CHARACTERIZATION OF DULOXETINE HCL API AND ITS PROCESS RELATED IMPURITIES

Evrykleia Karagiannidou*, Elli Vastardi Theodoros Panagiotidis, Theodoros Tsatsas, Efstratios Neokosmidis
Pharmathen, API Operations Unit, Building Thermi 1, 9th km Thessaloniki – Thermi, P.O. Box 60499, GR 570 01, Thessaloniki, Greece.

*corresponding author: Email: egkaragiannidou@pharmathen.com

ABSTRACT: The characterization of process related impurities associated with the synthesis of Duloxetine HCl active pharmaceutical ingredient was performed. This paper discusses the structure elucidation of Duloxetine HCl and its impurities on the basis of 1H-NMR, 13C-NMR, IR, Mass Spectroscopy and High Resolution Mass Spectroscopy. The polymorph of Duloxetine hydrochloride API was characterized with X-Ray Powder Diffraction analysis, Differential Scanning Calorimetry and Hot Stage Polarizing Optical Microscopy. A selective and stability indicating reversed phase high performance liquid chromatographic method (RP-HPLC) with PDA detector was developed for the purity determination of Duloxetine hydrochloride Active Pharmaceutical Ingredient in the presence of its ten process related impurities. Chromatography was carried out on a Symmetry C18 (250*4.6mm, 5µm) column, using a gradient elution of A: buffer/methanol / acetonitrile (35:52:13v/v/v) and B: methanol/ acetonitrile (80:20v/v) at a flow rate of 1.0ml/min.

Key words: Method development, Characterization, Impurities, Duloxetine HCl, RP-HPLC, Polymorphism, Microscopy.

INTRODUCTION
Duloxetine hydrochloride (N-Methyl-3-naphthalen-yloxy-3-thiophen-2-yl-propan-1-amine) is a selective serotonin and norepinephrine reuptake inhibitor (SSNRI) and it has been approved by the US Food and Drug administration for the treatment of major depressive disorder and anxiety. It is used for the treatment of neuropathic pain associated with peripheral neuropathy especially diabetic polyneuropathy for which it is first-line, and as an add-on treatment in stress urinary incontinence instead of surgery also indicated for the management of fibromyalgia. It restores the balance of neurotransmitters in the brain like serotonin and norepinephrine. Additionally it is also being used in the treatment of peripheral neuropathy caused by certain anticancer drugs [1-8]. The determination of a drug substance impurity profile, including potential degradation products and process-related impurities, is critical for the safety assessment of API and manufacturing process thereof. According to the guidelines issued by the International Conference on Harmonization (ICH) and European Pharmacopeia it is mandatory to identify and characterize the impurities in a pharmaceutical product if present above the accepted limits of 0.1% [9, 10]. A literature survey revealed different analytical methods for the analysis of Duloxetine hydrochloride API and its key intermediates/process related impurities by HPLC as well as pharmaceutical formulations of Duloxetine hydrochloride [11-31]. Reports were found regarding the characterization of phenolic impurities in Duloxetine samples by MS, NMR spectrometry and X-ray analysis and of impurities formed by interaction of Duloxetine with various enteric polymers. The proposed HPLC method has been also validated and applied for the determination of 1-fluoronaphthalene, key starting material for the synthesis of Duloxetine HCl API [32].

The aim of the present work is the characterization of Duloxetine hydrochloride and its process related impurities by infrared and nuclear magnetic resonance spectroscopy (IR, NMR), mass spectrometry (MS) / high resolution mass spectrometry (HRMS) and high performance liquid chromatography. Additionally, Duloxetine hydrochloride polymorph was characterized with X-Ray Powder Diffraction analysis (XRPD), Differential Scanning Calorimetry (DSC), Thermogravimetric analysis (TGA) and Hot Stage Polarizing Optical Microscopy.
EXPERIMENTAL

