NEW BIO-ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF NAPROXEN BY LCMS

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ABSTRACT
Bio analysis employed for the quantitative determination of drugs and their metabolites in biological fluids. Studies involving measurement of the drugs or metabolites in the biological fluids such as blood plasma, serum, urine, CSF, etc. requires the selective, sensitive, well characterized bio analytical method to yield reliable results which can be satisfactorily interpreted as well as to emphasize that each analytical technique has its own characteristics, which will vary from analyte to analyte. A research was carried out to develop and validate bioanalytical method by high performance liquid chromatography mass spectrometric method for the estimation of Naproxen in human plasma using Zidovudine as internal standard.

The method was validated over a concentration range of 500.1 ng/mL to 100028.5 ng/mL for Naproxen. The results of selectivity, carryover test, linearity, precision and accuracy, stabilities, recovery, and Concomitant Drug Effect presented in this report are within the acceptance range for bio-analytical batch acceptance criteria given in USFDA acceptance range as per ‘Guidance for Industry – Bio-analytical Method Validation. The analytical method described above is valid for the estimation of Naproxen in human plasma over a range of 500.1 ng/mL to 100028.5 ng/mL with the detection of Naproxen m/z – 229.00 (parent) and 185.00 (product) and internal standard Zidovudine m/z – 267.00 (parent) and 222.90 (product) in negative ion mode, it is concluded that the analytical method developed was novel, accurate, précised selective and
is suitable for application in routine analysis of pharmaceutical preparation and is within acceptance range for bio-analytical criteria in USFDA acceptance.

**KEYWORDS:** Naproxen, LCMS, Bio-Analytical Method.

**INTRODUCTION**

Bio analysis employed for the quantitative determination of drugs and their metabolites in biological fluids. Studies involving measurement of the drugs or metabolites in the biological fluids such as blood plasma, serum, urine, bile, CSF, etc. requires the selective, sensitive, well characterized bio analytical method to yield reliable results which can be satisfactorily interpreted as well as to emphasize that each analytical technique has its own characteristics, which will vary from analyte to analyte.\[^{1, 2}\] In today’s drug development environment, highly sensitive, selective and validated methods are required to quantify drugs in biological matrices such as blood, plasma, serum or urine using various techniques like High performance liquid chromatography (HPLC) with UV/PDA detector, High performance liquid chromatography (HPLC) with fluorescence detector, Liquid chromatography mass spectrometry (LC-MS), Liquid chromatography mass spectrometry (LC-MS/MS), Gas chromatography mass spectrometry (GC-MS), Gas chromatography tandem mass spectrometry (GC-MS/MS), capillary electrophoresis mass spectrometry (CE-MS) etc.

A bio analytical method consists of two main components Sample preparation which is a technique used to clean up and elimination of matrix effect on a sample with better recovery before analysis and/or to concentrate a sample to improve its detection & sensitivity and another is selection of chromatographic and mass spectrometric conditions.\[^{3, 4, 5, 6}\]

The following experimental design is drawn in order to prove the test method is capable to yield consistent, reliable and reproducible results within the predetermined acceptance limits. Acceptance criteria for the validation parameters are specified in individual experimental design.\[^{7, 8, 9}\]

**The following parameters have been validated**
- carryover test
- Selectivity
- sensitivity
matrix effect

Linearity

Precision and Accuracy

Recovery

Dilution integrity

Ruggedness

Re-injection stability

Concomitant Drug Effect

Stabilities
  o Room temperature stability
  o 10.2. Refrigerator stock solution stability
  o 10.3 Bench top stability
  o 10.4 Auto sampler stability
  o 10.5 Long term stability
  o 10.6 Freeze thaw stability
  o 10.7 Wet Extract Stability

MATERIALS AND METHODS

The study was approved by the Human Research Ethical committee, RIMS, Kadapa.
To develop and validate bioanalytical method by high performance liquid chromatography mass spectrometric method for the estimation of Naproxen in human plasma using Zidovudine as internal standard. The objective of this experiment was to optimize the method for estimation of naproxen in human plasma.

