High-Performance Liquid Chromatographic Analysis of Duloxetine and Its Metabolites in Rat and Characterization of Metabolites in Plasma, Urine, Feces and Bile through Retro-Synthesis Followed By NMR and MS Study

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ABSTRACT
A simple and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method for determination of duloxetine and twelve of its metabolites, Sulfate conjugate of 4-hydroxy duloxetine (M1), N-desmethyl duloxetine (M2), Glucuronide conjugate of 4-hydroxy duloxetine (M3), Glucuronide conjugate of 6-hydroxy duloxetine (M4), Glucuronide conjugate of 4,6,di-hydroxy duloxetine (M5), Glucuronide conjugate of 5-hydroxy-6-methoxy duloxetine (M6), 4-Hydroxy duloxetine (M7), 5-Hydroxy duloxetine (M8), 6-Hydroxy duloxetine (M9), Thiethyl alcohol (M10), Glucuronide conjugate of dihydrodiol duloxetine (M12) in Wistar rat plasma, urine, feces and bile was developed. 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) (7:3 v/v) as the mobile phase at a flow rate of 1ml/min. Detection was carried out at 221 nm with an UV detector. The above metabolites were characterized by retro-synthesis followed by 1H-NMR, 13C-NMR and M.S study for structure confirmation and finally injected separately into the HPLC system. All the twelve retention time matches with the metabolites present in the plasma, urine, feces and bile sample. This method has also been successfully applied in the pharmacokinetics study of duloxetine after orally administrating the duloxetine to Wistar rat.

Keywords: Duloxetine, Metabolites, HPLC, Retro-synthesis, Characterization.

INTRODUCTION
Duloxetine (N-Methyl-3-naphthlen-yloxy-3-thiophen-2-yl-propan-1-amine) a selective serotonin and norepinephrine reuptake inhibitor (SSNRI) is used for the treatment of major depressive disorder and anxiety. [1-3] 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 [4-5] also indicated for the management of fibromyalgia. [6-7] It restores the balance of neurotransmitters in the brain like serotonin and norepinephrine. [8] Moreover it is also being used in the treatment of peripheral neuropathy caused by certain anticancer drugs. [9]

A literature survey indicated few methods for the determination of duloxetine and its key intermediate, desmethyl-duloxetine, in human serum by HPLC. [10-11] Reports were found regarding the characterization of phenolic impurities in duloxetine samples by MS, NMR spectrometry and X-ray analysis [12] and of impurities formed by interaction of duloxetine with various enteric polymers. [13] A simple UV spectrophotometric method for the estimation of duloxetine in a formulation was reported. [14] An HPLC method to separate duloxetine and structurally related impurities using a combination of computer-based solvent strength optimization and solvent selectivity mixture design. [15] An HPTLC method for estimation of duloxetine in bulk and in tablet dosage form. [16] A capillary electrophoresis with laser-induced fluorescence detection method also reported for estimation of duloxetine in human plasma. [17] During our literature survey, very few articles related to the stability-indicating HPLC determination of duloxetine were found [18-24] but no article related to the measurement of duloxetine and its metabolites in rat plasma, urine, feces and bile 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 metabolites in rat plasma, urine, feces and bile and the structure of the metabolites, were characterized through retro-synthesis followed by MS and NMR study.

The probable in-vivo metabolites were divined through retro-synthesis (Fig. 1) and were synthesized in our own laboratory (Fig. 2, 3, 4). All these and other chemicals used in the experiments were of analytical grade and were purchased from Merck (India) Ltd., Mumbai, India.

Synthesis of in-vivo metabolites present in rats plasma, urine, feces and bile

The initial object was to prepare thiophene side chain i.e N-methyl-3-hydroxy-3-(2-thienyl) propylamine (M10) for that 2-Acetyltiophene (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 rises 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, paraformaldehyde, 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 solid. 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 white slurry and adds 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 (M10).

