DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF DULOXETINE

LIPSA SAMAL, AMARESH PRUSTY

Department of Pharmacy, College of Pharmaceutical Sciences, Puri 752002, Odisha, India
Email: amareshrusty@gmail.com

Received: 24 Nov 2018 Revised and Accepted: 22 Jan 2019

INTRODUCTION

Duloxetine is chemically (+)-(S)-N-methyl-1-naphthyloxy)-2-thiophenepropylamine hydrochloride belongs to the antidepressant class of drugs. Its Molecular formula is C_{18}H_{19}N_{2}OS representing Molecular mass: 297.4156 g/mol with Molecular weight: 333.88 [1-3]. It is administrated by oral route at a dose of 40-60 mg/day for major depressive disorder, 30 mg/day for fibromyalgia. It is available in tablet and capsule dosage forms and marketed under a brand name cymbalta. It is Soluble in water, methanol, DMSO (Dimethyl Sulfoxide), acetonitrile. It is Stored between the temperatures 25-30 °C and should be kept away from direct sunlight [2, 3]. Duloxetine inhibits the reuptake of serotonin and norepinephrine (NE) in the central nervous system. Duloxetine increases dopamine (DA) specifically in the prefrontal cortex where there are few DA reuptake pumps via the inhibition of NE reuptake pumps (NET) thus allowing greater diffusion of DA in this brain region [4-6]. However, duloxetine has no significant affinity for dopaminergic, cholinergic, histaminergic, opioid, glutamate, and GABA reuptake transporters and can, therefore, be considered to be a selective reuptake inhibitor at the 5HT and NE transporters. Duloxetine undergoes extensive metabolism, but the major circulating metabolites do not contribute significantly to the pharmacologic activity [6, 7].

Duloxetine has an elimination half-life of about 12 h (range 8 to 17 h) and its Pharmacokinetics is dose proportional over the therapeutic range. Steady-state plasma concentrations are typically achieved after 3 d of dosing. Elimination of duloxetine is mainly through hepatic metabolism involving two P450 isozymes, CYP2D6 and CYP1A2 [6-8].

Though determination of duloxetine hydrochloride by spectrofluorimetric method [9, 10] has been reported but there is no UV-visible spectrophotometric method for determination of duloxetine, which motivates us to develop and carry out this research work. This present research paper was found to be simple, precise, and accurate and validated as per ICH guidelines and rapid for the estimation of duloxetine in pure form. The solubility of duloxetine was analyzed using various solvents and it was found that the drug is freely soluble in acetonitrile and water. So the study was conducted using acetonitrile and water to optimize the analytical method. The chemical structure of duloxetine hydrochloride was shown in fig. 1.

MATERIALS AND METHODS

In instrumentation

Shimadzu UV-1800 and Shimadzu UV-1700 spectrophotometer (single beam detector) were used for this study.

Chemicals and reagents

All the chemicals were of good quality. Acetonitrile was obtained from Thomas Beaker (chemicals) Pvt. LTD., Mumbai and water used were double distilled water prepared in our college laboratory for this study. The solvents and reagents used in the study were of analytical grade.

Preparation of the solvent system

The solvent was prepared by mixing water and acetonitrile in the ratio of 2:8 v/v.

Preparation of the standard solution

Accurately weighed 10 mg of the powdered drug duloxetine was taken in a 10 ml volumetric flask and the prepared solvent water: acetonitrile (in ratio 20:80) was added up to the mark which gives the concentration of 1000 ppm. From the stock solution, 1 ml of the...
solution was taken in a 10 ml volumetric flask and then it was made up to the mark with the same solvent to prepare the concentration of 100 ppm. From the above solution, different aliquots of solution were prepared by taking 1, 2, 3, 4, 5 ml in each 10 ml of volumetric flask separately and it was made up to mark with the same solvent to produce 10, 20, 30, 40, 50 ppm respectively.

