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Analysis of Duloxetine Hydrochloride and Its Related Compounds in Pharmaceutical Dosage Forms and In Vitro Dissolution Studies by Stability Indicating UPLC

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Abstract

A reproducible gradient reversed-phase ultra-performance liquid chromatographic method is developed for quantitative determination of duloxetine hydrochloride in pharmaceutical dosage forms. The method is also applicable for analysis of related substances and for study of in vitro dissolution profiles. Chromatographic separation is achieved on a 50 mm × 4.6 mm, 1.8 µm C-18 column. Mobile phase A contains a mixture of 0.01 M KH_2PO_4 (pH 4.0) buffer, tetrahydro furan, and methanol in the ratio 67:23:10 (v/v/v), respectively, and mobile phase B contains a mixture of 0.01 M KH_2PO_4, (pH 4.0) buffer, and acetonitrile in the ratio 60:40 (v/v), respectively. The flow rate is 0.6 mL/min, and the detection wavelength is monitored at 236 nm. Resolution of duloxetine hydrochloride and three potential impurities is greater than 2.0 for all pairs of components. The drug was subjected to ICH prescribed hydrolytic, oxidative, photolytic, and thermal stress conditions. Method is validated for linearity, specificity, accuracy, precision, ruggedness, and robustness.

Introduction

Duloxetine hydrochloride is a selective serotonin and norepinephrine reuptake inhibitor (SSNRI) for oral administration. Its chemical designation is (+)-(*S*)-*N*-methyl-γ-(1-naphthyloxy)-2-thiophenepropylamine hydrochloride (Figure 1).

Duloxetine has been determined in pharmaceutical preparations by liquid chromatography (LC) (1,2), spectrophotometric (3), and liquid chromatography–mass spectrometry (LC–MS) (4,5,6) methods. No LC methods were reported in major pharmacopeias like United State Pharmacopeia, European Pharmacopeia, Japanese Pharmacopeia and British Pharmacopeia. Extensive literature survey revealed no stability-indicating LC method has been reported for determination of related substances and for quantitative estimation of duloxetine hydrochloride along with in vitro dissolution studies in pharmaceutical dosage forms.

Ultra-performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reductions in separation time and solvent consumption. Literature indicates that UPLC system allows approximately ninefold decrease in analysis time as compared to the conventional high-performance liquid chromatography (HPLC) system using 5-µm particle size analytical columns, and about threefold decrease in analysis time in comparison with 3 µm particle size analytical columns without compromise on overall separation (7–11).

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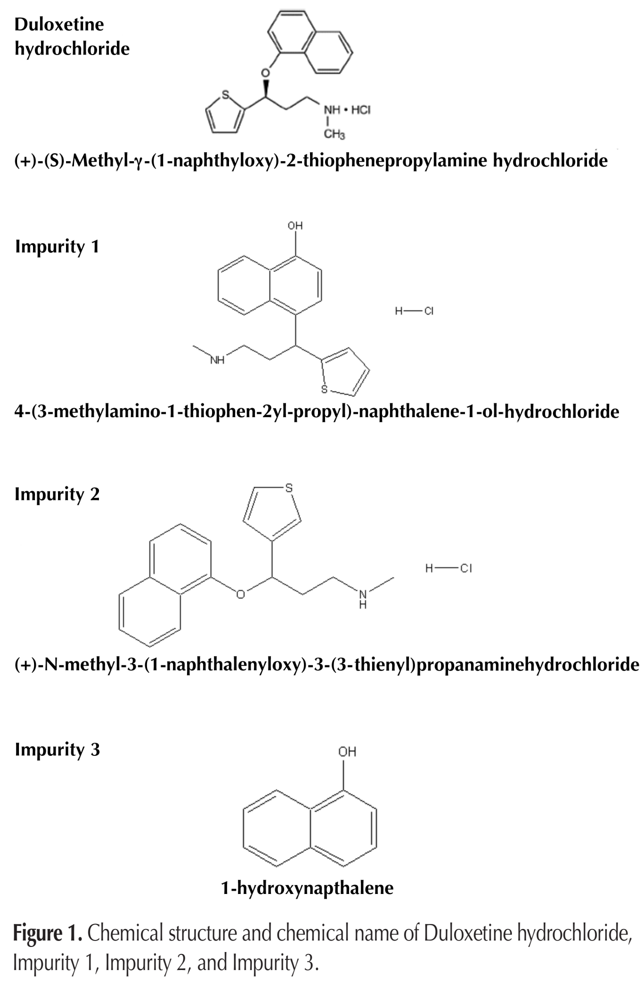

Figure 1. Chemical structure and chemical name of Duloxetine hydrochloride, Impurity 1, Impurity 2, and Impurity 3.