Second objective was to prepare corresponding fluoronaphthols. The first two compounds, 1-fluoronaphth-6-ol (B) and 1-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. 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-fluoronaphthalene (E1). Compound (E1) was formylated by stirring...
with 1, 1-dichloromethyl methyl ether and SnCl\textsubscript{4} at 0°C in methylene chloride to give 4-fluoro-1-naphthaldehyde (E2). [27] Baeyer Villiger rearrangement followed by saponification of the corresponding formate gave the desired product (E) in good overall yield. The hydroxy group on 1-fluorooxynaphth-6-ol (B) was protected as methyl ether. The resulting 1-fluoro-6-methoxynaphthalene (F1) was then formylated as described above. The formylation occurred exclusively at the C-5 position. [28-29] This aldehyde was then converted to the corresponding 1-fluoro-6-methoxynaphth-5-ol (B) by saponification with excess of LiOH in aqueous methanol at room temperature gave the conjugate M11. Saponification with excess of LiOH in methanol at room temperature gave the conjugate M12.

J5. Saponification with excess of LiOH in aqueous methanol at room temperature yielded J1. Treatment of J1 with trimethylamine sulfur trioxide to formate gave the desired product (E) in 89% yield. J5. Saponification with excess of LiOH in aqueous methanol gave J12.

ESI TOF: 393.07 (M+H).

M2: H NMR spectra (400 MHz, CDCl\textsubscript{3}): δ 5.34 (s, 1H, -NH), 2.691 (t, 2H, J=5.848 Hz, CH\textsubscript{2}CH\textsubscript{2}NH), 2.905 (m, 2H, CH\textsubscript{2}CH\textsubscript{2}NH), 4.965 (t, 1H, J=5.769 Hz, -CH=CH-CH\textsubscript{2}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=2.369 Hz, Ar-H), 7.597 (s, 1H, Ar-H), 1.821 (m,1H, Ar-H ), 8.292 (m,1H, Ar-H); Mass (m/z) ESI TOF: 284.11 (M+H).

M3: 2.431 (s, 3H,NH-CH\textsubscript{3}), 1.651 (m, 1H, -NH), 2.694 (t, 2H, J=5.842, -CH\textsubscript{2}-NH-), 2.027 (td, 2H, J=5.842, J=5.780, -CH\textsubscript{2}CH\textsubscript{2}NH), 7.633 (ddd, 1H, J=8.310, J=7.571, J=1.353, Ar-H), 7.617 (ddd, 1H, J=8.319, J=7.571, J=1.353, Ar-H), 7.401 (dd, 1H, J=8.063, J=5.518, Ar-H), 7.483 (dd, 1H, J=5.518, J=1.443, Ar-H), 7.052 (dd, 1H, J=8.063, J=1.443,Ar-H), 6.969 (dd, 1H, J=8.820, J=5.438, Ar-H), 8.304 (ddd, 1H, J=8.310, J=5.438, J=1.353, Ar-H), 8.291 (ddd, 1H, J=8.319, J=5.495, J=1.353, Ar-H), 7.650 (ddd, 1H, J=10.160, Cy-H), 3.834 (dd, 1H, J=10.160, J=3.450, Cy-H), 3.924 (dd, 1H, J=10.160, J=3.450, J=2.680, Cy-H), 4.038 (d1H, J=2.680,Cy-H), 5.212 (d, 1H, J=10.260, Cy-H), 3.631 (m, 1H, o, Cy-OH), 10.922 (m, 1H, Cy-COOH); Mass (m/z) ESI TOF: 490.15 (M+H+).

M4: 2.430 (s, -NH-CH\textsubscript{3}), 1.650 (m, 1H, -NH), 2.692 (t, 2H, J=5.857, -CH\textsubscript{2}NH-), 2.025 (td, 2H, J=5.857, J=5.731, -CH\textsubscript{2}CH\textsubscript{2}NH-), 5.408 (t, 1H, J=5.731, -CH=CH-CH\textsubscript{2}NH-), 7.276 (dd, 1H, J=8.085, J=5.429, Ar-H), 7.429 (ddd, 1H, J=8.651, J=7.899, J=4.275, Ar-H), 7.498 (dd, 1H, J=5.429, J=1.396, Ar-H), 6.987 (ddd, 1H, J=8.651, J=5.007, J=1.950, Ar-H), 7.391 (dd, 1H, J=8.085, J=1.396, Ar-H), 7.138 (dd, 1H, J=7.808, J=1.526, Ar-H), 7.402 (ddd, 1H, J=7.899, J=5.387, J=1.950, J=1.437, Ar-H), 7.872 (ddd, 1H, J=8.708, J=5.387, J=1.437, J=2.680, Cy-H), 3.834 (d, 1H, J=10.260, J=3.450, Cy-H), 3.924 (dd, 1H, J=10.160, J=3.450, J=2.680, Cy-H), 5.212 (d, 1H, J=10.260, Cy-H), 3.631 (m, 1H, o, Cy-OH), 10.921 (m, 1H, Cy-COOH); Mass (m/z) ESI TOF: 490.15 (M+H+).

