromethane ion pair complex fluoresces conveniently at 567 nm after excitation at 464 nm. Therefore, based on the literature background and our experimental findings, the reaction sequence of the proposed method is given in Fig. 3.3.

**Optimization of variables**

The optimization of variables for proposed spectrofluorimetric method was assessed by testing several parameters such as reaction time, concentration of eosin Y, buffer solutions of different pH, volume of buffer solution of pH 4.52, shaking time for extraction of complex and solvents.

**Effect of reaction time**

The effect of the reaction time on the development of fluorophore and its stability was investigated. The ion pair complex got stabilized immediately at 25 ± 1°C and remained stable for at least 1 hour.
Fig. 3.3: Reaction sequence of the proposed method.
**Effect of the concentration of eosin Y**

The effect of the concentration of eosin Y on the fluorescence intensity of the ion pair complex was investigated in the range of $5.78 \times 10^{-5} - 2.08 \times 10^{-3}$ M eosin Y. It was observed that the maximum fluorescence intensity was attained with $1.62 \times 10^{-3}$ M eosin Y and remained constant up to $2.08 \times 10^{-3}$ M eosin Y (Fig. 3.4). Therefore, $1.85 \times 10^{-3}$ M eosin Y was taken as the optimum concentration for the determination of the drug.

**Effect of pH**

The influence of pH on the fluorescence intensity of the ion pair complex was studied using sodium acetate-acetic acid buffer solution in the pH ranges of 4.05-5.57. The results are shown in Fig. 3.5. It is evident from the figure that the maximum fluorescence intensity of the complex was found at pH 4.52. Therefore, all fluorescence intensity measurements were made at pH 4.52 in the determination process.

**Effect of volume of pH 4.52 buffer solution**

The effect of volume of pH 4.52 buffer solution of sodium acetate-acetic acid on the fluorescence intensity of the complex was studied in the range of 0.5 - 3.0 ml. The highest fluorescence intensity was obtained with 2.0 ml of buffer (pH 4.52) solution, beyond this further increase in the volume of buffer solution, resulted in no change in the fluorescence intensity of the complex (Fig. 3.6). Therefore, 2.5 ml of pH 4.52 buffer solution was adopted as an optimum volume for measuring fluorescence intensity in the determination procedure.
Fig. 3.4: Effect of the concentration of eosin Y on the fluorescence intensity (0.6 μg ml⁻¹ doxepin hydrochloride).
Fig. 3.5: Effect of pH on the fluorescence intensity of the ion-pair complex (0.6 µg ml⁻¹ doxepin hydrochloride + 1.85 ×10⁻³ M eosin Y).
Fig. 3.6: Effect of the volume of pH-4.52 on the fluorescence intensity.
Effect of shaking time

The effect of the shaking time for the extraction of the ion pair complex was studied in the range of 0.5 – 3.0 min. The maximum fluorescence intensity of the complex was obtained at 2.0 min shaking and above this up to 3.0 min, the fluorescence intensity remained constant (Fig. 3.7), Therefore, 2.50 min was used as an optimum shaking time through out the determination process. The ion-pair complex was quantitatively recovered in one extraction only and was stable for at least 1 h.

Choice of organic solvent

A number of organic solvents such as chloroform, carbon tetrachloride, dichloromethane, dichloroethane, and ethyl acetate were examined for extraction of the ion pair complex in order to provide an applicable extraction procedure and maximum fluorescence intensity (Fig. 3.8). It is clear from the bar graph that the highest fluorescence intensity of the complex was attained in dichloromethane and there is no extraction of the complex in carbon tetrachloride and ethyl acetate. Therefore, dichloromethane was selected as the best solvent for extraction of the complex.

Solution stability

The UV absorption spectrum of aqueous solution of doxepin hydrochloride was recorded in the wavelength range of 200-320 nm. It was found that the drug solution absorbs maximally at 238 nm (0.706 absorbance value) and the absorbance value remained constant up to 5 h 30 min. This was also confirmed as TLC plate showed a single spot with \( R_f = 0.692 \) up to the duration of 5 h 30 min. Hence, it is clear from the absorbance value and \( R_f \) of the spot that the solution of doxepin hydrochloride in distilled water has considerable stability of 5 h 30 min.
Fig. 3.7: Effect of shaking time for the extraction of the ion-pair complex.
Fig. 3.8: Effect of the solvent on the fluorescence intensity of the ion-pair complex.
**Specificity and selectivity**

The proposed spectrofluorimetric conditions were found to be specific in the presence of tablet excipients (Table 3.1). It is clear from the table that common excipients present in capsule formulations did not cause any significant interference.