Materials and reagents

The HPLC grade solvents (acetonitrile, ACN and methanol MeOH) and analytical grade triethylamine (TEA), ortho phosphoric acid (H₃PO₄) and potassium dihydrogen phosphate (KH₂PO₄) were purchased from Merck (Germany). Deionized water was prepared by using Milli-Q plus purification system (Millipore). Duloxetine hydrochloride API (Purity 99.9%), (1S)-3-(Methylamino)-1-(thiophen-2-yl)propan-1-ol (Impurity B, Purity 99.98%), 4-[(1RS)-3-(Methylamino)-1-(thiophen-2-yl)propyl]naphthalene-1-ol (Impurity C, Purity 99.7%), 2-[(1RS)-3-(methylamino)-1-(thiophen-2-yl)propyl]naphthalene-1-ol (Impurity E, Purity 97.7%), (3RS)-N-Methyl-3-(naphthalene-1-yloxy)-3-(thiophen-3-yl)propan-1-amine HCl (Impurity F, Purity 97.1%) were synthesized in Pharmathen (API Operations Unit, Thessaloniki, Greece). 1-fluoronaphthalene was purchased from Sigma Aldrich and was recrystallized (Impurity G, Purity 99.9%). Naphthalen-1-ol (Impurity D) was purchased from Sigma Aldrich (33420, Purity 99.0%), 1-aminonaphthalene from Fluka (1360195, Purity 99.5%), 2-fluoronaphthalene from Supelco (LB61093, Purity 99.9%), naphthalene from Acros Organics (A0285904, Purity 99.0%) and 1-nitronaphthalene from Sigma Aldrich (103594, Purity 99.9%).

Chromatographic system

The chromatography was carried out using the Shimatzu Prominence HPLC system with photodiode array detector. The chromatographic separation was performed using a Symmetry C18 column (250*4.6mm i.d., 5µm, Waters). The mobile phase consisting of A (0.01M KH₂PO₄ buffer with 2ml triethylamine and pH adjustment at 2.5±0.1/MEOH/ACN, 35:52:13v/v/v, and B containing (MeOH/ACN, 80:20v/v) with the following time gradient program T (min)/B (%): 0/0, 22/35, 35/50, 55/50, 70/0, 85/0 and the flow rate of 1.00mL/min. The mobile phase solutions were filtered through a 0.45µm filter. The injection volume was 10µl and the detector wavelength was fixed at 230nm. The column temperature was kept at 20°C and the autosampler temperature at 5°C. The analyzed samples were dissolved and diluted in ACN/H₂O, (60:40v/v); the concentration was about 5.0mg/mL.

Preparation of stock solutions and standard solutions

25mg accurately weighed of Duloxetine hydrochloride API, (1S)-3-(Methylamino)-1-(thiophen-2-yl)propan-1-ol (Impurity B), 4-[(1RS)-3-(Methylamino)-1-(thiophen-2-yl)propyl]naphthalene-1-ol (Impurity C), 2-[(1RS)-3-(methylamino)-1-(thiophen-2-yl)propyl]naphthalene-1-ol (Impurity E), (3RS)-N-Methyl-3-(naphthalene-1-yloxy)-3-(thiophen-3-yl)propan-1-amine HCl (Impurity F), naphthylamine, 1-nitronaphthalene, naphthalene, 2-fluoronaphthalene, 1-fluoronaphthalene (Impurity G) and 1-naphthol (Impurity D) were diluted separately with 50mL acetonitrile: water 60: 40v/v to give separate solutions of 500µg/mL of Impurities B, C, D, E, F, G, naphthylamine, 1-nitronaphthalene, naphthalene, 2-fluoronaphthalene and Duloxetine hydrochloride API (stock solutions). With appropriate dilutions standard solution of Duloxetine HCl and Impurities in the concentration of 0.1% was obtained.
Nuclear magnetic resonance (NMR) spectroscopy
All the NMR measurements were performed on a Bruker DRX 500MHz spectrometer at N.C.S.R. Demokritos, Institute of Physical Chemistry, Athens. The $^1$H and $^{13}$C spectral data are given in relation to the TMS signal at 0.0ppm. The concentration of all solutions used for the measurements was about 20-30mg of compounds in the 0.6cm$^3$ of solvent.

Fourier Transform Infrared Spectroscopy (FT-IR)
FT-IR spectra were recorded on a Perkin Elmer instrument, Spectrum One model. The solids were ground with KBr and discs were prepared under compressed pressure. The spectra were collected in the range 400-4000cm$^{-1}$, by averagin 16scans with a resolution of 4cm$^{-1}$ with DTGS detector.

