DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF NAPROXEN IN BULK AND SEMI-SOLID FORMULATION

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ABSTRACT: A simple, accurate, precise and sensitive UV spectrophotometric method was developed for the determination of naproxen in bulk and semisolid formulation. The solvent used was methanol and the wavelength corresponding to maximum absorbance of the drug was found at 331 nm. Beer's law was obeyed in the concentration range of 10 - 60µg/mL with correlation coefficient 0.9984. The linear regression equation obtained by least square regression method was y = 0.0108 X - 0.028, where y is the absorbance and x is the concentration of the pure drug solution. The method was validated for several parameters like linearity, accuracy (recovery), precision, and specificity as per International Conference on Harmonization (ICH) guidelines. The values of relative standard deviation and % recovery were found to be satisfactory, indicating that the proposed method is precise and accurate and hence can be used for the routine analysis of naproxen in bulk and semi-solid formulation.

Key words: UV Spectrophotometric, Naproxen, Semi-Solid Formulation, Analytical Method, Validation

INTRODUCTION

Naproxen is chemically 2-(6-methoxynaphthalen-2-yl) propanoic acid (Figure 1). It is used in the treatment of inflammations, rheumatoid arthritis, musculoskeletal disorders and gout [1]. Naproxen is a non-steroidal anti-inflammatory drug (NSAID) commonly used for the reduction of moderate to severe pain, fever, inflammation and stiffness. It works by inhibiting both the COX-1 and COX-2 enzymes. Literature review revealed that some spectrophotometric and HPLC methods have been reported for the estimation of naproxen in tablet formulation [2, 3, 4, 5], raw material [6], plasma [7, 8], urine [9] and intestinal perfusion samples [10].

Since the nonspecific titrimetric assay method was specified for Naproxen API in the pharmacopoeia, hence, there was a need to develop a specific method which became the purpose of the further study [11]. The objective of the present work was to develop a simple, sensitive, precise and accurate UV spectrophotometric method for the determination of naproxen in bulk and semisolid formulations as per ICH Guidelines.
MATERIALS AND METHODS

Materials
Shimadzu UV-160 and UV-1800 UV/VIS Spectrophotometer were used with 1 cm matches quartz cell, CP224S analytical balance (Sartorius) and ultra-sonic cleaner (Fisher scientific FB15061) were used. Micropipette of Variable volume 10-1000 µL (Capp Ecopipette single channel) and Digital balance (Mettler Toledo XP 105). Naproxen (Ezo life sciences) (CAS 22204-53-1) was supplied by Sigma Aldrich. Naprosyn® USP gel (RPG Life sciences, India) was purchased from local market. All other chemicals and solvents used were of HPLC grade.

Preparation of stock solution
Standard stock solution of naproxen were prepared by dissolving accurately weighed 100mg of naproxen in methanol in a 100mL volumetric flask to give a concentration of 1000µg/mL. From this, 10ml of the solution was transferred to a 100mL volumetric flask and made up the volume with methanol to give a concentration of 100µg/mL which is the standard stock solution.

Determination of maximum wavelength (λ<sub>max</sub>)
The samples were scanned in UV spectrophotometer from a range of 200-400nm against methanol as blank and the wavelength corresponding to maximum absorbance in methanol were determined.

Preparation of standard calibration curve
For the preparation of standard calibration curve, concentration of 10-60µg/ml were prepared by pipetting out 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mL from the 100µg/mL solution into a 10ml volumetric flask and made up the volume with methanol. The absorbance of each solution was measured at maximum wavelength.

Validation
Validation can be defined as (ICH) establish documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method were validated for several parameters like linearity, accuracy, precision, ruggedness, robustness, Limit of detection (LOD), Limit of quantification (LOQ) according to ICH guidelines.

RESULTS AND DISCUSSION
Maximum wavelength (λ<sub>max</sub>), Linearity and range
The linearity of the analytical method was its ability to elicit test results which are directly proportional to analyte concentration in samples within a given range. To establish the linearity of the proposed method, various aliquots of the standard solution of the drug were prepared from stock solution and analysed. Each solution was analysed after filtration through 0.45 µm membrane filter after discarding first 2 mL. The prepared linearity dilutions were then analyzed in series by UV Spectrophotometer and their respective absorbance were recorded at the λ<sub>max</sub> a graph was plotted between absorbance and theoretical concentration. The drug showed linearity in the range of 10-60µg/mL with correlation coefficient of 0.9984 as maximum absorbance in methanol 331 nm was obtained (Figure 2 & 3).

