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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF DULOXETINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A fast, precise, and simple Reverse Phase High-Performance Liquid Chromatographic method (RP-HPLC) has been developed for the determination of Duloxetine Hydrochloride in the pharmaceutical dosage form. The chromatographic separation was done using Eclipse Plus C_{18} column (250 mm X 4.6 mm, 5 µm particle size) as a stationary phase with a mobile phase comprising of Phosphate Buffer:Acetonitrile: Methanol (50:30:20 v/v/v) adjust pH to 4.9 with dilute acetic acid at a flow rate of 1.0 mL/min., column temperature of 25 ± 1°C and UV detection at 231nm. The retention time of Duloxetine Hydrochloride was 11.03 minutes. The linearity was found to be in the range of 12-60 µg/mL with a correlation coefficient of 0.9997. The stated method was validated as per ICH Q2 (R1) guidelines and can be effectively applied for the determination of Duloxetine Hydrochloride in marketed formulations.

Keywords: Duloxetine Hydrochloride, RP-HPLC, Pharmaceutical Formulation, Validation

INTRODUCTION

The chemical name for Duloxetine Hydrochloride (3S)-N-methyl-3-(napthalen-1-yloxy)-3-(thiophen-2-yl) propan-1-amine hydrochloride. It has a molecular formula of

C18H19NOS•HCl, and a molecular weight of 333.88 g/mol. Duloxetine belongs to the drug class of anti-depressants. It is a selective serotonin inhibitor and norepinephrine reuptake inhibitor and hence it is used to treat depression. The chemical structure of Duloxetine Hydrochloride is shown in **Figure 1**. Literature survey revealed that few analytical methods have been reported for the estimation of Duloxetine Hydrochloride individually or in combination with other drugs. The reported methods are HPLC **[1-7]**, LC-MS **[8-9]**, HPTLC **[10, 11]** methods were reported.The present study aimed to develop a simple, sensitive, rapid, precise and accurate, and validated RP-HPLC method for the estimation of Duloxetine HCl in Pharmaceutical dosage form. The developed method was validated according to ICH guidelines by using high-performance liquid chromatography **[12-16]**.

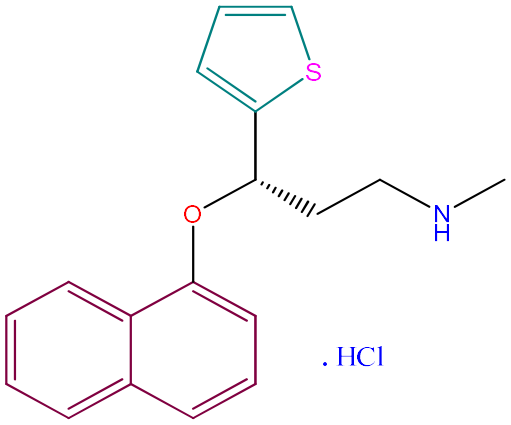

Figure 1: Chemical Structure of Duloxetine Hydrochloride

MATERIALS AND METHODS

Chemicals and Reagents:

The Sample of Duloxetine HCl was obtained as a gift sample from Hetero Labs Ltd., Hyderabad, India. The branded capsule (Duzac 40) containing 40 mg was procured from local pharmacy. Acetonitrile, Methanol, Ammonium Acetate, TEA, and glacial acetic acid used were of HPLC and analytical reagent grade, purchased from Merck Specialities Private Limited, Mumbai, India. Triple distilled water was used to carry out the analysis.

Instrumentation:

Chromatographic separation was performed on an Agilent HPLC quaternary-1260 Infinite – II series, Eclipse plus C_{18} column of 1260 series having diode array detector was used for higher data quality for more confidence. ELICO SL-210 UV Double beam spectrophotometer, having 1 cm matched quartz cells, was used for all spectral and absorbance measurements. Ultra-Sonicator of Spectral labs, model UCB 40, was used to sonicating the mobile phase. All the chemicals and drugs were weighed by using Essae vibra AJ analytical balance (0.001g) and pH of the mobile phase was adjusted by Systronics model – 802 pH meter. The data that was acquired, was processed by utilizing EZ chrome elite software.

