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Der Pharma Chemica, 2012, 4(4):1735-1741  
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ISSN 0975-413X  
CODEN (USA): PCHHAX

## Stability Indicating Nature of RP-HPLC method for Determination of Impurity profile and Degradation impurities in Duloxetine Hydrochloride

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### ABSTRACT

The objective of the present work was to develop stability indicating RP-HPLC method for Duloxetine Hydrochloride in the presence of its degradation products generated from forced degradation studies. Duloxetine Hydrochloride was subjected to the stress conditions and it is sensitive towards Acid and Oxidative degradation. Duloxetine Hydrochloride was found to be stable to Basic, Photolytic, Thermal and Humidity conditions attempted. Successful separation of Duloxetine Hydrochloride from degradation impurities under stress conditions was achieved on YMC Pack C8, 250 X 4.6 mm, 5 $\mu$ m column using a gradient mixture of solvent A (0.01 M of Sodium Di hydrogen Orthophosphate and 1.0g of 1-Heptane Sulfonic Acid Sodium Salt transfer in 1000mL of water, adjust the pH of the above solution to 3.0 + 0.1 using Orthophosphoric acid) and solvent B (Acetonitrile). The flow rate is 1.0ml/min and the detection wavelength is 217nm. Statistical analysis proved the method to be repeatable, specific and accurate for estimation of Duloxetine Hydrochloride and its degradation impurities. It can be used as a stability indicating method due to its effective separation of the drug from its degradation impurities.

**Keywords:** RP-HPLC, Duloxetine Hydrochloride, Degradants, Stress conditions, Validation.

### INTRODUCTION

The international conference on harmonization (ICH) guide-lines [1-3] emphasizes that the purity and assay of drug susceptible to change during storage, must be determined by using validated stability- indicating methods, which can selectively determine the drug in presence of its process and degradation impurities. Duloxetine Hydrochloride chemically. Known as (+)-(S)-N-methyl- $\gamma$ -(1-naphthoxy)-2-thiophenepropylamine hydrochloride [4], has an empirical formula of C<sub>18</sub>H<sub>19</sub>NOS.HCl and a molecular weight of 333.38 g/mol. It is a potent inhibitor of serotonin and norepinephrine reuptake and thus it is used for major depressive disorders [5-7]. Furthermore, it provides evidences of an effect on pain in the case of urinary incontinence [8, 9] independent of its effect on depression [10]. Therefore, Duloxetine Hydrochloride is an alternative to current therapeutic options in the treatment of the different symptoms of depression. Besides the reported impurities [11-20], we observed Amino alcohol chemically known as 3-Methylamino-1-thiophen-2-yl-propan-1-ol, Para-Isomer chemically known as 4-(3-Methylamino-1-thiophen-2-yl-propyl)-naphthalen-1-ol, Ortho-Isomer chemically known as 2-(3-Methylamino-1-thiophen-2-yl-propyl)-naphthalen-1-ol, Ring-Isomer chemically known as 2-[2-(3-Methylamino-propyl)-thiophen-3-yl]-naphthalen-1-ol,  $\alpha$ -Naphthol chemically known as 1-Naphthol and Ester chemically known as Methyl-[3-(naphthalene-1-yloxy)-3-thiophen-2-yl-propyl]-carbamic acid phenyl ester. Hence, a stability-indicating RP-HPLC method for determination

of Duloxetine Hydrochloride in presences of degradation impurities was developed and validated as per international conference on harmonization (ICH) guidelines [21].

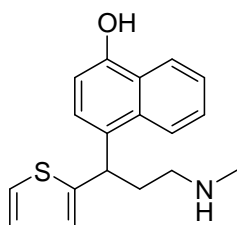
### MATERIALS AND METHODS

All reagents were obtained commercially and were of the highest commercial quality and used without further purification. Solvents were freshly distilled and used. Melting points were determined in open capillaries and are uncorrected. TLC or HPLC routinely checked the purity of all compounds. IR spectra were recorded on a Perkin-Elmer model spectrum100 instrument in KBr phase. <sup>1</sup>H-NMR (400 MHz) and <sup>13</sup>C-NMR (100MHz) spectra were recorded in CDCl<sub>3</sub> or DMSO using Bruker instrument and Mass spectra were recorded on a Perkin-Elmer mass spectrometer operating at 70 eV.