M5: 2.431 (s, 3H,NH-CH\textsubscript{3}), 1.653 (m, 1H, -NH), 2.695 (t, 2H, J=5.836, -CH\textsubscript{2}NH-), 2.027 (td, 2H, J=5.836, J=5.772, -CH\textsubscript{2}CH\textsubscript{2}NH-), 5.428 (t,1H, J=5.772, -CH=CH-CH\textsubscript{2}NH-), 7.247 (dd, 1H, J=8.080, J=5.399, Ar-H), 7.498 (dd, 1H, J=5.399, J=1.446, Ar-H), 7.052 (dd, 1H, J=8.080, J=1.446, Ar-H), 6.872 (dd, 1H, J=8.080, J=3.888, Ar-H), 6.798 (dd,1H, J=8.807, J=2.421, Ar-H), 7.034 (dd, 1H, J=8.822, J=2.747, Ar-H), 8.327 (ddd, 1H, J=8.822, J=3.888, J=1.052, Ar-H), 7.073 (ddd, 1H, J=2.747, J=2.421, J=1.052, Ar-H), 3.824 (dd, 1H, J=10.160, J=3.450,Cy-H), 3.251 (dd, 1H, J=10.260, J=10.160,Cy-H), 3.924 (dd, 1H, J=3.450, J=2.680,Cy-H), 5.094 (d, 1H, J=10.260,Cy-H), 4.038 (d, J=2.680,Cy-H), 5.094 (d, 1H, J=10.260,Cy-H), 4.038 (d,
1H, J=2.680, CY-H), 3.631 (m, 1H, o, CY-OH), 10.921 (m, 1H, CY-COOH); Mass (m/z) ESI TOF: 506.14 (M+H).

M6: 2.431 (s, 3H-NH-CH3), 3.871 (s, 3H, O-CH2), 1.652 (m, 1H, -NH), 2.693 (t, 2H, J=5.851, -CH2-NH2), 2.027 (td, 2H, J=5.851, -CH2-CH2-NH2), 5.428 (t, 1H, J=5.772, -CH2-CH2-CH2-NH2), 7.387 (dd, 1H, J=8.081, J=5.490, Ar-H), 7.320 (dd, 1H, J=8.095, J=0.895, Ar-H), 7.483 (dd, 1H, J=5.490, J=1.385, Ar-H), 7.057 (dd, 1H, J=8.095, J=1.748, Ar-H), 7.354 (dd, 1H, J=8.081, J=1.385, Ar-H), 6.504 (d, 1H, J=8.729, Ar-H), 7.407 (dd, 1H, J=8.081, J=1.437, Ar-H), 7.677 (d, 1H, J=10.160, J=1.496, Ar-H), 2.693 (t, 2H, J=5.851, CY-H), 1.652 (m, 1H, -NH), 2.693 (m, 2H, -CH2), 1.652 (m, 1H, -NH), 2.693 (m, 2H, -CH2)

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Animals
Three male and three female Wistar albino rats (body weight 200-250 g, obtained from M/s. Mahavir Enterprises, Hyderabad, Andhra Pradesh, India) were housed in nalgene metabolism cages (each cage one male and one female rat) for metabolite study. For conducting this experiment permission has taken from the University Animal Ethics Committee, Berhampur University, Berhampur, 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 the experiment. A single dose of duloxetine was administered to all animals orally at a dose of 10mg/kg body weight (via gavage). The dose was prepared by dissolving duloxetine in water at a concentration of 2mg/ml.

Biological sample preparation
Plasma
For identification of circulating metabolites, blood samples (0.5 ml) were collected sublingually at the intervals 1.0, 2.0, 4.0, 8.0, 12.0, 24.0, 48.0 and 72.0 h after dosing in heparinized tubes containing sodium heparin. After sampling, whole blood was centrifuged (ELTEK RC 4815 F, India) at 3000 rpm for 10 min, and plasma was transferred to Eppendorf snap-capped tubes and stored at -20°C for future analyses of duloxetine and metabolites. Physiological saline (0.5 ml) was administered to compensate for the blood loss after each blood was withdrawn. In order to ensure the effective separation of duloxetine and its circulating metabolites from plasma components, the deproteinization method was used. Three (3) ml of acetonitrile was added to1 ml of plasma. The mixture was vortex-mixed for 1 min and centrifuged for 15 min at 1600 rpm. The supernatant was transferred to a tube and evaporated to dryness (approximately 15 min), under a stream of nitrogen. The resulting residue was reconstituted with mobile phase (200μl) and injected into the HPLC system.

