Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 3, 2013

Research Article

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF DULOXETINE AND ITS METABOLITES IN RAT LIVER MICROSOMES AND CHARACTERIZATION OF *IN VITRO* MICROSOMAL METABOLITES THROUGH RETRO-SYNTHESIS FOLLOWED BY NMR AND MS STUDY

TAPAS KUMAR LAHA[a*], GITANJALI MISHRA[b] AND SUBRATA SEN[a]

[a]College of Pharmaceutical Sciences, Mohuda, Ganjam, Berhampur 760002, Orissa, India, [b]Berhampur University, Bhanja Bihar, Berhampur(Gm) 760007, Orissa, India. Email: tapaslaha80@rediffmail.com

Received: 20 Apr 2013, Revised and Accepted: 03 Jun 2013

ABSTRACT

Objective: A simple and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method for determination of duloxetine and four of its *in vitro* metabolites, 4-hydroxy duloxetine (M1), 5-Hydroxy duloxetine (M2), 6-hydroxy duloxetine (M3) and N-desmethyl duloxetine (M4) in Wistar rat liver microsomes was developed.

Method: Analysis was carried out on a µ-Bondapak C18 column (250mm × 4.6mm, 5µm particle size) using methanol: phosphate buffer (pH 7.8, 50 mM) (6:4 v/v) as the mobile phase at a flow rate of 1ml/min. Detection was carried out at 221 nm with an UV detector.

Results: The above metabolites were characterized by comparison of their retention time with synthetic standards. All the four retention time matches with the metabolites present in the microsomal sample.

Conclusion: A new HPLC method was developed for separation of *in vitro* metabolites present in rat liver microsomes. This method has also been successfully applied in routine analysis, stability study as well as pharmacokinetics study of duloxetine after orally administrating the duloxetine to Wistar rat.

Keywords: Duloxetine, *In vitro* metabolites, Liver microsomes, HPLC, Retro-synthesis, Characterization.

INTRODUCTION

Duloxetine (N-Methyl-3-naphthlen-yloxy-3-thiophen-2-yl-propan-1-amine) [1] a selective serotonin and norepinephrine reuptake inhibitor (SSNRI) is used for the treatment of major depressive disorder [2] and anxiety [3-5]. 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 [6-7] also indicated for the management of fibromyalgia [8-9]. It restores the balance of neurotransmitters in the brain like serotonin and norepinephrine [10]. Moreover it is also being used in the treatment of peripheral neuropathy caused by certain anti cancer drugs [11].

A literature survey indicated few methods for the determination of duloxetine and its key intermediate, desmethyl-duloxetine, in human serum by HPLC [12-13]. Reports were found regarding the characterization of phenolic impurities in duloxetine samples by MS, NMR spectrometry and X-ray analysis [14] and of impurities formed by interaction of duloxetine with various enteric polymers [15]. A simple UV spectrophotometric method for the estimation of duloxetine in a formulation was reported [16].

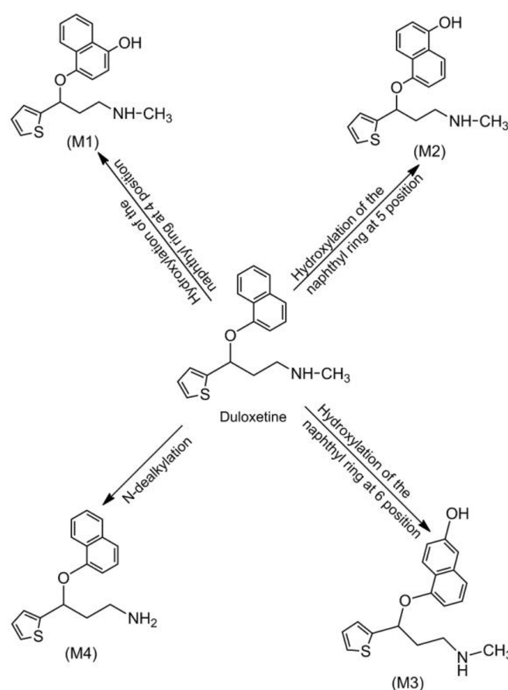

Fig. 1: Proposed metabolic pathways of duloxetine in rat liver microsomes.

