TITLE: Experimental

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2.1 MATERIALS, CHEMICALS AND INSTRUMENTATION

This section provides a list of materials, instrumentation, chemicals, solvents, solutions and reference or working standards of selected drugs used during the entire method development and validation and application of the validated method to the study sample analysis. The details are provided in Table 1, 2 and 3.

2.2 LIQUID CHROMATOGRAPHY CONDITIONS

The conditions optimized for liquid chromatography along with the type of columns used for separation of the drugs are summarized in Table 4. This table compiles the important variables for chromatographic study viz. the buffer solutions, mobile phase used, flow rates, chromatographic column, column oven temperature etc. during method development and validation.

| Table 1: Materials and instrumentation used in the study |
|---------------------------------------------|-----------------|-----------------|
| **Product**                                   | **Grade**       | **Manufacturer**         | **City, Country** |
| Materials                                    |                 |                            |                  |
| HLB Cartridge                                | SPE             | Waters                     | Milford, USA     |
| Betabasic, C-8, column                       | 100x4.6mm; 5μ   | Hypersil                   | USA              |
| Zorbax SB-C18 column                         | 50 mm x 4.6 mm i.d.; 3.5μ | Agilent Technologies       | USA              |
| Xterra, C-18, column                         | 30mm x 2.1mm i.d; 3.5μ  | Waters                     | Milford, Massachusetts, USA |
| HyPurity, C-18, column                       | 100mm x 2.1mm i.d; 5μ  | Hypersil                   | USA              |
| Betabasic, C-8, column                       | 100mm x 4.6mm i.d; 5μ  | Hypersil                   | USA              |
| Betasil, C-18, column                        | 100mm x 3.0mm i.d; 3μ  | Hypersil                   | USA              |
| Microtubes                                   | Polypropylene   | Axygen                     | Union city, California, USA |
| Micropipettes                                | Mechanical, variable volume | Eppendorf                 | USA              |
| Blank Human Plasma                           | CPD as anticoagulant | Green Cross blood bank     | Ahmedabad, India |
| Blank Human Plasma                           | Heparin as anticoagulant | Torrent Research Centre | Ahmedabad, India |
| Instruments                                   |                 |                            |                  |
| Heraeus HFU686                               | Deepfreezer, -86°C | Kendro                    | Langenselbold, Germany |
| Heraeus centrifuge                           | Multifuge 3S.R   | Kendro                     | Langenselbold, Germany |
| Turbobavp LV                                 | Concentration workstation | Zymark                   | Osterode, Germany |
| PE 200 Series HPLC                           | Low Pressure Gradient | Perkin Elmer   | Shelton, USA      |
| API-4000 (Sciex)                             | Triple Quadrupole | Applied Biosystems         | USA              |
### Table 2: Chemicals and solvents

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Solvents</th>
<th>Purity</th>
<th>Manufacturer</th>
<th>Location</th>
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</thead>
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<tr>
<td>Ammonium Acetate</td>
<td>Molecular biology</td>
<td>Sigma</td>
<td>Steinheim, Germany</td>
<td></td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>Suprapure</td>
<td>Merck</td>
<td>Darmstadt, Germany</td>
<td></td>
</tr>
<tr>
<td>Ammonium Formate</td>
<td>Molecular biology</td>
<td>Sigma</td>
<td>Steinheim, Germany</td>
<td></td>
</tr>
<tr>
<td>Formic Acid</td>
<td>Suprapure</td>
<td>Merck</td>
<td>Darmstadt, Germany</td>
<td></td>
</tr>
<tr>
<td>Ammonia Solution (25%)</td>
<td>AR</td>
<td>Merck</td>
<td>Mumbai, India ; Darmstadt, Germany</td>
<td></td>
</tr>
<tr>
<td>o-Phosphoric Acid</td>
<td>Suprapure</td>
<td>Merck</td>
<td>Darmstadt, Germany</td>
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</tr>
<tr>
<td>n-Propionic acid</td>
<td>Suprapure</td>
<td>Merck</td>
<td>Darmstadt, Germany</td>
<td></td>
</tr>
<tr>
<td>n-butyric acid</td>
<td>Suprapure</td>
<td>Merck</td>
<td>Darmstadt, Germany</td>
<td></td>
</tr>
<tr>
<td>MilliQ Water Type-I</td>
<td></td>
<td>Millipore</td>
<td>Bangalore, India</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>HPLC</td>
<td>JT Baker</td>
<td>Phillipsburg, USA</td>
<td></td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>Gradient</td>
<td>JT Baker</td>
<td>Phillipsburg, USA</td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>HPLC</td>
<td>Merck</td>
<td>Mumbai, India</td>
<td></td>
</tr>
<tr>
<td>t-butyl methyl ether</td>
<td>HPLC</td>
<td>Merck</td>
<td>Mumbai, India</td>
<td></td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>HPLC</td>
<td>Merck</td>
<td>Mumbai, India</td>
<td></td>
</tr>
<tr>
<td>n-Hexane</td>
<td>HPLC</td>
<td>Merck</td>
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</table>