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Hence, a reproducible stability-indicating reversed phase (RP) UPLC method was developed for the quantitative determination of duloxetine hydrochloride and its three impurities. The method was also applied for study of in vitro dissolution profiles in pharmaceutical dosage forms.

Experimental

Chemicals

Standards and capsules were supplied by Dr. Reddy's Laboratories Ltd., (Hyderabad, India). The HPLC-grade acetonitrile, methanol, tertahydro furan, and analytical grade KH_2PO_4 and ortho phosphoric acid were purchased from Merck (Darmstadt, Germany). Water was prepared by using Millipore Milli-Q Plus water purification system (Billerica, MA).

Chromatographic conditions

LC was carried out on a Waters Aquity UPLC with photodiode array detector (Milford, MA). The output signal was monitored and processed using empowers software. The chromatographic column used was Zorbax XDB C-18, 50 mm × 4.6 mm i.d. (Agilent, Santa Clara, CA), with 1.8 µm particles. The separation was achieved on a gradient method. Mobile phase A contained a mixture of 0.01 M KH_2PO_4 (pH 4.0) buffer, tetrahydro furan, and methanol in the ratio 67:23:10 (v/v/v), respectively and mobile phase B contained a mixture of 0.01 M KH_2PO_4, (pH 4.0) buffer, and acetonitrile in the ratio 60:40 (v/v), respectively.

The flow rate of mobile phase was 0.6 mL/min. The UPLC gradient program was set as: time (min) / % solution B: 0/0, 6/0, 8/100, 13/100, 14/0 and 16/0. The column temperature was maintained at 40°C, and the detection was monitored at a wavelength 236 nm. The injection volume was 5 µL.

Preparation of stock solutions

A stock solution of duloxetine hydrochloride (2.0 mg/mL) was prepared by dissolving an appropriate amount of drug in methanol–water 90:10 (v/v), respectively. Working solutions containing 200 and 20 µg/mL were prepared from this stock solution for determination of related substances and for assay determination, respectively. A mixed stock solution (0.5 mg/mL) of the impurities was also prepared in methanol.

Preparation of sample solution

Twenty duloxetine hydrochloride 60 mg capsules were weighed and transferred the pellets into a clean, dry mortar. Pellets equivalent to 20 mg drug was dissolved in 100 mL of methanol–water 90:10 (v/v) to give a solution containing 200 µg/mL. 10 mL of this solution was diluted to 100 mL with methanol–water 90:10 (v/v), to give a solution containing 20 µg/mL.

Stress studies

Forced degradation of duloxetine hydrochloride was performed to provide an indication of the stability-indicating properties and specificity of the method. The stress conditions used for the degradation study included light (14), heat (60°C), acid hydrolysis (1 N HCl), basic hydrolysis (1 N NaOH), aqueous hydrolysis, and oxidation (6% H_2O_2). The sample preparation for stress conditions is in Table I.

The purity of peaks obtained from stressed samples was checked by use of the PDA detector. The purity angle was within the purity threshold limit obtained in all stressed samples and demonstrated the analyte peak homogeneity. Assay of stressed samples was performed by comparison with reference standards and the mass balance (% assay + % impurities + % degradation products) for stressed samples was calculated.

Method validation

Precision

The repeatability of the related-substance method was checked by six-fold analysis of 200 µg/mL duloxetine hydrochloride spiked with 0.30% of each of the three impurities. The RSD (%) of peak area was calculated for each impurity.