Optimization of Instrument conditions (Tuning)

Naproxen chemical name is (+)-(S)-2-(6-methoxynaphthalen-2-yl) propanoic acid. It is easily deprotonized and gives Parent ion which is a Negative Ion. So I found below information regarding tuning parameters for Naproxen.

State file information (API 3000 in Negative ion mode)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection:</td>
<td>Negative ion mode (API 3000)</td>
</tr>
<tr>
<td>Naproxen m/z – 229.0 (parent) and 185.0 (product)</td>
<td></td>
</tr>
<tr>
<td>Zidovudine m/z – 266.00 (parent) and 222.90 (product)</td>
<td></td>
</tr>
</tbody>
</table>
Optimization of Chromatographic conditions
To get good peak shape and reproducible response for analyzing the drug, altered different buffers, mobile phases and columns.

**Buffer preparation:** 770.81mg of ammonium acetate transferred to a 500 mL reagent bottle and made up to the mark with Milli Q water. Mixed well and sonicated in an ultrasonicator for 5 minutes.

**Mobile phase:** 1800 mL HPLC grade Acetonitrile was transferred to a 2000 mL reagent bottle and 200 mL of HPLC 20mM Ammonium acetate buffer was added to it.

**Column:** Zorbax XDB Phenyl, 4.6 x 75 mm, 3.5 µm (Make: Agilent technologies)
The peak shape and reproducibility is good.

The above chromatographic conditions were suitable for analysis of drug.

**Step – 3: Selection of Internal standard (IS)**
Many comparative study trails were made with (diclofenac sodium (DS), lamovidine) selecting IS in order to reduce overlapping. Finally Zidovudine (ZD) was selected as Internal standard (IS) which was found to be symmetric, reproducible and intense.

**Selection of Sample Processing Procedure**
**METHOD-1**
- **Protein Precipitation Method**
  Take about 250µl of plasma (drug was spiked in plasma) in a ria vial and add 25µl of IS to it. Mix well and add buffer to it. Mix well the solution and centrifuge it for 20 mins. After Centrifugation take the Supernant liquid and inject into the loading vials and loaded into the auto sampler.

**Observation**
Low recovery and interference was observed with drug and internal standard. Peak shape was not good and column back pressure was increased. This method was not suitable for analysis of drug. Then, we processed with Liquid – Liquid Extraction.
METHOD-2

Liquid – Liquid Extraction

The samples were thawed in water bath and vortexed to ensure complete mixing of the contents. 100 µL of the plasma sample was pipetted into 15 mL stopper glass test tubes, 25 µL of 200µg/mL zidovudine dilution was added to it and vortexed except in blank plasma samples where 25 µL diluent was added and vortexed.5 mL of methyl tert-butyl ether (TBME) was added to it and shake for 20 mins on a reciprocating shaker at 200 rpm. Samples were centrifuged at 4000 rpm for 10 minutes at 4°C. Supernant organic layer (4.0 mL) was transferred to prelabelled dry test tubes and evaporated to dryness under nitrogen at 40°C. The samples were reconstituted in 4000 µL of reconstitution solution and vortexed all samples adequately. The reconstituted samples were transferred into loading vials and loaded into the auto sampler.

Observation

The peak shape and recovery was good. After long run time reproducibility of analyte and internal standard was decreased. So, this indicates that the ionization of analyte was not intact in sample. So, to overcome this problem buffer is added. 1% formic acid is added to the above solution before the addition of extractions solvent.

OPTIMIZED METHOD

Buffer-1 preparation: 770.81 mg of ammonium acetate was transferred to a 500 mL reagent bottle and made up to the mark with Milli Q water. Mixed well and sonicated in an ultrasonicator for 5 minutes.

Buffer-2 preparation

About 1 mL of Formic acid was transferred to a 100 mL reagent bottle and made up to the mark with HPLC grade water. Mixed well and sonicated in an ultrasonicator for 5 minutes.