Urine
Urine samples were collected from rats at 0 to 4, 4 to 10, and 10 to 24 h on day 1 and every 24 h over the next 3 days. The
urine collection tubes were frozen at -15°C to keep collected samples stable. Aliquot of urine sample (1 ml) was transferred to 1.5-ml microcentrifuge tube and centrifuged at 5,000 rpm for 10 min. The supernatant was injected (200 µL) directly into the HPLC system.

**Feces**

Fecal samples were collected in plastic bags and homogenized after the addition of water. All samples were kept on ice during the homogenization. The samples were then centrifuged and the supernatants were separated. All supernatants were mixed and evaporated to approximately 1 ml in Turbo Vap at 35°C under nitrogen. The concentrated residue was extracted with 10 ml of hexane to remove all lipophilic materials and the aqueous layer was evaporated to dryness in a Turbo Vap at 35°C under nitrogen. The residue obtained was reconstituted with mobile phase and a small volume was injected into the HPLC system.

**Bile**

For bile sampling, rats were anesthetized with ether, the bile duct was cannulated using polyethylene tubing, and bile was collected at 0 to 1, 1 to 3, 3 to 6, 6 to 12, 12 to 18, 18 to 24, 24 to 30, 30 to 36, 36 to 42, and 42 to 48 h after a single intravenous dose of duloxetine. Sodium taurocholate solution (pH 7.4) was infused into the duodenal cannula (~1 ml/h) during bile collection. The bile samples were stored at -70°C. All bile samples were mixed and fortified with 4 volumes of acetonitrile acidified with 1% formic acid to remove proteins. The mixture was vortex mixed and centrifuged, and the supernatant was transferred into new test tubes and evaporated to dryness, under a stream of nitrogen. The resulting residue was reconstituted with mobile phase (200µl) and injected into the HPLC system.
Chromatographic conditions
The experiment was performed on a Bondapak C18 (250mm × 4.6mm, 5µm particle size) column using methanol-phosphate buffer (pH 7.8; 50 mM) (7:3, 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 0.45µm) and degassed with a sonicator for 10 min. The chromatography was performed at room temperature at a flow rate of 1 ml/min. The column temperature was maintained at 25°C and eluents were monitored at a wavelength of 221 nm. The volume of each injection was 200µl. In all cases, metabolites were confirmed by co-injection.

RESULTS AND DISCUSSION
For identification of circulating metabolites, blood samples were collected at a particular time intervals followed by centrifugation, deproteinization and evaporation prior to HPLC analysis. Duloxetine, the sulfate conjugate of 4-hydroxy duloxetine (M1) and N-desmethyl duloxetine (M2) were identified from the extracted plasma sample 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 and M2 also matched that of the synthetic standards. These results confirmed the identification of the duloxetine, M1 and M2 in plasma. The major metabolite in plasma was the sulfate conjugate of 4-hydroxy duloxetine (M1) and second most abundant metabolite in plasma was the N-desmethyl duloxetine (M2). The glucuronide conjugate of 4-hydroxy duloxetine (M3), 6-hydroxy duloxetine (M4), 4, 6-dihydroxy duloxetine (M5) and 5-hydroxy-6-methoxy duloxetine (M6) were found in urine. Same retention times of M3, M4, M5 and M6 with synthesized standards confirmed the presence of M3, M4, M5 and M6 in urine sample. The comparison and matches of the retention times of M7, M8, M9 and M10 present in feces with that of synthesized standard confirmed that these metabolites were present in feces.

Similar structural analyses were performed for M3, M4, M6, M11 and M12 present in bile. Same retention times of M3, M4, M6, M11 and M12 with synthesized standards confirmed that the above metabolites were present in bile sample. In addition to unchanged parent drug, a total of 12 metabolites were identified in plasma, urine, feces and bile sample from male and female rats. The representative HPLC chromatograms of duloxetine and its metabolites in plasma, urine, feces and bile from rat are shown in Fig. 5.