**Linearity**

Under the optimized experimental conditions, the fluorescence intensity was plotted against the concentration of doxepin hydrochloride and found to be linear over the concentration range 0.1–0.8 μg ml⁻¹. Table 3.2 summarized the analytical parameters and the results of statistical analysis of the experimental data: regression equation computed from calibration graph, correlation coefficient (r), detection limit and quantitation limit. The high value of correlation coefficient (0.9999) for the proposed method indicated excellent linearity.

**Precision**

The intra- and inter day precisions were evaluated by determining the concentration of doxepin hydrochloride at lower, middle and upper concentration levels for five repeated times within the same day and on five consecutive days, respectively (Table 3.3). It can be seen from the table that the % recovery and % RSD were in the ranges of 100.01-100.20 % and 0.23-0.59 %, respectively for intra day and inter day precisions. The % recovery and % RSD values showed that the proposed method is very precise and can be used to analyze doxepin hydrochloride in pharmaceutical formulations.
Table 3.1: Effect of foreign species on the determination of 0.6 μg ml\(^{-1}\) doxepin hydrchloride.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Foreign species</th>
<th>Maximum tolerance limit (mg ml(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose</td>
<td>0.10</td>
</tr>
<tr>
<td>2</td>
<td>Fructose</td>
<td>0.16</td>
</tr>
<tr>
<td>3</td>
<td>Lactose</td>
<td>0.30</td>
</tr>
<tr>
<td>4</td>
<td>Starch</td>
<td>0.20</td>
</tr>
<tr>
<td>5</td>
<td>Sodium benzoate</td>
<td>0.30</td>
</tr>
</tbody>
</table>
Table 3.2: Optical and regression characteristics of the proposed method.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation wavelength ($\lambda_{ex}$):</td>
<td>464 nm</td>
</tr>
<tr>
<td>Emission wavelength ($\lambda_{em}$):</td>
<td>567 nm</td>
</tr>
<tr>
<td>Linear dynamic range ($\mu g$ ml$^{-1}$)</td>
<td>0.1 – 0.8</td>
</tr>
<tr>
<td>Linear regression equation</td>
<td>F.I. = 2.250 + 1729.99 C</td>
</tr>
<tr>
<td>Intercept, a</td>
<td>2.250</td>
</tr>
<tr>
<td>Slope, b</td>
<td>1729.99</td>
</tr>
<tr>
<td>Correlation coefficient ($r$)</td>
<td>0.9999</td>
</tr>
<tr>
<td>Detection limit ($\mu g$ ml$^{-1}$)</td>
<td>$2.947 \times 10^{-3}$</td>
</tr>
<tr>
<td>Quantitation limit ($\mu g$ ml$^{-1}$)</td>
<td>$8.929 \times 10^{-3}$</td>
</tr>
</tbody>
</table>
Table 3.3: Test of precision of the proposed method.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Intra day assay</th>
<th>Inter day assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration taken</td>
<td>0.160 0.400 0.800</td>
<td>0.16 0.400 0.800</td>
</tr>
<tr>
<td>(μg ml⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration found</td>
<td>0.160 0.401 0.800</td>
<td>0.1601 0.401 0.800</td>
</tr>
<tr>
<td>(μg ml⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.001 0.001 0.002</td>
<td>0.001 0.001 0.002</td>
</tr>
<tr>
<td>(μg ml⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>100.02 100.20 100.01</td>
<td>100.05 100.20 100.02</td>
</tr>
<tr>
<td>Relative standard deviation (%)</td>
<td>0.58 0.32 0.23</td>
<td>0.59 0.33 0.25</td>
</tr>
</tbody>
</table>

*Mean for five independent determinations.

