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# METHOD DEVELOPMENT AND VALIDATION OF DULOXETINE HYDROCHLORIDE IN BULK AND FORMULATION USING UV SPECTROPHOTOMETRIC METHOD

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**ABSTRACT**
New, simple and cost effective UV-spectrophotometric method was developed for the estimation of Duloxetine hydrochloride in bulk formulations. Duloxetine hydrochloride was estimated at 290 nm in 20% Acetonitrie. Linearity range was found to be 10–50 µg $ml^{-1}$ (regression equation: 0.017 + 0.016; $r^2$ = 0.999). The apparent molar absorptivity was found to be $5.922\times10^{-3}$ $mol^{-1}$ $cm^{-1}$ in 20% Acetonitrile. These methods were tested and validated for various parameters according to ICH guidelines and USP. The quantitation limits were found to be 0.2405 µg $ml^{-1}$ and 0.7289 µg $ml^{-1}$ in 20% Acetonitrile respectively. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation < 2%), while being simple, cheap and less time consuming and can be suitably applied for the estimation of Duloxetine hydrochloride in different dosage forms and dissolution studies.
**KEYWORDS:** Duloxetine hydrochloride, Acetonitrile Spectrophotometry; Validation

## INTRODUCTION

Duloxetine hydrochloride (+)-(s)-N-methyl-3-(1-napthyloxy) -3-(thiophen-2-yl)-propan-1-amine[1] is a potential dual inhibitor of the reuptake of serotonin and nor epinephrine. It has been approved by the US Food and Drug administration for the treatment of major depressive disorder and for the diabetic peripheral neuropathy pain. It belongs to the class narcoleptics. Literature survey reveals that only a few methods based on RP-HPLC method were developed and validated for the determination of duloxetine hydrochloride in pharmaceutical dosage forms[2]. Few others are: LC-tandem mass spectrometry method for the determination of duloxetine hydrochloride in human plasma[3], stability indicating RP-HPLC method for the duloxetine hydrochloride[4], metabolism, excretion and pharmacokinetics of duloxetine hydrochloride in healthy human subjects[5], duloxetine hydrochloride in Pharmaceutical formulation by HPLC with UV detection[6], spectrophotometric method in ultraviolet region for the determination of duloxetine hydrochloride in bulk and in pharmaceutical formulations[7], spectrophotometric method for quantification of duloxetine hydrochloride in capsule dosage form[8], liquid chromatography-mass spectrometric (LC/MS) method for the determination of duloxetine hydrochloride in human plasma using flupentixol as the internal standard[9], determination of duloxetine hydrochloride in human plasma by HPLC with column switching and ultraviolet spectroscopy[10], analysis of the duloxetine hydrochloride in human plasma after solid-phase extraction procedure (SPE)[11], HPTLC method for its estimation as bulk drug and its tablet dosage form[12] etc. Duloxetine hydrochloride is being marketed in both domestic and international market. No official method has been found in any of the pharmacopoeia. The present investigation by the author describes a rapid, accurate and precise UV-spectrophotometric method for the determination of the drug from bulk sample and pharmaceutical dosage form. The responses were linear in the concentration range of 20- 120 µg $ml^{-1}$ of drug. The method was validated as per ICH guideline

## MATERIALS AND METHODS

### Instrument

UV-Visible SpectrophotometerT60 (model), Analytical technologies Limited, connected to the digital system loaded with UVWin software ver.5.1.1 have an wavelength accuracy of ±5.0nm with quartz cells of 1cm path length.

### Absorption Maximum

In order to ascertain the wavelength of maximum absorbance ($\lambda_{max}$) the solution with particular concentration of drug 10 µg $ml^{-1}$ in 20% acetonitrile was scanned within the wavelength range of 200-400nm against a corresponding reagent blank. The resulting spectrum shows absorption curve with unique characteristic absorption maximum at 290nm. The absorption spectrum of Duloxetine hydrochloride is given in (**Fig-2**)

### Preparation of Stock Solutions

10 mg of Duloxetine hydrochloride was accurately weighed and dissolved in 100 ml of 20% acetonitrile in 100 ml volumetric flask to get the concentration about100 µg $ml^{-1}$ stock solution.