Method development and optimization of chromatographic conditions

To optimize the HPLC conditions, initially various mobile phases, stationary phases, flow rates and pH of buffers were tested. Finally, a mobile phase consisting of a mixture of phosphate buffer (adjusted to pH 4.9), acetonitrile and methanol in the ratio of 50:30:20 v/v/v and a stationary phase made up of the Eclipse plus C_{18} column (of 4.6 mm i.d. X 250 mm, 5µm particle size) column were found to be the most appropriate for analysis of Duloxetine HCl. The mobile phase flow rate and detection wavelength were adjusted to 1.0 min/mL and 230 nm, respectively at ambient column temperature.

Selection of detection wavelength

To estimate the maximum λ_{max}, Duloxetine Hydrochloride 10 µg/mL of working standard solution was prepared and scanned in a UV wavelength range of 200 - 400 nm utilizing as water a blank. The drug showed maximum absorbance at 231 nm.

Preparation of buffer and mobile phase:

A buffer solution was prepared from 4.5023 g ammonium acetate and 1.3 mL of TEA in 1200 mL water; the pH was adjusted to 5.0 with glacial acetic acid. Then, the mobile phase was prepared by mixing buffer (pH adjusted to 5.0 with dilute acetic acid), and acetonitrile and methanol in the ratio of 50:30:20 v/v/v. The solution was then passed through 0.45µm nylon filter and sonicated for 15 min. The prepared mobile phase was used as the diluent.

Preparation of Stock and Working standard solution:

About 60mg of Duloxetine Hydrochloride was weighed accurately and transferred into a 100 mL volumetric flask and to it 60 mL of Methanol was added. It was sonicated for 15 min. and made upto the mark with methanol. From the above solution, 1.0 ml was transferred into 10 mL volumetric flask and made up to the mark with diluents. The solution was filtered through 0.45 µm nylon filter paper.

Preparation of sample solution:

For the assay of pharmaceutical formulation, 20 capsules of Duloxetine Hydrochloride marketed formulation (Duzac 40 mg) were weighed; the average weight was calculated, and a quantity of capsular powder equivalent to 60 mg of Duloxetine Hydrochloride was accurately weighed and transferred into a 100 mL volumetric flask containing 60 mL of methanol. The solution was ultrasonicated for about 15 minutes with intermittent shaking, filtered through a Whattman filter paper 0.45 µm nylon filter, and the filtrate was made up to volume with mobile phase. The concentration was 10 mg/mL. Transfer

1 mL of the filtered sample solution to 100 mL volumetric flask and made up to volume with mobile phase to get a solution of 100 µg/mL. The solution is further diluted with mobile phase to obtained required concentrations. This is used as working solution for the preparation of the assay. Then 1 mL of this solution is transferred into a 10 ml volumetric flask and made up to volume to obtain 10 µg/mL which is used for the assay. The assay results are presented in **Table 1**.

Method development optimization:

The optimized HPLC conditions of several mobile phases with various compositions were tested to develop optimized chromatographic conditions like tailing factor, shape of the peak, and the number of theoretical plates. Optimized chromatographic conditions, system suitability parameters for estimation of Duloxetine Hydrochloride by proposed gradient RP-HPLC method are depicted in **Table 1**.

Table 1: Optimized chromatographic conditions for the proposed HPLC method

Parameter	Chromatographic conditions
Instrument	Agilent SPD 20A prominence UV- Vis detector LC-20AT prominence liquid chromatograph,1260 Quat Pump VL,1260 Diode Array Detector
Column	Thermo scientific model C18 column (4.6 mm i.d. X 250 mm, 5 µm particle size) (based on 99.999% ultra high purity silica).
Detector	1260 Diode Array Detector.
Mobile phase	Phosphate Buffer:Acetonitrile:Methanol (50:30:20 v/v)
Flow rate	1.0 mL/minute
Detection wavelength	UV at 231 nm
Run time	20 minutes
Temperature	Ambient Temperature (25±1 °C)
The volume of injection loop	20 µL
Retention time (R_t)	11.03 min.
Theoretical plates [th.pl]	10375
Tailing factor (asymmetry)	1.26

METHOD VALIDATION:

1. **System suitability:**

System suitability parameters are defined as the tests that are used to ensure that a method can generate results of acceptable precision and accuracy. The results below show system suitability parameters in **Table 2**.

2. **Precision**

Precision of the method was determined by elavuating intra-day and inter-day precisions and the results were expressed in terms of % relative standard deviation (%RSD). The results for intra-day and inter-day precision are shown in **Table 3**.