An HPLC method to separate duloxetine and structurally related impurities using a combination of computer-based solvent strength optimization and solvent selectivity mixture design [17]. An HPTLC method for estimation of duloxetine in bulk and in tablet dosage form [18]. A capillary electrophoresis with laser-induced fluorescence detection method also reported for estimation of duloxetine in human plasma [19]. During our literature survey, very few articles related to the stability-indicating HPLC determination of duloxetine were found [20-26] but no article related to the measurement of duloxetine and its metabolites in rat liver microsomes by HPLC method was reported. Therefore the aim of the present work is to develop a novel, isocratic, RP- HPLC method for the determination of duloxetine and its *in vitro* metabolites in rat liver microsomes and the structure of the metabolites were characterized through retro-synthesis followed by MS and NMR study.

MATERIALS AND METHODS

Apparatus

Experiments were performed using a Waters (India) 510 HPLC system with Waters 486 tunable absorbance detector. The samples were injected manually using a 200 µL sample loop. The Millennium-32 software was used for quantification and data processing.

Chemicals and reagents

Pure duloxetine (Fig. 1) was provided by Wockhardt Limited, Mumbai, India. Methanol and water of HPLC grade and were purchased from Merck (India) Ltd., Mumbai, India. The probable *in vitro* metabolites were divined through retro-synthesis (Fig. 1) and were synthesized in our own laboratory (Fig. 2). All these and other chemicals used in the experiments were of analytical grade and were purchased from commercial sources.