### Table 3: Working standards used in the study

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Purity</th>
<th>Manufacturer</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sibutramine hydrochloride</td>
<td>&gt; 99%</td>
<td>Nosch Lab Private Ltd</td>
<td>Hyderabad, India</td>
</tr>
<tr>
<td>Metabolite-M1 hydrochloride</td>
<td>&gt; 99%</td>
<td>Torrent Research Centre</td>
<td>Ahmedabad, India</td>
</tr>
<tr>
<td>Metabolite-M2 hydrochloride</td>
<td>&gt; 99%</td>
<td>Torrent Research Centre</td>
<td>Ahmedabad, India</td>
</tr>
<tr>
<td>Imipramine hydrochloride</td>
<td>&gt; 99%</td>
<td>Torrent Research Centre</td>
<td>Ahmedabad, India</td>
</tr>
<tr>
<td>Isosorbide-5-mononitrate</td>
<td>49.58%</td>
<td>JP Fine chemicals Ltd.</td>
<td>Mumbai, India</td>
</tr>
<tr>
<td>Topiramate</td>
<td>&gt; 99%</td>
<td>Hetero drugs</td>
<td>Hyderabad, India</td>
</tr>
<tr>
<td>Perindopril</td>
<td>&gt; 99%</td>
<td>Glenmark</td>
<td>Mumbai, India</td>
</tr>
<tr>
<td>Perindoprilat</td>
<td>&gt; 99%</td>
<td>Torrent Research Centre</td>
<td>Ahmedabad, India</td>
</tr>
<tr>
<td>Ramipril</td>
<td>&gt; 99%</td>
<td>Torrent Research Centre</td>
<td>Ahmedabad, India</td>
</tr>
<tr>
<td>Indapamid</td>
<td>&gt; 99%</td>
<td>Torrent Research Centre</td>
<td>Ahmedabad, India</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>&gt; 99%</td>
<td>Torrent Research Centre</td>
<td>Ahmedabad, India</td>
</tr>
<tr>
<td>Sertraline hydrochloride</td>
<td>&gt; 99%</td>
<td>Torrent Research Centre</td>
<td>Ahmedabad, India</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>&gt; 99%</td>
<td>Torrent Research Centre</td>
<td>Ahmedabad, India</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>&gt; 99%</td>
<td>Torrent Research Centre</td>
<td>Ahmedabad, India</td>
</tr>
</tbody>
</table>
2.3 MASS SPECTROMETRY PARAMETERS

The drugs studied in this work using MS as detector were successfully tuned to obtain intense and stable product ions in accordance with their polarity. The parent to product ion transitions studied for quantification of the selected drugs in biological matrices is presented in Table 5. A comprehensive summary of source dependent and compound dependent parameters set for mass spectrometric conditions of these molecules are also compiled in Table 6.

2.4 EXTRACTION METHODOLOGY

The protocols adopted for extraction of different drugs and their metabolites from plasma/whole blood sample are illustrated in a tabular form in Table 7. These include the extraction technique, matrix volume for processing, internal standards additives used during extraction and. The table also mentions the LLOQ achieved for all the drugs and their metabolites. The detailed extraction procedures are presented in the respective chapters 3-8.

