Journal of Pharmaceutical and Biomedical Analysis 51 (2010) 994–997

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Determination of duloxetine hydrochloride in the presence of process and degradation impurities by a validated stability-indicating RP-LC method

N.V.V.S.S. Raman[a], K.A. Harikrishna[a], A.V.S.S. Prasad[a], K. Ratnakar Reddy[a], K. Ramakrishna[b,*]

[a] Hetero Drugs Ltd. (R&D), Plot No. B. 80 & 81, APIE, Balanagar, Hyderabad 500018, India
[b] GITAM Institute of Science, GITAM University, Visakhapatnam 530045, India

ARTICLE INFO

Article history:
Received 16 July 2009
Received in revised form
25 September 2009
Accepted 30 October 2009
Available online 6 November 2009

Keywords:
Determination
Duloxetine
Degradants
Validation
Stability-indicating method

ABSTRACT

A stability-indicating gradient reverse phase liquid chromatographic purity and assay method for duloxetine hydrochloride (DUH) was developed and validated. DUH was subjected to the stress conditions and it is sensitive towards oxidative, acid and hydrolytic degradation. Successful separation of DUH from its two process impurities and one degradation impurity formed under stress conditions was achieved on a Symmetry C18, 250 × 4.6 mm, 5 μm column using a gradient mixture of solvent A (0.01 M potassium dihydrogen orthophosphate having 0.2% triethyl amine, pH adjusted to 2.5 with orthophosphoric acid) and solvent B (20:80 v/v mixture of acetonitrile and methanol). The flow rate is 1 ml/min and the detection wavelength is 230 nm. The mass balance was found to be in the range of 99.2–99.7% in all the stressed conditions.

1. Introduction

The International Conference on Harmonization (ICH) guidelines [1–3] emphasizes that the purity and assay of drugs, 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 (DUH), chemically known as (+)-(*S*)-*N*-methyl-γ-(1-naphthyloxy)-2-thiophenepropylamine hydrochloride [4], is an antidepressant. Besides the reported impurities [5], we have observed a potential impurity (Imp-1) in our synthetic process and impurity-3 in forced degradation. Hence, a stability-indicating LC method for determination of DUH in presence of process and degradation impurities was developed and validated as per International Conference on Harmonization (ICH) guidelines [6].

2. Experimental

2.1. Chemicals and reagents

DUH and its three impurities *viz.* Imp-1, Imp-2 and Imp-3 (Fig. 1) were obtained from Hetero Drugs Ltd. R&D division. Potassium dihydrogen orthophosphate monohydrate, orthophosphoric acid, acetonitrile, methanol, hydrochloric acid, sodium hydroxide and hydrogen peroxide (30%) were obtained from Merck, Mumbai, India. All the solutions were prepared in Milli Q water (Millipore, USA).

2.2. HPLC instrumentation and conditions

Waters Alliance 62695 separation module (Waters Corporation, Milford, USA) equipped with 2489 UV/visible detector or 2998 PDA detector (for specificity and forced degradation studies) with Empower 2 software was used for the analysis. Symmetry C18 column (250 × 4.6 mm, 5 μm, Waters corporation, Milford, USA) and a gradient mixture of solvent A and B were used as stationary and mobile phases, respectively. Buffer contains 0.01 M potassium dihydrogen phosphate and 0.2% triethyl amine and its pH was adjusted to 2.5 with orthophosphoric acid. Buffer was used as solvent A. Acetonitrile and methanol in 20:80 v/v ratio was used as solvent B. The gradient program (T/%B) was set as 0/35, 22/68, 35/90, 40/90, 45/35 and 55/35. 1.0 ml/min flow rate and 10 μl injection volume were maintained. The eluted compounds were monitored at 230 nm. The column oven temperature was maintained at 30 °C.

2.3. Preparation of stock and standard solutions

Buffer and acetonitrile in the ratio of 40:60 v/v was used as diluent. 0.5 mg/ml and 0.1 mg/ml stock solutions of DUH were prepared in diluent for impurities assay determination, respectively. A blend

* Corresponding author. Tel.: +91 98662 34551; fax: +91 0891 2790032.
E-mail address: karipeddi_rk@yahoo.com (K. Ramakrishna).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.10.025