### Preparation of Calibration curve

From the above stock solution prepare serial dilutions from 1 ml to 5 ml and transfer it to 10ml volumetric flasks. Dilute it with 20% acetonitrile to get the concentrations ranging from 10 µg $ml^{-1}$ to 50 µg $ml^{-1}$ respectively. The absorbances were measured at $\lambda_{max}$ 290 nm against 20% acetonitrile as a blank. Results are shown in (**Table-1).** The calibration curve was shown in (**Fig-2**)

### Method validation

### Specificity

Duloxetine hydrochloride solutions (10 µg $ml^{-1}$) were prepared in both the selected media along with and without common excipients (starch, dextrose, benzalkonium chloride, magnesium stearate) separately. All the solutions were

scanned from 450 to 200 nm and checked for change in the absorbance at respective wavelengths.

**Accuracy**

As a part of determining accuracy of the proposed method, drug concentrations were prepared from independent stock solution and analyzed ($N$ = 9). Accuracy was assessed as mean percentage recovery (Table 3).

**Precision**

Repeatability was determined by using different levels of drug concentrations (same concentration levels taken in accuracy study), prepared from independent stock solution and analyzed ($N$ = 9) (**Table 4**). Inter-day and intra-day variation and instrument variations were taken to determine intermediate precision of the proposed methods. Different levels of drug concentrations in triplicates were prepared three different times in a day and studied for intra-day variation. Same pro-tocol was followed for three different days to study inter-day variation ($N$ = 27)

**Linearity**

The linearity is established for the proposed method, nine separate series of solutions of the drug (10-50 µg $ml^{-1}$ in 20% acetonitrile medium) were prepared from the stock solutions and analyzed. Least square regression analysis is done for the obtained data. ANOVA test (one-way) was performed based on the absorbance values observed for each pure drug concentration during the replicate measurement of the standard solutions

**Detection limit (DL) and quantitation limit (QL)**

Detection limit (DL) and quantitation limit (QL) for the proposed method is determined by using calibration standards. DL and QL were calculated as 3.3r/S and 10r/S, respectively, where S is the slope of the calibration curve and r is the standard deviation of y-intercept of regression equation (**Table 5**).

**Robustness**

Robustness of the proposed method is determined by (a) changing strength of the media by ± 2% and (b) stability of the Duloxetine hydrochloride in the both selected medium at room temperature for 8 hrs. Three different concentrations (LQC, MQC and HQC) were prepared in both media with different strength. Mean percentage recovery was determined **(Table 6).**

**Estimation from formulations**

Twenty capsules were emptied and content was mixed properly. Amount of the powder equivalent to 10 mg of Duloxetine hydrochloride was taken and extracted with solvent for 30 min. These solutions were diluted to prepare a 100 µg $ml^{-1}$ concentration in respective media. Finally solution was filtered through Whatman filter paper number 40 and the filtrate was diluted to prepare a 10 µg $ml^{-1}$ concentration and the sample was analysed using proposed methods **(Table 7).**

## RESULTS AND DISCUSSION

For media optimization various aqueous media like acetate buffers (pH 3.6–5.8), phosphate buffers (pH 5.8–8.0) and 0.1N sodium hydroxide were investigated. Duloxetine hydrochloride showed UV absorption spectra in 20% Acetonitrile.The final decision of using 20% Acetonitrile as a media is based on criteria like; sensitivity of the method, cost, ease of preparation and applicability of the method to dissolution studies. The spectra of Duloxitine hydrochloride in the 20% Acetonitrile are shown in Fig. 2 the lambda max of Duloxitine hydrochloride in 20% Acetonitrile is found to be 290 nm. Apparent molar absorptivity of drug was found to be $2.79 \times 10^4$ $mol^{-1}$ $cm^{-1}$ in 20% Acetonitrile (Table 2). The linear regression equation obtained is: absorbance at 290 nm y = 0.0172 + 0.0164 with a regression coefficient of 0.9999, In 10% methanol in 0.1N hydrochloric acid, the linear regression equation obtained is :absorbance at 230 nm = 0.097+ 0.1277; with a regression coefficient of 0.9961 (Table 2).