Inter- and intra-day variation and analyst variation was studied to determine intermediate precision of the proposed method. Intra-day precision was determined by six-fold analysis of 200 µg/mL duloxetine hydrochloride spiked with 0.30% of each of the three impurities. The same protocol was followed for three different days to study inter-day variation (n = 18). Different analysts prepared different solutions on different days. The RSD (%)

Table I. Summary of Results from Forced Degradation Experiments

Stress condition	Amount of drug (mg)	Volume of regent (mL)	Stress period	Neutralized with	Volume of diluent* (mL)	Conc. of active substance (µg/mL)	Assay of active substance (%)	Mass balance†	Remarks: Major degradation products formed:
Acidic hydrolysis (1 N HCl)	50	25	3 h	25 mL of 1 N NaOH	200 200	200	58.9	99.1	Impurities 3 & 1
Basic hydrolysis (1 N NaOH)	50	25	3 h	25 mL of 1 N HCl	200	200	97.6	99.6	Impurity 3
Oxidation (6% H_2O_2)	50	25	3 h	NA	200	200	99.6	99.8	None
Aqueous hydrolysis	50	25	3 h	NA	200	200	84.6	99.3	Impurities 3 & 1
Thermal treatment (60°C)	20	NA	10 days	NA	100	200	99.5	99.6	None
Photolytic degradation	20	NA	10 days	NA	100	200	99.9	100.1	None

* Methanol–water 90:10 (v/v). † % Assay + % impurities + % degradation products.

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of peak area was calculated for each impurity.

The precision of the assay was evaluated by performing six independent assays of a test sample of duloxetine hydrochloride and comparison with a reference standard. The RSD (%) of the six results was calculated.

Limit of detection and quantitation

By injecting a series of solutions of known concentration (12), limit of detection (LOD) and limit of quantitation (LOQ) for duloxetine and for the three impurities were estimated at the amounts for which the signal-to-noise ratio was 3:1 and 10:1, respectively. Precision was also determined at LOQ level and % RSD was calculated for the peak area for each impurity and for duloxetine.

Response function

To test the response function of the method, solutions at six concentrations from LOQ to 150% of the analyte concentration (0.2, 10, 15, 20, 25, and 30 µg/mL) were prepared from the stock solution. Least-squares linear regression analysis was performed on peak areas and heights data. Solutions for testing linearity for the related substances were prepared by diluting the impurity stock solution to five different concentrations from the LOQ to 150% of the permitted maximum level of the impurity (i.e., the LOQ and 0.15%, 0.30%, 0.375%, and 0.45% for an analyte concentration of 200 µg/mL). The correlation coefficients, slopes, and *y*-intercepts of the peak areas and heights were reported.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the true value and the value found. The accuracy of the assay method for duloxetine hydrochloride was evaluated in triplicate at three concentrations, 10, 20, and 30 µg/mL on the drug product, and recovery was calculated for each added concentration.

For impurities, recovery was determined in triplicate for 0.15%, 0.30%, and 0.45% of the analyte concentration (200 µg/mL) on drug product and recovery of the impurities was calculated.

Robustness

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

To determine the robustness of the method, the experimental conditions were deliberately changed. The resolution of duloxetine hydrochloride and the three impurities were evaluated. The mobile phase flow rate was 0.6 mL/min; to study the effect of flow rate on resolution it was changed to 0.5 and 0.7 mL/min. The effect of pH was studied at pH 3.9 and 4.1 (instead of pH 4.0). The effect of column temperature was studied at 35°C and 45°C (instead of 40°C).

Solution stability and mobile phase stability

The stability of duloxetine hydrochloride and its impurities in solution was determined by leaving test solutions of the sample and reference standard in tightly capped volumetric flasks at room temperature for 48 h during which they were assayed at 24 h intervals. Stability in the mobile phase was determined by analysis of freshly prepared sample solutions at 24 h intervals for 48 h and compared the results with those obtained from freshly prepared reference standard solutions. The RSD (%) of the assay results was calculated for both the mobile phase and solution-stability experiments.

