Ultrafast Liquid Chromatographic Determination of Naproxen Sodium in Pharmaceutical Dosage Form

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Abstract

A simple, accurate and precise reverse phase ultrafast liquid chromatographic (RP-UFLC) method for determination of naproxen sodium (NPS) has been developed and validated. Separation was achieved on a Enable; C18G column (250 × 4.6mm i.d., 5μm) using methanol: 10mM TBAHS (tetra butyl ammonium hydrogen sulfate) (80:20, v/v) as mobile phase at flow rate of 1.2mL.min⁻¹. The detection wavelength was 231nm. The method is linear over a concentration range of 0.01-60.0 μg.mL⁻¹ with correlation coefficient of 0.9991. The proposed method is validated by determining accuracy, precision, stability and system suitability parameters. The method was found to be robust. Specificity of the method was determined by subjecting the drug to various stress conditions like acid, alkali, oxidation, thermal and photolytic degradation. The method was used successfully for the determination of NPS in tablet dosage form.

Keywords: Naproxen; UFLC; Forced Degradation; Stability indicating

1. Introduction

Naproxen sodium(NPS), (+)-2-(6-methoxy -2- naphthyl) propionic acid sodium salt, is indicated for pain management in rheumatic disorder, acute gout, migraine and other painful skeletomuscular conditions (Sweetmann, 2009). The structure of NPS is shown in Fig. 1.

Literature review reveals that no stability-indicating RP-UFLC (reverse phase-ultra fast liquid chromatography) method has been reported so far for determination of NPS in tablet dosage form using methanol: 10mM TBAHS (tetra butyl ammonium hydrogen sulfate) as the mobile phase. Some of the reported methods for determination of NPS includes titrimetric (Maheshwari et al., 2010), potentiometric (Lenik et al., 2002; Uysal et al.,2004),spectrophotometric (Maheshwari et al., 2009 and 2010; Yola et al., 2011; Kulsum et al.,2011; Dharmalingam et al., 2013), mass spectrometric (Pracz et al., 2012), electrophoretic (Macia et al.,2008),GC (Singh et al., 1991; ), HPLC (Tashtoush et
al, 2003; Mehta et al., 2012; Haque et al., 2012; Tanjin et al., 2013) and LC-MS (Elsinghorst et al., 2011) methods. The literature survey indicates there is only a single stability-indicating UPLC method for determination of NPS (Venkatarao et al., 2012). But the reported method has several drawbacks like preparation of a complex mobile phase composition having two organic phases, maintaining pH of buffer, gradient elution programming, need of column oven for maintenance of column temperature, lower sensitivity and narrow range of linearity etc.

![Chemical Structure of Naproxen sodium.](image)

**Fig. 1.** Chemical Structure of Naproxen sodium.

UFLC has been preferred as a better alternative analytical tool than HPLC for the determination of drugs in pharmaceutical products (Bandarkar et al., 2010), estimation of hallucinogenic agents in drug products (Min et al., 2008), determination of the drug in microdialysis samples (Sun et al., 2010), and in the determination of drugs in skin diffusate samples (Gannu et al., 2009). So, an attempt was made to overcome the drawbacks of reported method and develop a novel, simple, accurate and precise, stability-indicating RP-UFLC method for determination of NPS in tablet dosage form. To establish the stability-indicating nature of the method, forced degradation of drug was performed under stress conditions (acid, alkali, oxidation, thermal and photolysis), and the stressed samples were analyzed by the developed method. The method was also validated as per requirements of ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2005) guideline.

## 2. Experimental

### 2.1 Materials

Methanol used was of HPLC grade (Merck Ltd., Mumbai, India). Water for chromatography was obtained using a TKA water purification system (Germany). Standard drug of NPS (purity > 99.5%) was received from Cipla Ltd., India. AR grade Tetra Butyl Ammonium Hydrogen Sulfate (TBAHS) was procured from HiMedia Laboratories Pvt. Ltd., India. Marketed tablet formulation containing 500 mg of NPS was purchased from the local market.