**Analytical validation**

**Specificity and selectivity**

The UV-spectrum of Duloxetine hydrochloride was not changed in the presence of common excipients in the selected media. There was no significant difference between mean absorbance of solutions prepared from pure drug sample**.** Therefore proposed method was specific and selective for the drug.

**Accuracy**

The excellent % recovery values (nearly 100%) and their low standard deviation values (S.D. < 1.5) represent accuracy. In 20% Acetonitrile the mean percentage recoveries (% R.S.D.) for concentrations (**Table 3**) were found to be in the range of 99.46 to 100.25%(%RSD in range of 0.306 to 1.093). This result revealed that any small change in the drug concentration in the solution can be accurately determined by these proposed methods.

**Precision**

Precision determined by studying repeatability and intermediate precision. Repeatability (% R.S.D.) in 20% Acetonitrile, at all three levels of concentrations (**Table 4).** Repeatability results indicate the precision under the same operating conditions over a short interval of time and inter-assay precision. Intermediate precision expresses within-laboratory variations in different days and in different instruments. In intermediate precision study, % R.S.D. values were not more than 2.5% in all the cases (**Table 4**). R.S.D. values were within the acceptable range indicating that these methods have excellent repeatability and intermediate precision.

**Linearity**

In 20% Acetonitrile medium the linearity range was found to be 10–50 µg $ml^{-1}$ at 290 nm. Lower values of parameters like standard error of slope and intercept (**Table 2**) indicated high precision of the proposed methods. The mean slope and intercept values are within the 95% confidence interval. Goodness of fit of regression equations was supported by high regression coefficient values**.**

**DL and QL**

In 20% Acetonitrile DL and QL were found to be 0.2405 µg $ml^{-1}$ and 0.7289 µg $ml^{-1}$ respectively.

**Robustness**

Variation of strength in the selected media by ± 2% did not have any significant effect on absorbance. The mean % recovery (± S.D.) were found to be 100.39(± 0.284) in the 20% Acetonitrile respectively (**Table 6).**

**Estimation of formulations**

In 20% Acetonitrile the assay values of Duloxetine hydrochloride for capsule ranged from 99.6% to 102.29% with standard deviation not more than 0.87%. Assay values of formulations were same as mentioned in the label claim, this indicated that the interference of excipient matrix is insignificant in estimation of Duloxetine hydrochloride by

proposed methods. The estimated drug content with low values of standard deviation established the precision of the proposed methods (**Table 7**).

## CONCLUSION

In summary, the proposed method was simple, rapid, accurate, precise and inexpensive and can be used for routine analysis of Duloxetine hydrochloride in bulk, pharmaceutical formulations and for dissolution studies.