3. **Linearity**

The linearity of Duloxetine Hydrochloride was determined in the concentration range of

12 to 60 µg/mL. 7 serially diluted solutions containing 0 as blank, 12, 24, 36, 48, 54, 60 µg/mL of Duloxetine were injected in triplicates, separately into the optimized chromatograph system and chromatogram was recorded. The calibration curve of Duloxetine Hydrochloride is shown in **Figure 2a**. The linearity data is presented in **Table 4a**, and linear regression data is shown in **Table 4b**, and ANOVA studies are shown in **Figure 2b**.

4. Accuracy (Recovery studies)

A known amount of the drug, 10 µg/mL, was mixed with placebo at three different levels, 50%, 100%, 150%, in triplicate preparations. The samples were then assayed as per the proposed standard method. The accuracy studies are mentioned in **Table 5**.

5. Robustness

The robustness of the RP-HPLC method for Duloxetine Hydrochloride was determined for system suitability and assay value under different conditions. The robustness of this analytical method was confirmed by establishing its reliability against deliberate changes in the chromatographic conditions. The robustness of the RP-HPLC method of Duloxetine Hydrochloride is mentioned in **Table 6**.

6. Ruggedness

The ruggedness of the RP-HPLC method for Duloxetine Hydrochloride was determined under different conditions. The ruggedness of this method was determined by varying the normal test conditions. The ruggedness of the RP-HPLC method of Duloxetine Hydrochloride is mentioned in **Table 7**.

7. LOD and LOQ

Limit of Detection is the lowest concentration of the analyte in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The Limit of Quantitation is the lowest concentration of the analyte in a sample that can be determined with acceptable precision and accuracy. The LOD and LOQ are shown in **Table 8**.

8. Analysis Duloxetine Hydrochloride in tablet formulation

The developed and validated RP-HPLC method was successfully applied for the determination of Duloxetine Hydrochloride in their tablet dosage form. The assay result in **Table 9** shows that the amount of the drug was in great agreement with the labelled value of its formulation. The representative sample chromatogram of Duloxetine Hydrochloride is shown in **Figure 4**.

Table 2: System suitability parameters

System Suitability parameters	Limits	Duloxetine Hydrochloride
Tailing factor (T)	≤ 2.0	1.26
Number of theoretical plates	NLT 2000	10375
Retention time*	-	11.03 minutes
% RSD	NMT 2.0	0.0281

*** Average of five determinations, SD = Standard deviation, RSD = relative standard deviation.**
The results are well within the acceptance criteria, and the study concludes the suitability of the analytical system for the analysis

Table 3: Intra-day and Inter-day Precision data of Duloxetine Hydrochloride by HPLC

Initial amount taken (µg/ml)	Intra-day		Inter-day	
	Mean*	%RSD	Mean*	%RSD
50	50.17	0.653	50.21	0.815
60	60.38	0.852	60.14	0.654
70	70.08	0.137	70.43	0.734

***Results of Triplicate samples**

Table 4a: Linearity data of Duloxetine Hydrochloride by HPLC

S. No.	Concentration µg/mL	Retention time (R_t) minutes	Mean peak area (n=3)
1	0	0	0
2	12	11.03	1595
3	24	11.03	3191
4	36	11.03	4789
5	48	11.03	6220
6	54	11.03	6987
7	60	11.03	7808