Calibration curve
The prepared stock solution was scanned with water: acetonitrile (20:80) to construct Beer’s law plot for the pure drug. The overall Spectra of duloxetine by UV-Visible Spectroscopy using solvent water: acetonitrile (2:8) was shown in fig. 2. From the solution concentration of 20 ppm was then scanned in UV range. This showed an absorption maximum at 290 nm. The absorbance of each solution was measured at their respective λmax against acetonitrile (in the ratio of 20:80) as blank and results are shown in table 1. The calibration curve was plotted by taking the concentration of drug on x-axis and absorbance on the y-axis and the curve is shown in fig. 3.

Method validation
Accuracy
Accuracy of the method reveals the degree of similarity between the true value and the mean analytical value [11]. To determine the accuracy of the proposed method different sample solutions of same concentration 30 ppm were analyzed to determine percentage recovery of duloxetine by standard addition recovery method. The study was carried out by adding the known amount of the sample solution in the standard stock solution.

Precision
The precision of the proposed method was assessed by intra-day and inter-day variation studies using only one concentration of duloxetine (30 ppm) for several times [11]. During intra-day studies, five sample solutions of each concentration were analyzed on the same day whereas inter-day studies were determined by analyzing five sample solutions of each concentration for 5 consecutive days. The mean, standard deviation and % RSD were calculated.

Limit of detection (LOD) and limit of quantification (LOQ)
LOD and LOQ were calculated by using the following expressions:

LOD = 3.3 σ/S
LOQ = 10 σ/S

Where “σ” is the standard deviation of the regression line and “S” is the slope of the calibration curve.

Ruggedness
Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions [11-13]. The method's ruggedness was established by the determination of duloxetine by changing the different instruments of UV spectrophotometer i.e. Shimadzu UV-1800 and Shimadzu UV-1700 spectrophotometer. The data was then subjected to statistical analysis and the results are expressed in mean, standard deviation and % RSD.

Robustness
To verify the robustness of the method, three vital experimental variables such as composition of mobile phase, detection wavelength and flow rate were slightly varied. The analysis was performed by changing the different instruments of UV spectrophotometer i.e. Shimadzu UV-1800 and Shimadzu UV-1700 spectrophotometer. The data was then subjected to statistical analysis and the results are expressed in mean, standard deviation and % RSD.

RESULTS AND DISCUSSION
The proposed method for determination of duloxetine hydrochloride showed molar absorptivity 2.995×10⁻⁷ L/mmol cm. From the calibration curve it was found that it shows linearity in the range of 10-50µg/ml with regression coefficient 0.9999. Linear regression of absorbance on concentration gave the equation y = 0.020x+0.007 with a correlation coefficient (r) of 0.999. The detection wavelength showing λmax (maximum wavelength) at 290 nm.

Accuracy
The percentage recovery and % RSD were calculated. The mean percentage recovery and % RSD were found to be within limits and it is less than 2, which explains the present research paper is accurate in method development of duloxetine. The mean, standard deviation and percentage relative standard deviation (%RSD) were calculated. The results were shown in table 2.

Precision
Repeatability of the method was studied by precision experiments. The % RSD of duloxetine was found to be 0.811707 and 0.831657 in intra and inter-day precision respectively. The results for intra-day and inter-day were shown in table 3 and table 4 respectively.

LOD and LOQ
The LOD and LOQ were estimated from the standard deviation of the Y intercepts and slope of the calibration curve and values are 0.4 µg/ml and 1.32 µg/ml for LOD and LOQ respectively.

Ruggedness
The ruggedness results are shown in table 5 and table 6. The low percentage RSD value illustrates the ruggedness of the method.

Robustness
Robustness was evaluated by making deliberate changes to the chromatographic parameters of the method. The obtained results in table 7 and table 8 indicated the minor changes in each condition and did not affect the method.

The optical characteristics such as Beer's law limit, Sandell's sensitivity, Standard deviation, % RSD, LOD, LOQ were calculated and are summarized in table 9.