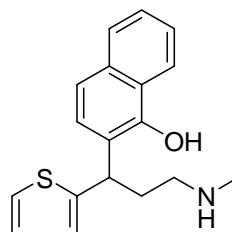
#### Typical procedure for Ring isomer preparation.

To the solution of Duloxetine 28.0 gms (1.0 mole) in Propylene glycol monomethyl ether (PGME) 280 ml potassium hydroxide 34.66 gms (5.58 mole) was added at 20-25°C and the reaction mass heated to 118-120°C. The reaction mixture was stirred for 177 hours at 118-120°C. (Reaction was monitored by TLC). At the end of this period, the solvent was removed under reduced pressure at 50-60°C to get crude compound, added water 115 ml and dichloromethane 115 ml at RT. The reaction was stirred for 2 hours at RT. The solids are filtered and washed with water 20ml. To obtain ring isomer was purified by recrystallization from ethyl acetate to yield ring isomer. Yield 15.0 gm (53%). White solid; MR: 161.7-165.2°C. IR (In KBr): 3427, 3054, 2944, 1643, 1550, 1377, 1350, 1294, 1244, 1214, 1035, 807 cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO, 400MHz) δ = 8.32 (d, 1H, *J*=7.92 Hz), 7.77 (d, 1H, *J*=7.72 Hz), 7.43-7.33 (m, 3H), 7.26 (d, 1H, *J*=8.28 Hz), 7.18 (d, 2H, *J*= 8.28 Hz), 6.99 (d, 1H, *J*=4.99 Hz), 2.73 (m, 2H), 2.49 (m, 2H), 2.17 (s, 3H), 1.17 (m, 2H) ppm; <sup>13</sup>C NMR(DMSO, 100MHz) δ = 152.73, 140.56, 136.63, 134.34, 130.60, 129.50, 127.54, 127.19, 125.95, 124.49, 123.57, 121.96, 118.11, 116.91, 50.54, 35.88, 30.79, 26.25 ppm. ESIMS [M+H]<sup>+</sup>m/z 298.1.

The following two molecules are prepared and characterization as per Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem. 46B (2007) 1695-1698. (The structure of para isomer was also confirmed by X-ray).



