Chapter 2

Experimental

2.1 Instrumentation

2.1.1 High performance liquid chromatography (HPLC)

Samples were analysed on a Waters alliance 2690 separation module equipped with quaternary gradient pump with degasser, auto sampler, column oven, PDA detector and 2487 UV detector (Waters Corporation, Milford, MA, USA). Data was acquired and processed by Empower software.

2.1.2 Liquid chromatography-Mass spectrometry (LC-MS)

The MS and MS³ studies were performed on Thermo LCQ-Advantage quadrupole ion trap mass spectrometer and Xcalibur software (Thermo Electron, San Jose, CA, USA). The mass analyzer is equipped with two types of external ion sources i.e. (i) electrospray ionization (ESI) interface and (ii) atmospheric pressure chemical ionization (APCI) interface. The MS and MS³/MS studies were performed on 3200 Q-trap mass spectrometer (AB Scienx, Foster city, CA, USA). The HPLC consisted of LC-20AD binary gradient pump, a SPD-10AVP UV detector, SIL-10 HTC auto sampler and a column oven CTO-10ASVP (Shimadzu Corporation, Kyoto, Japan).

2.1.3 Semi-preparative HPLC

A Shimadzu semi-preparative HPLC system consisted of LC-8A binary gradient pump, a SPD-10AVP UV detector, SIL-10AP auto sampler and FRC-10A fraction collector (Shimadzu Corporation, Kyoto, Japan). High vacuum using a Buchi rotavapor (Buchi Labortechnik AG, Flawil, Switzerland).

2.1.4 Nuclear magnetic resonance (NMR) spectroscopy

¹H and ¹³C spectra were recorded at 399.957 MHz using a Bruker AVANCE 400 MHz spectrometer (Bruker, Fallanden, Switzerland) equipped with a 5 mm BBO probe and a z-gradient shim system. The ¹H spectra were recorded with 1 s pulse repetition time using 30° flip angle, while ¹³C spectra were recorded at 100.432 MHz with power gated decoupling using 30° flip angle with
repetition time of 2 s. Samples were either dissolved in deuterated chloroform or in dimethyl sulfoxide-d6. The $^1$H and $^{13}$C chemical shift values were reported on the δ scale in ppm relative to CDCl3 (7.26 ppm), DMSO-d6 (2.50 ppm) and tetramethyl silane as applicable. All spectra were recorded with sample spinning.

For $^1$H, $^{13}$C NMR and DEPT-135 (Distortionless Enhancement by Polarization Transfer) spectra, the magnetic field was set at 9.3 tesla. The probe $^1$H/$^{13}$C, 5 mm was optimized for inverse detection. For DEPT spectral analysis, the pulse programme was DEPT 135. The IF pulse magnetic field duration was kept 5.0000 s. The $^1$H and $^{13}$C chemical shift values were reported on the δ scale (ppm).

### 2.1.5 Infrared (IR) spectroscopy

The IR spectrum was recorded in the solid state as KBr powder dispersion using Nicolet FT-IR model AVATAR 370 (Thermo Electron Scientific Instruments, Madison, WI, USA) with a DTGS KBr detector. Data were collected between 400 and 4000 cm$^{-1}$, with a resolution of 4.0 cm$^{-1}$. A total of 16 scans were obtained and processed using the OMNIC software version 6.0.

### 2.1.6 Elemental analysis (EA)

Elemental analysis (C, H, N, and S) was carried out using an elemental analyzer model Vario EL III with TCD detector (Elementar Analysensysteme GmbH, Hanau, Germany). Samples were weighed in a tin boat, to which tungsten oxide was added and neatly packed. The sample in tin boat was loaded in an auto sampler tray and was dropped into the combustion tube automatically at a temperature of 1200°C. Complete combustion of sample was ensured with a special oxygen jet injection.

### 2.1.7 Photo Stability

Photo stability studies were carried out using a photo stability chamber model TP 00000900 (Thermo Lab equipments Pvt. Ltd, Mumbai, India). 500 mg of each sample was kept in two separate LOD bottles. One bottle was covered with lid and then with aluminum foil (dark control). Another bottle (photolytic exposed sample) was covered with lid and kept into the photolytic chamber to
get a minimum exposure of 1.2 million lux hours for light and 200 Watt hours/square meter for ultraviolet region.

