Development and Validation of a Spectrophotometric Method for the Estimation of Duloxetine using Chromogenic agents in Bulk and Pharmaceutical Dosage forms

Ashok Gorja[1], Pani Kumar D. Anumolu[1], Koyyana Shirisha[1], Rachapudi Geetha rani sai supraja[1].

Department of Pharmaceutical Analysis, Gokaraju Rangaraju College of Pharmacy, Osmania University, Hyderabad, Telangana- 500090.

Corresponding Author: Koyyana Shirisha

Department of Pharmaceutical Analysis, Gokaraju Rangaraju College of Pharmacy, Osmania University, Hyderabad, Telangana- 500090.

ABSTRACT

A simple, precise and accurate colorimetric method was developed and validated for the antidepressant medication, duloxetine. The both methods estimates the duloxetine using chromogenic agents (potassium ferrocyanide and Ferric chloride) in bulk dosage forms. At the range between 400-800nm the absorption intensity of the chromogens formed upon reacting with chromogenic agents were determined. The linearity was established for both method-I and method-II within the concentration range of 1-5 and 5-25 µg/ml respectively with R^2 values of 0.999. The developed method was validated as per ICH guidelines. The results suggests that both methods are accurate and precise.

Key words: Duloxetine, potassium ferrocyanide, Picric acid, colorimetric method

INTRODUCTION

Duloxetine is an antidepressant which is used to treat depression and anxiety. It is also used in the treatment for the pain caused by nerve damage associated with diabetes (diabetic peripheral neuropathy). Duloxetine is also used to treat fibromyalgia (muscle pain and stiffness) and chronic (long-lasting) pain that is related to muscles and bones. Duloxetine belongs to a group of medicines known as selective serotonin and norepinephrine reuptake inhibitors (SSNRIs). These medicines are thought to work by increasing the activity of chemicals called serotonin and norepinephrine in the brain.

Several methods have been reported for the development and validation of duloxetine, but only a few colorimetric methods have been reported. So in this present study, chromogenic agents such as picric acid and potassium ferrocyanide have been used to develop and validate two methods for the estimation of duloxetine.

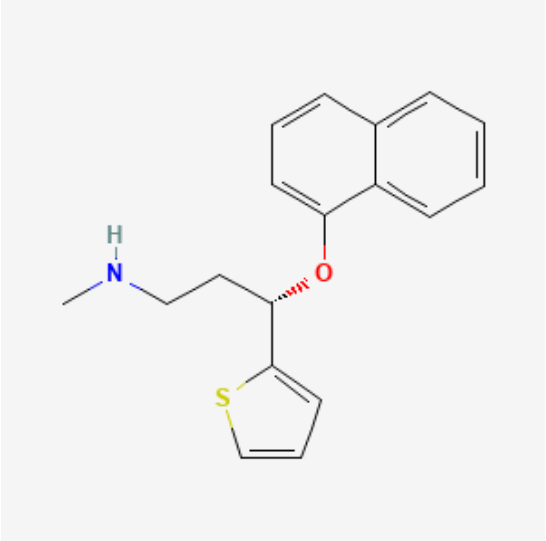

Fig.1 Structure of duloxetine

METHODOLOGY:

Apparatus:

All absorption measurements were observed by using Shimadzu UV 1800 model digital spectrophotometer with 1cm path length quartz cells.

Materials:

Duloxetine (API) procured from Hetero drugs private Ltd, Duvanta-20, Potassium ferrocyanide, picric acid, hydrochloric acid, chloroform. All the above chemicals were purchased from S.D. Fine chemicals Ltd, Mumbai, India. Distilled water was used whenever required and all the chemicals are of analytical grade.

Reagents:

0.3%w/v Ferric chloride: Take 3g of Ferric chloride and dissolved in 100ml of 0.1 N hydrochloric acid.

0.3%w/v Potassium ferrocyanide: Take 300mg of potassium ferrocyanide and dissolved in 100ml distilled water.

1N hydrochloric acid: Take 8.9 ml concentrated HCl and transfer it to 100ml volumetric flask containing few ml of water and make up the volume with the same solvent.

0.2%w/v Picric acid: Take 0.2 g of Picric acid and dissolved it in 100 ml chloroform.