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## REFERENCES

1. The Merck Index, 13th edition, Merck & co., INC., Whitehouse station, NJ, USA, 2001; 3498.
2. Dasari, S., Viriyala, R.K., Santosh, K., Kumari, A., Ravikumar B.V.V., Bisht, S.P.S., A validated RP-HPLC method for the analysis of duloxetine hydrochloride in pharma-ceutical dosage forms, Pharmacie Globale (IJCP), 2010; 1(3): 1-3.
3. Senthamil, S.P., Gowda K.V., Mandal U., Sam S.W.D., Pal, T.K.. Determination of duloxetine in human plasma by liquid chromatography with atmospheric pressure io-nization – tandem mass spectrometry and its application to pharmacokinetic study. J Chromatogr B Analyt Technol Biomed Life Sci., 2007; 858(1-2): 269-275.
4. Dahivelkar, P.P. Redasani, V.K., Bari S.B. Study of hydrolytic and oxidative behavior of duloxetine in aqueous solution by RP-HPLC. Journal of Global Pharma Technology, 2010; 2(8):19-23.
5. Lantz, R.J., Gillespie, T.A., Rash, T. J. Kuo, F., Skinner, M., Kuan, H-Y., Knadler, M.P. Metabolism, Excretion and pharmacokinetics of duloxetine in healthy human subjects. The American Society for Pharmacology and Ex-perimental Therapeutics, 2003; 31(9): 1142-1150.
6. .Lakshmana, P.S., Srinivasan, M., Thiyagarajan, S., Marina, Q. Determination of duloxetine hydrochloride in pharmaceutical formulation by HPLC with UV detection. International Journal of Chem Tech Research, 2010; 2(3): 1441-1444.
7. Yunoos, M., Sankar, D.G., Kumar, B.P., Hameed, S., Hussain, A. Simple UV Spectrophotometric determination of duloxetine hydrochloride in bulk and in pharmaceutical formulations. E-Journal of Chemistry, 2010; 7(3): 785-788.
8. Raj, R.V.A., Ramesh, T., Kumar, A.P., A validated UV Spectrophotometric determination of an antidepressant drug – duloxetine hydrochloride from capsule formula-tions. International Journal of Pharma and Bio Sciences, 2011; 2 (1): 716-720.
9. Ma, N., Zhang, B.K., Li, H.D., Chen, B.M., Xu, P., Wang, F., Zhu, R.H., Feng, S., Xiang, D.X., Zhu, Y.G. Deter-mination of duloxetine in human plasma via LC/MS and subsequent application to a pharmacokinetic study in healthy Chinese volunteers. Clinica Chimica Acta, 2007;380(1-2): 100-105.
10. Waldschmitt, C., Vogel, F., Maurer, C., Hiemke, C. (). Measurement of duloxetine in blood using High-Performance Liquid Chromatography with Spectrophotometric detection and column switching. Ther Drug Monit, 2007; 29(6): 767-772.
11. Mercolini, L., Mandrioli, R., Cazzolla, R., Amore, M., Raggi, M.A., HPLC analysis of the novel antidepressant duloxetine in human plasma after an original solid-phase extraction procedure. Journal of Chromatography B, 2007; 856: 81-87.
12. Dhaneshwar, S.S., Deshpande, P., Patil, M., Vadnerkar, G., Dhaneshwar, S.R. Development and validation of HPTLC method for estimation of duloxetine hydrochlo-ride in bulk drug tablet dosage form. Indian Journal of Pharmaceutical Sciences, 2008; 70(2): 233-236.
13. Bhimanadhuni, C.N., Garikapati, D.R., Karamsetty, S. Development and validation of RP-HPLC method for de-termination of Modafinil in bulk and dosage form. International Current Pharmaceutical Journal, 2012; (4): 77-80.
14. Olsen, B.A., Argentine, M.D. (). HPLC method develop-ment for duloxetine hydrochloride using a combination of computer-based solvent strength optimization and solvent selectivity mixture design. Journal of Liquid Chromato-graphy & Related Technologies, 1996; 19(12): 1993-2007.