2.2 HPLC analysis (Chromatographic Conditions, sample preparation and validation procedure)

2.2.1 Rivastigmine tartrate

2.2.1.1 Chromatographic conditions

XTerra RP-18 column (250 mm × 4.6 mm, 5 μm, Waters corporation, Milford, MA, USA). A mobile phase consisted of A, 10 mM dipotassium hydrogen phosphate adjusted to pH 7.60 ± 0.05 with orthophosphoric acid-acetonitrile (90:10, v/v) and B, acetonitrile-methanol (60:40, v/v) with a timed gradient mode T (min)%B: 0/5, 3/10, 16/20, 20/35, 55/55 60/5 70/5. Flow rate was set at 1.0 mL/min throughout the analysis. The injection volume was 10 μL for a sample concentration of 0.5 mg/mL prepared in diluent (mobile phase A-methanol, 40:60, v/v). Detector wavelength was fixed at 210 nm and the column temperature was maintained at 40°C.

2.2.1.2 Preparation of stock solutions for method validation

A test preparation of 1500 μg/mL of rivastigmine tartrate API sample was prepared by dissolving the appropriate amount in diluent (mobile phase A and methanol 80:120, v/v). A stock solution of impurities was prepared by dissolving 5 mg each of Imp-A, Imp-B, Imp-C, Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-7, Imp-8, Imp-9, Imp-10, Imp-11 and 5 mg of rivastigmine tartrate in 10 mL of diluent. From this solution, 4.5 mL was diluted to 100 mL with diluent. A standard solution containing 2.25 μg/mL of each impurity and rivastigmine tartrate was prepared from the stock solution for checking solution stability and robustness parameters.

2.2.2 Galantamine hydrobromide

2.2.2.1 Chromatographic conditions

Samples were analysed on a Waters alliance 2690 separation module equipped with 2487 UV detector (Waters Corporation, Milford, MA, USA) using a XTerra MS-C18, (100 mm × 4.6 mm, 3.5 μm, Waters Corporation, Milford, MA, USA). Method as per Ph.Eur. consisted of mobile phase A, 4.4 mM disodium hydrogen phosphate, 15.8 mM sodium dihydrogen phosphate-methanol (95:5
v/v) and mobile phase B consisted of acetonitrile in gradient mode (T<sub>ini</sub>A:B) T<sub>i</sub>100:0, T<sub>i</sub>2000:0, T<sub>i</sub>955:5, T<sub>i</sub>85:15, T<sub>i</sub>80:20 at a flow rate of 1.5 mL/min. The injection volume was 20 μL for a sample concentration of 1 μg/mL prepared in mobile phase A. Detector wavelength was fixed at 230 nm and the column temperature was maintained at 55°C throughout the analysis.

2.2.3 Monohydroxy carbazepine & Escobarbazepine acetate

2.2.3.1 Chromatographic conditions

A symmetry shield RP-8, (250 mm × 4.6 mm, 5 μm, Waters Corporation, MA, USA). Mobile phase A consisted, 10 mM potassium dihydrogen phosphate adjusted to pH 5.0± 0.05 with sodium hydroxide solution–acetonitrile (95:5, v/v) and mobile phase B consisted acetonitrile-water (80:20, v/v) in gradient mode (T<sub>ini</sub>A:B) T<sub>i</sub>70:30, T<sub>i</sub>65:35, T<sub>i</sub>50:50, T<sub>i</sub>30:70, T<sub>i</sub>20:80 T<sub>i</sub>70:30. The flow rate was set at 1.0 mL/min. The injection volume was 10 μL for a sample concentration of 400 μg/mL prepared in diluent (mobile phase A-acetonitrile, 50:50, v/v). Detector wavelength was fixed at 215 nm and the column temperature was maintained at 35°C throughout the analysis.

2.2.3.2 Preparation of stock solutions for method validation

A test preparation of 400 μg/mL of escobarbazepine acetate API sample was prepared by dissolving in diluent (mobile phase-acetonitrile-50:50). A stock solution of impurities was prepared by dissolving 5 mg each of Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-7, Imp-8, Imp-9, Imp-10, Imp-11, Imp-A, Imp-B, Imp-C and Imp-D and 5 mg of escobarbazepine acetate in diluent and made up to 25 mL with diluent. Transferred 5 mL of each individual stock solution into a 100 mL volumetric flask and made up to volume with diluent. From this stock solution, standard solution of 0.60 μg/mL of each impurity and 0.60 μg/mL of escobarbazepine acetate was prepared. This standard solution was also used for checking solution stability and robustness parameters.