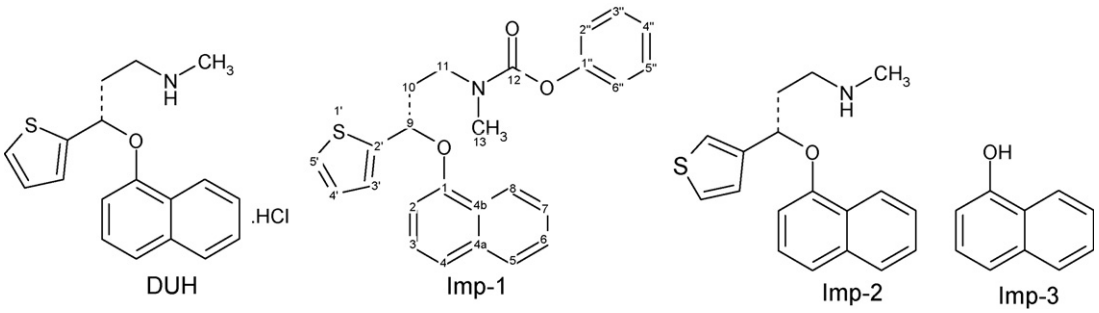

Fig. 1. Structures of DUH and its three impurities.

of three 0.15% DUH impurities was prepared in diluent with respect to 0.5 mg/ml of DUH.

3. Results and discussion

3.1. Characterization of Imp-1

FT-IR spectrum of Imp-1 contains absorption bands at 3053 (=C—H stretch), 2928 (—C—H stretch), 1720 (—C=O stretch), 1595 & 1578 (C=C stretch), 1462 and 1397 (—C—H bending), 1264 (C—N stretch), 1204 (C—O stretch) and 1094 and 1019 (=C—H in plane bending) and 772 and 690 cm^{-1} (=C—H out plane bending). ^1H NMR spectrum displays the peaks in ppm as δ 8.28–8.30 (d, 1H, H^8), 7.71–7.74 (d, 1H, H^5), 7.15–7.36 (m, 9H, $H^{2,3,4,6,7,2'',3'',5'',6''}$), 6.82–6.93 (m, 4H, $H^{3',4',5',4''}$), 5.85–5.86 (t, 1H, H^9), 3.51–3.80 (t, 2H, H^{11}), 2.98–3.09 (s, 3H, H^{13}) and 2.31–2.58 (q, 2H, H^{10}). ^{13}C NMR spectrum consists the peaks in ppm as 156.39–156.49 (C^{12}), 154.22–154.34 (C^1), 152.53–152.66 ($C^{1''}$), 145.97 ($C^{2'}$), 136.05 (C^{4a}), 130.14 ($C^{5',3'',5''}$), 128.50 ($C^{5,4b}$), 127.31–127.62 ($C^{3,6,4'}$), 125.95–126.68 ($C^{3',4'',7}$), 122.62–122.99 ($C^{4,8}$), 121.70 ($C^{2'',6''}$), 108.15–108.32 (C^2), 74.96–75.50 (C^9), 46.97–47.66 (C^{11}), 37.55–38.39 (C^{13}) and 35.17 (C^{10}). The mass spectrum shows a parent peak at *m/z* 417 and 440 (M + Na). The determined percentages of carbon (71.92%), hydrogen (5.55%), nitrogen (3.35%), oxygen (11.50%) and sulphur (7.68%) match with the values calculated from the molecular formula $C_{25}H_{23}NO_3S$.

3.2. HPLC method development

The HPLC method was optimized so as to obtain a stability-indicating method that can resolve two process impurities and one degradation impurity from DUH. 0.01 M potassium dihydrogen orthophosphate was initially chosen as buffer. The pH of buffer was studied over a range (pH 2.5–6.0) and buffer having pH 2.5 was adopted, because, it was suitable to separate the impurities and degradants from DUH. However, tailing for DUH peak was observed and hence 0.2% triethyl amine was introduced to buffer to reduce it. Initially, 90% acetonitrile in water was used as solvent B. The resolution between Imp-2 and DUH was less than 1.5. Hence, acetonitrile was replaced with methanol and Imp-1 was eluted at longer retention time. Therefore acetonitrile and methanol in the ratio of 20:80 v/v was used as solvent B. Among different makes of C18 columns tried, Symmetry C18 (250 × 4.6 mm, 5 μm) column allowed a rapid resolution between Imp-2 and DUH and showed the best values of theoretical plates and asymmetry for DUH. A typical chromatogram of DUH spiked with 0.15% of impurities is shown in Fig. 2.