In vitro dissolution studies

Dissolution tests are one of the tests most used in the characterization of drugs and in the quality control of dosage forms. It was introduced in the 1960s, and it has evolved into a test that pharmaceutical manufacturers hope will better predict the in vivo performance of drug products. In addition, the reliability and discriminatory capabilities of dissolution methods for delayed release products has gained much attention in recent years. The most widely used dissolution tests for delayed release products use 900 or 1000 mL of an acid medium followed by 900 or 1000 mL of aqueous buffer medium with USP apparatus I (basket) or apparatus II (paddle) at rotation speeds of 100 or 50 rpm, respectively. It was desired to have an in vitro dissolution method that is sensitive to formulation factors that affect the dissolution process and in consequence bioavailability. In our study, release characteristics of duloxetine hydrochloride capsules were studied according to the FDA dissolution method (basket) at 37°C ± 0.5°C using 1000 mL of 0.1 N HCl (2 h) followed by 1000 mL of 6.8 phosphate buffer (pH 6.8) at 100 rpm rotation speed (13). In vitro dissolution was conducted on duloxetine hydrochloride 60 mg test product and on duloxetine hydrochloride 60 mg innovator (Cymbalta) product. At scheduled time intervals, 1 mL of sample was withdrawn and replaced with fresh medium. The final solution was filtered through 0.45 µm nylon membrane filter and then analyzed by the proposed HPLC method.

Results and Discussion

Method development and optimization

The main objective of the chromatographic method was to separate critical closely eluting impurities Impurity 1, unknown (0.87 RRT) and Impurity 2 and to elute duloxetine hydrochloride as a symmetrical peak. Attempts were made using different C18 (Inertsil ODS-3, 50 mm × 2.1 mm, 2 µm particles and Waters Aquity BEH C_{18} 50 mm × 2.1 mm, containing 1.7 µm particles) UPLC columns, using different buffer pH (7.0) condition and using isocratic mobile phase elution. But at all above conditions, separation of impurities and degradation products was not satisfactory (Table II and Figures 2A–2D). But separation was satisfactory in the adopted chromatographic conditions only (Figure 2E). There was no interference of excipients with impurities peaks and duloxetine peak

The unknown impurity (0.87 RRT) which was formed in acid hydrolysis and aqueous hydrolysis was investigated with LCMS study. The mass of unknown impurity was 298.4 and the mass of Impurity 2 (4-(3-methylamino)-1-(thiophen-2-yl) propyl) naphthalin-1-ol) was 298 and the UV spectra of Impurity 2 was similar

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to that of unknown impurity. Hence, based LC–MS data and UV spectra, the possible chemical name of the unknown impurity could be '2-(3-methylamino)-1-(thiophen-2-yl) propyl) naphthalin-1-ol. The proposed structure of unknown impurity was positional isomer of Impurity 2.

System suitability parameters were evaluated for duloxetine and its three impurities. Tailing factor for all three impurities and duloxetine was found less than 1.3. Resolution of duloxetine hydrochloride and three potential impurities was greater than 2.0 for all pairs of compounds.

Validation of the method

Precision

RSD (%) in the study of the repeatability of the assay of duloxetine hydrochloride was within 0.15%. The % RSD of peak area for the three impurities namely Impurity 1, Impurity 2, and Impurity 3, in the study of the repeatability was 1.3%, 0.9%, and 1.8%, respectively. RSD (%) results of duloxetine hydrochloride and its impurities for intermediate precision (intra and inter day repeatability) are presented in Table III. These results confirmed the method was highly precise.

LOD and LOQ

The limit of detection for duloxetine, Impurity 1, Impurity 2, and Impurity 3 were 0.01%, 0.005%, 0.01%, and 0.005%; respectively. The limit of quantification for duloxetine, Impurity 1, Impurity 2 and Impurity 3 were 0.04%, 0.013%, 0.027%, and 0.014%, respectively. RSD (%) for precision at the LOQ concentration for duloxetine, Impurity 1, Impurity 2, and Impurity 3 were, 0.8%, 2.3%, 2.1%, and 1.3%; respectively.

Response function

For assay, the response of the duloxetine using the test method was linear over the narrow range studied, that was 0.2 to 30 µg/mL (LOQ to 150%). Correlation coefficient for peak areas and heights were 0.9999. Typically, the regression equation for the calibration curve was found to be $y = 37892702\,x + 3713$.

The response of the duloxetine and its three impurities using this test method was linear over the narrow range studied, that was LOQ to 0.45% of the concentration of duloxetine hydrochloride. Correlation coefficients for peak areas and heights were 0.998 or greater for the impurities in the stated range.

Accuracy

Recovery of duloxetine hydrochloride from pharmaceutical dosage forms ranged from 99.6% to 101.2%. Recovery of the three impurities from pharmaceutical dosage forms ranged from 93.3 to 104.5. An UPLC chromatogram obtained from a sample of duloxetine hydrochloride spiked with all three impurities at the 0.30% level is shown in Figure 2E.