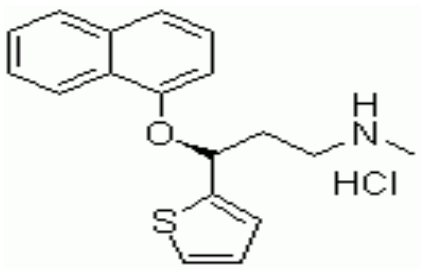
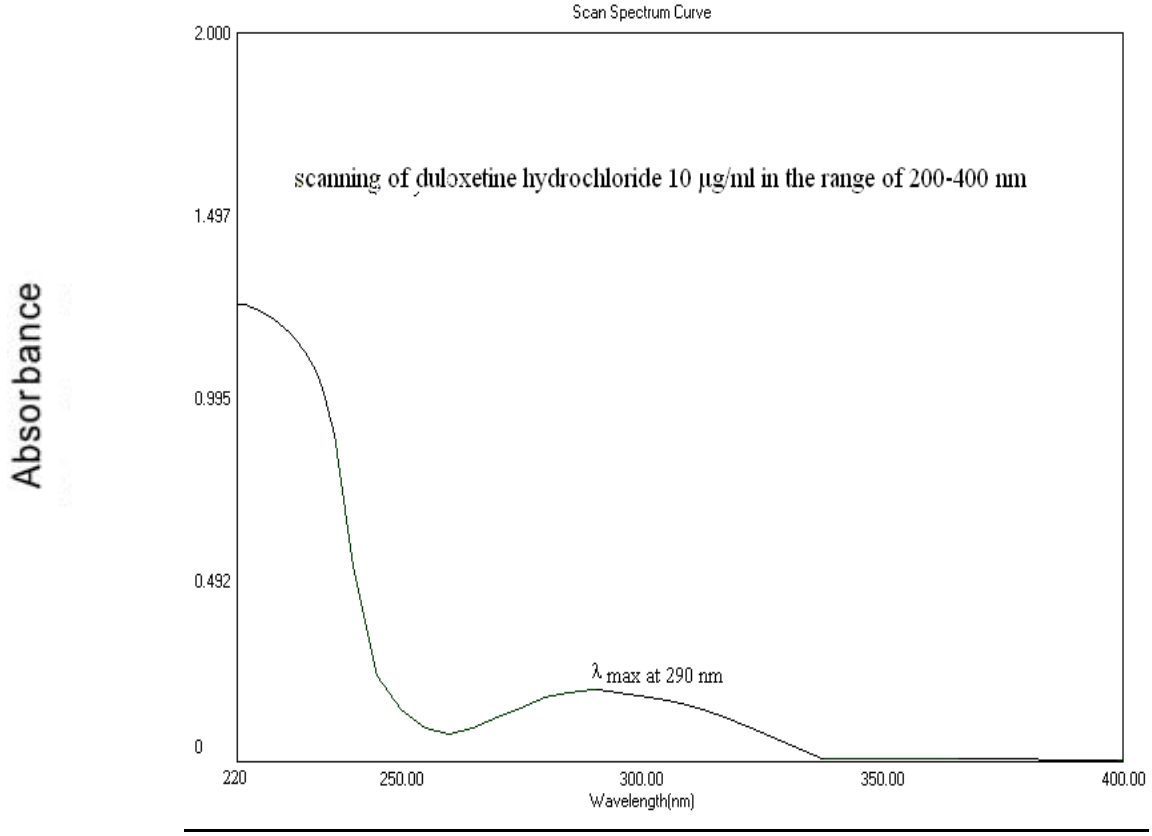