Para isomer

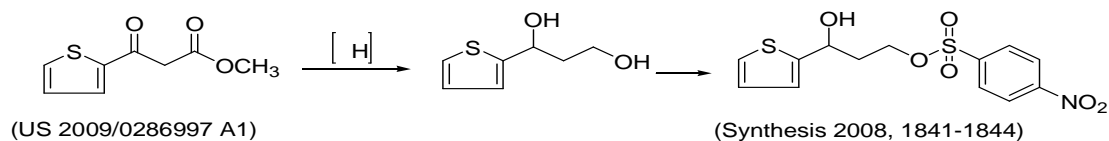


Ortho isomer

#### Typical procedure for amino alcohol preparation:

To the solution of 4-Nitro-benzenesulfonic acid 3-hydroxy-3-thiophen-2-yl-propyl ester\* 5.0 gms (1.0 mole) in methanol 5 ml, 40% aqueous monomethyl amine 15 ml (10 mole) was added at 20-25°C and the reaction mass heated to 50-55°C. The reaction mixture was stirred for 2 hours at 50-55°C. (Reaction was monitored by TLC). At the end of this period, the solvent was removed under reduced pressure at 40-45°C to get crude compound, added water 5 ml and dichloromethane 10 ml at RT, separate both layers. The organic layer was washed with water 5 ml and concentrated under reduced pressure. The crude product was purified by column chromatography to yield pure 3-Methylamino-1-thiophen-2-yl-propan-1-ol (Amino alcohol). Yield 1.0 gm (40%). Pale yellow color liquid. IR (In Neat): 3307, 3108, 1568, 1374, 1310, 1035, 853, 703cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz) δ = 7.22 (d, 1H, *J*=4.90 Hz), 6.97 (m, 2H), 5.2 (dd, 1H, *J*<sub>1</sub>=3.04, *J*<sub>2</sub>=3.01Hz), 2.98 (m, 2H), 2.90 (m, 2H), 2.44 (s, 3H), 1.98 (m, 2H) ppm; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100MHz) δ = 149.25, 126.52, 123.73, 122.72, 70.72, 49.28, 36.36, 35.23ppm. ESIMS [M-H]<sup>+</sup>m/z 202.

\* Prepared as follows:



### Chemicals and reagents

Duloxetine Hydrochloride and its impurities viz. Amino alcohol, Para isomer, Ortho isomer, Ring isomer,  $\alpha$ -Naphthol and Ester (Fig.1) were obtained from Suven Life sciences Ltd [22-24]. Sodium dihydrogen ortho phosphate, 1-Heptane Sulfonic acid sodium salt, Ortho-phosphoric acid, Acetonitrile, hydrochloric acid, sodium hydroxide, hydrogen peroxide (30%), sodium chloride and barium chloride were obtained from Rankem, New delhi, India. All solution are prepared in Milli Q water (Millipore USA).

### HPLC instrumentation and conditions.

Waters Alliance 2695 separation module (Waters Corporation, Milford, USA) equipped with 2996 PDA detector (for specificity and forced degradation studies) and 2487 UV/visible detector with Empower software was used for the analysis. YMC Pack C8 column (250 X 4.6 mm, 5 $\mu$ m, YMC Corporation, Japan) and a gradient mixture of solvent A and B were used as stationary and mobile phases, respectively. Buffer contains 0.01 M of Sodium Di hydrogen Orthophosphate and 1.0g of 1-Heptane Sulfonic Acid Sodium Salt transfer into 1000mL of water, adjust the pH of the above solution to  $3.0 \pm 0.1$  using Orthophosphoric acid. Buffer was used as solvent A. Acetonitrile was used as solvent B. The gradient program (T/%B) was set as 0/40, 5/40, 20/75, 30/75, 30.1/40 and 40/40. 1.0ml/min flow rate and 5.0 $\mu$ l injection volume were maintained. The eluted compounds were monitored at 217nm. The column oven temperature was maintained at 25°C.

### Preparation of standard solutions

Buffer and Acetonitrile in the ratio of 30:70 v/v were used as diluent. 0.001mg/ml standard solution of Duloxetine Hydrochloride was prepared in diluent for impurities assay determination. A blend of six 0.10% Duloxetine Hydrochloride impurities was prepared in diluent with respect to 2.0mg/ml of Duloxetine Hydrochloride

