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Stability Indicating RP-HPLC Method for Estimation of Duloxetine in Tablet Dosage Form

Jinkal R. Daraji[1]*, Chiragkumar Panchal[1], Bhumika Sakhreliya[2], Mandev B. Patel[3]

1 Student of M.Pharm, Departement of Quality Assurance A-One Pharmacy College Anasan Dascroi Ahmedabad Gujarat India

2 Assistant Professor, Departement of Quality Assurance A-One Pharmacy College Anasan Dascroi Ahmedabad Gujarat India

3 Principal, A-One Pharmacy College Anasan Dascroi Ahmedabad Gujarat India

ABSTRACT:

Development And Validation Of Stability Indicating RP-HPLC Method For Estimation Duloxetine In Tablet Dosage Form Chromatography was performed on a C-18, 150 X 4.6 mm, 5 m (Dionex), column with mobile phase containing Water : Methanol : Acetonitrile: TEA (40:40:24:1.8) with Triethylamine (0.1%). The flow rate was 1 ml/min and the eluent was monitored at 230 nm. The selected chromatographic conditions were found effectively to seperate Duloxetine at 4.38 min. Linearity for Duloxetine was found in the range of 5-40 µg/ml.

KEYWORDS: RP-HPLC, Duloxetine, Stability Indicating, Methanol,ACN, Triethylamine.

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For Correspondence:

Jinkal R. Daraji

Student of M.Pharm, Departement of Quality Assurance A-One Pharmacy College Anasan Dascroi Ahmedabad Gujarat India.

(www.jpsbr.org)

INTRODUCTION:

Pharmacological action of Duloxetine HCl [1]

Duloxetine is a potent inhibitor of serotonin (5-hydroxytryptamine, 5-HT) and noradrenaline (NE) uptake in vitro and in vivo and is 3- to 5-times more effective at inhibiting 5-HT uptake. Duloxetine is a weak inhibitor of dopamine (DA) uptake and the binding of radioligands to neurotransmitter receptors. Upon administration of Duloxetine in vivo, the inhibitory effects on uptake of 5-HT and NE persist for up to 8 hr. Desmethylduloxetine, a potential metabolite, is also an inhibitor of 5-HT and NE uptake. Consistent with the ability to inhibit the uptake of 5-HT, Duloxetine blocks p-chloroamphetamine induced depletion of mouse and rat brain 5-HT. Duloxetine also blocks the 6-hydroxydopamine induced depletion of mouse heart NE and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced depletion of NE in frontal cortex but does not block the MPTP induced depletion of DA in rat striatum. Electrophysiological studies show that Duloxetine decreases the activity of 5-HT neurones in dorsal raphe and at 5-times higher dose also decreases the activity of NE neurones in the locus coeruleus. [2-5]

MATERIALS AND METHODS:

Instrumentation:

High performance liquid chromatography including a thermo scientific product, UV detector, and C-18, 150 X 4.6 mm, 5 m (Dionex)

Materials and reagents: Duloxetine was obtained as a gift sample from Reliance pharmaceutical

Chromatographic condition:

A mobile phase consisted of Water : Methanol : Acetonitrile: TEA (40:40:24 : 1.8: adjusted pH 3 with opa) was pumped at a flow rate of 1 mL/min. The elution was monitored at 238 nm and the injection volume was 20 µL. The validation of the method was done following the ICH guidelines.[5-6]

1) Preparation of Mobile Phase

A mixture containing 40 volumes of water ,40 volumes of methanol and 24 volumes of ACN, at $_{P}$H-3 adjusted with 10% Ortho phosphoric acid was prepared and sonicated for 10 minutes. The mobile phase was filtered through 0.45 µm Cellulose acetate filter and was sonicated for 15 min before use.[7]

2) Preparation of Standard stock solution

2.5 mg of duloxetine was transferred in 25 ml volumetric flask, dissolved and diluted up to the mark with methanol HPLC grade, to get 100µg/ml of Duloxetine From this standard stock solution, different aliquots were transferred into 10 ml volumetric flask and volume was made up to the mark with methanol. This solution was used as a working standard solution.[8]

3) Preparation of Sample solution

An accurately weighed tablet powder equivalent to 2.5 mg of duloxetine was transferred in to 25ml volumetric flask. To this 20 ml of methanol was added and sonicated for 15 min. Volume was made up to the mark with methanol then solution was filtered through whatman filter paper no.41. Then the sample solution was filtered through 0.45 µm cellulose acetate filter paper (0.45 µm) before diluting further. From this standard stock solution, different aliquots were transferred into 10 ml volumetric flask and volume was made up to the mark with methanol.