Confidence limit at 95% confidence level and four degrees of freedom (t = 2.776)
The accuracy of the proposed method was tested by performing recovery experiments through standard addition technique. The results of analyses are summarized in Table 3.4 and 3.9. It is evident from the table and the graph that the linearity of the regression line of standard addition method was good. Hence, the proposed method being a precise one is accurate too. The most attractive feature of the spectrofluorimetric method using standard addition method is its relative freedom from pharmaceutical additives and excipients. Mostly pharmaceutical additives and adjuvants are not fluorescent in nature and did not interfere in the proposed method.

Ruggedness

Ruggedness of the proposed method was established by deliberately changing the reaction conditions of the proposed method. The operational parameters challenged to prove the ruggedness of the proposed method includes; volume of $2.891 \times 10^{-3}$ M eosin Y, 3.2 ml (± 0.4 volume of pH 4.52, 2.5 ml (± 0.5 ml) and shaking time, 2.5 min (± 0.5 min.)

Under these optimal conditions, the doxepin hydrochloride solution containing 0.8 μg (Spectra 25 capsule) was analyzed by the proposed method. The results showed the % recovery ± RSD of 99.99 ± 0.23 %. The results indicated the ruggedness of the proposed method.
<table>
<thead>
<tr>
<th>Concentration (µg ml⁻¹)</th>
<th>Coefficients of linear regression equation of standard addition</th>
<th>Recovery(^b) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectra-25 capsule added</td>
<td>Nominal Intercept slope (r^a) (0.200) (0.1, 0.2, 0.3) (0.2005) (347.21) (1731.55) (0.9999) (100.26)</td>
<td></td>
</tr>
<tr>
<td>Spectra-25 capsule added</td>
<td>Nominal Intercept slope (r^a) (0.400) (0.1, 0.2, 0.3) (0.3999) (693.90) (1734.91) (0.9999) (99.99)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.4: Accuracy of the proposed method: Recovery of doxepin hydrochloride from spectra 25 capsule by standard addition technique.
Fig. 3.9: Recovery of doxepin hydrochloride from spectra-25 capsule by standard addition technique: (a) 0.202 and (b) 0.399 \( \mu g \text{ ml}^{-1} \).
Evaluation of bias

The applicability of the proposed method for the quantitative analysis of doxepin hydrochloride in Spectra 25 and Doxetar-25 capsule has been tested. The results of the proposed method were statistically compared with those of the reference spectrofluorimetric method [14] using point and interval hypothesis tests. Table 3.5 shows that the calculated t-(paired) and F-values at 95% confidence level are less than the tabulated t-value (2.036 at \( v = 8 \)) and F-value (6.39 at \( v = 4,4 \)), thus confirming no significant difference between the performance of the proposed method and the reference method. It is also clear from the table that the bias evaluated by interval hypothesis test by means of lower limit (\( \theta_L \)) and upper limit (\( \theta_U \)) are in the range of 0.98-1.02. Therefore, it is concluded that the proposed spectrofluorimetric method is applicable for routine quality control analysis of doxepin hydrochloride in commercial dosage forms with acceptable recovery results which are within the acceptable limit of \( \pm 2\% \).
Table 3.5: Point and Interval Hypothesis Tests: Applicability of the proposed method in pharmaceutical formulations and its comparison with the reference method at 95% confidence level.

<table>
<thead>
<tr>
<th>Pharmaceutical formulations</th>
<th>Proposed method</th>
<th>Reference method</th>
<th>t- &amp; F values$^b$</th>
<th>$\theta_U^c$</th>
<th>$\theta_L^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsule</td>
<td>Recovery (%)</td>
<td>RSD$^a$ (%)</td>
<td>Recovery (%)</td>
<td>RSD$^a$ (%)</td>
<td>t = 0.956</td>
</tr>
<tr>
<td>Spectra 25</td>
<td>99.99</td>
<td>0.232</td>
<td>100.17</td>
<td>0.338</td>
<td>F = 1.458</td>
</tr>
<tr>
<td>Doxetar-25</td>
<td>99.99</td>
<td>0.236</td>
<td>100.17</td>
<td>0.338</td>
<td>t = 0.956</td>
</tr>
</tbody>
</table>

$^a$Mean for 5 independent analyses.

$^b$Theoretical $t$ ($v= 8$) and $F$-values ($v= 4, 4$) at 95% confidence level are 2.306 and 6.39, respectively.

$^c$A bias, based on recovery experiments, of ± 2% is acceptable.
REFERENCES