Mobile phase: 1800 mL HPLC grade Acetonitrile was transferred to a 2000 mL reagent bottle and 200 mL of HPLC 20mM Ammonium acetate buffer was added to it.

Diluent: HPLC Methanol and Milli-Q water were mixed in the ratio of 50:50 and sonicated to degas.

Rinsing solution: HPLC Methanol and Milli-Q water were mixed in the ratio of 50:50 and sonicated to degas.
INSTRUMENT EMPLOYED
Make: WATERS HPLC
Model number: API – 3000 LCMS/MS
Software: EMPOWER-2
Column: Agilent zorbax XDB Phenyl, 4.6x75mm, 3.5 µm
(Make: Agilent technologies)
Detector: Quadrapole mass Analyser.

BIO ANALYTICAL CONDITIONS
Mobile phase: HPLC grade Acetonitrile: 20mM Amoniumacetate in water (90:10, v/v)
Rinsing solution: HPLC Methanol: Milli-Q water (50:50, v/v)
Flow rate: 0.500 mL/minute (direct)
Sample Cooler Temperature: 10°C
Injection volume: 15 µL
Needle Rinsing Volume: 1000 µL
Dip time: 2 sec
Retention time: Naproxen 2.0 ± 0.3 minutes
Zidovudine 1.75 ± 0.3 minutes
Run Time: 3.00 minutes

NAPROXEN STOCK SOLUTION
Weigh accurately about 40.00 mg of naproxen sodium working standard and transfer to a 10 ml clean glass volumetric flask and dissolve in HPLC grade Methanol and make up the volume with the same to produce a solution of 4000000.0000 ng/ml. The stock solution was stored in refrigerator at 2-8°C. The stock solutions were diluted to suitable concentrations using diluent for spiking into plasma to obtain calibration curve (CC) standards, quality control (QC) samples and DIQC samples. All other final dilutions (system suitability dilutions, aqueous mixture, etc.) were prepared in mobile phase.

ZIDOVUDINE STOCK SOLUTION (INTERNAL STANDARD)
Weigh accurately about 5.0000 mg of zidovudine and transfer to a 5 mL volumetric flask and dissolve in HPLC grade methanol and make up the volume with the same to produce a solution of 1000000.0000 ng/ml. The stock solution was stored in refrigerator at 2-8°C. The
stock solution was diluted to suitable concentration using diluent for internal standard dilution.

**BIOLOGICAL MATRIX**

Eight lots of \( K_2 \)EDTA human plasma including one hemolytic and one lipemic plasma were screened for selectivity check. Eight human plasma-lots including hemolytic and lipemic plasmas were found free of any significant interference for naproxen and zidovudine. Two plasma lots were used to prepare calibration standards, quality control samples. Selectivity and matrix effect tests was performed according to Bio analytical Method Validation.

After bulk spiking, aliquots of 200 µL for CCs and 200 µL for QCs of spiked plasma samples were pipetted out into a prelabelled polypropylene micro centrifuge tubes and then all the bulk spiked samples were stored to deep freezer at \(-70 \, ^\circ C \pm 10 \, ^\circ C\), except twelve replicates each of LQC and HQC, which were stored in deep freezer at \(-20 \, ^\circ C \pm 5 \, ^\circ C\) for generation of stability data at \(-20 \, ^\circ C\).

**RESULTS AND DISCUSSION**

**Chromatography**

Representative chromatograms of aqueous standard analytes and internal standard mixture, blank plasma, blank plasma with internal standard, LLOQQC:0.2014 mg/ml sample, LQC:0.5640 mg/ml sample, MQC-l:2.0141 mg/ml sample MQC-2: 10.070 mg/ml sample, and HQC:17.667 mg/ml samples of naproxen.