Metabolites M3, M4 and M6 were common in urine and bile sample, except M4 all were present in approximately similar amount. A list of all the metabolites of duloxetine detected in rats, together with the associated information used for quantitative work, is summarized in Table 1.

The free form of duloxetine and its metabolites after oral administration of a single dose of duloxetine were characterized in this study. Duloxetine was rapidly and extensively metabolized to form multiple oxidative and conjugated metabolites. Only a small portion of duloxetine (approximately 3% for AUC) was present in plasma only. The most abundant metabolite, M1, appears in plasma may be due to initial oxidation of duloxetine at 4-position of the naphthyl ring, this hydroxyl group can undergo further sulfation to form Sulfate conjugate of 4-hydroxy duloxetine (M1). The presence of trace amount of N-desmethyl duloxetine (M2) in the plasma sample is due to demethylation of secondary amine of duloxetine. The glucuronide conjugate of 4-hydroxy duloxetine (M3), 6-hydroxy duloxetine (M4), 4, 6-dihydroxy duloxetine (M5) and 5-hydroxy-6-methoxy duloxetine (M6) were found in urine. This may be due to initial biotransformation of duloxetine appears to be oxidation at either the 4-, 5-, or 6-position of the naphthyl ring. These hydroxyl compounds can then be conjugated or they can undergo further oxidation to form a catechol intermediate or another dihydroxy. The catechol can then undergo methylation to form a methyl catechol, which undergoes glucuronidation. In feces a probable pathway was the cleavage of duloxetine at the chiral center to form a thienyl alcohol and naphthol. The thienyl alcohol (M10) was detected with a very small peak but naphthol was not detected may be due to very low concentration in feces. The glucuronide conjugate of 4-
Table 1: HPLC peak area percentages of unchanged duloxetine and its metabolites in rat plasma, urine, feces and bile

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plasma</th>
<th>Urine</th>
<th>Feces</th>
<th>Bile</th>
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</thead>
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<tr>
<td>Duloxetine</td>
<td>04</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sulfate conjugate of 4-hydroxy duloxetine (M1)</td>
<td>37</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N-desmethyl duloxetine (M2)</td>
<td>28</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glucuronide conjugate of 4-hydroxy duloxetine (M3)</td>
<td>-</td>
<td>18</td>
<td>-</td>
<td>17</td>
</tr>
<tr>
<td>Glucuronide conjugate of 6-hydroxy duloxetine (M4)</td>
<td>-</td>
<td>03</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td>Glucuronide conjugate of 4,6-dihydroxy duloxetine (M5)</td>
<td>-</td>
<td>46</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glucuronide conjugate of 5-hydroxy-6-methoxy duloxetine (M6)</td>
<td>-</td>
<td>09</td>
<td>-</td>
<td>07</td>
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<tr>
<td>4-Hydroxy duloxetine (M7)</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
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<td>-</td>
<td>-</td>
<td>26</td>
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<tr>
<td>6-Hydroxy duloxetine (M9)</td>
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<td>-</td>
<td>04</td>
<td>-</td>
</tr>
<tr>
<td>Thieryl alcohol (M10)</td>
<td>-</td>
<td>-</td>
<td>02</td>
<td>-</td>
</tr>
<tr>
<td>Glucuronide conjugate of 5-hydroxy duloxetine (M11)</td>
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<td>-</td>
<td>-</td>
<td>05</td>
</tr>
<tr>
<td>Glucuronide conjugate of dihydrodiol duloxetine (M12)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>07</td>
</tr>
</tbody>
</table>

Fig. 5: Representative HPLC chromatograms of duloxetine and its metabolites in plasma, urine, feces and bile

- Hydroxy duloxetine (M3), 6-hydroxy duloxetine (M4), 5-hydroxy-6-methoxy duloxetine (M6), 5-hydroxy duloxetine (M11) and dihydrodiol duloxetine (M12) were present in bile. Metabolites M3, M4 and M6 were formed in similar...
metabolites present in rat plasma, urine, feces and bile metabolites. The method was found to be specific, accurately applied in the pharmacokinetics study of all followed by spectroscopic analysis. Characterization of the metabolites was carried by synthesis sample. These metabolites were identified by HPLC analysis. providing all the facilities required to accomplish the work. 

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