Mass Spectroscopy (MS) and High Resolution Mass Spectroscopy (HRMS)
The LC-MS analysis was performed on a Schimatzu quardropole LC-MS-2010EV with two Prominence LC pumps (LC-20AD), a degasser (DGU-20A3), a diode array detector (SPD-M20A), an autosampler (SIL-20AC) and a column oven (CTO-20AC). A 4.6*100mm C18 5µm analytical column (Symmetry Shield, Waters, USA) was used for the chromatographic separation. Both the autosampler and the column oven temperature were set at 25°C. The mobile phase had a flow rate of 0.8ml/min and consisted of methanol and water in the proportion of 90:10, v/v respectively. All samples were eluted isocratically. The Schimatzu mass selective detector operated in atmospheric pressure chemical ionization (APCI) and/or electron spray ionization (ESI) and scanning monitoring was performed in positive and negative polarity, with scan speed at 4000amu/sec. The following mass parameters were applied: detector voltage at 1.7kV, drying gas at 0.02MPa, Interface temperature at 400°C, CDL temperature at 250°C, Heat block temperature at 200°C and nebulizing gas flow at 2.5L/min. High resolution mass spectrometry was performed on a Bruker BioApex II ESI-FTICR Instrument with a 7.4 Tesla Magnet at Universitat Leipzig, Inst. f. Analyt. Chemie, Leipzig Germany.

X-Ray Powder Diffraction Analysis (XRPD)
The XRPD were recorded at room temperature using the D500 Siemens with Cu Ka radiation ($\lambda_1 = 1.54060$, $\lambda_2 = 1.54439$), running at 40kV and 30mA. The 2theta range was covered from 5.00 to 40.00 degrees with a step size of 0.040 degrees and scan step of 2.00 second per each step.

Differential Scanning Calorimetry (DSC)
Differential Scanning Calorimetry (DSC) measurements were made on a Perkin Elmer, Diamond DSC instrument using aluminum sealed pans. A constant nitrogen flow (50ml/min) was maintained to provide a constant thermal blanket within the DSC cell, thus eliminating thermal gradients and ensuring the validity of the applied calibration standard from sample to sample. The instrument was calibrated with high purity indium and zinc standards. Samples of about 10mg were used. The scans were recorded between 25°C and 250°C at a constant heating rate of 10°C/min.

Thermogravimetric Analysis (TGA)
Thermogravimetric analysis (TGA) measurements were made on a Perkin Elmer, Pyris 1 TGA thermal analyzer. The scans were recorded between 25°C and 250°C, under a 20ml/min nitrogen flow and at a constant heating rate of 10°C/min. The %weight loss was calculated using Pyris 1 TGA software.

Hot Stage Polarizing Optical Microscopy (HSPOM)
A polarizing light microscope (Nikon, Optiphot-2) equipped with a Linkam THMS 600 heating stage, a Linkam TP 91 control unit and a Jenoptic Progress C10 plus camera with the Capture Pro 2.1 software was used for polarizing light microscopy (PLM) observations.

RESULTS AND DISCUSSION
Structural confirmation of Duloxetine HCl and its impurities by NMR, FT-IR and Mass Spectrometry
During the evaluation of the manufacturing process all compounds described in Table 1 were taken into account as potential impurities of the final Duloxetine hydrochloride Active Pharmaceutical Ingredient. A detailed analysis and comparison of $^1$H-NMR and $^{13}$C-NMR spectra of all investigated compounds, as well as the mass spectral data, were crucial for their structure elucidation. The analysis of the NMR, FT-IR and mass data confirmed the structure of Duloxetine hydrochloride and its relevant impurities. The characteristic IR band positions, NMR shifts and mass spectral results for all studied compounds are tabulated (Tables 2, 3 and 4).
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<th>Structural formula</th>
<th>Chemical Formula/MW</th>
<th>Name</th>
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<td>(1S)-3-(Methylamino)-1-(thiophen-2-yl)propan-1-ol (Impurity B)</td>
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<tr>
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<td>C₁₀H₇OH</td>
<td>1-naphthol (Impurity D)</td>
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<td>C₁₈H₁₉NOS</td>
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<td>C₁₈H₂₀CINOS</td>
<td>(3RS)-N-Methyl-3-(naphthalene-1-yloxy)-3-(thiophen-3-yl)propan-1-amine HCl (Impurity F)</td>
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<td>C₁₀H₇F</td>
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<td>C₁₀H₉N</td>
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<td>C₁₀H₇NO₂</td>
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<td></td>
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<td>2-fluoronaphthalene</td>
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Chromatography