3.3. Method validation

The developed method was validated as per ICH guidelines [6] and the results are given in Table 1. 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 LC method for DUH was determined in the presence of its process and degradation impurities. All the stressed samples of DUH were spiked with its impurities (0.15% with respect to DUH concentration) and all the process and degradation impurities were well resolved from one another and from DUH. The analysis was carried out by HPLC with PDA detector. The chromatographic peak purity tool, applied for DUH and its impurities peaks, demonstrated that all the peaks were pure in all cases, confirming 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 quantitation limit (QL) for Imp-1, Imp-2 and Imp-3 were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentration. Precision study was carried at specification and QL level by injecting six individual preparations of Imp-1, Imp-2 and Imp-3 and calculating the percentage of R.S.D. of the area. Assay precision was evaluated by carrying out six independent assays of test concentration of DUH against qualified reference standard. The percentage of R.S.D. of six assay values was found as 1.6. The intermediate precision in all the cases was also evaluated on six different days. Linearity test solutions for purity determination were at six concentration levels from QL to 200% of the specification level (0.15%). Linearity test solutions for assay determination were prepared from stock solution at five concentration levels from 50% to 150% of assay analyte concentration (0.1 mg/ml). The peak area versus concentration data was performed by least-squares linear regression analysis. Standard addition and recovery experiments were conducted to determine accuracy of the impurities quantitation in bulk drug samples. The study was carried out in triplicate at QL, 100% and 150% level with respect to specification 0.15%. The percentage of recoveries for Imp-1, Imp-2 and Imp-3 were calculated. The accuracy of DUH assay was evaluated in triplicate at three concentration levels *viz.* 50%, 100% and 150% with respect to 0.1 mg/ml of DUH test concentration. The assay of DUH ranged from 99.9% to 100.2%. The robustness of the developed method was determined by altering experimental

Table 1
Validation data of the developed method.

Parameter	Imp-1	Imp-2	Imp-3
DL (%)	0.006	0.006	0.003
QL (%)	0.02	0.02	0.01
Precision (% RSD)#	3.2	0.3	0.3
Intermediate precision (% RSD)#	1.8	0.4	0.2
Accuracy[a] (% recovery) at			
QL	103.3 ± 1.56	103.7 ± 1.65	106.7 ± 1.93
100%	98.4 ± 0.72	98.3 ± 0.49	103.3 ± 0.52
150%	91.7 ± 0.45	98.2 ± 0.72	102.8 ± 0.85

[a] Carried at QL, 100% and 150% level with respect to specification (0.15%).