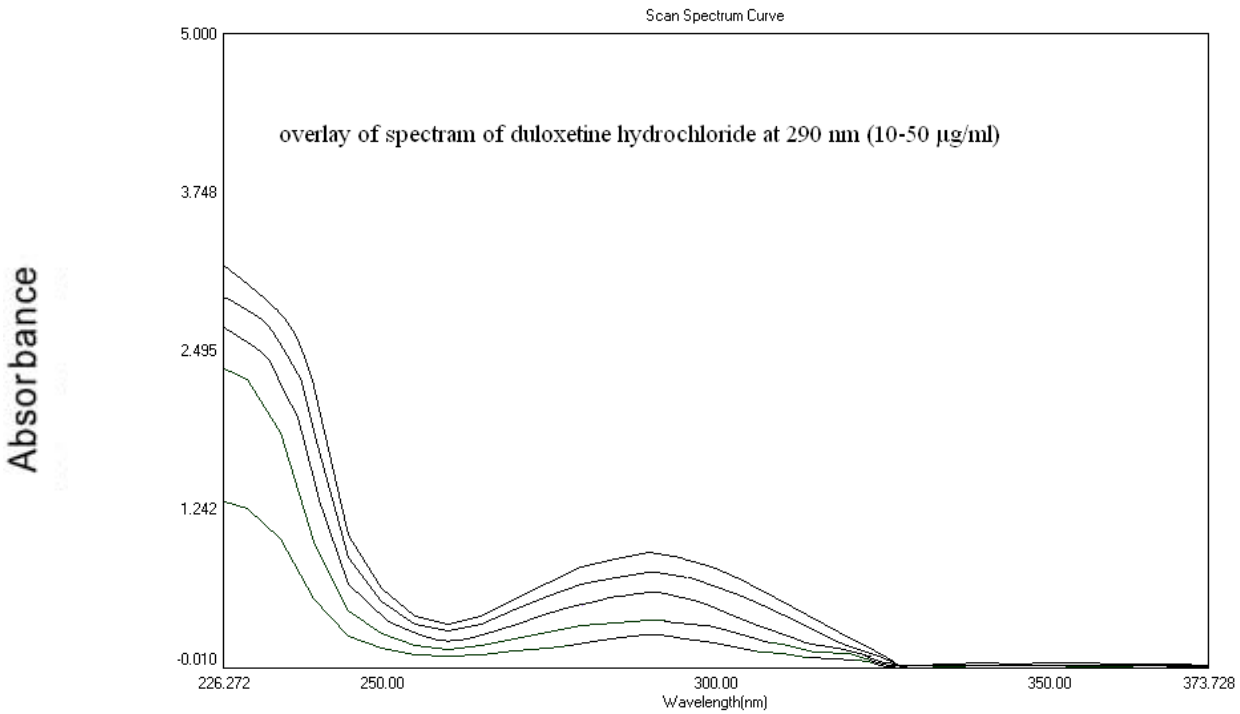