The developed HPLC method allowed the determination of the chemical purity of the final API product of Duloxetine hydrochloride. The specificity and selectivity of the HPLC method was determined by analyzing the Duloxetine hydrochloride sample containing impurities at the level of 0.1%. A representative chromatogram generated to demonstrate the specificity and selectivity of the HPLC method is shown in Figure 1. The resolution between Duloxetine HCl and impurities peaks meets the established criterion (>1.5). The detection limit was estimated by the analysis of the spiked solution of Duloxetine hydrochloride with impurities at the level of 0.1% (Table 5). The stability-indicating status of the developed HPLC method was demonstrated based on the force degradation studies of Duloxetine hydrochloride.

Table 2: NMR assignment for Duloxetine HCl API and its Impurities

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<tr>
<th>Compound</th>
<th>Chemical shift δ ppm</th>
<th>Multiplicity</th>
<th>Integration</th>
<th>Assignment</th>
<th>¹H-NMR</th>
<th>Assignment</th>
<th>¹³C-NMR</th>
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<td></td>
<td></td>
<td>ppm</td>
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<td>1.89-1.96</td>
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<td>2H</td>
<td>H-2</td>
<td>35.9</td>
<td>C-2</td>
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<td></td>
<td>2.35</td>
<td>Singlet</td>
<td>3H</td>
<td>H-6</td>
<td>37.2</td>
<td>C-6</td>
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<td>2.73-2.86</td>
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<td>H-4</td>
<td>49.8</td>
<td>C-4</td>
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<tr>
<td></td>
<td>3.94</td>
<td>Singlet</td>
<td>1H</td>
<td>H-3, H-5</td>
<td>71.2</td>
<td>C-1</td>
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<td></td>
<td>5.07-5.13</td>
<td>Doublet of doubles $(J_1=8.0, J_2=3.8Hz)$</td>
<td>1H</td>
<td>H-1</td>
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<td>C-10</td>
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<td>6.87-6.93</td>
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<td>H-8, H-9</td>
<td>126.4</td>
<td>C-9</td>
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<td>7.14-7.16</td>
<td>Doublet of doubles $(J_1=4.9, J_2=1.2Hz)$</td>
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<td>H-10</td>
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<td>C-7</td>
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<td>C-6</td>
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<td>2.65</td>
<td>Singlet</td>
<td>3H</td>
<td>H-6</td>
<td>34.4</td>
<td>C-1</td>
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<td>5.03-5.06</td>
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<td>6.82-6.84</td>
<td>Triplet $(J=7.62Hz)$</td>
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<td>6.92-6.94</td>
<td>Doublet $(J=7.87Hz)$</td>
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<td>H-9</td>
<td>124.0</td>
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<td>Doublet $(J=3.10Hz)$</td>
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<td>H-18</td>
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<td>H-20</td>
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Available online at [www.ijapbs.com](http://www.ijapbs.com)
| H & 1-H & 3-H & 4-H | H-7 & H-10 & H-13’ & H-21,H-13 | 33.2 & C-7 |
|---|---|---|---|---|---|---|---|---|---|
| 5.70-5.72 | Doublet of doublets $(J=4.67, J_1=7.27Hz)$ | 1-H | H-6 | 109.4 | C-2 |
| 6.71-6.73 | Doublet $(J=5.65Hz)$ | 1-H | H-17 | 109.7 | C-2 |
| 7.07-7.08 | Doublet $(J=4.24Hz)$ | 3-H | H-1,H-15,H16 | 120.6 | C-9 |
| 7.21-7.24 | Doublet $(J=8.21Hz)$ | 1-H | H-4 | 120.7 | C-9 |
| 7.26-7.28 | Doublet $(J=3.2Hz)$ | 2-H | H-19,H-20 | 123.8 | C-4 |
| 7.47-7.52 | Doublet $(J=5.65Hz)$ | 1-H | H-21 | 123.8 | C-4 |
| 7.77-7.78 | Doublet $(J=7.2Hz)$ | 1-H | H-18 | 124.0 | C-10 |
| 8.30-8.32 | Doublet $(J=4.2Hz)$ | 1-H | H-3 | 125.7 | C-3 |