Fig. 2: Overlay spectra of duloxetine by UV-visible spectroscopy using solvent water: acetonitrile (2:8)
Table 1: Calibration table of UV-Vis spectrophotometric method for duloxetine

| Concentration in µg/ml | Absorbance 1 | Absorbance 2 | Absorbance 3 | Absorbance 4 | Absorbance 5 | Absorbance 6 | Mean | Standard deviation % Relative standard deviation |
|------------------------|--------------|--------------|--------------|--------------|--------------|--------------|------|-----------------------------------------------|
| 10                     | 0.21         | 0.213        | 0.212        | 0.214        | 0.206        | 0.202        | 0.2095| 0.004637                                      |
| 20                     | 0.412        | 0.423        | 0.414        | 0.412        | 0.411        | 0.415        | 0.4145| 0.004416                                      |
| 30                     | 0.611        | 0.602        | 0.601        | 0.603        | 0.618        | 0.621        | 0.609333| 0.008687                                      |
| 40                     | 0.819        | 0.822        | 0.919        | 0.916        | 0.824        | 0.821167     | 0.008971| 0.483547                                      |
| 50                     | 1.023        | 1.019        | 1.014        | 1.018        | 1.016        | 1.015        | 1.0175 | 0.003271                                      |

*values given in the table are the mean±SD of six observations.

Fig. 3: Calibration curve was plotted using the concentration on X-axis and mean absorbance on Y-axis

Table 2: Accuracy data of UV-Vis spectrophotometric method for duloxetine

| S. No. | Concentration (µg/ml) | Absorbance | Calculated amount | Statistical analysis |
|--------|------------------------|------------|-------------------|----------------------|
| 1.     | 30                     | 0.611      | 30.2              | Mean = 30.11667      |
| 2.     | 30                     | 0.602      | 29.75             | Standard deviation = 0.434358 |
| 3.     | 30                     | 0.601      | 29.7              | % RSD = 1.44225      |
| 4.     | 30                     | 0.603      | 29.8              |                      |
| 5.     | 30                     | 0.618      | 30.55             |                      |
| 6.     | 30                     | 0.621      | 30.7              |                      |

*values given in the table are the mean±SD. % RSD: Relative standard deviation

Table 3: Interday precession data of UV-Vis spectrophotometric method for duloxetine

| S. No. | Concentration (µg/ml) | Absorbance (1) | Absorbance (2) | Absorbance (3) | Average | Statistical analysis |
|--------|------------------------|----------------|----------------|----------------|---------|----------------------|
| 1.     | 30                     | 0.611          | 0.61           | 0.615          | 0.613   | Mean = 0.6081        |
| 2.     | 30                     | 0.601          | 0.607          | 0.608          | 0.605333| Standard deviation = 0.004936 |
| 3.     | 30                     | 0.603          | 0.604          | 0.605          | 0.604333| % RSD = 0.811707     |
| 4.     | 30                     | 0.602          | 0.607          | 0.604          | 0.604333|                      |
| 5.     | 30                     | 0.618          | 0.607          | 0.617          | 0.614   |                      |

*values given in the table are the mean±SD for 5 samples. % RSD: Relative standard deviation

Table 4: Intraday precession data of UV-Vis spectrophotometric method for duloxetine

| S. No. | Concentration (µg/ml) | Day 1 | Day 2 | Day 3 | Average | Statistical analysis |
|--------|------------------------|-------|-------|-------|---------|----------------------|
| 1.     | 30                     | 0.615 | 0.618 | 0.617 | 0.616667| Mean = 0.612067      |
| 2.     | 30                     | 0.608 | 0.609 | 0.611 | 0.609333| Standard deviation = 0.00509 |
| 3.     | 30                     | 0.605 | 0.607 | 0.619 | 0.610333| % RSD = 0.831657     |
| 4.     | 30                     | 0.604 | 0.606 | 0.608 | 0.606   |                      |
| 5.     | 30                     | 0.617 | 0.618 | 0.619 | 0.618   |                      |