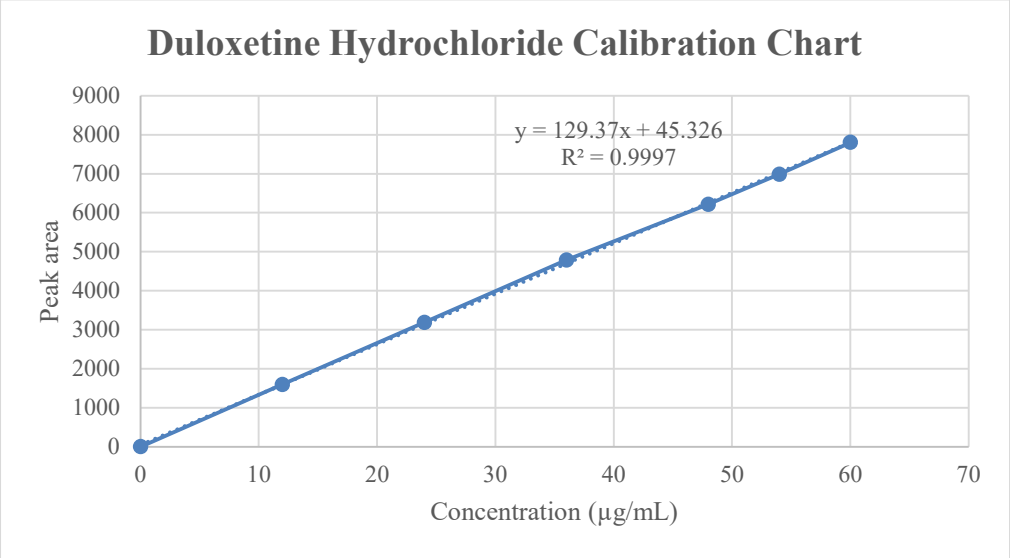

Figure 2a: Calibration graph of Duloxetine Hydrochloride by RP- HPLC

Table 4b: Regression analysis of the linearity of Duloxetine Hydrocholride

S. No.	Parameters	Duloxetine Hydrochloride
1	Calibration (µg/mL)	12.0 – 60.0
2	Regression equation	y=129.37x + 45.326
3	Slope (m)	129.37
4	Intercept (c)	45.326
5	Correlation Coffecient (r^2)	0.9997

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.999857509
R Square	0.999715038
Adjusted R Square	0.999658045
Standard Error	53.62374608
Observations	7

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	50439782.47	50439782.47	17541.18404	4.6547E-10
Residual	5	14377.53072	2875.506143		
Total	6	50454160			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	45.32593857	38.43187646	1.179383958	0.29130109	-53.46634497	144.1182221	-53.46634497	144.1182221
X Variable 1	129.3705916	0.976801036	132.4431351	4.6547E-10	126.8596446	131.8815386	126.8596446	131.8815386

Figure 2b: The summary output of ANOVA study of Duloxetine Hydrochloride

Table 5: Accuracy study of Duloxetine Hydrochloride

S No.	Concentration % of spiked level	Amount taken (µg/mL)	Amount Found conc. (µg/mL)	% recovery*	% RSD
1	50 %	31.20+10=41.20	40.84	98.81344	1.02
2	100 %	60.40+10=70.40	70.73	100.6541	1.04
3	150 %	89.70+10=99.70	98.72	99.9505	0.69

Table 6: Robustness results of Duloxetine Hydrochloride.

S. No	Parameter	Used	% Assay (n=3)	Retention time (t_R), min	Plate count[$]	Tailing Factor
1.	Change in flow rate (±0.1 mL/minute)	1.1 mL/min	100.18	10.97	8880	1.2
		0.9 mL/min	99.10	10.83	8954	1.11
2.	Mobile phase in v/v/v (Buffer:ACN:MeOH)	50:30:20	99.12	11.09	8480	1.10
		50:20:30	100.23	10.72	8932	1.21
3.	Buffer pH (±0.1)	5.1	101.01	10.98	8950	1.15
		4.9	98.18	11.12	8898	1.01

Acceptance criteria (Limits): [#]Peak Asymmetry < 1.5, [$] Plate count > 2000, * Significant change in Retention time

Table 7: Ruggedness results of Duloxetine Hydrochloride.

S. No.	Variables	% Assay (n=3)
1.	Analyst-I	99.85
2.	Analyst-II	99.84
3.	Day-I	99.79
4.	Day-II	99.83
Mean ± SD		99.055 ± 0.59
RSD (%)		10.596

Table 8: LOD and LOQ results of Duloxetine Hydrochlorideby HPLC

Limit of Detection (LOD)	0.4320µg/ml
Limit of Quantitation (LOQ)	1.1120µg/ml

Table 9: Assay results of Duloxetine Hydrochloride by HPLC

S. No.	Formulation	Labelled claim	Mean% recovery ± SD	% Recovery	% RSD*
1	Duzac tablets	40mg capsules	93.79 ± 0.673	99.87	0.041

*** Average of six determinations, SD denotes standard deviation; RSD denotes % relative standard deviation**

RESULTS AND DISCUSSION

Since the above mentioned Duloxetine Hydrochloride is relatively polar, an RP-HPLC method was used. The column for the separation was a Inertosil column that has an internal diameter of 4.5mm, length of 250 mm and 5µm particle size. A multiple number of trials were performed using various buffer solutions with various compositions of methanol, ethanol, acetonitrile, and HPLC grade water with variable flow rates. Eventually, optimum separation was obtained with a mixture of Phosphate Buffer:Acetonitrile:Methanol (50:30:20 v/v/v). The mobile phase flow rate was adjusted at 1mL/min, and the detection wavelength was set at 231 nm. Thus, a proper chromatographic peak was obtained with excellent symmetry and least peak tailing. The chromatogram of standard concentration was shown in **Figure 3**.