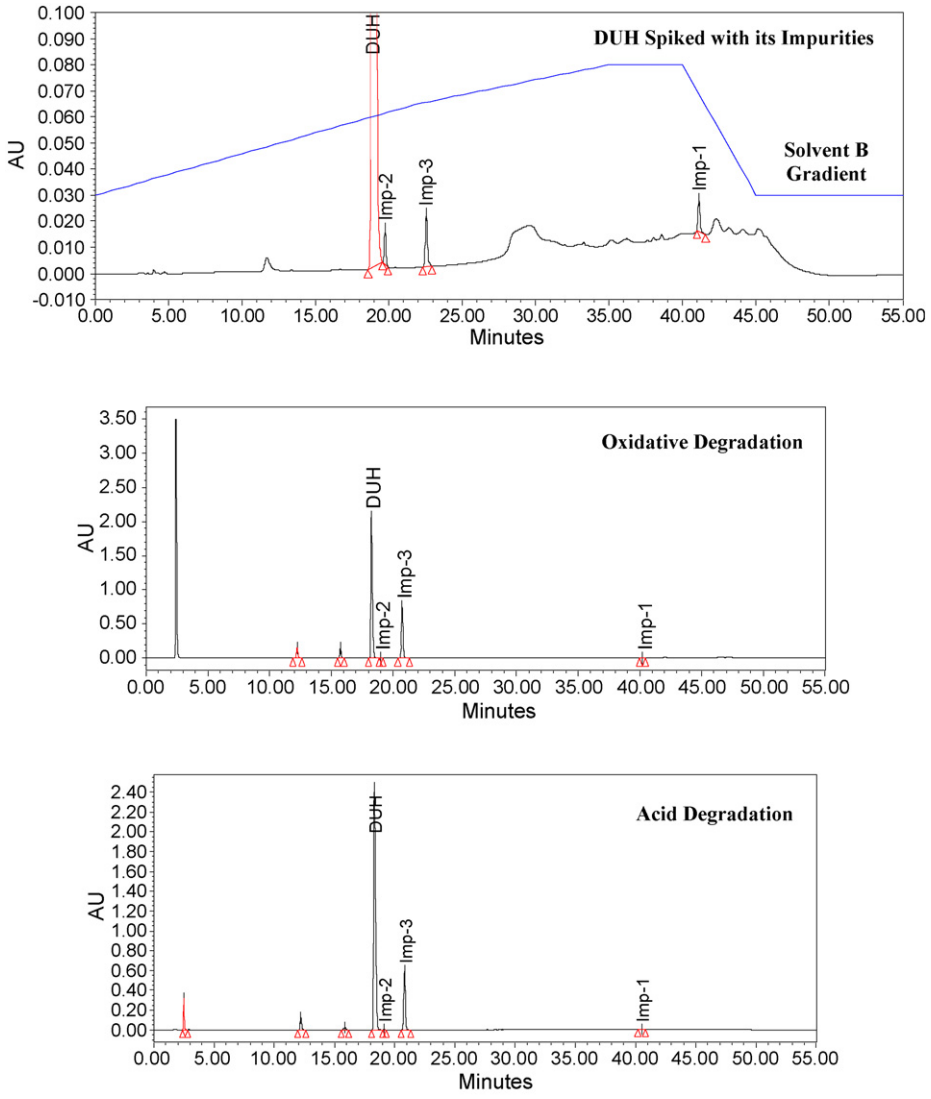

Fig. 2. Typical chromatograms of DUH spiked with its impurities and its oxidative and acid degradation samples.

conditions purposely and evaluating the resolution between DUH, Imp-1, Imp-2 and Imp-3. Flow rate was changed by 0.2 units, pH was varied by ± 0.2 units and column temperature was studied at 28 °C and 32 °C instead of 30 °C. In all the 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 Imp-1, Imp-2, Imp-3 and DUH assay during solution and mobile phase stability experiments. The stability data confirmed that sample solutions were stable up to 48 h. The system suitability was established in terms of resolution between Imp-2 and DUH which was more than 2.0, when a 0.5 mg/ml DUH solution spiked with 0.15% of Imp-2 was injected.

3.4. Forced degradation studies results

The stability-indicating power of the developed method was studied by conducting forced degradation studies on DUH. Forced degradation samples were injected at regular intervals and the final stress conditions were established so as to obtain 5–20% degra-

Table 2
Summary of forced degradation studies.

Stress type	% Degradants formed					% Assay	Mass balance (%)
	Imp-1	Imp-2	Imp-3	MSUI	Total		
Unstressed	0.03	0.07	0.01	0.01	0.12	–	–
Oxidative degradation	0.03	0.09	10.57	3.56	18.12	81.1	99.2
Acid degradation	0.08	0.10	7.99	3.60	14.94	84.5	99.4
Base degradation	0.06	0.10	0.01	0.04	0.21	99.5	99.7
Hydrolytic degradation	0.03	0.11	0.65	1.38	3.11	96.4	99.5
Thermal degradation	0.03	0.07	0.02	0.02	0.20	99.5	99.7
Photolytic degradation	0.03	0.07	0.02	0.02	0.18	99.5	99.7

MSUI: Maximum single unknown impurity.

dation of DUH. LC–MS/MS system (Waters 2695 Alliance liquid chromatograph coupled with Quattro micro mass spectrometer with MassLynx software, Waters Corporation, Milford, USA) was also used for the identification of unknown compounds formed during forced degradation studies. DUH was degraded and resulted in the formation of Imp-3, when it was exposed to oxidative degradation in 20% hydrogen peroxide at 80 °C for 1 h or acid degradation in 0.1N hydrochloric acid at 80 °C for 15 min or hydrolytic degradation in water at 80 °C for 2 h. When DUH was exposed to base degradation in 1N NaOH at 80 °C for 2 h or kept in oven at 80 °C for 7 days or in photo stability chamber/200 Wh/m^2 in UV light and 1.2 million lux hours in visible light for 7 days, it did not give any degradation products. The mass balance of stressed samples was in the range of 99.2–99.7% (Table 2).

4. Conclusions

A stability-indicating HPLC method has been developed and validated for the purity and assay determination of DUH. The behavior of DUH under various stress conditions was studied. The advantage of this method is that it can resolve the DUH and its positional isomer (Imp-2). Since the method is able to separate the DUH from its process and degradation impurities, it can be conveniently applied for the testing of batch release and stability studies.

Acknowledgement

The authors are highly thankful to Dr. Bandi Parthasarathi Reddy, CMD, Hetero Drugs Limited, Hyderabad for providing facilities.

References

[1] International Conference on Harmonisation Guideline on Stability Testing of New Drug Substances and Products, Q1A (R2), 2003.
[2] International Conference on Harmonisation Guideline on Photostability Testing of New Drug Substances and Products, Q1B, 1996.
[3] International Conference on Harmonisation Guideline on Impurities in New Drug Products, Q3B (R2), 2006.
[4] The Merck Index, Merck & Co., Inc., Whitehouse Station, NJ, USA, 2006, 14:587.
[5] P. Srinivasulu, K.S. Srinivas, R.S. Reddy, K. Mukkanti, R. Buchireddy, Pharmazie 64 (2009) 10–13.
[6] International Conference on Harmonisation Guideline on Validation of Analytical Procedures: Text and Methodology, Q2 (R1), 2005.