**Figure 1:** Structure of duloxetine hydrochloride




**Wave length**

**Figure 2:** Absorption spectrum of duloxetine hydrochloride




**Wave length**

**Overlay spectram of duloxetine hydrochloride at 290 nm**

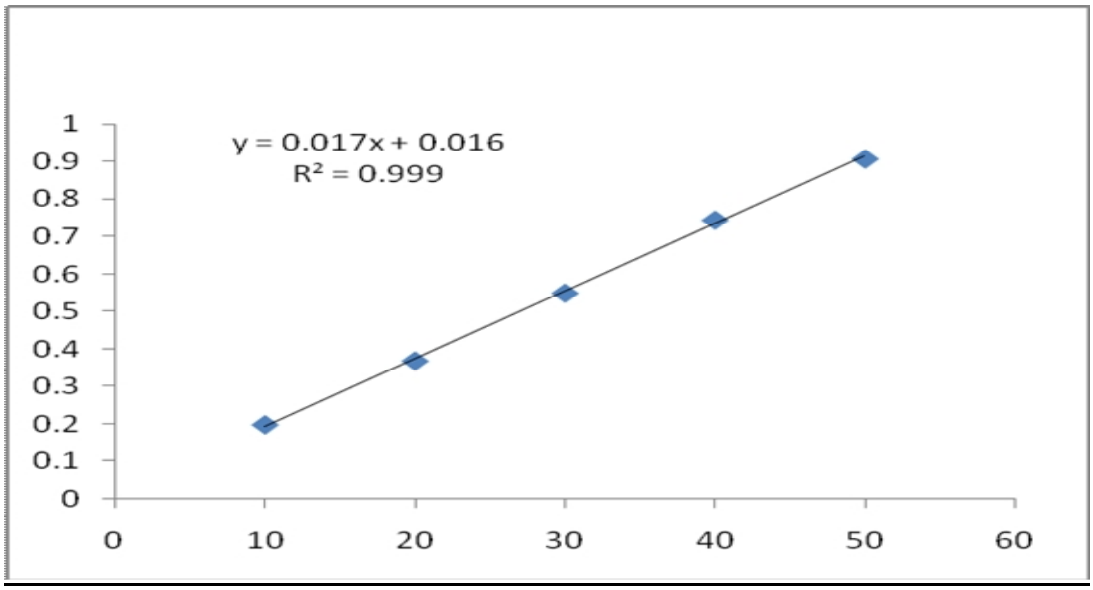



**Figure 3:** Calibration curve of duloxetine hydrochloride

**Table 1:** Calibration data of the develop

| S.no | Concentration(µg $ml^{-1}$) | Absorbance at 263nm±(SD) | %RSD |
|---|---|---|---|
| 1 | 10 | 0.199±0.0022 | 1.093 |
| 2 | 20 | 0.369±0.0025 | 0.673 |
| 3 | 30 | 0.550±0.0041 | 0.745 |
| 4 | 40 | 0.745±0.0037 | 0.490 |
| 5 | 50 | 0.908±0.0028 | 0.306 |

S.D: Standard deviation, %RSD: Relative standard deviation
(Each value is result of nine separate determinations)

**Table 2:** Optical Characteristics and Regression Equation of duloxetine hydrochloride

| Parameters | values |
|---|---|
| Λmax | 290 nm |
| Beer's law limit (µgm/ml) | 10-50 |
| Molar absorptivity (lit/mol.cm) | $= 5.922\times10^{-3}$ |
| Regression equation(y=mx+c) | y = 0.017x + 0.016 |
| Slope (m) | 0.017 |
| Intercept(c) 0.0233 | 0.016 |
| Correlation coefficient(r2) | 0.999 |
| Limit of detection(LOD) (µgm/ml) | 0.2405 |
| Limit of quantification (LOQ)(µgm/ml) | 0.7289 |

Y* = ax+b, where 'x' is concentration in µg/ml and Y is absorbance$10^{-3}$

**Table 3:** Accuracy data of the developed method

| Sno | Concentration(µg $ml^{-1}$) | Mean Absorbance | S.D | %RSD | %Recovery |
|---|---|---|---|---|---|
| 1 | 10 | 0.199 | 0.0022 | 1.093 | 99.83 |
| 2 | 20 | 0.371 | 0.0025 | 0.673 | 99.46 |
| 3 | 30 | 0.549 | 0.0041 | 0.745 | 100.12 |
| 4 | 40 | 0.747 | 0.0037 | 0.490 | 99.70 |
| 5 | 50 | 0.905 | 0.0028 | 0.306 | 100.25 |

S.D-Standard Deviation, %RSD-Relative Standard Deviation

**Table 4:** Precision data of the developed method

| Sno | Concentration( µg $ml^{-1}$) | Intra-day repeatability % R.S.D(*N* = 9) | | | Inter-day repeatability % R.S.D (*N* = 27) |
|---|---|---|---|---|---|
| | | Day 1 | Day 2 | Day 3 | |
| 1 | 15 | 0.314 | 0.315 | 0.315 | 0.631 |
| 2 | 35 | 0.670 | 0.672 | 0.670 | 2.23 |
| 3 | 55 | 0.980 | 0.978 | 0.979 | 0.978 |

**Table 5:** Detection limit (DL) and quantitation limit (QL) data for the developed method

| Sno | | Mean of calibration curve (N=9) | SD | DL | QL |
|---|---|---|---|---|---|
| 1 | Slope | 0.0171 | | | |
| 2 | Intercept | 0.017 | 0.0012 | 0.2405 | 0.7289 |
| 3 | $R^2$ | 0.9987 | | | |

**Table 6 :** Robustness data for the developed method

| S. no | Concentration( µg $ml^{-1}$ ) | Mean Absorbance (*N=9)* | S.D | %Recovery | %Recovery of SD |
|---|---|---|---|---|---|
| 1 | 15 | 0.316 | 0.002 | 100.17 | 0.284 |
| 2 | 35 | 0.664 | 0.018 | 100.39 | |
| 3 | 55 | 0.975 | 0.012 | 100.73 | |

**Table 7:** Assay of duloxetine hydrochloride

| Sample | Labeled amount(mg) | Amount found(mg) | %Purity (range) |
|---|---|---|---|
| Duzela | 20 | 19.84 | 99.6 -102.2 |