2.2.4 Carbamazepine

2.2.4.1 Chromatographic conditions

A Nucleosil cyanop column (250 cm × 4.6 cm, i.d 5 μm particles, Macherey Nagel GmbH & Co. KG, Duren, Germany). For chromatographic separations as per USP method, tetrahydrofuran-methanol-water were mixed
(3:12:85, v/v/v). To 1000 mL of this solution, 0.2 mL of anhydrous formic acid and 0.5 mL of triethylamine were added and a flow rate was set at 1.5 mL/min. 0.10 g of sample was dissolved in methanol and diluted to 50 mL with the same solvent. 10 mL of this solution was diluted to 20 mL with water. The injection volume was 20 μL and the detector wavelength was set at 230 nm. The column temperature was maintained at 25°C throughout the analysis.

### 2.2.5 Darifenacin hydrobromide

#### 2.2.5.1 Chromatographic conditions

Samples were analysed on a Waters alliance 2690 separation module equipped with 2487 UV detector (Waters corporation, Milford, MA, USA) using an X Terra RP-18, (250 mm × 4.6 mm, 5 μm, Waters corporation, Milford, MA, USA). Mobile phase A consisted of, 10 mM sodium dihydrogen phosphate-0.2 mM, 1-Octane sulfonic acid sodium salt-acetonitrile (95:5, v/v) adjusted to pH 6.10 ± 0.05 with orthophosphoric acid and mobile phase B consisted of acetonitrile-methanol (90:10, v/v) with a timed gradient mode 

#### 2.2.5.2 Preparation of stock solutions for method validation

A test preparation of 1000 μg/mL of darifenacin hydrobromide sample was prepared by dissolving the appropriate amount in diluent (mobile phase A-mobile phase B, 80:20, v/v). A stock solution of impurities was prepared by dissolving 5 mg each of Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-A, Imp-B, Imp-C, Imp-D and 5 mg of darifenacin hydrobromide in 20 mL of diluent and further diluted, 2.5 mL to 50 mL with diluent. From this stock solution, a standard solution containing 1.5 μg/mL of each impurity and 1.5 μg/mL of darifenacin hydrobromide was prepared. This standard solution was also used for checking solution stability and robustness parameters.

### 2.2.6 Deferasirox

#### 2.2.6.1 Chromatographic conditions
Samples were analysed on a Waters alliance 2690 separation module equipped with 2487 UV detector (Waters corporation, MA, USA) using an Inertsil ODS-3V column (250 mm × 4.6 mm, 5 μm, GL Sciences Inc., Tokyo, Japan). Mobile phase A consisted of water-trifluoroacetic acid (100:0.05, v/v) and mobile phase B consisted of acetonitrile-methanol-trifluoroacetic acid (50:50:0.05,v/v/v) in gradient mode (Tmin:A:B 7.50:50, T20:20:80, T30:20:80, T40:50:50, T50:50:50 with a flow rate of 1.0 mL/min, detector wavelength was set at 245 nm and the column temperature was maintained at 25°C throughout the analysis. Injection volume was 10 μL for a sample concentration of 400 μg/mL prepared in mobile phase-B.

2.2.6.2 Preparation of stock solutions for method validation

A test preparation of 400 μg/mL of deferasirox sample was prepared by dissolving appropriate amount in methanol. A stock solution of impurities were prepared by dissolving 10 mg each of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E and Imp-1 and 10 mg of deferasirox in 100 mL of methanol. From this stock solution a solution containing 0.6 μg/mL each of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-1.

2.2.7 Ottalopram hydrobromide

2.2.7.1 Chromatographic conditions

For chromatographic separations as per USP method-1 a Phenomenex Luna C8(2) column (250 mm × 4.6 mm, 5 μm, Phenomenex Inc. Torrance, CA, USA) was used. The mobile phase consisted of 10 mM sodium acetate and 6.0 mL of triethylamine, adjusted to pH 4.60 ± 0.05 with acetic acid-acetonitrile (80:20, v/v) in isocratic mode. The apparent pH of mobile phase is 5.0 ± 0.1. The flow rate was set at 1.0 mL/min, with detector wavelength fixed at 239 nm. The injection volume was 20 μL for a sample concentration of 0.625 mg/mL prepared in diluent (methanol-water 1:1 v/v). Column temperature was maintained at 50°C throughout the analysis. As per USP method-2 a Symmetry-C18 column (250 mm × 4.6 mm, 5 μm, Waters Corporation, MA, USA) was used. The mobile phase-A consisted of 2.7 g of potassium hydrogen phosphate in 1 L of water, 1 mL of N,N-dimethylectyamine and adjusted the pH to 3.0 ± 0.1 with phosphoric acid-methanol-tetrahydrofuran (70:24:6, v/v/v) and mobile phase-B consisted of buffer-acetonitrile (70:30, v/v) in gradient
mode (T<sub>in-out</sub>): T<sub>1</sub>=100:0, T<sub>2</sub>=10:90, T<sub>3</sub>=10:90, T<sub>4</sub>=100:0 T<sub>5</sub>=100:0. The flow rate was set at 0.8 mL/min with detector wavelength was fixed at 224 nm. The injection volume was 20 μL for a sample concentration of 1.5 mg/mL prepared in diluent (methanol-water 1:1 v/v). Column temperature was maintained at 40°C throughout the analysis.