Robustness

In all the deliberate varied chromatographic conditions (flow rate, column temperature, and pH variation), all analytes were adequately resolved and elution orders remained unchanged. Resolution between Impurity 1 and duloxetine hydrochloride was greater than 3.5 and resolution between duloxetine hydrochloride and Impurity 2 was greater than 2.4 for all flow rate (0.5, 0.7 mL/min), column temperature (35°C, 45°C) and pH (3.9, 4.1) variation conditions.

Table II. Results from Different Trials

Trial no.		UPLC Conditions	Remarks
1	*Column:*	Inertsil ODS-3, 50 mm × 2.1 mm, 2 µm particles	Impurity 2 and unknown (0.87 RRT) peeks were co-eluting with duloxetine peak
	Mobile phase:	0.01 M KH_2PO_4 buffer, tetra hydro furan, and methanol (67:23:10, v/v/v), pH adjusted to 4.0 with ortho phosphoric acid	
	Flow rate:	0.6 mL/min	
2	*Column:*	Waters AQUITY BEH C18, 50 mm × 2.1 mm, 1.7 µm particles	Impurity 2 peak was co-eluting with duloxetine and unknown peak at RRT 0.87 is partially merged with duloxetine peak
	Mobile phase:	0.01 M KH_2PO_4 buffer, tetra hydro furan, and methanol (67:23:10, v/v/v), pH adjusted to 4.0 with ortho phosphoric acid	
	Flow rate:	0.6 mL/min	
3	*Column:*	Zorbax XDB C-18 column, 50 mm × 2.1 mm, 1.8 µm particles	Impurity 1, unknown (0.87 RRT), Impurity 2 and duloxetine peaks are well separated from each other. But Impurity 3 retention time was 29 min
	Mobile phase:	0.01 M KH_2PO_4 buffer, tetra hydro furan, and methanol (67:23:10, v/v/v), pH adjusted to 4.0 with ortho phosphoric acid.	
	Flow rate:	0.6 mL/min	
4	*Column:*	Zorbax XDB C-18 column, 50 mm × 2.1 mm, 1.8 µm particles	Peak distortion of duloxetine peak and co-elution of Impurity 2 with duloxetine was observed
	Mobile phase:	0.01 M ammonium acetate buffer, tetra hydro furan and methanol (67:23:10, v/v/v), pH adjusted to 7.0 with aqueous ammonia solution	
	Flow rate:	0.6 mL/min	
5	*Column:*	Zorbax XDB C-18 column, 50 mm × 2.1 mm, 1.8 µm particles	The tailing factor for duloxetine is 1.1, Resolution (RS) between Impurity 1 and unknown (0.87 RRT) was 6.7, RS between unknown (0.87 RRT) and duloxetine was 1.1 and RS between duloxetine and Impurity 2 was 2.6
	Mobile phase:	.01 M KH2PO4 buffer, tetra hydro furan and methanol (67:23:10, v/v/v), pH adjusted to 4.0 with ortho phosphoric acid	
	Flow rate:	0.6 mL/min	
	Gradient:	Time (min) / % solution B: 0/0, 6/0, 8/100, 13/100, 14/0, and 16/0	

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Stability in solution and in the mobile phase

RSD (%) for assay of duloxetine hydrochloride during solution stability and mobile phase stability experiments was within 1%. No significant changes in the amounts of the three impurities were observed during solution stability and mobile phase experiments when performed using the related substances method. The results from solution stability and mobile phase stability experiments confirmed that standard solutions and solutions in the mobile phase were stable for up to 48 h during assay and determination of related substances.