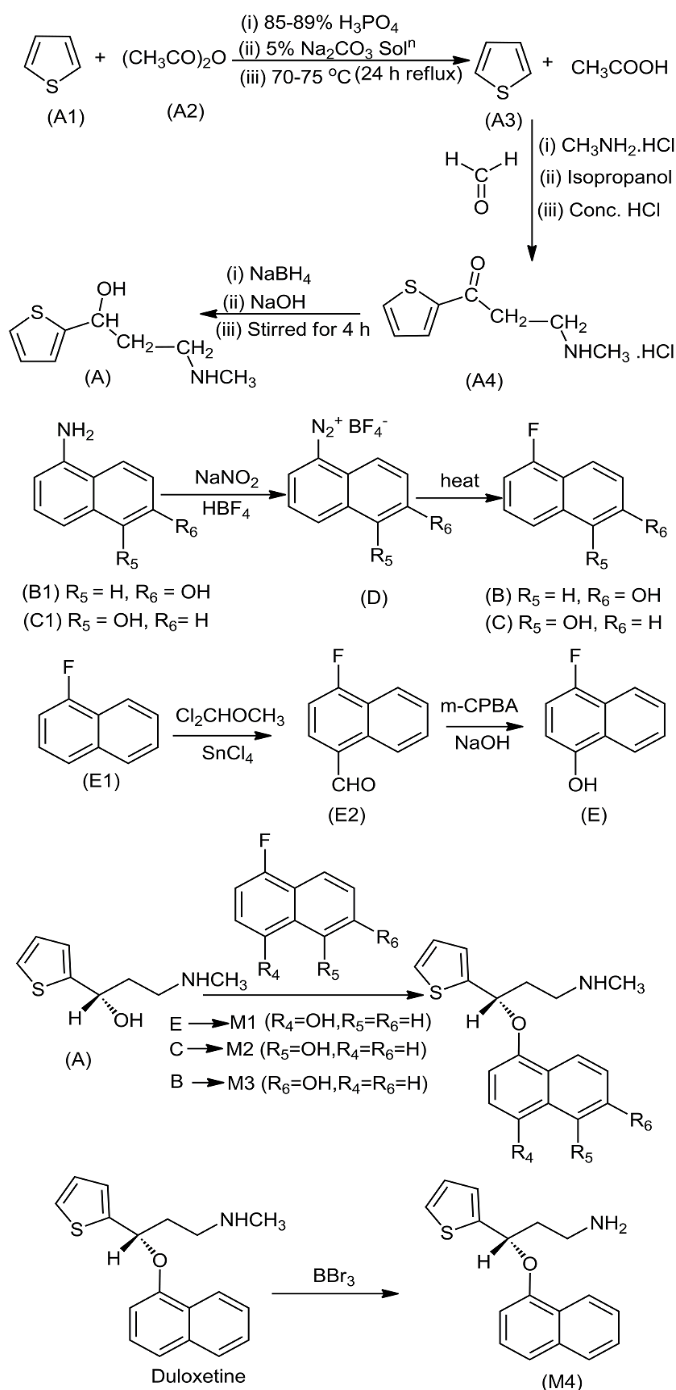

Fig. 2: Different synthetic scheme for the microsomal metabolites of duloxetine.

Synthesis of *in vitro* metabolites present in rat liver microsomes

The initial object was to prepare thiophene side chain i.e N-methyl-3-hydroxy-3-(2-thienyl) propylamine (A) for that 2-Acetylthiophene (A3) was prepared by the acetylation of thiophene (A1) with acetic anhydride (A2) in the presence of orthophosphoric acid. Placed (A1) and (A2) in a three necked flask, fitted with a thermometer, mechanical stirrer and reflux condenser. Heated the stirred solution to 70-75 °C, removed the source of heat, added 87 percent orthophosphoric acid. An exothermic reaction occurred after 2-3 minutes and temperature raised up to 90 °C; Immersed the flask in a bath of cold water to control the reaction. When the boiling subsides (ca. 5 minutes) refluxed the mixture for 2 h at 175-190 °C, added water, stirred for 5 minutes, transferred the cold reaction mixture to a separatory funnel, removed the water layer, washed with 5 percent sodium carbonate solution and dried over anhydrous magnesium sulphate. Distilled the orange-red liquid through a short fractionating column at atmospheric pressure and recovered some portion of unchanged thiophene at 83-84 °C. Distilled the residue under reduced pressure to collect (A3) at 89-90 °C/10mm; this solidifies on cooling in ice, with this methylamine hydrochloride, paraformaldahyde, concentrated hydrochloric acid and isopropanol were added and this mixture was heated to reflux and stirred for 6 h. The mixture was then cooled to 0 °C and stirred for one hour more. The slurry was then filtered, and the solid was washed with cold ethanol. The washed solid was dried for 16 hrs at 50 °C to obtain 2-thienyl 2-methylaminoethyl ketone hydrochloride (A4), as a white sold. This intermediate product (A4) was stirred in presence of ethanol at ambient temperature, and the pH of the solution was raised to 11-12 by slow addition of sodium hydroxide. A required portion of sodium borohydride was added, and the mixture was stirred at ambient temperature for 4 h. Then acetone was added, and the mixture was stirred for 20 minutes more. The mixture was then concentrated by evaporation to a white slurry and add methyl t-butyl ether. The mixture was acidified to pH 1-1.5 by addition of concentrated hydrochloric acid, and the solution was stirred for ten minutes. The pH was then made basic to pH 12 by slow addition of sodium hydroxide. The layers were then separated, the aqueous phase was extracted with methyl t-butyl ether and the organic phases were combined and washed once with water. The organic phase was concentrated by evaporation to get a solid product (A).