2.5 ACCEPTANCE CRITERIA FOR METHOD VALIDATION

The results of the method validation parameters studied for the drugs, met the acceptance criteria defined in Table 8. [1] The formulas used in calculating these parameters are given in Table 9.
<table>
<thead>
<tr>
<th>Name of drug</th>
<th>Buffer solutions</th>
<th>Solvent</th>
<th>Mobile phase ratio (Buffer: Solvent)</th>
<th>Flow rate (ml/min)</th>
<th>Column make, type and dimensions</th>
<th>Column oven temp. (°C)</th>
<th>% Split ratio of column eluate (MS: Waste)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sibutramine and its metabolites M1 and M2</td>
<td>10mM Ammonium Formate, pH=3.5 with formic acid</td>
<td>Acetonitrile</td>
<td>10: 90</td>
<td>0.8</td>
<td>Betabasic, C-8, 100x4.6mm; 5µ</td>
<td>45</td>
<td>33: 67</td>
</tr>
<tr>
<td>Isosorbide-5-mononitrate</td>
<td>2mM Ammonium Acetate, pH=9.0 with ammonia</td>
<td>Acetonitrile</td>
<td>10: 90</td>
<td>0.6</td>
<td>Zorbax, SB C-18, 50x4.6mm; 3.5µ</td>
<td>45</td>
<td>33: 67</td>
</tr>
<tr>
<td>Perindopril and its metabolite Perindoprilat</td>
<td>0.1% Ammonia (v/v)</td>
<td>Methanol</td>
<td>20: 80</td>
<td>0.3</td>
<td>Xterra, C-18, 30x2.1mm; 3.5µ</td>
<td>35</td>
<td>100</td>
</tr>
<tr>
<td>Indapamide</td>
<td>10mM Ammonium Acetate, pH=3.5 with acetic acid</td>
<td>Acetonitrile</td>
<td>10: 90</td>
<td>0.3</td>
<td>HyPurity, C-18, 100x2.1mm; 5µ</td>
<td>35</td>
<td>100</td>
</tr>
<tr>
<td>Sertraline</td>
<td>5mM Ammonium Acetate, pH=3.0 with acetic acid</td>
<td>Acetonitrile</td>
<td>20: 80</td>
<td>0.5</td>
<td>Betabasic, C-8, 100x4.6mm; 5µ</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>5mM Ammonium Acetate, pH=3.2 with acetic acid</td>
<td>Acetonitrile</td>
<td>20: 80</td>
<td>0.5</td>
<td>Betasil, C-18, 100x3.0mm; 3µ</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>Drug Name</td>
<td>Polarity (Positive / Negative)</td>
<td>Parent Ion (m/z)</td>
<td>Q1 Resolution</td>
<td>Product Ion (m/z)</td>
<td>Q3 Resolution</td>
<td>Dwell time (msec)</td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------------------</td>
<td>------------------</td>
<td>---------------</td>
<td>------------------</td>
<td>---------------</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>Sibutramine</td>
<td>Positive</td>
<td>280.20</td>
<td>Unit</td>
<td>125.00</td>
<td>Unit</td>
<td>200</td>
<td></td>
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<tr>
<td>Metabolite M1</td>
<td>Positive</td>
<td>266.50</td>
<td>Unit</td>
<td>125.10</td>
<td>Unit</td>
<td>200</td>
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</tr>
<tr>
<td>Metabolite M2</td>
<td>Positive</td>
<td>252.50</td>
<td>Unit</td>
<td>125.10</td>
<td>Unit</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Imipramine (IS)</td>
<td>Negative</td>
<td>281.00</td>
<td>Unit</td>
<td>86.00</td>
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<td>200</td>
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<tr>
<td>Isosorbide-5-mononitrite</td>
<td>Negative</td>
<td>250.20</td>
<td>Unit</td>
<td>59.00</td>
<td>Unit</td>
<td>200</td>
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<tr>
<td>Topiramate (IS)</td>
<td></td>
<td>337.88</td>
<td>Unit</td>
<td>77.94</td>
<td></td>
<td>200</td>
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<tr>
<td>Perindopril</td>
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<td>368.10</td>
<td>Unit</td>
<td>168.10</td>
<td>Unit</td>
<td>200</td>
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<tr>
<td>Perindoprilat</td>
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<td>339.30</td>
<td>Unit</td>
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<tr>
<td>Ramipril (IS)</td>
<td>Positive</td>
<td>415.30</td>
<td>Unit</td>
<td>166.10</td>
<td></td>
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<tr>
<td>Indapamide</td>
<td>Positive</td>
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<td>Low</td>
<td>132.20</td>
<td>Unit</td>
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<tr>
<td>Glimepiride (IS)</td>
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<td>Low</td>
<td>352.30</td>
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<td>Low</td>
<td>81.00</td>
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<td>Unit</td>
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<td>Drug Name</td>
<td>Source Parameters</td>
<td>Compound Parameters</td>
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<td>Turbo Ion Spray</td>
<td>DP (V)</td>
<td>CE (V)</td>
<td>CXP (V)</td>
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<td>Voltage (V)</td>
<td>GS1</td>
<td>GS2</td>
<td>CUR</td>
<td>TEM</td>
<td>CAD</td>
<td>EP (V)</td>
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<td>20</td>
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<td>-10</td>
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<td>35</td>
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<td>20</td>
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<td>Ramipril (IS)</td>
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<td>50</td>
<td>20</td>
<td>450</td>
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<td>Indapamide</td>
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<td>20</td>
<td>40</td>
<td>10</td>
<td>400</td>
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<td>Glimepiride (IS)</td>
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<td>40</td>
<td>50</td>
<td>20</td>
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<td>Sertraline</td>
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<td>5500</td>
<td>40</td>
<td>50</td>
<td>20</td>
<td>450</td>
<td>5</td>
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<tr>
<td>Imipramine (IS)</td>
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<td>40</td>
<td>50</td>
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<td>Levetiracetam</td>
<td></td>
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<td>20</td>
<td>40</td>
<td>10</td>
<td>400</td>
<td>5</td>
</tr>
<tr>
<td>Clonazepam (IS)</td>
<td></td>
<td>5500</td>
<td>20</td>
<td>40</td>
<td>10</td>
<td>400</td>
<td>5</td>
</tr>
<tr>
<td>Sr. No.</td>
<td>Name of drug</td>
<td>Biological matrix</td>
<td>Biological matrix volume for processing (ml)</td>
<td>Internal Standard</td>
<td>Extraction additives</td>
<td>Extraction technique</td>
<td>Eluting solvent</td>
</tr>
<tr>
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<td>-------------------</td>
<td>---------------------</td>
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<td>-----------------</td>
</tr>
<tr>
<td>1</td>
<td>Sibutramine and its metabolites M1 and M2</td>
<td>Plasma</td>
<td>0.5</td>
<td>Imipramine</td>
<td>H₃PO₄</td>
<td>SPE</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>2</td>
<td>Isosorbide-5-mononitrate</td>
<td>Plasma</td>
<td>0.5</td>
<td>Topiramate</td>
<td>MilliQ Water</td>
<td>SPE</td>
<td>0.1% Ammonia in acetonitrile</td>
</tr>
<tr>
<td>3</td>
<td>Perindopril and its metabolite Perindoprilat</td>
<td>Plasma</td>
<td>0.75</td>
<td>Ramipril</td>
<td>H₃PO₄ and MilliQ Water</td>
<td>SPE</td>
<td>Methanol</td>
</tr>
<tr>
<td>4</td>
<td>Indapamide</td>
<td>Whole Blood</td>
<td>0.5</td>
<td>Glimepiride</td>
<td>5% ZnSO₄ and 0.1M NaOH</td>
<td>LLE</td>
<td>Water: Acetonitrile (10:90)</td>
</tr>
<tr>
<td>5</td>
<td>Sertraline</td>
<td>Plasma</td>
<td>0.5</td>
<td>Imipramine</td>
<td>MilliQ Water</td>
<td>SPE</td>
<td>0.1% Formic acid in methanol</td>
</tr>
<tr>
<td>6</td>
<td>Levetiracetam</td>
<td>Plasma</td>
<td>0.2</td>
<td>Clonazepam</td>
<td>H₃PO₄ and MilliQ Water</td>
<td>SPE</td>
<td>Methanol</td>
</tr>
</tbody>
</table>
# Table 8: Acceptance criteria of validation parameters performed for the drugs studied