For chromatographic separations as per Ph.Eur. method, a Phenomenex Synergy Hydro RP 18 column (250 mm x 4.6 mm, 5 μm, Phenomenex Inc. Torrance, CA, and USA) was used. The mobile phase-A consisted of, 1.58 g of ammonium formate in 320 mL of water-160 mL of methanol and 20 mL of acetonitrile and mobile phase-B consisted of, 1.58 g of ammonium formate in 160 mL of water-340 mL of acetonitrile in gradient mode (T<sub>in-out</sub>): T<sub>1</sub>=100:0, T<sub>2</sub>=00:10, T<sub>3</sub>=40:60, T<sub>4</sub>=60:40, T<sub>5</sub>=100:0 T<sub>5</sub>=100:0. The flow rate was set to 1.0 mL/min with detector wavelength was fixed at 230 and 254 nm. The injection volume was 40 μL for a sample concentration of 0.5 mg/mL prepared in mobile phase-A. Column temperature was maintained at 40°C throughout the analysis.

2.3 Preparative LC condition

2.3.1 Rivastigmine tartrate

An Inertsil ODS-3 column (250 mm x 2.1 cm, particle size 10 μm, GL Sciences Inc., Tokyo, Japan) was used for semi-preparative isolation. LC isocratic method consisted of a mixture of 0.1 mM ammonium formate-acetonitrile (75:25, v/v) was used as mobile phase at a flow rate of 20 mL/min for a sample concentration of 150 mg/mL was prepared using methanol as diluent. The injection volume was 1.0 mL and the detection was monitored at 210 nm. The collected fractions were combined and concentrated to about 10 mL by evaporation under high vacuum using a Buchi rotavapor (Buchi Labortechnik AG, Flawil, Switzerland).

2.3.2 Galantamine hydrobromide

The unknown impurity was isolated from oxidative stressed sample of galantamine hydrobromide using Shimadzu semi-preparative HPLC system consisting of LC-8A binary gradient pump, a SPD-10AVP UV detector, SIL-10AP and an auto sampler (Shimadzu Corporation, Kyoto, Japan). An Inertsil
ODS-3 column (25 ×2.0 cm, particle size 10 μm, GL Sciences Inc., Tokyo, Japan) was used for semi-preparative isolation. LC method consisted of mobile phase A, 0.1% trifluoroacetic acid in water and mobile phase B consisted of acetonitrile in gradient mode (T mó:A:B) T<sub>0</sub>20:10, T<sub>60</sub>40:60, T<sub>90</sub>90:10 as the mobile phase at a flow rate of 25 mL/min. The injection volume was 1.0 mL for a sample concentration of 100 mg/mL prepared in diluent (methanol). Detector wavelength was fixed at 230 nm and the column temperature was maintained at 35°C throughout the analysis. The fractions of unknown impurity were collected manually. The collected fractions were pooled and freeze dried under high vacuum in a Virtis advantage lyophilizer (SP Scientific, NY, USA).

2.3.3 Carbamazepine

An Inertial ODS-3 column (GL Sciences Inc., Tokyo, Japan) (250 cm×1.9 cm, particle size 10 μm) was used for semi-preparative isolation. LC isocratic method was used, consisting of a mixture of water-methanol (30:70, v/v) as the mobile phase at a flow rate of 18 mL/min. A sample solution of 50 mg/mL was prepared using methanol as the diluent. The injection volume was 1.0 mL and the detection was monitored at 230 nm. The collected fractions were concentrated to dryness by lyophilization under high vacuum consisting of Virtis advantage lyophilizer (SP Scientific, New York, USA).