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<td>33.1 &amp; C-21</td>
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<td>H-13’</td>
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<td>34.9 &amp; C-13</td>
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<td>1-H</td>
<td>H-12</td>
<td>46.2 &amp; C-19</td>
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<tr>
<td>7.26-7.28</td>
<td>Singlet</td>
<td>1-H</td>
<td>H-5,H-15</td>
<td>122.0</td>
<td>C-7</td>
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<tr>
<td>7.47-7.52</td>
<td>Doublet $(J=8.2Hz)$</td>
<td>2-H</td>
<td>H-10</td>
<td>125.5</td>
<td>C-17</td>
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<tr>
<td>7.77-7.78</td>
<td>Doublet $(J=6.5Hz)$</td>
<td>1-H</td>
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<td>C-15</td>
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<tr>
<td>8.29-8.30</td>
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<td>2-H</td>
<td>H-20 (±NH2)</td>
<td>125.8</td>
<td>C-16,C-4</td>
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<td>9.81</td>
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<td>126.0</td>
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International Journal of Analytical, Pharmaceutical and Biomedical Sciences
Available online at www.ijapbs.com
### COSY-NMR

<table>
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<th>Assignment</th>
<th>COSY Signal</th>
<th>Assignment</th>
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<td>2.45-2.62, 5.06-5.12</td>
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<td>H-2, H-4’</td>
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<td>2.45-2.62</td>
<td>H-6, H-2’, H-4</td>
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<td>H-1</td>
<td>2.15-2.24</td>
<td>H-2</td>
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<td>H-21, H-5</td>
<td>7.14-7.16</td>
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<td>H-8, H-9</td>
<td>7.23-7.26</td>
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<td>6.93-6.94</td>
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<td>2.55-2.59</td>
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<td>7.21-7.24</td>
<td>H-16</td>
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<td>6.71-6.73</td>
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<td>H-19, H-20</td>
<td>7.77-7.8, 8.30-8.32</td>
<td>H-21, H-18</td>
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<td>7.47-7.52</td>
<td>H-20</td>
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<td>H-18</td>
<td>7.47-7.52</td>
<td>H-19</td>
</tr>
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<td>H-20 (-+NH2)</td>
<td>2.64-2.65, 3.19-3.21</td>
<td>H-13’, H-19, H-12, H-20</td>
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<td>H-13’</td>
<td>2.64-2.65, 3.19-3.21, 5.95-5.97, 9.81</td>
<td>H-21, H-13, H-19, H-12</td>
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<td></td>
<td>H-19</td>
<td>2.64-2.65, 2.80-2.84, 9.81</td>
<td>H-21, H-13, H-13’, H-20</td>
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<td></td>
<td>H-12</td>
<td>2.64-2.65, 2.80-2.84</td>
<td>H-21, H-13, H-13’</td>
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<td>H-3</td>
<td>6.88-6.90, 7.17-7.18</td>
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<td>H-17</td>
<td>7.24-7.28</td>
<td>H-16</td>
</tr>
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<td></td>
<td></td>
<td>H-8, H-9</td>
<td>7.77-7.78, 8.29-8.30</td>
<td>H-10, H-7</td>
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<td></td>
<td></td>
<td>H-10</td>
<td>7.49-7.51</td>
<td>H-8, H-9</td>
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<td>H-7</td>
<td>7.49-7.51</td>
<td>H-8, H-9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H-20 (-+NH2)</td>
<td>2.64-2.65, 3.19-3.21</td>
<td>H-21, H-13, H-19</td>
</tr>
</tbody>
</table>