STABILITY STUDY (Force Degradation)[9-10]

1. Preparation of hydrochloric acid solution (0.1 N):

Concentrated hydrochloric acid (0.85 ml) was transferred in 100 ml volumetric flask and diluted up to the mark with water.

2. Preparation of sodium hydroxide solution (0.1 N):

Accurately weighed 0.4 gm of sodium hydroxide was transferred in 100 ml volumetric flask and diluted up to mark with water.

Table 1 RP-HPLC optimized chromatographic conditions

Column	**C-18, 150 X 4.6 mm, 5 µ (Dionex)**
Mobile Phase	Water : Methanol : Acetonitrile: TEA (40:40:24:1.8 Adjusted pH 3 with OPA)
Flow rate	1.0 mL/min
Injection volume	20 µl
Detection λ	230 nm
Column temp.	25 °C
Sample temp.	25 °C
Run time	06 min.

3. Preparation of hydrogen peroxide solution (3% w/v):

Hydrogen peroxide (10 ml, 30%) was transferred in 100 ml volumetric flask and diluted up to the mark with water.

4. Acid hydrolysis:

Accurately measured 1 ml of Duloxetine (100 µg/ml) and 1 ml 0.1N HCl was transferred in to 10 ml volumetric flask solution was heated for 2 hours at 80^{o}C for acid hydrolysis. neutralize with 0.1N NaOH Make up the volume with methanol up to mark to get 10 µg/ml . Filtered through 0.45 µm membrane filter paper and injected in to HPLC system.

5. Base hydrolysis:

Accurately measured 1 ml of Duloxetine (100 µg/ml) and 1 ml 0.1N NaOH was transferred in to 10 ml volumetric flask, solution was heated for 2 hours at 80^{o}C for base hydrolysis. .neutralize with 0.1N HCl Make up the volume with methanol up to mark to get 10 µg/ml and Filtered through 0.45 µm membrane filter paper and injected in to HPLC system.

6. Oxidative hydrolysis:

Accurately measured 1 ml of Duloxetine (100 µg/ml) and 1 ml of 3% H_2O_2 was transferred in to 10 ml volumetric flask solution was heated for 2 hours at 80^{o}C for oxidative hydrolysis. Than up the volume with methanol up to mark to get 10 µg/ml Filtered through0.45 µm membrane filter paper and injected into HPLC system.

7. Thermal Degradation:

For dry heat degradation study, Tablet powder equivalent to 5 mg Duloxetine was spread over petri dish and exposed to dry heat (80˚C) for 12 hour in an oven then from that powder was transferred to 50 ml volumetric flask and dissolved in 30 ml of mobile phase. The flask was sonicated for 5 min and volume was made up to mark with mobile phase to get 100 µg/ml of Duloxetine. Further dilution was carried out by diluting 1 ml of solution in 10 ml volumetric flask with mobile phase to get 10 µg/ml of Duloxetine.

Calibration curve of standard of Duloxetine

A calibration curve was plotted over a concentration range of 5-40 µg/ml for Duloxetine. Accurately measured working standard stock solution of Duloxetine (0.5,1, 1.5, 2, 3, and 4 ml) were transferred to a series of 10 ml volumetric flasks and the volume in each flask made up to the mark with methanol to get concentration range of 5-40 µg/ml for Duloxetine. The resulting solution was injected into the column and the peak area obtained at 4.38 minute at flow rate 1 ml/min were measured at 230 nm for Duloxetine. Calibration curve was constructed by plotting peak area versus concentration at 230 nm.

Method validation for duloxetine

A. Linearity

The linear response of Duloxetine was determined by analyzing six independent levels of the calibration curve in the range of 5-40 µg/ml for Duloxetine

B. Precision volumetric flask and dissolved in 30 ml of mobile phase. The flask was sonicated for 5 min and volume was made up to mark with mobile phase to get 100 µg/ml of Duloxetine. Further dilution was carried out by diluting 1 ml of solution in 10 ml volumetric flask with mobile phase to get 10µg/ml of Duloxetine.