2.3.4 Citalopram hydrobromide

An Inertial ODS-3 column (250 mm× 20 mm, 10 μm, GL Sciences Inc., Tokyo, Japan) was used for semi-preparative isolation. An isocratic method consisting of a mixture of water-acetonitrile-trifluoroacetic acid (60:40:0.1, v/v/v) as the mobile phase at a flow rate of 18 mL/min was used. A sample solution of 100 mg/mL was prepared using methanol as diluent. The injection volume was 1.0 mL and the detection was monitored at 239 nm. The collected fractions containing citalopram were pooled and freeze dried under high vacuum consisting of Virtis advantage lyophilizer (SP Scientific, New York, USA).
2.4 Liquid Chromatography-Mass Spectrometry (LC-MS) Conditions

2.4.1 Rivastigmine tartrate

The instrument was operated in enhanced product ion mode in positive polarity mode with the following settings: collision energy of 40V, collision energy spread 10V and de-clustering potential 10V. Nitrogen was used as curtain gas at a pressure of 12 psi and as collision associated dissociation (CAD) gas. Zero air was used as nebulizer gas and heater gas at pressure of 50 psi. The ion spray voltage was 5500 V. The HPLC consisted of LC-20AD binary gradient pump, a SPD-10AVP UV detector, SIL-10HTC auto sampler and a column oven CTO-10ASVP (Shimadzu Corporation, Kyoto, Japan). The injection volume was 10 µL for a sample concentration of 0.5 mg/mL prepared in methanol. Detector wavelength was fixed at 210 nm and the column temperature was maintained at 40°C.

An XTerra RP-18, (250 mm × 4.6 mm, 5 µm) was used for chromatographic separation. Mobile phase A consisted of, 20 mM ammonium bicarbonate adjusted to pH 7.60 ± 0.05 with ammonia and mobile phase B consisted of, acetonitrile–methanol (60:40, v/v) in gradient mode; T (min)/ %B: 0/5, 3/10, 20/20, 30/35, 60/65 65/5 70/5. Column temperature was maintained at 50°C and the flow rate was at 1.0 mL/min throughout the analysis.

2.4.2 Galantamine hydrobromide

The MS and MS/MS studies were performed on Thermo LCQ-Advantage (Thermo Electron, San Jose, California, USA) using electrospray ionization source and ion trap mass spectrometer. The source voltage was maintained at 3.0 kV and the capillary temperature at 250°C. Nitrogen was used as both sheath and auxiliary gas. The mass to charge ratio was scanned across the range of m/z 50–1000. MS/MS studies were carried out by keeping normalized collision energy at 25% and an isolation width of 4 amu.

The HPLC consisted of Waters alliance 2690 separation module equipped with 2487 UV detector and column oven. A C18 column (Inertsil ODS-3, 250 mm × 4.6 mm, 5 µm) was used for chromatographic separation. The mobile phase A consisted of, 10 mM ammonium acetate adjusted to pH 6.50 ± 0.05 with formic acid and mobile phase B consisted of acetonitrile in gradient
mode ($T_{m=A:B}$) $T_{s}=80:20$, $T_{c}=60:20$, $T_{b}=60:40$, $T_{o}=30:70$, $T_{e}=80:20$, $T_{o}=80:20$ at a flow rate of 1.5 mL/min. The injection volume was 10 µL for a sample concentration of 0.5 mg/mL prepared in diluent (acetone-trile-water, 26:80, v/v). Detector wavelength was fixed at 230 nm and the column temperature was maintained at 30°C throughout the analysis.

2.4.3 Elicarbasepine acetate

The data acquisition was under the control of Xcalibur software (Thermo Electron, San Jose, CA, USA). The typical source conditions were: spray voltage-5 KV, capillary voltage-15-20 V, heated capillary temperature-250°C, tube lens offset voltage 20V, sheath gas (N₂) pressure-20 psi and helium was used as damping gas. For the ion trap analyser, the automatic gain control (AGC) setting was $2 \times 10^7$ counts for a full-scan mass spectrum and $2 \times 10^7$ counts for a full product ion mass spectrum with a maximum ion injection time of 200 ms. In the full scan MS³ mode, the precursor ion of interest was first isolated by applying an appropriate waveform across the end-cap electrodes of the ion-trap to resonantly eject all trapped ions, except those ions of m/z interest. The isolated ions were then subjected to a supplementary AC signal to resonantly excite hence causing collision induced dissociation (CID). The collision energies of 15-35 eV and isolation width of 5 amu were used. The excitation time was 30 ms. All the spectra were recorded under identical experimental conditions for isomers and average of 20-25 scans was performed.