*values given in the table are the mean±SD. % RSD: Relative standard deviation

Table 5: Ruggedness data of the UV-Vis spectrophotometric method by UV-1700 for duloxetine

| S. No. | Concentration (µg/ml) | Absorbance | Calculated amount | Statistical analysis |
|--------|------------------------|------------|-------------------|----------------------|
| 1.     | 30                     | 0.606      | 29.95             | Mean = 30.2          |
| 2.     | 30                     | 0.607      | 30.0              | Standard deviation = 0.23184 |
| 3.     | 30                     | 0.611      | 30.2              | % RSD = 0.767694     |
| 4.     | 30                     | 0.614      | 30.35             |                      |
| 5.     | 30                     | 0.617      | 30.5              |                      |

*values given in the table are the mean±SD. % RSD: Relative standard deviation
Table 6: Ruggedness data of UV-Vis spectrophotometric method by UV-1800 for duloxetine

| S. No. | Concentration (µg/ml) | Absorbance | Calculated amount | Statistical analysis |
|--------|-----------------------|------------|-------------------|---------------------|
| 1.     | 30                    | 0.608      | 30.05             | Mean= 30.24         |
| 2.     | 30                    | 0.609      | 30.1              | Standard deviation= 0.178185 |
| 3.     | 30                    | 0.612      | 30.25             | %RSD = 0.589237     |
| 4.     | 30                    | 0.613      | 30.3              |                     |
| 5.     | 30                    | 0.617      | 30.5              |                     |

*values given in the table are the mean±SD. % RSD: Relative standard deviation

Table 7: Robustness data of UV-Vis spectrophotometric method by UV-1700 instruments for duloxetine

| S. No. | Concentration (µg/ml) | Absorbance | Calculated amount | Statistical analysis |
|--------|-----------------------|------------|-------------------|---------------------|
| 1.     | 30                    | 0.608      | 30.05             | Mean= 30.39         |
| 2.     | 30                    | 0.611      | 30.2              | Standard deviation= 0.270185 |
| 3.     | 30                    | 0.615      | 30.4              | %RSD = 0.89059      |
| 4.     | 30                    | 0.619      | 30.6              |                     |
| 5.     | 30                    | 0.621      | 30.7              |                     |

*values given in the table are the mean±SD. % RSD: Relative standard deviation

Table 8: Robustness data of UV-Vis spectrophotometric method by UV-1800 instruments for duloxetine

| S. No. | Concentration (µg/ml) | Absorbance | Calculated amount | Statistical analysis |
|--------|-----------------------|------------|-------------------|---------------------|
| 1.     | 30                    | 0.605      | 29.9              | Mean= 30.22         |
| 2.     | 30                    | 0.607      | 30                | Standard deviation= 0.270647 |
| 3.     | 30                    | 0.612      | 30.25             | %RSD = 0.89559      |
| 4.     | 30                    | 0.615      | 30.4              |                     |
| 5.     | 30                    | 0.618      | 30.55             |                     |

*values given in the table are the mean±SD. % RSD: Relative standard deviation

Table 9: Optical characteristics of duloxetine

| Beer's law limit (µg/ml) | 10-50 µg/ml |
|-------------------------|-------------|
| ƛ max                   | 240 nm      |
| Molar extinction co-efficient (E 1% 1cm) | 2036.66 |
| Molar absorptivity (L mole⁻¹ cm⁻¹) | 2.995×10⁻³ |
| Sandell’s sensitivity (µg cm⁻²/0.001 absorbance unit) | 0.540 |
| Standard deviation     | 0.004996    |
| % Relative standard deviation | 1.101867 |
| Confidence limits      |             |
| Correlation coefficient | 0.999       |
| Regression equation (Y) | 0.020x+0.007 |
| Slope (a)              | 0.020       |
| Intercept (b)          | 0.007       |
| LOD                    | 0.4 µg/ml   |
| LODQ                   | 1.52 µg/ml  |

CONCLUSION
The proposed method UV-Vis Spectrophotometric method was found to be simple, precise, and accurate and validated as per ICH guidelines and rapid for the estimation of duloxetine. The mobile phase is simple to prepare, inexpensive solvent. Hence, this method can be easily and conveniently adopted for routine analysis of duloxetine in quality control laboratories and the method can also be extended for the routine assay of duloxetine in formulations.