System suitability study was conducted according to the methodology. System suitability solution, and six replicate of standard preparation were injected into HPLC. The tailing factor was found to be 1.26. The number of theoretical plates was 10375. The retention time was found out to be 11.03 minutes, and the % RSD was calculated to 0.0281. The results were well within the acceptance norms, and the study concludes the suitability of the analytical system for analysis. The system suitability parameters were expressed in **Table 2**. The precision of the method was examined by using Intra-day and Inter-day precisions. Various levels of concentration were taken in six replicate samples. The %RSD was found to be 0.852 and 0.654. The precision was mentioned in **Table 3**. The results are well within the acceptance criteria, and the %RSD observed for the replicate injections indicates the precision of the HPLC used, assay values indicates the precision of the method. The linearity of Duloxetine Hydrochloride was determined in the concentrations of 12 – 60 µg/mL. The squared correlation coefficient value was found to be 0.9997, which is well within the limit. The results of the linearity studies were mentioned in **Table 4**. To determine the accuracy of the Duloxetine Hydrochloride, the drug was spiked with placebo at three different levels in triplicate preparations. The results of accuracy are mentioned in the **Table 5**. The mean % recovery at each level was found out to be within limits i.e., 98.0 % to 102.0 %.The robustness of the HPLC was determined for the suitability and assay value under multiple variable conditions like Flow rate change, Buffer pH change, and change in mobile phase composition. The results are

mentioned in **Table 6**. The ruggedness of thr HPLC was determined for the suitability and assay value under various normal test conditions during different days and different analysts. The data is mentioned in **Table 7**. The Limit of Detection (LOD) and Limit of Quantitation (LOQ) of Duloxetine Hydrochloride were found out to be 0.4320 µg/mL and 1.1120 µg/mL, respectively. The results are mentioned in **Table 8**. The % assay of the Duloxetine Hydrochloride was found to be 93.79 ± 0.673, which was in good agreement with labelled claim. The method was specific and has no interference observed when the Duloxetine Hydrochloride were determined in presence of excipients. The results are mentioned in **Table 9**. The chromatogram of the Duloxetine marketed formulation is seen in **Figure 4**.

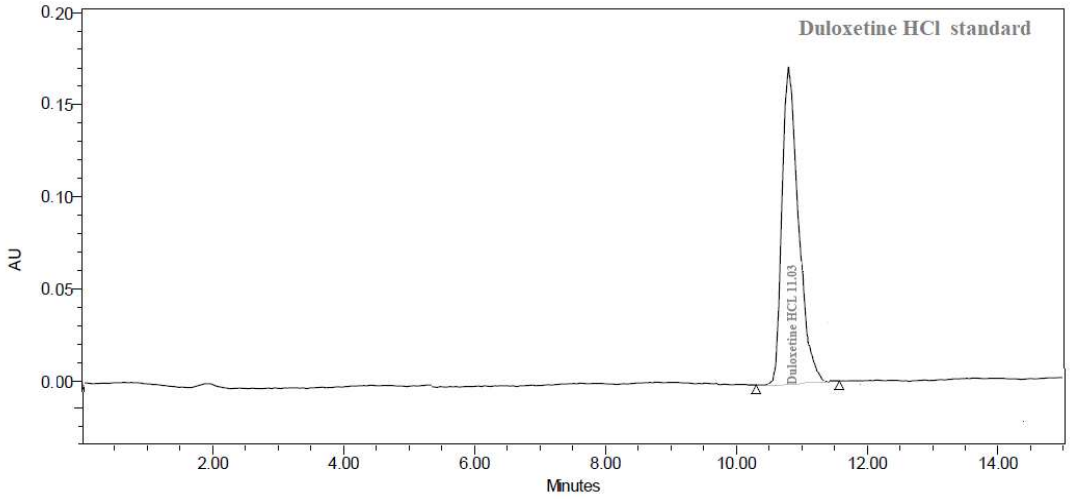

Figure 3: Standard chromatogram of Duloxetine Hydrochloride (10 µg/mL)