A symmetry shield RP-8, (250 mm x 4.6 mm, 5 µm, Waters Corporation, MA, USA) was used for chromatographic separation. Mobile phase-A consisted of, 10 mM ammonium bicarbonate-acetonitrile (95:5, v/v) and B, acetonitrile-water (80:20, v/v) in gradient mode ($T_{m=A:B}$) $T_{s}=79:30$, $T_{c}=65:35$, $T_{b}=50:50$, $T_{o}=30:70$, $T_{e}=35:70:30$ $T_{o}=70:30$. The flow rate was set to 1.0 mL/min with detector wavelength was fixed at 215 nm. The injection volume was 10 µL for a sample concentration of 0.4 mg/mL prepared in diluent (mobile phase A-acetonitrile, 50:50, v/v). Column temperature was maintained at 35°C throughout the analysis.
2.4.4 Carbamazepine

The source voltage was maintained at 3.0 kV and the capillary temperature at 250°C. Nitrogen was used as both sheath and auxiliary gas. The mass to charge ratio was scanned across the range of m/z 50-1000. MS/MS studies were carried out by keeping normalized collision energy at 25% and an isolation width of 1 amu.

The HPLC consisted of Waters alliance 2690 separation module equipped with 2487 UV detector and column oven. A C18 column (Inertsil ODS-3 250 mm × 4.6 mm i.d. 5 μm particles) was used for chromatographic separation. The mobile phase consisted of a mixture of water- methanol-trifluoroacetic acid (30:70:0.05, v/v/v). The flow rate was maintained at 1.0 mL/min. 0.10 g of sample was dissolved in methanol and diluted to 50 mL with the same solvent. 10 mL of this solution was diluted to 20 mL with water. The injection volume was 20 μL and the detector wavelength was set at 230 nm. The column was maintained at 25°C throughout the analysis.

2.4.5 Darifenacin hydrobromide

MS and MS² studies were performed on Thermo LCQ-Advantage (Thermo Electron, San Jose, CA, USA) using electrospray ionization source and ion trap mass spectrometer. The typical source conditions were: spray voltage-5 kV, capillary voltage-15-20 V, heated capillary temperature, 250°C, tube lens offset voltage 20V, sheath gas N₂ 20psi and helium was used as damping gas. In the full scan MS² mode, the precursor ion of interest was first isolated by applying an appropriate wave form across the end cap electrodes of the ion-trap to resonantly eject all trapped ions, except those ions of m/z interest. The isolated ions were then subjected to a supplementary AC signal to resonantly excite hence causing collision induced dissociation (CID). The collision energy of 15–35 eV, isolation width of 5 amu and excitation time 30 ms were used. All the spectra were recorded under identical experimental conditions for isomers and average of 20–25 scans was performed.

The HPLC consisted of Waters alliance 2690 separation module equipped with 2487 UV detector, column oven, using an XTerra RP-18 column (250 × 4.6) mm, 5 μm, (Waters Corporation, Milford, MA, USA). Mobile phase A consisted of, 20 mM ammonium bicarbonate-acetonitrile (95:5, v/v) adjusted to
pH 5.5±0.05 with formic acid and mobile phase B consisted of acetonitrile-methanol (90:10, v/v) with a timed gradient mode T_{min}A:B, T_{0}:80:20, T_{75:25}, T_{35:65}, T_{60:40}, T_{70:35:65}, T_{20:80:20}, T_{80:20}. The flow rate was set at 1.0 mL/min with detector wavelength was fixed at 210 nm. The injection volume was 10 μL for a sample concentration of 1.0 mg/mL prepared in diluent (mobile phase A-mobile phase B, 80:20, v/v). Column temperature was maintained at 43°C throughout the analysis.

2.4.6 Deroxasirox

The MS and MS/MS studies were performed on 3200 Q-trap mass spectrometer (AB Sciex, CA, USA) using electrospray ionization source (-ive mode). The instrument was operated in enhanced product ion mode with the following settings: collision energy of -40V, collision energy spread-10 V declustering potential -10V and the capillary ion spray voltage was -4500 V. Nitrogen was used as curtain gas and CAD gas at a pressure of 15 Psi. Zero air at a pressure of 45 psi was used as nebulizer gas and heater gas. The HPLC consisted of LC-20AD binary gradient pump, SPD-10AVP UV detector, SIL-10HTC auto sampler and a column oven CTO-10ASVP (Shimadzu Corporation, Kyoto, Japan).