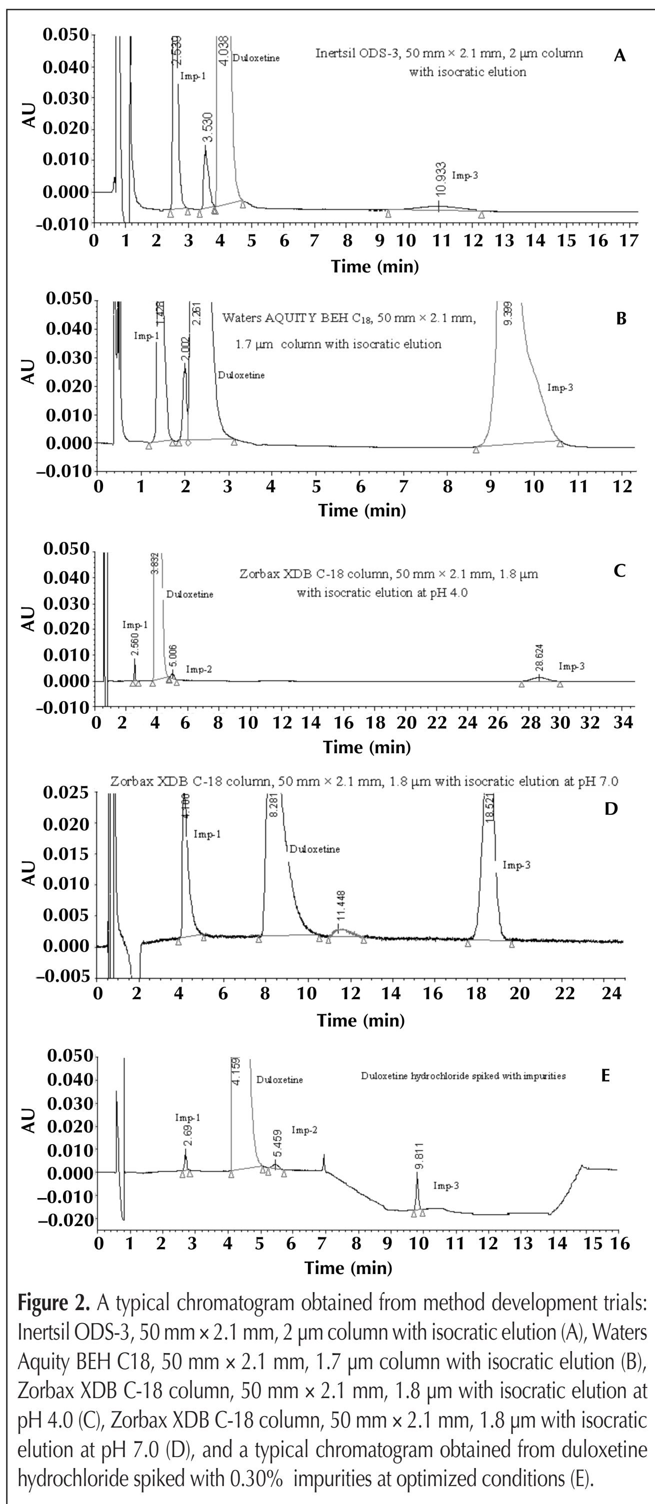

Figure 2. A typical chromatogram obtained from method development trials: Inertsil ODS-3, 50 mm × 2.1 mm, 2 µm column with isocratic elution (A), Waters Aquity BEH C18, 50 mm × 2.1 mm, 1.7 µm column with isocratic elution (B), Zorbax XDB C-18 column, 50 mm × 2.1 mm, 1.8 µm with isocratic elution at pH 4.0 (C), Zorbax XDB C-18 column, 50 mm × 2.1 mm, 1.8 µm with isocratic elution at pH 7.0 (D), and a typical chromatogram obtained from duloxetine hydrochloride spiked with 0.30% impurities at optimized conditions (E).

Results from forced degradation studies

Degradation was not observed when duloxetine hydrochloride was subjected to oxidative conditions, light and heat. Degradation was observed when the drug was subjected to acidic, basic, and aqueous hydrolysis (Figure 3A–3D). Results from force degradation studies were presented in Table I.

The mass balance for the stressed samples was close to 99.1%. Assay of duloxetine hydrochloride was unaffected by the presence of the three impurities/degradation products, confirming the stability-indicating power of the method.