Second objective was to prepare corresponding fluoronaphthols. The first two compounds, 1-fluoronaphth-6-ol (B) and1-fluoronaphth-5-ol (C) were synthesized from commercially available 1-aminonaphth-6-ol (B1) and 1-aminonaphth-5-ol (C1) using a modified Schiemann reaction [27-28]. The diazonium tetrafluoroborate intermediate (D) was isolated and dried. The dried powder was then heated in decahydronaphthalene at 160 °C to give the desired fluoronaphthols, B and C. The 1-fluoronaphth-4-ol (E) was synthesized from the commercially available 1-fluoro-naphthalene (E1). Compound (E1) was formylated by stirring with 1,1-dichloromethyl methyl ether and $SnCl_4$ at 0°C in methylene chloride to give 4-fluoro-1-naphthaldehyde (E2) [29]. Baeyer Villiger rearrangement followed by saponification of the corresponding formate gave the desired product (E) in good overall yield.

To synthesize some of the duloxetine metabolites, M1, M2 and M3 were accomplished by condensing the thiophene side chain (A) with the corresponding fluoronaphthols, E, C and B. The hydroxyl groups were protected as ketals or as acetals, under the conditions for the synthesis of duloxetine [30]. The protecting groups were then removed by acetic acid. Demethylation of pure duloxetine with BBr_3 at room temperature yielded M4.

^{1}H NMR spectra (in ppm, 400 MHz, $CDCl_3$): M1: δ 2.381 (s, 3H, -NH-C**H₃**), 1.650 (m, 1H, -N**H**), 2.691 (t, 2H, J=5.848 Hz, -CH₂-C**H₂**-NH), 2.905 (m, 2H, -C**H₂**-CH₂-NH-), 4.965 (t, 1H, *J*= 5.769 Hz, -**CH**-CH₂-CH₂-NH-), 6.821(m,1H, Ar-H), 6.902 (1H, dd, J=8.633 Hz, J=4.870 Hz, Ar-H), 7.362 (dd, 1H, J=8.633 Hz, J=3.556 Hz, Ar-H), 6.495 (q, 1H, *J*= 7.489 Hz, Ar-H), 6.564 (q, 1H, *J*= 7.289 Hz, Ar-H), 5.36 (s, 1H, -OH), 7.629 (ddd, 1H, J=8.327 Hz, J=7.579 Hz, J=1.396 Hz, Ar-H), 7.650 (ddd, 1H, J=8.340 Hz, J=7.579 Hz, J=1.393 Hz, Ar-H), 8.216 (m,1H, Ar-H), 8.292 (m,1H, Ar-H); Mass (m/z) ESI TOF: 314.12 (M+H).

M2: δ 2.381 (s, 3H, -NH-C**H₃**), 1.650 (m, 1H, -N**H**), 2.691 (t, 2H, J=5.848 Hz, -CH₂-C**H₂**-NH), 2.905 (m, 2H, -C**H₂**-CH₂-NH-), 4.965 (t, 1H, *J*= 5.769 Hz, -**CH**-CH₂-CH₂-NH-), 6.821(m,1H, Ar-H), 6.902 (1H, dd, J=7.613 Hz, J=4.812 Hz, Ar-H), 7.362 (dd, 1H, J=8.633 Hz, J=3.556 Hz, Ar-H), 6.495 (q, 1H, *J*= 7.489 Hz, Ar-H), 7.564 (q, 1H, *J*= 7.289 Hz, Ar-H), 7.829 (ddd, 1H, J=8.386 Hz, J=7.479 Hz, J=1.496 Hz, Ar-H), 5.36 (s, 1H, -OH), 6.650 (ddd, 1H, J=8.310 Hz, J=7.479 Hz, J=1.293 Hz, Ar-H), 7.456 (m,1H, Ar-H), 7.972 (m,1H, Ar-H); Mass (m/z) ESI TOF: 314.13 (M+H).