A Symmetry shield RP18 column (Waters Corporation, MA, USA, 250 × 4.6 mm, 5 μm) was used for chromatographic separation. Mobile phase A consisted of 0.1% (v/v) formic acid and mobile phase B consisted of 0.1% formic acid in acetonitrile-methanol mixture (50:50:0.1,v/v/v) in gradient mode (T_{min} A:B) T_{50:50}, T_{20:80}, T_{30:70}, T_{40:60}, T_{50:50} and a flow rate of 1.0 mL/min was used. Injection volume was 10 μL for a sample concentration of 400 μg/mL prepared in mobile phase-B. The column was maintained at 25°C throughout the analysis. Wavelength was adjusted to 245 nm.

2.4.7 Citralopram hydrobromide

The source voltage was maintained at 3.0 kV and the capillary temperature, at 250°C. Nitrogen was used as both sheath and auxiliary gas. The mass to charge ratio was scanned across the range of m/z 50-1000. MS/MS studies were carried out by keeping normalized collision energy at 25%
and an isolation width of 4 amu. Injection volume was 10 μL for a sample concentration of 0.5 mg/mL and detector wavelength was adjusted to 230 nm.

The HPLC consisted of Waters Alliance 2690 separation module equipped with 2487 UV detector and column oven. An Inertial ODS-3 column (GL Sciences Inc., Tokyo, Japan 250 mm × 4.6 mm, 5 μm) was used for chromatographic separation. The mobile phase consisted of a mixture of water-acetonitrile-urifluoroacetic acid (67:33:0.05, v/v/v). The flow rate was 1.0 mL/min and the column temperature was maintained at 25°C throughout the analysis.

2.5 NMR Conditions for galantamine hydrobromide

$^1$H and $^{13}$C NMR spectra were recorded at 399.957 and 100.432 MHz respectively, using a Bruker AVANCE 400 MHz spectrometer (Bruker, Fällanden, Switzerland) equipped with a 5mm BBO probe and a z-gradient shim system. The $^1$H spectra were recorded with 1 s pulse repetition time using 30° flip angle, while $^{13}$C spectra were recorded with power gated decoupling using 30° flip angle with repetition time of 2 s. Samples were dissolved in deuterated chloroform. The $^1$H and $^{13}$C chemical shift values were reported on the δ scale in ppm relative to tetramethyl silane as internal standard. All spectra were recorded with sample spinning. Attached proton test (APT) was done by j-modulated spin–echo for x–nuclei coupled to H–1 to determine the number of attached protons with 90 degree and 180 degree high power pulse. 2D homonuclear shift correlation experiments were performed with 20-90 degree and 90 degree with high power gradient pulse. Heteronuclear multiple quantum coherence (HMQC) correlation experiments were performed by heteronuclear zero and double quantum coherence with decoupling during acquisition with 90, 180 and 90 degree high power gradient pulse. Heteronuclear multiple bond coherence (HMBC) were done by optimized on long range couplings with low pass j-filter to suppress one band correlations, no decoupling during acquisition with 90, 180 and 90 degree high power gradient pulse.

2.6 Materials and reagents

2.6.1 Solvents and reagents
Deionized water was prepared using a Mill-Q plus water purification system from Millipore (Bedford, MA, USA). HPLC grade methanol, acetonitrile, isopropyl alcohol, tetrahydrofuran, triethylamine, N,N-dimethyloctylamine were purchased from Merck India Limited (Mumbai, India) and Qualigen Ltd (Mumbai, India). Analytical reagent grade orthophosphoric acid, sodium dihydrogen phosphate, 1-octane sulfonic acid sodium salt, dipotassium hydrogen phosphate, ammonium bicarbonate, ammonium acetate, potassium dihydrogen phosphate, sodium acetate, and formic acid were purchased from Merck India Limited (Mumbai, India). Analytical reagent grade trifluoroacetic acid, triethylamine and formic acid were purchased from Spectrochem (Mumbai, India) and Qualigen Ltd (Mumbai, India). Spectrochem (Mumbai, India). Analytical reagent grade orthophosphoric acid, Dimethyl sulfoxide-d6 and deuterated chloroform (for NMR) was purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Potassium bromide PT-IR grade was purchased from Merck KGaA (Darmstadt, Germany). Nitrogen, used as carrier gas for GC and auxiliary gas for ESI source, is obtained from Dominick Hunter H2 generator. Laboratory grade hydrochloric acid, sodium hydroxide and hydrogen peroxide were used for forced degradation study.