Standard stock solutions: 100 µg/ml solution of drug was prepared by dissolving 10 mg of drug in 100 ml distilled water for method-I and same concentration of solution was prepared by using chloroform for method-II.

Preparation of calibration curve:

For method-I: From the standard stock solution, transfer 0.1–0.5 ml aliquots of the standard drug solution into 10-ml volumetric flasks. After adding 0.5 ml of $FeCl_3$, 0.5 ml of potassium ferrocyanide, and 0.5 ml of 1N HCl, this was thoroughly shaken and left for a few minutes. Distilled water was used to adjust the volume in order to produce a series of standard solutions ranging from 1 to 5 µg/ml. Next, the absorbance of the bluish green chromogen was measured at 400–800 nm.

For method-II: 0.5–2.5 ml aliquots of the standard solution were put into a series of 10-milliliter volumetric flasks. 1 ml of picric acid was added, stirred for 5 minutes, and the volume was adjusted with chloroform to produce a series of standard solutions with 5–25 µg/ml. The absorbance of the yellow-coloured complex was then measured at wavelengths ranging from 400 to 800 nm.

RESULTS AND DISCUSSION:

Linearity: Linear relation was established by plotting absorbance versus concentration.

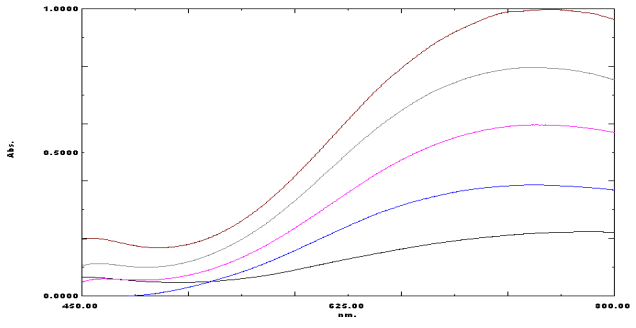

Figure 2: Overlay spectra of duloxetine for method-I

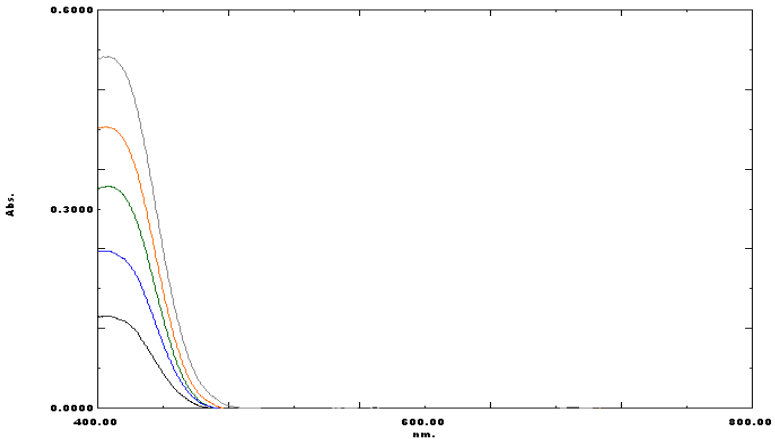

Figure 3: Overlay spectra of Duloxetine for method-II

The regression equation for the results were:

Method-I: $y = 0.1977x + 0.0058$, $R^2 = 0.9994$

Method-II: $y = 0.0214x + 0.0073$, $R^2 = 0.9994$

Precision:

One concentration of standard solutions was run through six replicates in one day and alternate days, respectively, to report intraday and interday precision in terms of percentage RSD.