## RESULTS AND DISCUSSION

### Characterization of Impurities

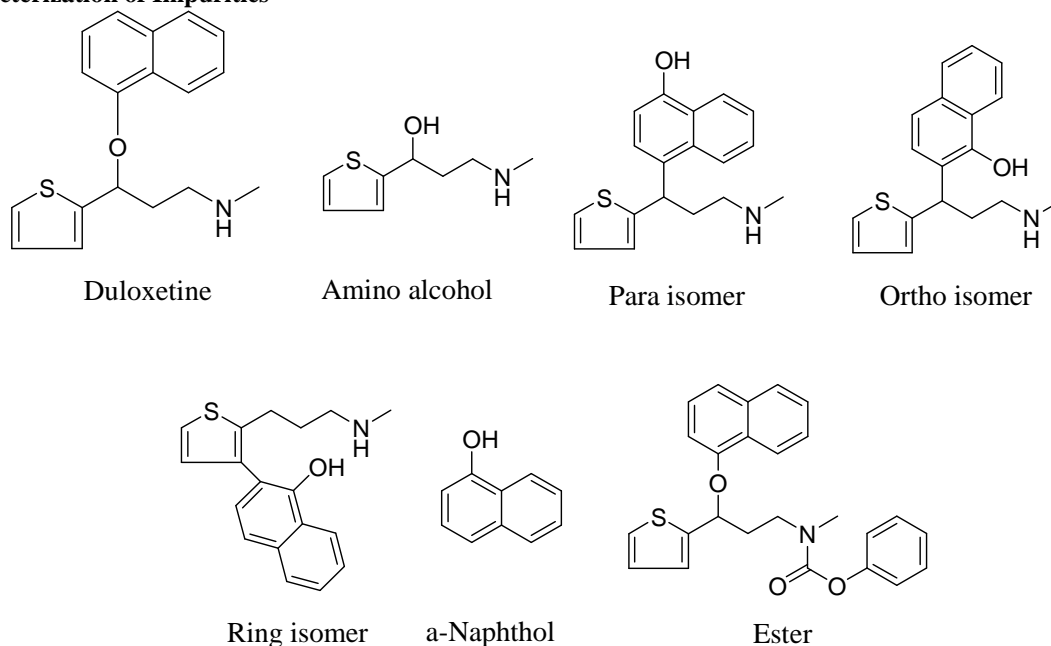


Fig-1: Structures of Duloxetine and its related impurities.

**HPLC method development**

The HPLC method was optimized so as to obtain a stability- indicating method that can resolve degradation impurities from Duloxetine Hydrochloride. 0.01 M of Sodium Di hydrogen Orthophosphate and 1.0g of 1-Heptane Sulfonic Acid Sodium Salt was initially chosen as buffer. The buffer having pH 3.0 was adopted, because, it was suitable to separate the degradation impurities from Duloxetine Hydrochloride. YMC Pack C8 column (250 X 4.6 mm, 5 $\mu$ m) column allowed to a rapid resolution between Ortho isomer and Ring isomer and showed the best values of theoretical plates and symmetry for Duloxetine Hydrochloride. A typical chromatogram of Blank, Mother Sample and Duloxetine Hydrochloride spiked with 0.10% of impurities (Fig.2).