Calibration curve of standard of Duloxetine

A calibration curve was plotted over a concentration range of 5-40 µg/ml for Duloxetine. Accurately measured working standard stock solution of Duloxetine (0.5,1, 1.5, 2, 3, and 4 ml)volumetric flasks and the volume in each flask made up to the mark with methanol to get concentration range of 5-40 µg/ml for Duloxetine. The resulting solution was injected into the column and the peak area obtained at 2.3 minute at flow rate 1 ml/min were measured at 230 nm for Duloxetine. Calibration curve was constructed by plotting peak area versus concentration at 230 nm.

Method validation for DULOXETINE

A. Linearity

The linear response of Duloxetine was determined by analyzing six independent levels of the calibration curve in the range of 5-40 µg/ml for Duloxetine

It provides an indication of random error. The precision of an analytical method is usually expressed as the standard deviation, relative standard deviation or coefficient of variance of a series of measurements.

1) Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Repeatability of method was performed by preparing the test solution of Duloxetine for six times from tablet dosage form and analyzed as per the proposed method. Percentage relative standard deviation (RSD) should be less than 2% (Table 5.4).

2) Intermediate Precision

It expresses within laboratory variations as on different days analysis or equipment within the laboratory

a. Intra-day precision

Variation of results within same day is called Intra-day precision. The Intra-day precision for HPLC method was

determined for three concentration of Duloxetine solution for the three times on the same day

b. Inter-day precision

Variation of results amongst days called Inter-day precision. The Inter-day precision for HPLC method was determined for Duloxetine for three days

D. Limit of Detection

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantify under the stated experimental conditions.

Limit of detection can be calculated using following equation as per ICH guidelines

Where , = Standard deviation of the y intercept S = Slope of the calibration curve

E. Limit of Quantification

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guideline.

Where , = Standard deviation of the y intercept S = Slope of the calibration curve

F. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Robustness of the method was studied by changing the flow rate; change in wavelength, change in pH, and composition of mobile phase and change in response of drugs were studied.

G. System Suitability

System suitability parameter is established to ensure that the validity of the analytical method is maintained whenever used.

The parameters used in these were asymmetry of chromatographic peak, Theoretical plates, resolution, retention time and repeatability as RSD of peak area for replicate injections.

RESULT AND DISCUSSIONS:

Several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was obtained by using the mobile phase containing Water : Methanol : Acetonitrile: TEA (40:40:24 : 1.8: Adjusted pH 3 with OPA). Quantification was achieved with UV detection at 230 nm based on peak area.

Table 2 RP-HPLC optimized chromatographic conditions

Column	**C-18, 150 X 4.6 mm, 5 µ (Dionex)**
Mobile Phase	Water : Methanol : Acetonitrile: TEA (40:40:24 : 1.8: Adjusted pH 3 with OPA)
Flow rate	1.0 mL/min
Injection volume	20 µl
Detection λ	230 nm
Column temp.	25 °C
Sample temp.	25 °C
Run time	06 min.

LINEARITY

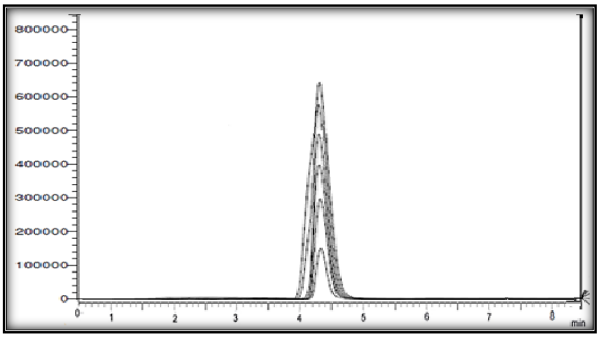

Figure 1 **Calibration curve of Duloxetine 5-40 µg/ml**

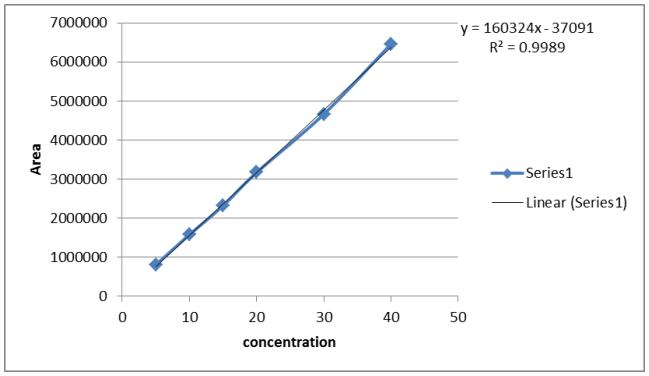

Figure 2 Calibration graph of Duloxetine

Table 3 Result of Calibration reading of Duloxetine

Sr. No.	Conc. (µg/ml)	Area (Mean ± S.D, n=2)	%RSD
1	5	1583290±10344	0.619816
2	10	1583290±10344	0.653323
3	15	2322715±14831	0.63852
4	20	3172972.5±25899	0.816222
5	30	4666050±16295	0.349225
6	40	6460852.5±33504	0.518562