For method-I:

Table 1: Precision data for method-I

	INTRA-DAY		**INTER-DAY**	
SAMPLE NAME	ABSORBANCE	% ASSAY	ABSORBANCE	% ASSAY
1	0.6010	99.88	0.6023	99.80
2	0.6021	100.07	0.6024	99.82
3	0.5999	99.70	0.6022	99.78
4	0.5998	99.68	0.6022	99.78

5	0.6001	99.73	0.6023	99.80
6	0.6001	99.73	0.6025	99.83
AVERAGE	0.6005	99.80	0.6023	99.80
%RSD	0.15	0.15	0.02	0.02

For method-II:

Table 2: Precision data for method-II

	INTRA-DAY PRECISION		**INTER-DAY PRECISION**	
SAMPLE NAME	ABSORBANCE	%ASSAY	ABSORBANCE	%ASSAY
1	0.3322	99.26	0.3567	98.75
2	0.3332	99.56	0.3592	99.44
3	0.3325	99.35	0.3589	99.36
4	0.3348	100.04	0.3616	100.10
5	0.3351	100.13	0.3633	100.58
6	0.3362	100.46	0.3634	100.60
AVERAGE	0.3340	99.80	0.3605	99.81
%RSD	0.48	0.48	0.75	0.75

This assay gave satisfactory results as the %RSD was found to be less than 2.

Accuracy:

To confirm the accuracy of the method, a recovery study with multiple stages was reported. Three standard addition levels—50, 100, and 150%—were used. A standard drug was added to a previously examined sample in a predefined amount in order to determine the accuracy.

For method-I:

Table 3: Accuracy data for method-I

Spike level (in %)	Amount added (mg)	Amount found (mg)	% Recovery	Mean% Recovery
50	3.00	2.98	99.33	100.11
	3.00	3.02	100.67	
	3.00	3.01	100.33	
100	6.00	5.94	99.00	99.67
	6.00	5.98	99.67	
	6.00	6.02	100.33	
150	9.00	9.01	100.11	99.93
	9.00	8.99	99.89	
	9.00	8.98	99.78	

For method-II:

Table 4: Accuracy data for method-II

Spike level (in %)	Amount added (mg)	Amount found (mg)	% Recovery	Mean% Recovery
50	7.50	7.52	100.27	100.09
	7.50	7.49	99.87	
	7.50	7.51	100.13	
100	15.00	15.01	100.07	99.93
	15.00	14.99	99.93	

	15.00	14.97	99.80	
150	22.50	22.54	100.18	98.96
	22.50	22.09	98.18	
	22.50	22.17	98.53	

This method was found to be accurate as the percentage recovery at each level was found to be between 98% and 102

Sensitivity: Limit of quantification (LOQ) and limit of detection (LOD) were used to evaluate the method's sensitivity. Estimates are based on the following equations from the ICH guidelines

$$LOD: 3.3\sigma/S$$

$$LOQ: 10\sigma/S$$

Where,

σ = response standard deviation

S = slope of the calibration study

For method-I:

Parameters for Validation	LOD	LOQ
Values (µg/ml)	0.18	0.55

For method-II:

Parameters for Validation	LOD	LOQ
Values (µg/ml)	0.34	1.04

CONCLUSION:

A comprehensive review of the literature showed that while there were numerous techniques for estimating duloxetine, there were relatively few colorimetric techniques. These developed techniques, which use potassium ferrocyanide and picric acid as chromogenic agents, are simple to use, accurate, and precise.

- With R^2 values of 0.999 for each method, we demonstrated linearity from concentration ranges of 1–5 µg/ml and 5–25 µg/ml, respectively.
- As per the guidelines provided by ICH, these methods have been verified.
- Since the %RSD was less than 2, these techniques were determined to be exact and accurate.
- LOD and LOQ values for method-I were found to be 0.18 and 0.55 µg/ml
- LOD and LOQ values for method-II were found to be 0.34 and 1.04 µg/ml

REFERENCES:

1. Alarfaj NA, Ammar RA, El-Tohamy MF. Cationic-enhanced spectrofluorimetric method for determination of selective serotonin reuptake inhibitor duloxetine hydrochloride in its dosage forms. Asian J. Chem. 2013 Sep 1;25:6416-20.

2. Misiuk W. Spectrofluorimetric study on inclusion interaction of β-cyclodextrin with duloxetine and its analytical application.

3. Prabu SL, Shahnawaz S, Kumar CD, Shirwaikar A. Spectrofluorimetric method for determination of duloxetine hydrochloride in bulk and pharmaceutical dosage forms. Indian journal of pharmaceutical sciences. 2008 Jul;70(4):502.