**Table No. 1: Selectivity Data for Naproxen**

<table>
<thead>
<tr>
<th>SEL-LLOQ:0.2014 mg/ml</th>
<th>Naproxen Area</th>
<th>IS Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7924</td>
<td>1184429</td>
</tr>
<tr>
<td>2</td>
<td>8960</td>
<td>1206661</td>
</tr>
<tr>
<td>3</td>
<td>8814</td>
<td>1190596</td>
</tr>
<tr>
<td>4</td>
<td>9021</td>
<td>1236173</td>
</tr>
<tr>
<td>5</td>
<td>7726</td>
<td>1186449</td>
</tr>
<tr>
<td>6</td>
<td>8742</td>
<td>1245520</td>
</tr>
<tr>
<td>Mean</td>
<td>8531.2</td>
<td>1208304.7</td>
</tr>
<tr>
<td>SD</td>
<td>559.50</td>
<td>26554.25</td>
</tr>
<tr>
<td>% CV</td>
<td>7.56</td>
<td>2.20</td>
</tr>
</tbody>
</table>
There was no significant interference from endogenous components observed at the mass transitions of Naproxen and internal standard.

**Table No. 2: Within Batch Precision and Accuracy for Naproxen**

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>LLOQ</th>
<th>LQC</th>
<th>MQC1</th>
<th>MQC2</th>
<th>HQC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Max</strong></td>
<td>600.5</td>
<td>1725.1</td>
<td>17250.8</td>
<td>57502.5</td>
<td>97760.1</td>
</tr>
<tr>
<td><strong>Min</strong></td>
<td>400.3</td>
<td>1275.1</td>
<td>12750.6</td>
<td>42501.9</td>
<td>72257.5</td>
</tr>
<tr>
<td><strong>QC</strong></td>
<td>500.4</td>
<td>1500.1</td>
<td>15000.7</td>
<td>50002.2</td>
<td>85008.8</td>
</tr>
<tr>
<td>1</td>
<td>553.3</td>
<td>1707.2</td>
<td>15558.9</td>
<td>55202.6</td>
<td>93187.2</td>
</tr>
<tr>
<td>2</td>
<td>605.3*</td>
<td>1844.4*</td>
<td>16274.2</td>
<td>55083.3</td>
<td>96187.8</td>
</tr>
<tr>
<td>3</td>
<td>541.5</td>
<td>1539.0</td>
<td>16617.0</td>
<td>55772.3</td>
<td>94139.3</td>
</tr>
<tr>
<td>4</td>
<td>564.9</td>
<td>1561.8</td>
<td>17624.4*</td>
<td>57397.4</td>
<td>96344.6</td>
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<tr>
<td>5</td>
<td>568.2</td>
<td>1459.1</td>
<td>16385.1</td>
<td>57808.3*</td>
<td>94301.3</td>
</tr>
<tr>
<td>6</td>
<td>553.4</td>
<td>1510.0</td>
<td>16797.9</td>
<td>56959.2</td>
<td>99924.9*</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>564.43</td>
<td>1603.42</td>
<td>16542.58</td>
<td>56370.35</td>
<td>95680.85</td>
</tr>
<tr>
<td><strong>S.D.</strong></td>
<td>22.164</td>
<td>144.273</td>
<td>678.949</td>
<td>1170.045</td>
<td>2417.913</td>
</tr>
<tr>
<td><strong>C.V.%</strong></td>
<td>3.93</td>
<td>9.00</td>
<td>4.10</td>
<td>2.08</td>
<td>2.53</td>
</tr>
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</table>
### Table No. 3: Intra-day Precision and Accuracy for Naproxen