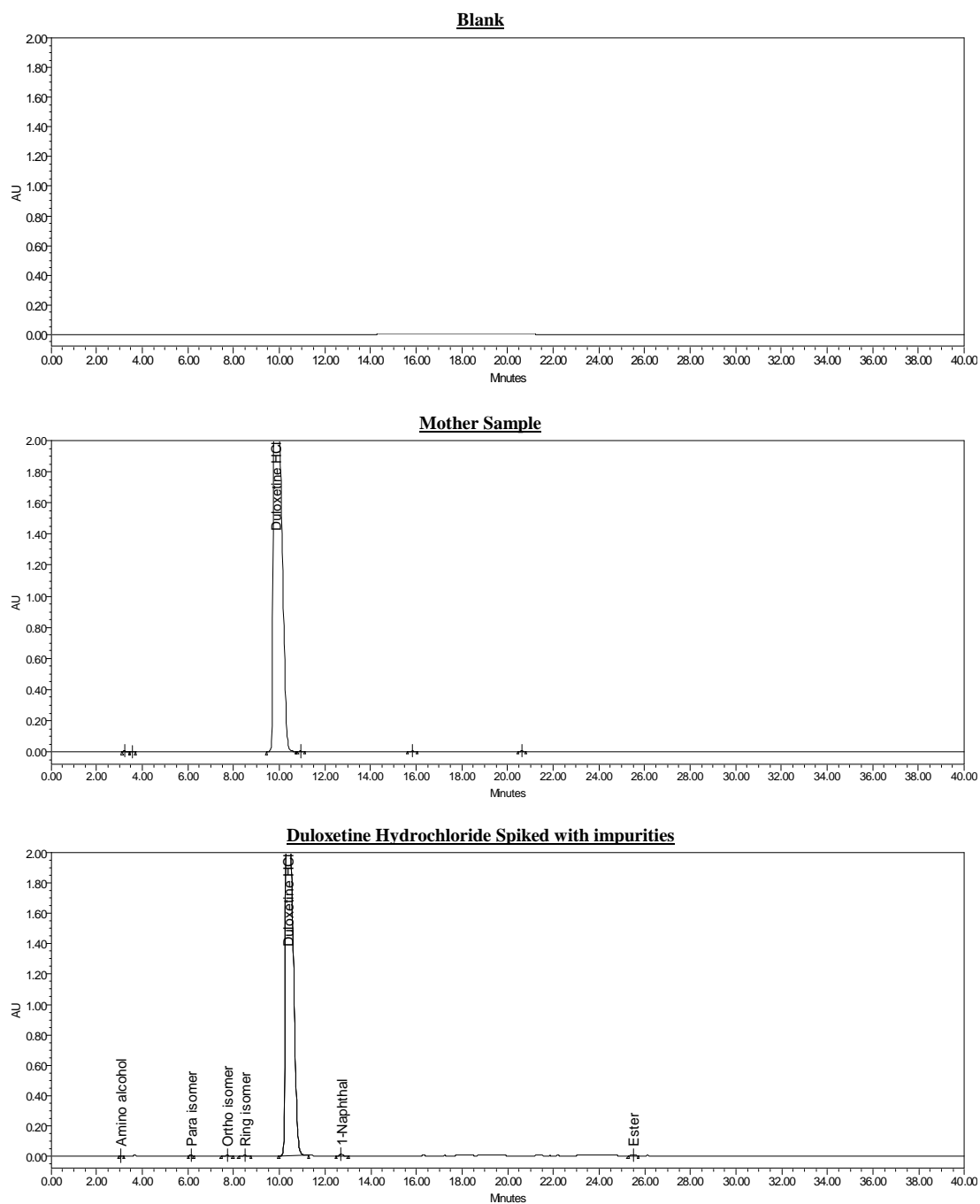


Fig-2: Typical chromatograms of Blank, Mother sample and Duloxetine Hydrochloride spiked with its impurities .

### Method Validation

The developed method was validated as per ICH guidelines and the results are given (Table I). Stress testing of the drug substance can help to identify the degradants, which in turn help to evaluate the stability-indicating nature of the developed method. The specificity of the developed HPLC method for Duloxetine Hydrochloride was determined in the presence of its process and degradation impurities. All the stressed samples of Duloxetine Hydrochloride and all degradation impurities were well resolved from one another and from Duloxetine Hydrochloride. The analysis was carried out by HPLC with PDA detector. The chromatographic peak purity tool, applied to Duloxetine Hydrochloride and its impurities peaks, demonstrated that all the peaks were pure in all cases conform the absence of other impurities co-eluting in the same retention time and there by signifying the specificity and stability indicating nature of the method. The detection limit (DL) and quantification limit (QL) for Amino alcohol, Para isomer, Ortho isomer, Ring isomer,  $\alpha$ -Naphthol and Ester were determined at a signal to noise ratio of 3:3 and 10:1 respectively, by injecting a series of dilute solutions with known concentration. Precision study was carried at QL level by injecting six times and calculating the percentage of R.S.D of area of Amino alcohol, Para isomer, Ortho isomer, Ring isomer,  $\alpha$ -Naphthol and Ester. Linearity test solutions for purity determination were at six concentration levels from QL to 150 % of the specification level (0.10%). Peak area versus concentration data was performed by least-squares linear regression analysis. Standard addition and recovery experiments were conducted to determine accuracy of impurities quantitation in bulk drug samples. The study was carried out in triplicate at QL, 100% and 150% level with respect to specification 0.1%. The percentages of recoveries for impurities were calculated. The robustness of developed method was determined by altering experimental conditions purposely and evaluating the resolution between Duloxetine Hydrochloride, Amino alcohol, Para isomer, Ortho isomer, Ring isomer,  $\alpha$ -Naphthol and Ester. Flow rate was changed by  $\pm 0.1$  units, pH was varied by  $\pm 0.2$  units, wave length was changed by  $\pm 2$ nm and column temperature was studied at 23°C and 28°C instead of 25°C in all above varied conditions the components of the mobile phase were held constant and no significant change (relative error less than 5%) of relative retention time was observed. Significant changes were not observed in the contents of Amino alcohol, Para isomer, Ortho isomer, Ring isomer,  $\alpha$ -Naphthol and Ester. The stability data confirmed that sample solutions were stable up to 24hrs. The system suitability was established in terms of resolution between Ortho isomer and Ring isomer which was more than 2.0, when a 2mg/ml Duloxetine Hydrochloride solution spiked with 0.10% of Amino alcohol, Para isomer, Ortho isomer, Ring isomer,  $\alpha$ -Naphthol and Ester were injected.