4. Samal L, Prusty A. Development and validation of UV-Visible spectrophotometric method for determination of Duloxetine. Int J Pharm Pharm Sci. 2019;11(3):27-31

5. Ramesh T, Kumar AP, Raj RV. A validated UV spectrophotometric determination of an antidepressant drug–duloxetine hydrochloride from capsule formulations. Int J Pharma Bio Sci. 2011;2(1):717-20.

6. Prashanthi M, Venkateshwaralu G. Spectrophotometric estimation of drugs using nbromosuccinamide and amarnth dye couple. Int J Pharm Res Anal. 2014;4:58-64.

7. Sasikala M, Sayanna K, Venkateshwarlu G. Spectrophotometric determination of drugs by using Chloramine-T and methyl orange. World J Pharm Res. 2015;4(2):709-22.

8. Ganesh M, Aziz AS, Peng MM, Ravikumar R, Sakthimanigandan K, Cha SE, Jang HT. Evaluation of tropaeolin 000-1 as a colorimetric reagent for assay of duloxetine and escitalopram in solid dosage form. Tropical Journal of Pharmaceutical Research. 2016 Apr 8;15(3):613-21.

9. Derayea SM, Ali R, Abu-Hassan AA. Two facile approaches based on association complex with erythrosine-B for nano-level analysis of duloxetine: application to content uniformity. Royal Society Open Science. 2021 Sep 29;8(9):210922.

10. Mani Ganesh MG, Aziz AS, Peng MeiMei PM, Ramaswamy Ravikumar RR, Kalaimani Sakthimanigandan KS, Cha SeungEun CS, Jang HyunTae JH. Evaluation of tropaeolin 000-1 as a colorimetric reagent for assay of duloxetine and escitalopram in solid dosage form.

11. Pushparaj Hemalatha PH, Mani Ganesh MG, Peng MeiMei PM, Jang HyunTae JH. Facile colorimetric determination of duloxetine in formulations using methyl orange as ion-pairing agent.

12. Reddy DC, Bapuji AT, Rao VS, Himabindu V, Rama Raju D, Syedba S, Ravikiran HL. Development and validation of a LC/MS/MS method for the determination of duloxetine in human plasma and its application to pharmacokinetic study. Journal of Chemistry. 2012;9(2):899-911.

13. Globale p. A validated RP-HPLC method for the analysis of Duloxetine hydrochloride in pharmaceutical dosage forms.

14. Chauhan CK. Development and Validation of Analytical Method for Simultaneous Estimation of Duloxetine and Methylcobalamin in Pharmaceutical Dosage Form. An International Journal of Advances in Pharmaceutical Science. 2015;6(2):2815-7.

15. Puranik M, Wadherb S, Sharmaa K. A Simple, novel validated stability indicating RP-HPLC method for estimation of duloxetine HCl in capsule pharmaceutical formulation. methods. 2014 Jul 1;3:5.

16. Bhimanadhuni CN, Garikapati DR, Srinivas C. Development and validation of RP-HPLC method for determination of Duloxetine hydrochloride in bulk and dosage form. International Current Pharmaceutical Journal. 2012 Apr 7;1(5):98-102.

17. Boopathy D, Jawarkar RD, Prakash M, Mathew B, Perumal P. New RP-HPLC method development and validation determination for estimation of duloxetine HCl in enteric coated capsules. Int J Chem Tech Res. 2010 Jan;2(1):239-41.

18. Sinha VR, Anamika, Kumria R, Bhinge JR. Stress degradation studies on duloxetine hydrochloride and development of an RP-HPLC method for its determination in capsule formulation. Journal of chromatographic science. 2009 Aug 1;47(7):589-93.

19. Goyal MK, Yadav TC. Analytical Method Development and its Validation for Estimation of Duloxetine Hydrochlorideby Reversed Phase High Performance Liquid Chromatography (RP-HPLC).

20. Rao DD, Sait SS, Reddy AM, Chakole D, Reddy YR, Mukkanti K. Analysis of duloxetine hydrochloride and its related compounds in pharmaceutical dosage forms and in vitro dissolution studies by stability indicating UPLC. Journal of chromatographic science. 2010 Nov 1;48(10):819-24.

21. Guideline IH. Validation of analytical procedures: text and methodology. Q2 (R1). 2005 Nov;1(20):05.