### 2.2 Instrumentation and Chromatographic Conditions

Quantitative UFLC was performed on a binary gradient UFLC with two Shimadzu LC-20AD pumps, with a 20 µL sample injection loop (manual) and SPD M20A PDA detector. The output signal was monitored and integrated using Shimadzu LC Solution Software. An Enable C18G column (250 × 4.6mm i.d., 5µm) was used for separation. Methanol: 10mM TBAHS in the ratio (80:20, v/v) was used as the mobile phase. The 10mM TBAHS solution was prepared by accurately weighing 3.3954gms of TBAHS salt and dissolving it in 1000mL of HPLC grade water. Afterwards, both the methanol and TBAHS solution were ultrasonicated up to 30 min for degassing and were filtered through a 0.45µm membrane filter, prior to use. The flow rate was 1.2mL.min⁻¹ and PDA detection was carried out at 231nm. The separation was achieved at room temperature.

### 2.3 Preparation of Standard Stock Solutions and Calibration Curve

Standard stock solution was prepared by transferring 10mg of drug to 10mL volumetric flask. The
drug was then dissolved in 5mL of methanol, shaken and finally volume was made up with methanol, to get a concentration of 1000µg.mL⁻¹. The standard drug solution was filtered through a 0.2µm membrane filter. The stock solution was stored at 2-8°C.

Appropriate aliquots of the standard stock solution of NPS (1000µg.mL⁻¹) were transferred into a series of 10mL volumetric flasks to obtain final concentrations of 0.01-60µg.mL⁻¹ (13 points). Final volume was made up with mobile phase. Each standard solution was injected and chromatograms were recorded. The calibration curve was plotted for peak area of each drug against concentrations of drug.

2.4 Analysis of Pharmaceutical Dosage Form

Twenty tablets were weighed accurately and finely powdered. A quantity of tablet powder equivalent to 10mg of NPS was transferred into a 10mL volumetric flask coning 5mL of methanol. Ultra sonication was performed for 25 minutes and volume was made up with the methanol, followed by filtration through 0.22µm membrane filter. The tablet solution was further diluted with the mobile phase to obtain test solutions within the linearity range. The test solutions were injected and chromatograms were obtained. The amount of NPS present in the test solution was calculated using the calibration curve of NPS.

2.5 Forced Degradation Study

Specificity of the method was determined by checking the interference of any of the possible degradation products produced during the forced degradation study. The forced degradation was carried out with 0.1M HCl, 0.1M NaOH, 3% v/v hydrogen peroxide, thermal (80°C) and photolysis (365nm). The degradation samples were prepared by taking suitable aliquots of the drug solution, and then undertaking the respective stress testing procedures for each solution. After the fixed time period the treated drug solutions were diluted with mobile phase. For every stress condition drug solution was prepared as 30 µg.mL⁻¹ of NPS. The specific stress conditions are described as follows.

Acidic degradation was carried out by adding 1.0 mL of 0.1M HCl, and after 45min neutralizing the mixture by adding 0.1M NaOH. Alkali degradation was carried out by adding 1.0mL of 0.1M NaOH, and after 45min neutralizing the mixture by adding 0.1M HCl. Oxidative degradation was performed by exposing the drug to 1.0mL of 3% (v/v) H₂O₂. 45min. Thermal degradation was performed by heating the drug solutions at 80°C on a thermostatically controlled water bath for 45min. Photolytic degradation was carried out by exposing the drug solutions to UV light (365nm) inside an UV chamber for 45min.

2.6 Accuracy and Precision

To check the accuracy of the proposed method, recovery studies were carried out at 80, 100 and 120 % of the test concentration as per ICH guidelines. The recovery study was performed three times at each level.

The precision (intraday and interday) of the method was ascertained from the peak areas obtained by determination of six separately prepared replicates of fixed concentration of NPS. System precision was assessed by six injections of a NPS (fixed concentration) solution. The percent relative standard deviations were calculated.

2.7 Robustness

Robustness of the method was studied by deliberately changing the detection wavelength (±5nm),
strength of TBAHS solution (±0.5mM) and organic phase proportion (±2%). The effect was studied in terms of various system suitability parameters like retention time, theoretical plates and tailing factor. Solution stability study was also carried out by keeping the sample solutions at 25±2°C for 24hrs.