---

### Table 3 FT-IR bands for Duloxetine HCl API and its Impurities

<table>
<thead>
<tr>
<th>Compound</th>
<th>Typical IR bands (cm⁻¹)</th>
<th>Interpretation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>3376</td>
<td>Amine N-H stretching vibrations</td>
</tr>
<tr>
<td></td>
<td>3289</td>
<td>Phenol O-H stretching vibrations (non bonded)</td>
</tr>
<tr>
<td></td>
<td>3102, 2941</td>
<td>Aromatic C=H stretching vibrations</td>
</tr>
<tr>
<td></td>
<td>2892-2642</td>
<td>Aliphatic C-H stretching vibrations</td>
</tr>
<tr>
<td></td>
<td>1490</td>
<td>Aromatic C=C stretching vibrations</td>
</tr>
</tbody>
</table>
Amine N-H stretching vibrations
Phenol O-H stretching vibrations (non bonded)
Aromatic C=H stretching vibrations
Aliphatic C-H stretching vibrations
Aromatic C=C stretching vibrations

Table 4: Mass Spectral results

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical formula</th>
<th>Exact Mass</th>
<th>Mass Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="C8H13NNaOS+" alt="Image" /></td>
<td>[M+Na]+</td>
<td>Calculated 194.06101</td>
<td>[(194.06101-194.06088)/194=0.0000007=0.7ppm]</td>
</tr>
<tr>
<td><img src="C16H26N2NaO2S2" alt="Image" /></td>
<td>[2M+Na]+</td>
<td>Calculated 365.13279</td>
<td>[(365.13279-365.13256)/365=0.0000006=0.6ppm]</td>
</tr>
<tr>
<td><img src="C18H20NOS" alt="Image" /></td>
<td>[M+H]+</td>
<td>Calculated 298.12601</td>
<td>[(298.12601-298.12625)/298=0.0000008=0.8ppm]</td>
</tr>
</tbody>
</table>
The stress studies conducted under alkaline conditions (at 80°C for 1 hour with 1 M NaOH), under daylight and UV light exposure for 24 hours and under thermal conditions (for 24 hours at 105°C, without vacuum) revealed no change in the purity of the final API. The stress studies conducted under acid conditions (with 1 M HCl at 80°C for 1 hour) showed the complete decomposition of Duloxetine hydrochloride mainly to Impurities C, D and E, whereas the stress studies under oxidative conditions (3% w/v H₂O₂, at 80°C for 1 hour) revealed the degradation of Duloxetine hydrochloride to mainly Impurities B, C and D.

Figure 1: Representative chromatogram of Duloxetine HCl and Impurities
X-Ray Powder Diffraction Analysis

X-Ray powder diffraction analysis was performed on Duloxetine hydrochloride Active pharmaceutical ingredient. The crystalline form of Duloxetine hydrochloride obtained (Figure 2, Table 6) is matching with Form I reported in the Research Disclosure by Synthon BV [33, 34]. According to the stability data the crystalline form remains stable under normal and accelerated conditions for a period of 24 and 6 months respectively (Figure 3, Table 7).

**Table 6: Characteristic peaks of Duloxetine Hydrochloride API polymorph**

<table>
<thead>
<tr>
<th>2theta value</th>
<th>d-spacing</th>
<th>% Intensity</th>
</tr>
</thead>
<tbody>
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<td>9.64</td>
<td>9.17</td>
<td>6.3</td>
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<tr>
<td>13.9</td>
<td>6.36</td>
<td>11.6</td>
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<tr>
<td>14.49</td>
<td>6.11</td>
<td>6.0</td>
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<tr>
<td>16.02</td>
<td>5.53</td>
<td>5.6</td>
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<tr>
<td>18.07</td>
<td>4.90</td>
<td>17.4</td>
</tr>
<tr>
<td>18.88</td>
<td>4.70</td>
<td>30.1</td>
</tr>
<tr>
<td>20.92</td>
<td>4.24</td>
<td>100</td>
</tr>
<tr>
<td>22.15</td>
<td>4.01</td>
<td>8.3</td>
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<tr>
<td>23.35</td>
<td>3.81</td>
<td>20.1</td>
</tr>
<tr>
<td>26.44</td>
<td>3.37</td>
<td>10.7</td>
</tr>
<tr>
<td>27.99</td>
<td>3.18</td>
<td>44.5</td>
</tr>
</tbody>
</table>

**Table 7: Characteristic peaks of Duloxetine HCl at zero time and stability 24 months**

<table>
<thead>
<tr>
<th>2theta value</th>
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<tbody>
<tr>
<td>Zero time</td>
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<td>13.90</td>
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<tr>
<td>22.15</td>
</tr>
<tr>
<td>23.35</td>
</tr>
<tr>
<td>26.44</td>
</tr>
<tr>
<td>27.99</td>
</tr>
</tbody>
</table>
Thermal Analysis (DSC/TGA)