M3: ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.381 (s, 3H, -NH-C**H₃**), 1.650 (m, 1H, -N**H**), 2.691 (t, 2H, J=5.848 Hz, -CH₂-C**H₂**-NH), 2.905 (m, 2H, -C**H₂**-CH₂-NH-), 4.965 (t, 1H, *J*= 5.769 Hz, -**CH**-CH₂-CH₂-NH-), 6.821(m,1H, Ar-H), 7.214 (1H, dd, J=8.623 Hz, J=4.870 Hz, Ar-H), 7.4 (m, o, 1H, Ar-H), 6.614 (dd, 1H, *J*= 7.356 Hz, J=4.270 Hz, Ar-H),7.565 (dt,1H, *J*= 7.232 Hz, J=3.247 Hz, Ar-H), 7.19 (t, 1H, J=8.213 Hz, Ar-H), 5.271 (s, 1H, -OH), 7.021 (dd, 1H, J=8.093, J=5.325, Ar-H), 8.416 (m,1H, Ar-H); Mass (m/z) ESI TOF: 314.09 (M+H).

M4: δ 5.34 (s, 1H, -N**H₂**), 2.691 (t, 2H, J=5.848 Hz, -CH₂-C**H₂**-NH₂), 2.905 (m, 2H, -C**H₂**-CH₂-NH-), 4.965 (t, 1H, *J*= 5.769 Hz, -**CH**-CH₂-CH₂-NH-), 6.821(m,1H, Ar-H), 6.902 (1H, dd, J=8.633 Hz, J=4.870 Hz, Ar-H), 7.362 (dd, 1H, J=8.633 Hz, J=3.556 Hz, Ar-H), 6.495 (q, 1H, *J*= 7.489 Hz, Ar-H), 6.564 (q, 1H, *J*= 7.289 Hz, Ar-H), 7.629 (ddd, 1H, J=8.327 Hz, J=7.579 Hz, J=1.396 Hz, Ar-H), 7.650 (ddd, 1H, J=8.340 Hz, J=7.579 Hz, J=1.393 Hz, Ar-H), 8.216 (m,1H, Ar-H), 8.292 (m,1H, Ar-H); Mass (m/z) ESI TOF: 284.11 (M+H).

Animals

Male Wistar albino rats (body weight 230-250 gm) were supplied by M/s. Mahavir Enterprises, Hyderabad, Andhra Pradesh, India. Before starting the experiment Wistar rats were kept in an environmentally controlled room for one week and fed with standard laboratory food and water *ad libitum*. Rats were fasted overnight before used for preparation of liver microsomes. For conducting this experiment permission has taken from the University Animal Ethics Committee, Berhampur University, Berhampur, India.

Preparation of microsomes

In order to minimize degradation of enzymes, all apparatus and solutions were cooled and stored at 4 ºC prior to the start of the experiment. Rats were weighed and then killed by cervical dislocation. The livers were rapidly removed and immediately placed in ice-cold saline to wash off excess blood and to cool the tissue. The livers were then blotted dry, minced, weighed and transferred to a homogenizer. With this added 4 times of their weight of KCl-sucrose buffer (pH 7.4) consisting of 0.154M KCl and 0.25M sucrose in 0.05M phosphate buffer (pH 7.5). The livers were then homogenized at 23 000 rpm for 1 min using FJ-200 homogeniser (Shanghai, China). The homogenate was centrifuged (Sorvall Super T21, Dupont, Wilmington, USA) at 20000 rpm at 4 ºC for 20 min. The supernatant was further ultracentrifuged (Sorvall Ultracen- trifuge OTD 55B, Dupont, Wilmington, USA) at 100 000 rpm at 4 ºC for 60 min. Pour off supernatant portion and the remaining microsomal pellets were suspended in phosphate buffer 0.05 M (pH 7.5) and ultracentrifuged again at 100000 rpm at 4 ºC for 60 min. The supernatant was discarded and the washed microsomal pallets were resuspended in Glycerol/Phosphate buffer (20% glycerol (v/v), 80% 0.1M phosphate buffer pH 7.5) and stored at -70 ºC until used.

Incubation study

Incubation study was performed using 500 µL microsome extract, 500 µL cofactor solution (2mM NADPH), duloxetine (250µg/mL, 500µL) and 100 mM sodium phosphate buffer (pH 7.4) added to bring a total volume of 2.0 mL. Incubation was done at 37°C for 30 min. After 30 min reactions were terminated using an equal volume (2mL) of cold acetonitrile.