Table-1 Validation data of the developed method

Parameter	Amino alcohol	Para isomer	Ortho isomer	Ring isomer	$\alpha$ - Naphthol	Ester
DL (%)		0.02	0.01	0.01	0.01	0.01
QL (%)		0.05	0.03	0.01	0.02	0.03
Method Precision(%RSD)#		1.48	1.05	0.46	0.22	0.34
Intermediate precision(%RSD)#		2.59	0.65	0.94	0.48	0.17
Accuracy <sup>a</sup> (% recovery) at:-						
QL		97.15	98.74	99.68	99.10	100.20
100%		99.06	92.29	101.92	99.32	100.91
150%		95.27	95.69	100.02	97.32	98.71

<sup>a</sup> Carried at QL, 100% and 150% level with respect to specification (0.10%)

### Forced degradation studies result

The stability –indicating power of the developed method was studied by conducting forced degradation studies on Duloxetine Hydrochloride. Forced degradation samples were injected at regular intervals and the final stress conditions were established so as to obtain 10-30% degradation of Duloxetine Hydrochloride. Duloxetine Hydrochloride was degraded and resulted in formation of Amino alcohol, Para isomer, Ortho isomer, Ring isomer,  $\alpha$ -Naphthol and Ester, when it was exposed to acid degradation in 0.1N hydrochloric acid at room temperature for 3hrs or oxidative degradation in 3% hydrogen peroxide at 90°C for 1hr (Fig.3). when Duloxetine Hydrochloride was exposed to base degradation in 0.1N NaOH at 90°C for 3hrs or kept in oven at 100°C for 48hrs or in photo stability chamber/200Wh/m<sup>2</sup> in UV light or humidity degradation at 75-85% RH, it did not give any degradation products.