ACKNOWLEDGMENT
Authors are thankful to the College of Pharmaceutical Sciences, Puri, Odisha for providing the facilities for carrying out this research work.

AUTHORS CONTRIBUTIONS
For preparing this research paper L. Samal gave a substantial contribution by data collection, data analysis and interpretation by executing the experimental work in our laboratories. A. Prusty drafted the manuscript and extensively revised to improve the quality of the manuscript. Conception, design, critical revision of the article and supervision of the work has been done by A. Prusty.

CONFLICT OF INTERESTS
Authors declare that there is no conflict of interest

REFERENCES
1. Remington. The science and practice of pharmacy. Edited by Loyd V, Allen Jr, PhD, RPh, Published by Pharmaceutical Press, 1 Lambeth High Street, London SE1 7JN, UK, University city science Center, 3624 Market Street, Suite 5E, Philadelphia, PA 19104, USA; volume-1, Twenty-second edition, 1416; 2013.
2. The Merck Index: an encyclopedia of chemicals, drugs, biological. Edited. MJO Neil, Merck and Co., Inc. Merck Research laboratories, Whitehouse station, NJ, USA. 14th edition; 2006. p. 3466.
3. Drug Today, July-September; 2017. p. 502.
4. HP Rang, JM Ritter, RJ Flower. Rang and Dale's pharmacology, Elsevier Churchill Livingstone publication, china. 8th edition; 2016. p. 569-77.
5. Tripathy KD. Essentials of medical pharmacology, jaypee brothers medical publishers (P) Ltd. 7th edition. Newdelhi, Ansari Road, Daryaganj; 2013. p. 441-3.
6. Skinner MH, Kuan HY, Pan A, Sathirakul K, Knadler MP, Gonzales CR, et al. duloxetine is both an inhibitor and a substrate of cytochrome P4502D6 in healthy volunteers. Clin Pharmacol Ther 2003;73:170–7.
7. Lantz RJ, Gillespie TA, Rash Tj, Kuo F, Skinner M, Kuan HY, et al. Metabolism, excretion, and pharmacokinetics of duloxetine in healthy human subjects. Drug Metab Dispos 2003;31:142–50.
8. Bymaster FP, Beedle EE, Findlay J, Gallagher PT, Krushinski JH, Mitchell S, et al. Duloxetine (Cymbalta), a dual inhibitor of serotonin and norepinephrine reuptake. Bioorg Med Chem Lett 2003;13:4477–80.
9. Xiangping Liu, Yingxiang Du, Xiulan Wu. Simple UV spectrophotometric determination of duloxetine hydrochloride in bulk and in pharmaceutical formulations. Spectrochim Acta Part A 2008;71:915-20.
10. Prabhul SL, Shahnawaz S, Dinesh Kumar C, Shirwaikar A. Spectrofluorimetric method for determination of duloxetine hydrochloride in bulk and pharmaceutical dosage forms. Indian J Pharm Sci 2008;70:502-3.
11. Yunoos M, Sangkar DG, Kumar BP, Hameed S. Simple UV spectrophotometric determination of duloxetine hydrochloride in bulk and in pharmaceutical. E-J Chem 2010;7:785-8.
12. Sydabi P, Muneer S, Ishaq BM, Kumar ES. Development and validation of an analytical method for duloxetine hydrochloride in capsule formulations by HPLC-UV. Indo Am J Pharm Sci 2014;1:1:11-6.
13. Methuku K, Aarely K, Raghunandan N. Simple UV spectrophotometric determination of duloxetine hydrochloride in bulk and in pharmaceutical formulations. J Pharm Sci Innovation 2012;1:81-6.