HPLC analysis and characterization of metabolites

Above sample was centrifuged at 14,000 rpm for 10 min and the supernatant was evaporated to dryness under a stream of nitrogen at 40 ºC. Residue was redissolved in 1000 µL of LC mobile phase,

and an aliquot of 250 µL was injected into the HPLC system. Experiment was performed on a Bondapak C18 (250mm ×4.6mm, 5µm particle size) column using methanol-phosphate buffer (pH 7.8; 50 mM) (6:4 v/v) as the mobile phase at a flow rate of 1 ml/min. The mobile phase was filtered through a nylon membrane filter paper (pore size 0.45 µm) and degassed with a sonicator for 10 min. Ambient column temperature was maintained and eluents were monitored at a wavelength of 221 nm. The volume of each injection was 200 µL. In all cases, metabolites were confirmed by co-injection.

RESULTS AND DISCUSSION

Table 1 shows data for the *in vitro* metabolism of duloxetine using rat liver microsomal preparation. The experimental findings indicate that a rat liver microsomal preparation (in the presence of NADPH) metabolizes an essential portion of the parent compound in thirty minutes. The detectable metabolites were 4-hydroxy duloxetine (M1), 5-Hydroxy duloxetine (M2), 6-hydroxy duloxetine (M3) and N-desmethyl duloxetine (M4). The metabolites were formed by the hydroxylation of the naphthyl ring and N-dealkylation of the parent duloxetine. Duloxetine and above metabolites were identified by comparison with the synthetic standards. The retention time of the duloxetine peak matched that of the authentic duloxetine standard and the retention time of M1, M2, M3 and M4 also matched that of the synthetic standards. These results confirmed the identification of the duloxetine, M1, M2, M3 and M4 in microsomal sample. The major metabolite in microsomal sample was the N-desmethyl duloxetine (M4) 17.34% and second most abundant metabolite was the 6-hydroxy duloxetine (M3) 15.02%. In addition to unchanged parent drug, a total of 4 metabolites were identified in microsomal sample. The representative HPLC chromatograms of duloxetine and its metabolites in microsomal sample are shown in figure 3.

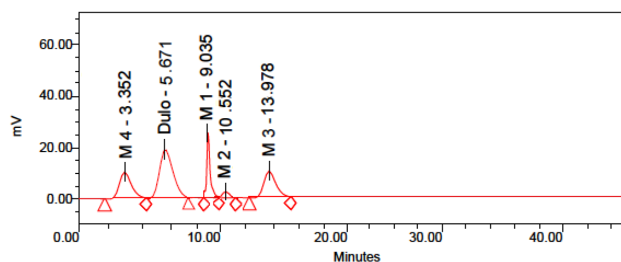

Fig. 3: Representative HPLC chromatograms of duloxetine and its metabolites in rat liver microsomes incubated with 250µg/mL duloxetine for 30 min.

In vitro incubation with human or animals liver microsomes has been an usually method in drug metabolism studies. In the present study, duloxetine was incubated with rat liver microsomes for 30 min, and the percentage of unmetabolized duloxetine was 57.14 %.

Table 1: Retention time and HPLC peak area percentages of unchanged duloxetine and its metabolites in rat liver microsomes.

Compound	Retention time (min)	Peak Area %
Duloxetine	5.671	57.14
4-Hydroxy duloxetine (M1)	9.035	9.11
5-Hydroxy duloxetine (M2)	10.552	1.24
6-Hydroxy duloxetine (M3)	13.978	15.02
N-desmethyl duloxetine (M4)	3.352	17.34

The ratio of metabolites to parent drug in incubates were very low, which were due to the limited formation of metabolites *in vitro*. A total of four metabolites of duloxetine were found in rat liver microsomal incubates by HPLC method. The identities of four major metabolites were confirmed by chromatographic comparison with synthetic standards. Based on the results, hydroxylation and *N*-dealkylation were the main metabolic pathways of duloxetine in rat liver microsomes. Due to very low concentration of M2, initially it was not detected. To detect M2, we investigated various elution systems and chromatographic procedures. In addition, two different HPLC columns, Novapak C18 and Bondapak C18 from Waters India, USA, were utilized for this study. A spectrum of different wavelengths was tested for this purpose (data not shown). The satisfactory detection was obtained by using Bondapak C18 column at a wavelength of 221 nm.