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>LLOQ QC</th>
<th>LQC</th>
<th>MQC1</th>
<th>MQC2</th>
<th>HQC</th>
</tr>
</thead>
<tbody>
<tr>
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<td>72257.5</td>
</tr>
<tr>
<td>QC</td>
<td>500.4</td>
<td>1500.1</td>
<td>15000.7</td>
<td>50002.2</td>
<td>85008.8</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Within-batch precision for LLOQ QC, LQC, MQC1, MQC2 and HQC ranged from 3.25 % to 7.43 %, 3.33 % to 9.00 %, 2.31 % to 4.33 %, 2.08 % to 2.64 % and 1.67 % to 4.13 % respectively. Within-batch accuracy for LLOQ QC, LQC, MQC1, MQC2 and HQC ranged from 91.36% to 112.80%, 89.43% to 107.89%, 91.29% to 110.28%, 98.89% to 112.74% and 98.54% to 112.55% respectively.
<table>
<thead>
<tr>
<th></th>
<th>LQC Response</th>
<th>MQC2 Response</th>
<th>HQC Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extracted QC</td>
<td>Non Extracted QC</td>
<td>Extracted QC</td>
</tr>
<tr>
<td>REC QC</td>
<td>LQC (07-12)</td>
<td>LQC (1-6)</td>
<td>MQC2 (07-12)</td>
</tr>
<tr>
<td>1</td>
<td>33433</td>
<td>52941</td>
<td>1550832</td>
</tr>
<tr>
<td>2</td>
<td>37090</td>
<td>50427</td>
<td>1532466</td>
</tr>
<tr>
<td>3</td>
<td>40116</td>
<td>50399</td>
<td>1560886</td>
</tr>
<tr>
<td>4</td>
<td>39507</td>
<td>52836</td>
<td>1649310</td>
</tr>
<tr>
<td>5</td>
<td>39031</td>
<td>50434</td>
<td>1520889</td>
</tr>
<tr>
<td>6</td>
<td>36437</td>
<td>49834</td>
<td>1472106</td>
</tr>
<tr>
<td>Mean</td>
<td>37602.3</td>
<td>51145.2</td>
<td>1547748.2</td>
</tr>
<tr>
<td>SD</td>
<td>2489.63</td>
<td>1369.77</td>
<td>58607.51</td>
</tr>
<tr>
<td>CV%</td>
<td>7.62</td>
<td>2.68</td>
<td>3.79</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>% Recovery</td>
<td>73.52</td>
<td>80.75</td>
<td>87.62</td>
</tr>
</tbody>
</table>

Table No. 4: Recovery of Naproxen from Human Plasma

Intra-day precision for LLOQ QC, LQC, MQC1, MQC2 and HQC was 12.73%, 11.58%, 10.63%, 7.19% and 7.24% respectively. Intra-day accuracy for LLOQ QC, LQC, MQC1, MQC2 and HQC was 102.08%, 98.16%, 100.78%, 105.81% and 105.55% respectively.

Table No. 5: Overall Recovery of Naproxen from Human Plasma

<table>
<thead>
<tr>
<th>Overall Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>SD</td>
</tr>
<tr>
<td>%CV</td>
</tr>
<tr>
<td>% Difference</td>
</tr>
</tbody>
</table>
Table No. 6: Within Batch Precision and Accuracy of Naproxen for Ruggedness

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>LLOQ QC</th>
<th>LQC</th>
<th>MQC1</th>
<th>MQC2</th>
<th>HQC</th>
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<td>85008.8</td>
</tr>
<tr>
<td>13</td>
<td>529.8</td>
<td>1759.2*</td>
<td>13705.7</td>
<td>50624.6</td>
<td>94965.8</td>
</tr>
<tr>
<td>14</td>
<td>517.3</td>
<td>1591.7</td>
<td>13687.4</td>
<td>49718.4</td>
<td>87957.6</td>
</tr>
<tr>
<td>15</td>
<td>502.3</td>
<td>1568.6</td>
<td>14460.6</td>
<td>51097.5</td>
<td>88244.0</td>
</tr>
<tr>
<td>16</td>
<td>507.7</td>
<td>1535.7</td>
<td>14101.1</td>
<td>53237.7</td>
<td>87677.8</td>
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<tr>
<td>17</td>
<td>482.0</td>
<td>1657.9</td>
<td>14145.5</td>
<td>52161.9</td>
<td>92733.6</td>
</tr>
<tr>
<td>18</td>
<td>519.5</td>
<td>1433.8</td>
<td>13683.6</td>
<td>50960.1</td>
<td>84965.3</td>
</tr>
<tr>
<td>Mean</td>
<td>509.60</td>
<td>1591.15</td>
<td>13963.82</td>
<td>51299.70</td>
<td>89423.85</td>
</tr>
<tr>
<td>S.D.</td>
<td>17.551</td>
<td>110.489</td>
<td>322.724</td>
<td>1233.551</td>
<td>3691.624</td>
</tr>
<tr>
<td>C.V.%</td>
<td>3.25</td>
<td>7.94</td>
<td>2.31</td>
<td>2.40</td>
<td>4.13</td>
</tr>
<tr>
<td>% Nominal</td>
<td>101.84</td>
<td>107.07</td>
<td>93.09</td>
<td>102.59</td>
<td>105.19</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