2.6.2 Materials and reagents used for synthesis

All the materials, reagents, solvents used for study were obtained from Chemical Research Department, Jubilant Life Sciences Limited (C, 26, Sector 59, Noida, India).

2.6.3 Materials

2.6.3.1 Rivastigmine tartrate

2.6.3.2 Eslicarbazepine acetate

Eslicarbazepine acetate API (batch No. SLB-crude) and standards of Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-7, Imp-8, Imp-9, Imp-10 and Imp-11, methoxy iminostilbene, toluene, sodium cyanate, benzoic acid, hydrochloric acid, sodium borohydride, trimethylamine, dimethylaminopyridine, acetic anhydride, pyridine, dichloromethane, zinc chloride, sodium bicarbonate, isopropyl alcohol. Imp-A, Imp-B, Imp-C and Imp-D were synthesized.

2.6.3.3 Galantamine hydrobromide

Sample of galantamine hydrobromide (Batch No. GMN/011/002) was obtained from Jubilant Life Sciences Limited (Mysore, India). Analytical reagent grade trifluoroacetic acid and hydrogen peroxide were purchased from Qualigens India Limited (Mumbai, India). Imp-A was isolated by semi-preparative HPLC.

2.6.3.4 Carbamazepine

Sample of carbamazepine API (Batch No. CBZ18/RD/09-crude) and iminodibenzyl standard (Batch No. IDB-std), sulphur, sodium cyanate, monochloro acetic acid diphenyl oxide, were obtained from Jubilant Life Sciences Limited (Mysore, India). Imp-B was isolated by semi-preparative HPLC.

2.6.3.5 Darifenacin hydrobromide

Sample of darifenacin hydrobromide API (batch No. DRF-crude) and standards of Imp-1 (2,2-Diphenyl-2-[[3S]-pyrroolidin-3-yl] acetamide tetrurate), Imp-2 (1-[2-(2,3-Dihydro-1-benzofuran-5-yl)]ethyl[pyrroolidin-3-yl](diphenyl acetic acid), Imp-3(5-Bromoacetyl)-1,2-dihydro-1-benzofuran), Imp-4 (5-Vinyl-2,3-Dihydro-1-benzofuran) 5-(5-Bromoethyl)-2,3-dihydro-1-benzofuran, N-bromo succinimide, dibenzoyl peroxide, carbon tetrachloride, 2,2-diphenyl-2-[[3S]-pyrroolidin-3-yl] acetamide tetrurate, potassium carbonate, toluene, acetone, hydrobromic acid, 2,2-Diphenyl-2-[[3S]-pyrroolidin-3-yl] acetamide tetrurate, 2-bromo-1(2,3-dihydro-1-benzofuran-5-yl)ethenone, potassium carbonate, ethyl acetate, isopropyl alcohol, hydrogen
peroxide, acetic acid, conc. hydrochloric acid, chloroform, sodium bicarbonate, dichloromethane, 2,2-Diphenyl-2-[(3S)-pyrrolidin-3-yl] acetamide tetratate, 7-bromo-5-(2-bromoethyl)-2,3-dihydro-1-benzofuraz. potassium carbonate, toluene, acetone, hydrobromic acid. Imp-A, Imp-B, Imp-C and Imp-D were synthesised.

2.6.3.6 Deferasirox

Sample of deferasirox API (Batch No. DFX/RD-crude), Imp-A (Salicylamide), Imp-B (Salicylic acid), Imp-C (2-Hydroxy-N-[2-hydroxyphenyl] carbonyl]benzamide), Imp-D(2-(2-Hydroxyphenyl)-4H-1,3-benzoxaz-4-one) and Imp-E (\{-3,5-bis[2-Hydroxyphenyl]-1-H,1,2,4-triazol-1-yl\}methylbenzoate were obtained from Jubilant Life Sciences Limited (Mysore, India), pyridine, o-xylene, triethylamine, ethyl alkol. Imp-I was synthesised.

2.6.3.7 Citalopram hydrobromide

Samples of citalopram hydrobromide, 1-(3-Dimethylaminopropyl)-1-(4-fluoro-phenyl)-1.3-dihydro-isobenzofuran-5-carboxamide (Imp-A), desmethyl Citalopram (Imp-B) were obtained from Jubilant Life Sciences Limited (Mysore, India). Ammonium chloride, methylene chloride, sodium borohydride, methanol, toluene, hydrochloric acid, cuprous cyanide, N,N-dimethylaminopropyl chloride, sodium hydride, toluene, dimethyl sulphoxide. Imp-B was isolated by semi-preparative HPLC.