CONCLUSION

A new HPLC method was developed for separation of *in vitro* metabolites present in rat liver microsomes. These metabolites were identified by HPLC analysis. Characterizations of the metabolites were confirmed by chromatographic comparison with synthetic standards. The newly developed HPLC method confirms the suitability of HPLC with UV detection for analysis of *in vitro* microsomal metabolites of duloxetine and does not re-quire use of hyphenated HPLC–MS. The method was found to be specific, accurate and precise, and can also be used for the routine analysis as well as to monitor the stability studies.

ACKNOWLEDGEMENTS

The authors are grateful to Wockhardt Limited, Mumbai, India for providing gift sample of pure duloxetine. Above all, the authors would like to offer their gratitude to the authorities of college of pharmaceutical sciences, Mohuda and P.G. Dept. of Zoology, Berhampur University for providing all the facilities required to accomplish the work.

REFERENCES

1. Maryadele JO. The Merck Index. 14th ed., Whitehouse Station, New Jersey, USA: Merck and Co. Inc.; 2006.
2. Devias M, Varghese AK, Sriram S, Rajalingam B, SivasankarV, Chitra B et al. Evaluation of *in vitro* interactions of warfarin and duloxetine with selected coadministered NSAIDS in bovine serum albumin, Int J Pharm Pharm Sci 2011; 3(1): 196-199.
3. Dasari S, Viriyala RK, Santosh K, Dnss AK, Ravikumar BVV, Bishat SPS. A validated RP-HPLC method for the analysis of duloxetine hydrochloride in pharmaceutical dosage forms. Pharmacie Globale (IJCP) 2010; 1(3): 1-3.
4. Mishra L. Duloxetine hydrochloride is a newer selective serotonin and norepinephrine reuptake inhibitor (SSNRI) used for major depressive disorders. Drugs today 2006; 1: 489.
5. Stephan AC, Luc AG, Francois RV, Peter RB, Frank PB, Melissa JJ et al. Duloxetine increases serotonin and norepinephrine availability in healthy subjects: A double-blind controlled study. J Neuropsychopharmacol 2003; 28: 1685-1693.
6. National Institute for Health and Clinical Excellence. Urinary incontinence the management of urinary incontinence in women. NW, London; 2006.
7. National Institute for Health and Clinical Excellence. The pharmacological management of neuropathic pain in adults in non-specialist settings. High Holborn, London; 2010.
8. http://www.drugbank.ca/drugs/DB00476.
9. Wolfe F, Smythe HA, Yunus MB. Criteria for the Classification of Fibromyalgia. Arthritis Rheum 1990; 33: 160–72.
10. Douglas RD. Treating Patients for Comorbid Depression, Anxiety Disorders and Somatic Illnesses. J Am Osteopath Assoc 2006; 106: 51-58.
11. http://www.cancer.gov/dictionary/CdrID=589399.
12. Johnson JT, Oldham SW, Lantz RJ, Delong AF. High performance liquid chromatographic method for the determination of duloxetine and desmethyl duloxetine in human plasma. J Liq Chromatogr Rel Technol 1996; 19: 1631–1641.
13. Pankaj S, Mariappan TT, Banerjee UC. High-performance liquid chromatographic method for the simultaneous estimation of the key intermediates of duloxetine. Talanta 2005; 67: 975–978.
14. Brenna E, Frigoli S, Fronza G, Fuganti C, Malpezzi L. Isolation and characterization of a phenolic impurity in a commercial sample of duloxetine. J Pharm Biomed Anal 2007; 43: 1573–1575.
15. Jansen PJ, Oren PL, Kemp CA, Maple SR, Baertschi SW. Characterization of impurities formed by interaction of duloxetine HCl with enteric polymers hydroxypropyl