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Validation Parameter</th>
<th>Acceptance Criteria</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Calibration curve range</td>
<td>----</td>
</tr>
<tr>
<td>2</td>
<td>Specificity</td>
<td>Area response at the rt of analyte in control plasma should be &lt; 20% of LLOQ area response &amp; area response at the rt of IS in control plasma &lt; 5% of IS area response</td>
</tr>
<tr>
<td>3</td>
<td>Sensitivity (LLOQ)</td>
<td>Area response of analyte in LLOQ should be at least five times compared to control plasma area response. % CV of concentration should be ≤ 20 % Nominal concentration should be 80-120</td>
</tr>
<tr>
<td>4</td>
<td>Linearity</td>
<td>% Nominal concentration:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For LLOQ: 80-120</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For LQC: 85-115</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For MQC: 85-115</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For HQC: 85-115</td>
</tr>
<tr>
<td>5</td>
<td>Within-batch or Intra-assay accuracy</td>
<td>% Nominal concentration:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For LLOQ: 80-120</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For LQC: 85-115</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For MQC: 85-115</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For HQC: 85-115</td>
</tr>
<tr>
<td>6</td>
<td>Between-batch or Inter-assay accuracy</td>
<td>% Nominal concentration:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For LLOQ: 80-120</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For LQC: 85-115</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For MQC: 85-115</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For HQC: 85-115</td>
</tr>
<tr>
<td>7</td>
<td>Within-batch or Intra-assay precision</td>
<td>% CV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For LLOQ: ≤ 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For LQC: ≤ 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For MQC: ≤ 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For HQC: ≤ 15</td>
</tr>
<tr>
<td>8</td>
<td>Between-batch or Inter-assay precision</td>
<td>% CV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For LLOQ: ≤ 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For LQC: ≤ 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For MQC: ≤ 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For HQC: ≤ 15</td>
</tr>
</tbody>
</table>