The thermal analysis was performed for Duloxetine hydrochloride API. The DSC curve of Duloxetine hydrochloride is shown in Figure 5 with the onset melting point at 169.12°C. Duloxetine hydrochloride exhibited an endothermic peak at 175.49°C due to melting, which was followed by an exothermic peak at 190.26°C (Figure 4). Upon thermogravimetric analysis there was no weight loss of Duloxetine hydrochloride up to 200°C and after 200°C decomposition of the material was observed (Figure 5).
In order to explain the exothermic peak of Duloxetine hydrochloride above its melting condition, a Nikon Hot Stage Polarizing Optical Microscope with Digital Camera was used. In Figure 6 images from the Hot Stage Polarizing Microscope are illustrated during the heating of Duloxetine hydrochloride reference standard with a heating rate of 10°C/min. After the heating of the compound up to 200°C, cooling was performed up to room temperature (25°C) and reheating of the compound. During the second heating a new melting point was not observed, confirming our first assumption that the exothermic peak observed after the melting point of the compound at 175.49°C was due to decomposition.
Figure 6: Representative crystal images from Hot Stage Polarizing Optical microscope of Duloxetine HCl API upon heating.

Table 5: The chromatographic parameters for the mixture solution of Duloxetine HCl and its impurities at the level of 0.1%.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time (min)</th>
<th>Resolution</th>
<th>Tailing Factor</th>
<th>Theoretical Plates</th>
<th>Signal to noise (s/n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1S)-3-(Methylamino)-1-(thiophen-2-yl)propan-1-ol (Impurity B)</td>
<td>2.75</td>
<td>0.0</td>
<td>1.24</td>
<td>4712</td>
<td>251</td>
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<tr>
<td>4-[(1RS)-3-(Methylamino)-1-(thiophen-2-yl)propyl]naphthalene-1-ol (Impurity C)</td>
<td>18.00</td>
<td>9.1</td>
<td>1.04</td>
<td>51290</td>
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<tr>
<td>1-naphthol (Impurity D)</td>
<td>28.44</td>
<td>2.4</td>
<td>1.02</td>
<td>102842</td>
<td>333</td>
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<tr>
<td>2-[(1RS)-3-(methylamino)-1-(thiophen-2-yl)propyl]naphthalene-1-ol (Impurity E)</td>
<td>22.96</td>
<td>15.2</td>
<td>1.03</td>
<td>74898</td>
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<tr>
<td>3RS)-N-Methyl-3-(naphthalene-1-yloxy)-3-(thiophen-3-yl)propan-1-amine HCl (Impurity F)</td>
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<td>3.5</td>
<td>1.05</td>
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<tr>
<td>1-fluoronaphthalene (Impurity G)</td>
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<td>0.93</td>
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<td>10.5</td>
<td>1.06</td>
<td>102895</td>
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<td>1.03</td>
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<td>2-fluoronaphthalene</td>
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</table>

CONCLUSION

Structure elucidation of the main process related impurities was discussed and the polymorph characterization of the final Duloxetine hydrochloride Active Pharmaceutical Ingredient was presented. The developed high-performance liquid chromatography method proved to be selective and stability indicating and allowed to determine the chemical purity of Duloxetine hydrochloride. The determination of the impurity profile and elucidation of the structures of the main impurities is very important to comply with the regulatory norms as well as for assessing the quality of Duloxetine hydrochloride as an API. The presented studies can be also helpful in preparing pharmacopoeial monograph of this substance.
ACKNOWLEDGMENTS

The analytical work was conducted with the support of the laboratory of Organic Chemical Technology, Chemistry Department of the Aristotle University of Thessaloniki, the N.C.S.R. Demokritos, Institute of Physical Chemistry Athens and the Universität Leipzig, Inst. f. Analyt. Chemie, Germany.

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[1] Mishra L. Duloxetine hydrochloride is a newer selective serotonin and norepinephrine reuptake inhibitor (SSNRI) used for major depressive disorders. Drugs today 1 2006. 489