methylcellulose acetate succinate and hydroxypropyl methylcellulose phthalate. J Pharm Sci 1998; 87: 81–85.
16. Kamila MM, Mondal N, Ghosh LK. A validated UV spectrophotometric method for determination of duloxetine hydrochloride. Pharmazie 2007; 62: 414–415.
17. Olsen BA, Argentine MD. HPLC method development for duloxetine hydrochloride using a combination of computer-based solvent strength optimization and solvent selectivity mixture design. J Liq Chromatogr Rel Technol 1996; 19: 1993–2007.
18. Dhaneshwar SS, Deshpande P, Patil M, Vadnerkar G, Dhaneshwar SR. Development and validation of a HPTLC method for estimation of duloxetine hydrochloride in bulk drug and in tablet dosage form. Ind J Pharm Sci 2008; 70: 233–236.
19. Musenga A, Amore M, Mandrioli R, Kenndler E, Martino L Raggi MA. Determination of duloxetine in human plasma by capillary electrophoresis with laser-induced fluorescence detection. J Chromatogr B 2009; 877: 1126–1132.
20. Patel SK, Patel NJ, Prajapati AM, Patel DB, Patel SA. Stability-indicating RP-HPLC Method development and validation for Duloxetine Hydrochloride in Tablets. J AOAC Int 2010; 93: 123–132.
21. Sinha VR, Kumria R, Bhinge JR. Stress degradation studies on duloxetine hydrochloride and development of an RP-HPLC method for its determination in capsule formulation. J Chromatogr Sci 2009; 47: 589-93.
22. Ramana NVVSS, Harikrishnaa KA, Prasada AVSS, Reddya KR, Ramakrishna K. Determination of duloxetine hydrochloride in the presence of process and degradation impurities by a validated stability-indicating RP-LC method. J Pharm Biomed Anal 2010; 51: 994–997.
23. Chhalotiya UK, Bhatt KK, Shah DA, Baldania SL. Development and validation of a stability-indicating RP-HPLC method for duloxetine hydrochloride in its bulk and tablet dosage form. Sci Pharm 2010; 78: 857–868.
24. Reddy PB.Validation and stability indicating reverse phase-high performance liquid chromatography for the determination of duloxetine in tablets. Int J Chem Tech Res 2009; 1: 602-605.
25. Reddy PRM, Sreeramulu1 J, Naidu PY, Reddy AR. Stability indicating fast LC for the Simultaneous estimation of Intermediates and degradants of duloxetine hydrochloride. Chromatographia 2010; 71: 95–100.
26. Laha TK, Mishra G, Sen S. A Validated Stability Indicating Reversed Phase High Performance Liquid Chromatographic Method of Duloxetine and Characterization of its Degradation Products through Retro-Synthesis. Pharm Anal Acta (Scientific Reports) 2012; 1(6): 1-6.
27. Adcock W, Alste J, Rizvi SQA, Aurangzeb M. Substituent effects in the naphthalene ring system by fluorine-19 NMR. J Am Chem Soc 1976; 98: 1701-1711.
28. Adcock W, Dewar MJS. Substituent Effects VIII - Synthesis of Substituted α- and β-Fluoronaphthalenes. J Am Chem Soc 1967; 89(2): 386–390.
29. Boswell GE, Licause JF. A Convenient Large-Scale Synthesis of 4-Fluoro-1-naphthaldehyde and Its Aromatic Nucleophilic Substitution Reactions. J Org Chem 1995; 60(20): 6592–6594.
30. Wheeler WJ and Kuo F. An asymmetric synthesis of duloxetine hydrochloride, a mixed uptake inhibitor of serotonin and norepinephrine and its C-14 labeled isotopomers. J Labelled Compd Radiopharm 1995; 36: 213–223.