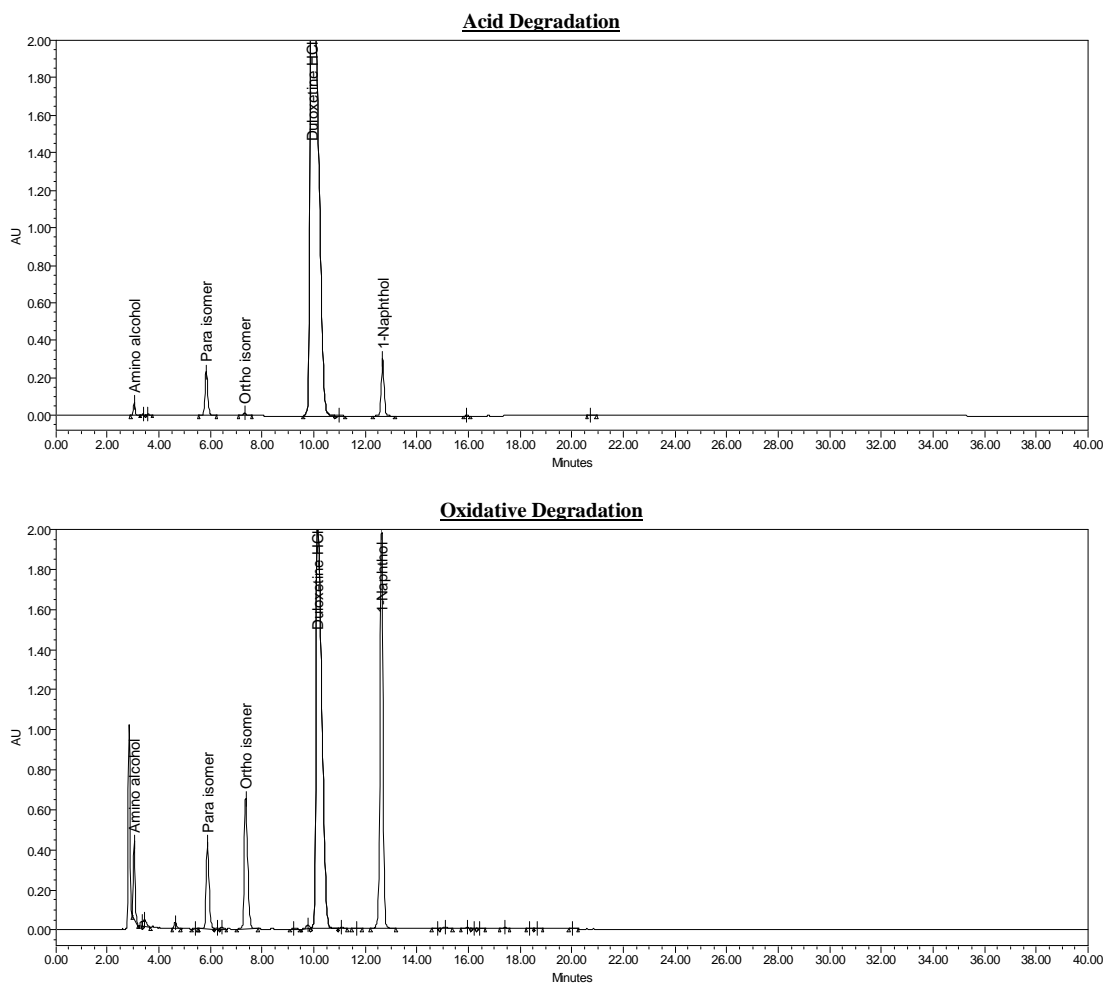


Fig-3: Typical chromatograms of oxidative and acid degradation samples.

CONCLUSION

A stability indicating HPLC method has been developed and validated for the purity determination of Duloxetine Hydrochloride. The behavior of Duloxetine Hydrochloride under various stress conditions was studied. This method is able to separate the Duloxetine Hydrochloride from its degradation impurities, it can be conveniently applied for the testing of batch release and stability studies (Table II).

Table II Summary of forced degradation studies

Stress type	% Degradants formed							
	Amino alcohol	Para isomer	Ortho isomer	Ring isomer	α-Naphthol	Ester	MUI	TI
Unstressed	BDL	BDL	BDL	BDL	BDL	BDL	0.06	0.17
Acid degradation	3.25	3.52	0.16	BDL	1.90	BDL	0.05	9.00
Oxidative degradation	18.86	6.44	8.85	BDL	12.53	BDL	0.47	48.64
Base degradation	0.10	0.34	0.67	0.07	0.09	BDL	0.11	1.74
Photolytic degradation	0.20	BDL	0.07	BDL	0.03	BDL	0.03	0.41
Thermal degradation	BDL	BDL	0.03	BDL	BDL	BDL	0.05	0.22
Humidity degradation	BDL	BDL	BDL	BDL	BDL	BDL	0.04	0.15

MUI: Major unknown impurity, TI: Total impurities, BDL: Below detection limit

**Acknowledgments**

The authors greatly acknowledge their thanks to Mr. Venkat Jasti, CEO, Suven Life Sciences Ltd, Hyderabad for providing excellent facilities to carry out this work.

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