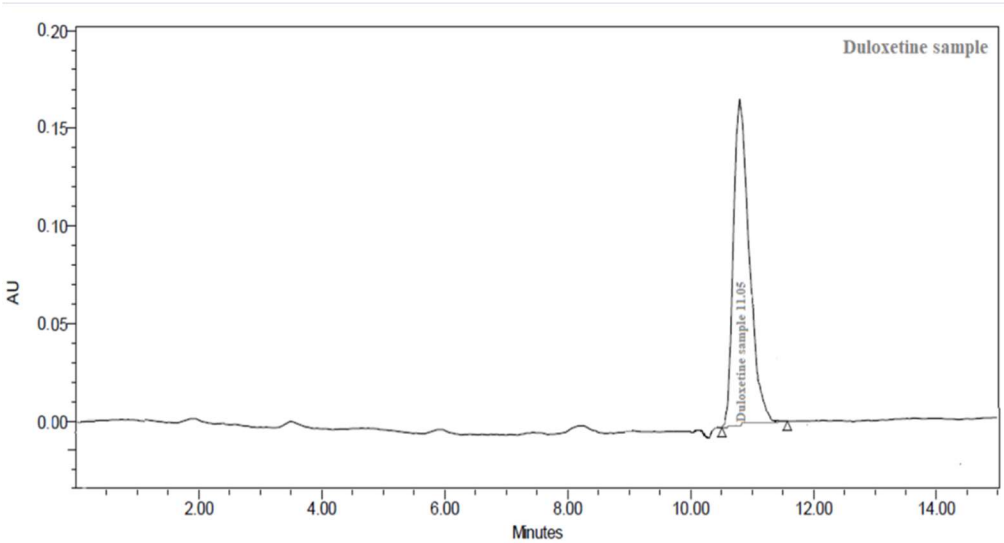

Figure 4: Sample chromatogram of Duloxetine Hydrochloride (Duzac)

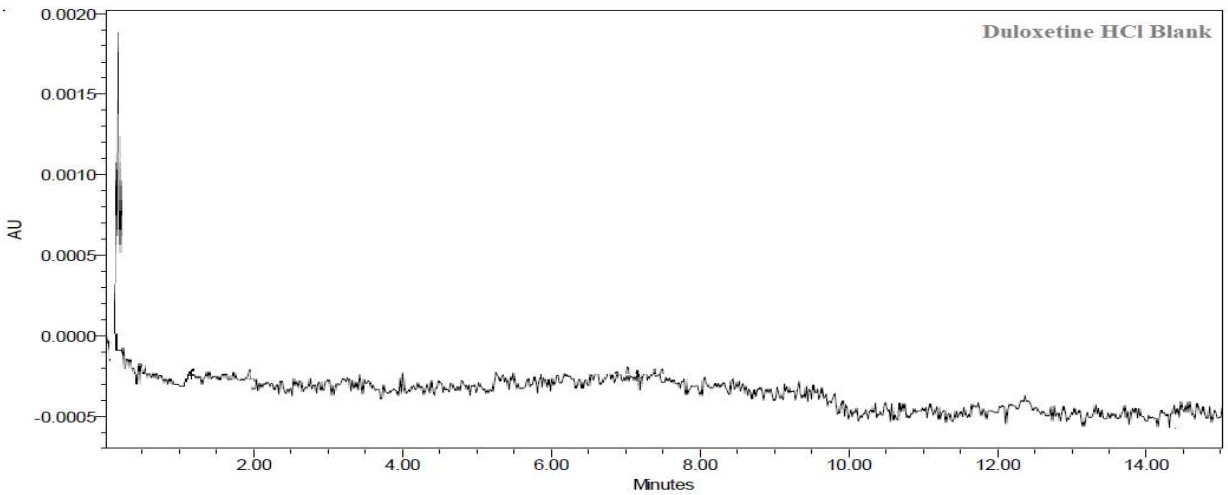

Figure 5: Chromatograph of the blank solution

CONCLUSION

The present study explained the validated Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the estimation of Duloxetine Hydrochloride which is available as tablet dosage form.The scope of this work is to build up the linearity and optimization of the chromatographic conditions, to develop the RP-HPLC method for the estimation of drug in tablet dosage form. The method was completely validated and showed satisfactory results. This RP-HPLC method was free from interference of other active ingredients and additives used in the formulation. The RP-HPLC method for the estimation of Duloxetine Hydrochloride has various advantages like low solvent consumption; less retention time; good peak symmetry; being accurate, precise and robust. The results of this study indicate that the developed method was found to be simple, sensitive, accurate, precise, linear, economical, and reproducible, having a short run time, making the method rapid. Thus, it can be concluded that this method can be employed for the routine quality control analysis of Duloxetine Hydrochloride in active pharmaceutical preparations.

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