2.8 System Suitability

The System Suitability was calculated for different parameters like Retention time, theoretical plates, tailing factor, LOD and LOQ. The LOD and LOQ were separately determined based on the Signal to Noise ratio. For LOD the S/N ratio is 3:1. For LOQ the S/N ratio is 10:1.

3. Results and Discussion

3.1 UFLC Method Development and Optimization

The method development process was carried out by examining different conditions like flow rate (0.8mL·min⁻¹, 1.0mL·min⁻¹ and 1.2mL·min⁻¹), mobile phase compositions like acetonitrile: water, acetonitrile: buffer, methanol: water, methanol: 10mM TBAHS and ratios (50:50, 60:40, 70:30 and 80:20, v/v) were used. PDA detection was carried out at 231nm. By use of a C18 column it was found that the mobile phase consisting of methanol: 10mM TBAHS (80:20, v/v) provided well defined peak shape. NPS was eluted at 4.512 min. The representative chromatograms of NPS in pure drug and tablet are shown in Fig. 2 (A) and (B), respectively.

![Representative chromatograms of NPS](image)

**Fig. 2.** Representative chromatograms of NPS (A) standard drug (B) tablet dosage form.

3.2 Method Validation

NPS has linearity over concentrations ranging from 0.01 to 60.0µg·mL⁻¹. The slope (a) and intercept (b) were found to be 243851 and 94446, respectively. Correlation coefficient ($r^2$) was found to be
0.9991. These results indicate a good linear relationship between peak area and the amount of analyte in the range studied.

The % recovery of NPS in tablet dosage form was found to be more than 99%. The good % recovery and non-interference in the separation NPS due to formulation components suggests that; the excipients present in the formulation have no effect in the determination process and the method is selective. The results are given in Table 1.

**Table 1 Analysis of tablet dosage form**

<table>
<thead>
<tr>
<th>Formulation Type</th>
<th>Labeled Amount (mg)</th>
<th>Recovery by proposed method(a), %±S.D.; R.S.D,%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet</td>
<td>500</td>
<td>99.71±0.36,0.36</td>
</tr>
</tbody>
</table>

\(a\) average of three determinations

Specificity of the method was ascertained by checking any interference due to excipients or degradation products produced after forced degradation of NPS. The stress conditions like 0.1M HCl, 0.1M NaOH, 3% v/v H\(_2\)O\(_2\), 80°C temperature and 365nm UV radiation were applied on NPS. Some well separated extra peaks were noticed (resolution > 2.0) after the forced degradation of NPS. Hence the method was found to be specific and stability-indicating. NPS undergoes significant degradation (42.27%) in thermal stress and moderate degradation (11.03%) in photolysis stress conditions. However, it was found to be stable in the applied acidic, alkali and oxidation stress conditions. Fig. 3 (A), (B), (C), (D), (E) and (F) represents the chromatograms of untreated; acid, alkali, oxidation, thermal and photolysis degraded NPS, respectively.

![Fig. 3A Representative chromatogram of untreated NPS (30μg.mL\(^{-1}\))](image)

![Fig. 3B. Representative chromatogram of acid degraded NPS (30μg.mL\(^{-1}\)).](image)
Fig. 3C. Representative chromatogram of alkali degraded NPS (30μg.mL⁻¹).

Fig. 3D. Representative chromatogram of oxidation degraded NPS (30μg.mL⁻¹).

Fig. 3E. Representative chromatogram of thermally degraded NPS (30μg.mL⁻¹).