In vitro dissolution studies

The dissolution characteristics of duloxetine hydrochloride are given in Figure 4. Analyzing the in vitro dissolution profile of test and innovator, it could be concluded that test showed the faster release rate than that of innovator. Initially at 15 min and at 30 min, dissolution rate of test product slightly faster than the innovator product, but from 45 min to 90 min both test and

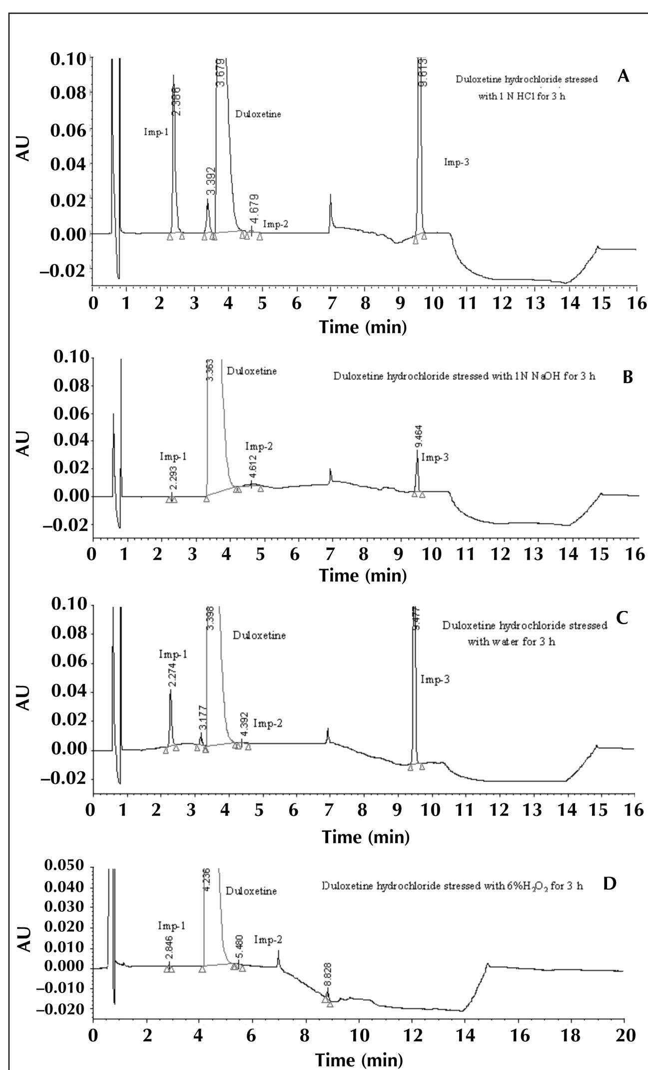

Figure 3. A typical chromatogram obtained from duloxetine hydrochloride sample and from stressed duloxetine hydrochloride samples: Duloxetine HCl stressed with 1N HCl for 3 h (A), Duloxetine HCl stressed with 1N NaOH for 3 h (B), Duloxetine HCl stressed with water for 3 h (C), and Duloxetine HCl stressed with 60% H_2O_2 for 3 h (D).

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Table III. Results from Determination of Intermediate Precision*

Day	RSD (%) for assay (*n* = 6)	RSD (%) for impurities (*n* = 6)			Resolution between	
		Imp. 1	Imp. 2	Imp. 3	Imp. 1 and DH	DH and Imp. 2
Intra-day repeatability						
Day 1	0.15	1.3	0.9	1.8	3.7	2.5
Day 2	0.21	0.8	1.2	1.6	3.7	2.6
Day 3	0.18	1.5	1.1	1.9	3.7	2.5
Inter-day repeatability						
	0.13	1.9	0.6	1.1	3.5	2.5

* Number of determinations (*n*); duloxetine hydrochloride (DH).

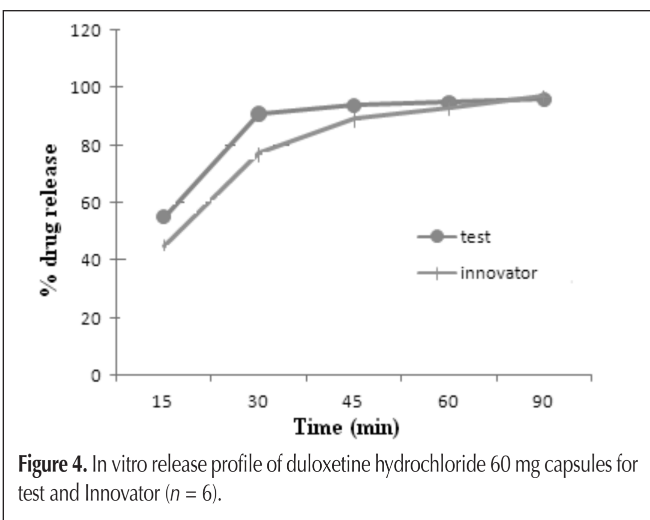

Figure 4. In vitro release profile of duloxetine hydrochloride 60 mg capsules for test and Innovator (*n* = 6).

innovator products were releasing in a similar way. The release of the total amount of duloxetine was achieved at 90 min for innovator product; test product was released completely at 45 min in the medium. Based these observations from test and innovator products, it could be concluded that initial time points up to 30 min were critical for in vitro dissolution mapping. This result was in agreement with the literature and the dissolution method mentioned above would be appropriate to simulate the in vitro release of duloxetine hydrochloride for innovator and test products.