5.4.2 PRECISION

Repeatability, Relative standard deviation of all the parameters is less than 2%

Table 4 Result of Repeatability

Sr. No.	Concentration(µg/ml)	Area	% Amount found
1	10	1559294	99.5727
2	10	1567421	100.0796
3	10	1549542	98.96443
4	10	1533870	97.9869
5	10	1567146	100.0625
6	10	1513486	96.71547
	MEAN	1548460	98.89693
	S.DEV	21275.72	
	%RSD	1.373992	

Intermediate Precision

The low % RSD values of intraday and interday precision reveals that the proposed method is precise

Table 5 Intraday Precision data for RP-HPLC method

Drug	Conc. (µg/ml)	Area (n = 3) 0 hr	2 hr	4 hr	% Amount found	SD	% RSD
Duloxetine	5	801234	802432	806398	104.844	2206.84	0.2747
	10	1583470	1586628	1588749	101.256	2168.96	0.13673
	15	2365434	2311765	2322715	98.5672	2315 4.5	0.99235

Table 6 Interday Precision data for RP-HPLC method

Drugs	Conc. (µg/ml)	Area (n = 3) DAY 1	DAY 2	DAY 3	% Amount found	SD	% RSD
Duloxetine	5	809271	792531	800285	104.512	6840.24	0.85429
	10	1568541	1555628	1576027	100.037	8425.52	0.53778
	15	2345646	2361087	2335467	99.1533	10532.6	0.44869

LOD and LOQ

Based on calibration curve LOD and LOQ was calculated for

Table 7 LOD and LOQ for Duloxetine

DRUG	LOD(µg/ml)	LOQ(µg/ml)
Duloxetine	1.24657	3.77748

ACCURACY

Table 8 Result of Accuracy

% Amt Estimate	Conc. of Std. Solution used (µg/mL)	Conc. Of Sample Solution added (µg/mL)	Total Amt	Amount Recovered (µg/mL)	% Recovery ± SD	%RSD
50	10	5	15	14.7234	98.16±0.2372	0.2417
100	10	10	20	19.7875	98.93±1.4436	1.4591
150	10	15	25	24.9153	99.66±0.6446	0.6468

System Suitability Parameters

Statistical analysis of parameters required for system suitability testing of the HPLC method

Table 9 System Suitability Parameters for RP-HPLC method

System Suitability Parameters	Duloxetine
Tailing Factor	1.39
Theoretical Plates	27325
Retention Time (min.)	4.38
Area ± SD	1583290±10344
% RSD	0.653323

Robustness

Table 10 Robustness parameter for RP-HPLC method

Sr	Parameter	Normal Condition	Condition 1	Condition 2
1	Mobile Phase Composition	40:40:24	39:39:23	41:41:25
	Area± SD (n=3)	1574374 ±4985.11	1572870±92 50.33	1571652±8 932.26
	%RSD	0.316641	0.588119	0.568336
2	Flow Rate	1 ml/min.	0.8 ml/min.	1.2 ml/min.
	Area ± SD (n=3)	1572660 ±5877.35	1585852±10 863.22	1563906±1 4595.9
	%RSD	0.373721	0.685008	0.933297
3	Wavelength	238	236	240
	Area ± SD (n=3)	1572070 ±5761.17	1581600±13 548.52	1578179±9 319.51
	%RSD	0.366471	0.856634	0.590523
4	pH	3	2.8	3.2
	Area ± SD (n=3)	1572424 ±4125.50	1585167±10 185.32	1578454±5 850.45
	%RSD	0.262366	0.642539	0.370645

Application of Proposed Method for analysis of Marketed formulation

The proposed method was applied successfully for analysis of marketed formulation and results obtained are shown in following table.

Table 11 Analysis of marketed formulation by RP-HPLC method

Parameter	Duloxetine
Linearity	5-40(µg/ml)
Precision(% RSD, NMT 2)	
Repetability	1.37
Intermediate pricision	0.13
Accuracy (% recovery)	98.94
LOD	1.246
LOQ	3.777
Assay ±%RSD	101.174±0.6217

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