Fig. 3F. Representative Chromatogram of photolyzedNPS (30μg.mL⁻¹)
Table 2 Results of forced degradation study

<table>
<thead>
<tr>
<th>Stress Applied</th>
<th>Retention Time (min)</th>
<th>Degradation, %</th>
<th>Peak Purity</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>4.536</td>
<td>...</td>
<td>1.00000</td>
<td>...</td>
</tr>
<tr>
<td>0.1 M HCl</td>
<td>4.517</td>
<td>0.14</td>
<td>1.00000</td>
<td>4.564</td>
</tr>
<tr>
<td>0.1 M NaOH</td>
<td>4.517</td>
<td>0.7</td>
<td>1.00000</td>
<td>3.217</td>
</tr>
<tr>
<td>3% H₂O₂</td>
<td>4.536</td>
<td>1.98</td>
<td>1.00000</td>
<td>2.335</td>
</tr>
<tr>
<td>80 °C</td>
<td>4.528</td>
<td>42.27</td>
<td>0.99999</td>
<td>2.154</td>
</tr>
<tr>
<td>UV radiation (365 nm)</td>
<td>4.604</td>
<td>11.03</td>
<td>1.00000</td>
<td>9.423</td>
</tr>
</tbody>
</table>

Accuracy was checked by preparing mixtures containing different amounts of pure drug and fixed concentration of formulation; and analyzing the mixtures by use of developed method. Percentage recovery and relative standard deviation were also calculated. The results obtained indicate that the recoveries were excellent, and relative standard deviations were less than 2%. The results of recovery study are given in Table 3.

Table 3 Accuracy of the method

<table>
<thead>
<tr>
<th>Concentration levels comparing to test concentration, %</th>
<th>Amount Added Pure Drug (µg.mL⁻¹)</th>
<th>Pure Drug Recovery, % ±SD; RSD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>16</td>
<td>100.01±0.0.0784;0.08</td>
</tr>
<tr>
<td>100</td>
<td>20</td>
<td>99.66±0.8339;0.84</td>
</tr>
<tr>
<td>120</td>
<td>24</td>
<td>99.93±0.863;0.86</td>
</tr>
</tbody>
</table>

Average of three determinations at each level

The RSD for method (intraday and interday) and system precision were found to be less than 2% showing high degree of preciseness as shown in Table 4.

Table 4 Intraday, interday and system precision studies (n=6)

<table>
<thead>
<tr>
<th>Type of Precision</th>
<th>Concentration Taken (µg.mL⁻¹)</th>
<th>Peak Areab ± Standard Deviation; RSD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraday precision</td>
<td>10</td>
<td>2550157 ± 18173.06;0.71</td>
</tr>
<tr>
<td>Interday precision</td>
<td>10</td>
<td>2547373.16 ± 19698.05;0.77</td>
</tr>
<tr>
<td>System precision</td>
<td>10</td>
<td>2553301.83 ± 29484.22;1.20</td>
</tr>
</tbody>
</table>

Average of six determinations

Robustness was assured in accordance with deliberate changes made in strength of TBAHS solution (mM), composition of organic phase and detection wavelength. Various system suitability parameters were evaluated to determine the robustness of the method. The results for system suitability parameters are presented in Table 5. The result of solution stability was satisfactory with a recovery more than 99 % after 24 hr, indicating no significant loss of the analyte in the selected mobile phase.
A critical evaluation of the method was performed. Also the LOD and LOQ values show superior sensitivity of the method. The system suitability parameters are shown in Table 6.

**Table 6** System suitability

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Obtained Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>4.512</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>6274</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.442</td>
</tr>
<tr>
<td>LOD (µg.mL⁻¹)</td>
<td>0.005</td>
</tr>
<tr>
<td>LOQ (µg.mL⁻¹)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

### 4. Conclusion

A reversed phase ultrafast liquid chromatographic method has been developed and validated for the determination of NPS in tablet dosage form. The mobile phase and sample preparation technique are simple, making it suitable for routine laboratory testing. The method is linear over a wide range of concentrations with better correlation coefficient. Specificity study carried out in terms of forced degradation ensures that the method is specific and able to describe the stability nature of drug. Results of precision study demonstrate the superior preciseness of the method as the RSD values were well within the limits. The recovery value of more than 99% shows higher levels accuracy of the method. So it can be concluded that the developed RP-UFLC method is fast, simple, accurate, precise, sensitive, and stability-indicating and can be employed successfully for the determination of NPS in tablet dosage form. Further the developed method may be applied for bioavailability and bioequivalence study of NPS in different biological matrix.
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None

Conflict of Interest

None

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