One precision and accuracy batch (PA BATCH-3) was processed and analyzed by different analyst, different column of same make and different solutions. Within batch precision for LLOQ QC, LQC, MQC1, MQC2 and HQC was 3.25%, 7.94%, 2.31%, 2.40%, and 4.13% respectively. Within batch accuracy for LLOQ QC, LQC, MQC1, MQC2 and HQC was 101.84%, 107.07%, 93.09%, 102.59%, and 105.19% respectively.

Table No. 7: Room Temperature Stock Solution Stability of Naproxen (0, 9 hours)

<table>
<thead>
<tr>
<th>Stock ID</th>
<th>Assay Date</th>
<th>Hours</th>
<th>%Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>NST-01A</td>
<td>22/12/11</td>
<td>0</td>
<td>100.00</td>
</tr>
<tr>
<td>NST-02</td>
<td>22/12/11</td>
<td>9</td>
<td>99.38</td>
</tr>
</tbody>
</table>

Table No. 8: Room Temperature Stock Solution Stability of Zidovudine (0, 9 hours)

<table>
<thead>
<tr>
<th>Stock ID</th>
<th>Assay Date</th>
<th>Hours</th>
<th>%Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZST-01A</td>
<td>22/12/11</td>
<td>0</td>
<td>100.00</td>
</tr>
<tr>
<td>ZST-02</td>
<td>22/12/11</td>
<td>9</td>
<td>97.93</td>
</tr>
</tbody>
</table>

CALCULATIONS

\[
\text{% Stability} = \frac{\text{Mean response of stability samples} \times \text{Comparison stock concentration} \times 100}{\text{Mean response of comparison stock samples} \times \text{Stability stock concentration}}
\]

Room temperature (20 ± 5 °C) stock solution stability was carried out at 9 hours for Naproxen and Zidovudine (Internal Standard) by injecting six replicates of prepared stock dilutions of Naproxen equivalent to the final MQC2 quality control concentration and
Zidovudine at final working concentration. Comparison of the mean area response stability samples of Naproxen and Zidovudine at 9 hours was carried out against the comparison samples (freshly prepared). The precision of room temperature (20 ± 5 °C) stock solution stability of Naproxen at 0 hours and 9 hours was 1.78% to 2.13%, respectively and percentage of stability was found to be 99.38%. The precision of room temperature (20 ± 5 °C) stock solution stability of Zidovudine at 0 hours and 9 hours was 3.18% to 3.84%, respectively and percentage of stability was found to be 97.93%.

**CALCULATIONS**

\[
\text{% Stability} = \frac{\text{Mean response of stability samples} \times \text{Comparison stock concentration}}{\text{Mean response of comparison stock samples} \times \text{Stability stock concentration}} \times 100
\]

Room temperature (20 ± 5 °C) spiking solution stability was carried out at 9 hours for Naproxen and Zidovudine (Internal Standard) by injecting six replicates of prepared stock dilutions of Naproxen equivalent to final MQC2 quality control concentration and Zidovudine at final working concentration from spiking solution. Comparison of the mean area response of stability samples of Naproxen and Zidovudine at 9 hours was carried out against the comparison samples (freshly prepared). The precision of room temperature (20 ± 5 °C) spiking solution stability of Naproxen at 0 hours and 9 hours 1.97% to 2.13%, respectively and percentage of stability was found to be 98.77%. The precision of room temperature (20 ± 5 °C) spiking solution stability of Zidovudine at 0 hours and 9 hours was 3.86% to 3.84% respectively and percentage of stability was found to be 99.64%.