(contd.)
<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Validation Parameter</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Recovery of analyte</td>
<td>% CV across the QC level should be ≤ 20</td>
</tr>
<tr>
<td>10</td>
<td>Recovery of internal standard</td>
<td>% CV across the QC level should be ≤ 15</td>
</tr>
<tr>
<td>11</td>
<td>Matrix effect</td>
<td>% Bias / % In accuracy For LQC: ± 15 For HQC: ± 15</td>
</tr>
<tr>
<td>12</td>
<td>Dilution integrity</td>
<td>% Nominal concentration: For ½ of 2ULOQ: 85-115 For ¼ of 2ULOQ: 85-115 %CV at ½ of 2ULOQ: ≤ 15 %CV at ¼ of 2ULOQ: ≤ 15</td>
</tr>
<tr>
<td>14</td>
<td>Stock solution stability of drug at room temperature and at refrigerated condition (2-8°C) { hours / days }</td>
<td>Mean % change at ULOQ: ± 5 %</td>
</tr>
<tr>
<td>15</td>
<td>Internal standard stock solution stability at room temperature and at refrigerated condition (2-8°C) { hours / days }</td>
<td>Mean % change for IS: ± 5 %</td>
</tr>
<tr>
<td>16</td>
<td>Bench top stability of drug in biological matrices at room temp { hours }</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Process sample stability of drug at refrigerated condition / room temperature { hours }</td>
<td>Mean % change For LQC: ± 15 For HQC: ± 15</td>
</tr>
<tr>
<td>18</td>
<td>Dry state sample stability of drug at refrigerated condition / room temperature { hours }</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Freeze thaw stability of drug in biological matrices after 3rd FT cycle at -70°C</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Long term stability of drug in biological matrices at -70°C for { days }</td>
<td>Mean % change: For LQC: ± 15 For MQC: ± 15 For HQC: ± 15</td>
</tr>
</tbody>
</table>
Table 9: Common formulae used for calculating the various validation parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>% CV</td>
<td>( \text{standard deviation} \times 100 )</td>
</tr>
<tr>
<td></td>
<td>( \text{mean} )</td>
</tr>
<tr>
<td>% Accuracy</td>
<td>( \frac{\text{calculated conc.}}{\text{theoretical conc.}} \times 100 )</td>
</tr>
<tr>
<td>% Inaccuracy</td>
<td>( \frac{(\text{calculated conc.} - \text{theoretical conc.})}{\text{theoretical conc.}} \times 100 )</td>
</tr>
<tr>
<td>% Recovery</td>
<td>( \frac{\text{peak area for standard spiked before extraction}}{\text{peak area for standard spiked after extraction into plasma extracts}} \times 100 )</td>
</tr>
</tbody>
</table>

Note: The formula used to calculate % Inaccuracy is same as that for % Relative Error, % Bias and % Change

2.6 STABILITY OF DRUGS IN MATRIX

The stability of drugs and their metabolites in plasma/whole blood was assessed under different conditions viz. bench top, in autosampler, dry state, freeze and thaw cycles and long term at – 70°C. The solution stability was also evaluated at room temperature and by storing at 2-8°C. The details are presented in Table 10.