Conclusions

The reproducible gradient RP-UPLC method developed for quantitative analysis of duloxetine hydrochloride, its related substances and study of in vitro dissolutions in pharmaceutical dosage forms is precise, accurate, linear, robust, rugged, and specific. Satisfactory results were obtained from validation of the method. The method is stability-indicating and can be used for routine analysis of production samples and to check the stability of samples of duloxetine hydrochloride.

References

1. P. Srinivasulu, K.S. Srinivas, R.S. Reddy, Mukkanti K, and R. Bucchireddy. A validated stability indicating rapid LC method for duloxetine HCl. *Pharmazie.* **64(1):** 10–3 (2009).
2. J. Yang, X. Lu, Y. Bi, F. Qin, and F. Li. Chiral separation of Duloxetine and its R –Enantiomer. *LC. Chromatogr.* **66:** 389–393 (2007).
3. M.M. Kamila, N. Mondal, and L.K. Ghosh. A validated UV spectrophotometric method for determination of duloxetine hydrochloride. *Pharmazie.* **62(6):** 414–415 (2007).
4. P.S. Selvan, V. Gowda, U. Mandal, W.D. Sam Solomon, and T.K. Pal. Determination of duloxetine in human plasma by liquid chromatography with atmospheric pressure ionization–tandem mass spectrometry and its application to pharmacokinetic study. *J. Chromatogr. B.* **858(1):** 269–275 (2007).
5. D.K. Satonin, J.D. McCulloch, F. Kuo, and M.P. Knadler. Development and validation of a liquid chromatography—tandem mass spectrometric method for the determination of the major metabolites of duloxetine in human plasma. *J. Chromatogr. B* **852(1):** 582–589 (2007).
6. P.J. Jansen, P.L. Oren, C.A. Kemp, S.R. Maple, and S.W. Baertschi. Characterization of impurities formed by interaction of duloxetine HCl with enteric Polymers hydroxypropyl methylcellulose acetate succinate and hydroxypropyl methylcellulose phthalate. *J. Pharm. Sci.* **87(1):** 81–5 (1998).
7. R. Russo, D. Guillarme, T-T. D. Nguyen, C. Bicchi, S. Rudaz, and J.L. Veuthey. Pharmaceutical applications on columns packed with sub-2 μm particles. *J. Chromatogr. Sci.* **46(3):** 199–208 (2008).
8. D.T. Nguyen, D. Guillarme, S. Rudaz, and J.L. Veuthey. Fast analysis in liquid chromatography using small particle size and high pressure. *J. Sep. Sci.* **29(12):** 1836–1848 (2006).
9. J.R. Mazzeo, U.V. Neue, K. Marianna, and R.S. Plumb. Advancing LC performance with smaller particles and higher pressure. *Anal. Chem.* **77(23):** 460A–467A (2005).
10. A.D. Villiers, F. Lestremau, R. Szucs, S. Gelebart, F. David, and P. Sandra. Evaluation of ultra performance liquid chromatography. Par I. Possibilities and limitations. *J. Chromatogr. A* **1127(1-2):** 60–69 (2006).
11. S.A.C. Wren and P. Tchelitcheff. Use of ultra-performance liquid chromatography in pharmaceutical development. *J. Chromatogr. A* **1119(1–2):** 140–146 (2006).
12. International Conference on Harmonization (1995) ICH guidelines on validation of analytical procedures: text and methodology Q2 (R1): FDA. *Federal Register* **60:**11260.
13. http://www.accessdata.fda.gov/scripts/cder/dissolution/index.cfm.
14. International Conference on Harmonization (1996) ICH guidelines on stability testing: Photo stability testing of new drug substances and products Q1B.

Manuscript received July 31, 2009;
revision received September 7, 2009.