**Table No. 9: Within Batch Precision and Accuracy of Naproxen for Concomitant Drug Effect**

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>LLOQ QC</th>
<th>LQC</th>
<th>HQC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max</td>
<td>601.7</td>
<td>1727.5</td>
<td>97875.2</td>
</tr>
<tr>
<td>Min</td>
<td>401.1</td>
<td>1277.1</td>
<td>72342.6</td>
</tr>
<tr>
<td>CCDE QC</td>
<td>501.4</td>
<td>1501.3</td>
<td>85108.9</td>
</tr>
<tr>
<td>1</td>
<td>517.8</td>
<td>1528.9</td>
<td>95353.2</td>
</tr>
<tr>
<td>2</td>
<td>557.2</td>
<td>1594.8</td>
<td>90850.0</td>
</tr>
<tr>
<td>3</td>
<td>517.3</td>
<td>1419.2</td>
<td>87904.7</td>
</tr>
<tr>
<td>4</td>
<td>485.9</td>
<td>1577.3</td>
<td>88955.5</td>
</tr>
<tr>
<td>5</td>
<td>537.7</td>
<td>1304.2</td>
<td>84769.6</td>
</tr>
<tr>
<td>6</td>
<td>517.6</td>
<td>1579.3</td>
<td>91049.2</td>
</tr>
<tr>
<td>Mean</td>
<td>521.42</td>
<td>1500.45</td>
<td>89813.70</td>
</tr>
</tbody>
</table>
Concomitant drug effect was investigated to ensure the precision and accuracy is not compromised with potentially interfering concomitant medication. Paracetmol, Ibuprofen, Diphenhydramine, Caffeine, Nicotine and Pantoprazole drugs were spiked in screened plasma at the concentration equivalent to their individual $C_{\text{max}}$ concentration level (15067.2 ng/mL for Caffeine, 25067.9 ng/mL for Ibuprofen, 10043.6 ng/mL for Paracetmol, 103.1 ng/mL for Diphenhydramine, 5043.7 ng/mL for Pantoprazole and 50.7 ng/mL for Nicotine). Within-batch precision and accuracy for concomitant drug spiked QC’s: Within batch precision for LLOQ QC, LQC and HQC was 4.52%, 7.70% and 3.96% respectively. Within batch accuracy for LLOQ QC, LQC and HQC was 103.99%, 99.94% and 105.53% respectively.

Figure No. 1: A Representative Chromatogram of an Aqueous Standard and Internal Standard Mixture of Naproxen
CONCLUSION

In this present study we have made and attempted to develop a novel analytical method for estimation to zidovudine by LCMS/MS method in the tablet dosage form. The method was validated over a concentration range of 500.1 ng/mL to 100028.5 ng/mL for Naproxen. This validation report provides the results of selectivity, matrix effect. Sensitivity determinations, calibration standards and quality control samples data, precision and accuracy data, the results of recovery, various stabilities and dilution integrity. The results of selectivity, carryover test, matrix effect, sensitivity, linearity, precision and accuracy, stabilities, recovery, dilution integrity and Concomitant Drug Effect presented in this report are within the acceptance range for bioanalytical batch acceptance criteria given in USFDA acceptance range as per ‘Guidance for Industry - Bioanalytical Method Validation (May 2001)’ given by CDER. The analytical method described above is valid for the estimation of Naproxen in human plasma over a range of 500.1 ng/mL to 100028.5 ng/mL with the detection of Naproxen m/z – 229.00 (parent) and 185.00 (product) and internal standard Zidovudine m/z – 267.00 (parent) and 222.90 (product) in negative ion mode. From the forgoing it is concluded that the analytical method developed was novel, accurate, precise, selective and is suitable
for application in routine analysis of pharmaceuticual preparation and is within acceptance range for bioanalytical criteria in USFDA acceptance.

REFERENCES