2.7 BIOEQUIVALENCE STUDY CONDITIONS

The application of the developed and validated methods was done in the study of bioequivalence in healthy human volunteers with the selected drug formulation under fast and /or fed conditions. The results were compared with a reference of identical formulation. A summary of the studies is presented in Table 11.

2.8 REFERENCE

### Table 10: Summary of established stability in different conditions during method validation

<table>
<thead>
<tr>
<th>Activity</th>
<th>Molecules</th>
<th>Parameters</th>
<th>Sibutramine, M1 and M2</th>
<th>5-Isosorbide mononitrate</th>
<th>Perindopril and Perindoprilat</th>
<th>Indapamide</th>
<th>Sertraline</th>
<th>Levetiracetam</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solution stability</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Room temp (RT)</td>
<td></td>
<td></td>
<td>16 h</td>
<td>8 h</td>
<td>24 h</td>
<td>24 h</td>
<td>24 h</td>
<td>24 h</td>
</tr>
<tr>
<td>2-8°C</td>
<td></td>
<td></td>
<td>12 days</td>
<td>14 days</td>
<td>10 days</td>
<td>48 h</td>
<td>21 days</td>
<td>17 days</td>
</tr>
<tr>
<td><strong>Stability in matrix</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bench top at RT</td>
<td></td>
<td></td>
<td>8 h</td>
<td>6 h</td>
<td>6 h</td>
<td>6 h</td>
<td>6 h</td>
<td>6 h</td>
</tr>
<tr>
<td>Autosampler</td>
<td></td>
<td></td>
<td>24 h at RT</td>
<td>28 h at RT</td>
<td>48 h at 5°C</td>
<td>43 h at 10°C</td>
<td>40 h at 5°C</td>
<td>46 h at 5°C</td>
</tr>
<tr>
<td>Dry state</td>
<td></td>
<td></td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>20 h at 2-8°C</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Freeze and thaw (F&amp;T)</td>
<td></td>
<td></td>
<td>3rd cycle</td>
<td>3rd cycle</td>
<td>3rd cycle</td>
<td>3rd cycle</td>
<td>3rd cycle</td>
<td>3rd cycle</td>
</tr>
<tr>
<td>Long term at -70°C (LT)</td>
<td></td>
<td></td>
<td>36 days</td>
<td>14 days</td>
<td>222 days</td>
<td>40 days</td>
<td>71 days</td>
<td>66 days</td>
</tr>
</tbody>
</table>
### Table 11: Summary of bioequivalence study conditions for selected drug molecules

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Dose (mg)</th>
<th>Dosage form</th>
<th>Type of formulation</th>
<th>No of volunteers</th>
<th>Study condition</th>
<th>No period</th>
<th>Treatments</th>
<th>Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sibutramine</td>
<td>15</td>
<td>CAPSULE</td>
<td>Immediate release</td>
<td>40</td>
<td>Fast</td>
<td>2</td>
<td>2</td>
<td>Crossover</td>
</tr>
<tr>
<td>5-ISMN</td>
<td>60</td>
<td>TABLET</td>
<td>Extended release</td>
<td>24</td>
<td>Fast</td>
<td>2</td>
<td>2</td>
<td>Crossover</td>
</tr>
<tr>
<td>Perindopril</td>
<td>4</td>
<td>TABLET</td>
<td>Immediate release</td>
<td>24</td>
<td>Fast</td>
<td>2</td>
<td>2</td>
<td>Crossover</td>
</tr>
<tr>
<td>Indapamide</td>
<td>1.5</td>
<td>TABLET</td>
<td>Sustained release</td>
<td>14</td>
<td>Fast &amp; Fed</td>
<td>2</td>
<td>2</td>
<td>Crossover</td>
</tr>
<tr>
<td>Sertraline</td>
<td>100</td>
<td>TABLET</td>
<td>Immediate release</td>
<td>18</td>
<td>Fast</td>
<td>2</td>
<td>2</td>
<td>Crossover</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>1000</td>
<td>TABLET</td>
<td>Immediate release</td>
<td>12</td>
<td>Fed</td>
<td>2</td>
<td>2</td>
<td>Crossover</td>
</tr>
</tbody>
</table>