![Fig-2: Absorbance](image-url)
Precision studies were carried out to ascertain the reproducibility of the proposed method. Repeatability was determined by preparing six replicates of same concentration of the sample and the absorbance was measured. Intraday precision study was carried out by preparing drug solution of same concentration and analyzing it at three different times in a day. The values of the precision (% RSD) of repeatability along with intraday and interday precision were displayed in Table 1 and Table 2.

### Table 1: Intraday precision

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Absorbance - 1</th>
<th>Absorbance - 2</th>
<th>Absorbance - 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.2521</td>
<td>0.2525</td>
<td>0.2528</td>
</tr>
<tr>
<td>20</td>
<td>0.2522</td>
<td>0.2531</td>
<td>0.2531</td>
</tr>
<tr>
<td>20</td>
<td>0.2531</td>
<td>0.2523</td>
<td>0.2514</td>
</tr>
<tr>
<td>20</td>
<td>0.2543</td>
<td>0.2538</td>
<td>0.2531</td>
</tr>
<tr>
<td>20</td>
<td>0.2528</td>
<td>0.2514</td>
<td>0.2538</td>
</tr>
<tr>
<td>Avg</td>
<td>0.2526</td>
<td>0.2527</td>
<td>0.2529</td>
</tr>
<tr>
<td>StdDev</td>
<td>0.0010</td>
<td>0.0008</td>
<td>0.0008</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.4169</td>
<td>0.34820</td>
<td>0.3216</td>
</tr>
</tbody>
</table>

### Table 2: Interday precision

<table>
<thead>
<tr>
<th>Labeled Claim (mg)</th>
<th>Level of Addition (%)</th>
<th>Amount of pure naproxen added (mg)</th>
<th>Amount of drug Recovered (mg)</th>
<th>% Recovery</th>
<th>Statistical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>80</td>
<td>1.2</td>
<td>2.650</td>
<td>98.72</td>
<td>98.72 0.3863 0.8952</td>
</tr>
<tr>
<td>1.5</td>
<td>80</td>
<td>1.2</td>
<td>2.718</td>
<td>100.45</td>
<td>101.15 1.1236 1.1108</td>
</tr>
<tr>
<td>1.5</td>
<td>100</td>
<td>1.5</td>
<td>3.052</td>
<td>101.15</td>
<td>100.25 1.01 1.0074</td>
</tr>
<tr>
<td>1.5</td>
<td>100</td>
<td>1.5</td>
<td>2.961</td>
<td>99.12</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>120</td>
<td>1.8</td>
<td>3.281</td>
<td>99.24</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>120</td>
<td>1.8</td>
<td>3.313</td>
<td>100.25</td>
<td></td>
</tr>
</tbody>
</table>
Accuracy
Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (80%, 100% and 120%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % recovery was calculated. The results are shown in Table 3.

Table 3: Accuracy results of naproxen

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Absorbance Day -1</th>
<th>Absorbance Day -2</th>
<th>Absorbance Day -3</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.2532</td>
<td>0.2524</td>
<td>0.2517</td>
</tr>
<tr>
<td>20</td>
<td>0.2526</td>
<td>0.2522</td>
<td>0.2544</td>
</tr>
<tr>
<td>20</td>
<td>0.2543</td>
<td>0.2548</td>
<td>0.2531</td>
</tr>
<tr>
<td>20</td>
<td>0.2528</td>
<td>0.2546</td>
<td>0.2538</td>
</tr>
<tr>
<td>20</td>
<td>0.2549</td>
<td>0.2536</td>
<td>0.2526</td>
</tr>
<tr>
<td>Avg</td>
<td>0.2535</td>
<td>0.2535</td>
<td>0.2534</td>
</tr>
<tr>
<td>StdDev</td>
<td>0.0008</td>
<td>0.0010</td>
<td>0.0012</td>
</tr>
<tr>
<td>RSD</td>
<td>0.3540</td>
<td>0.4274</td>
<td>0.4881</td>
</tr>
<tr>
<td>Average %RSD</td>
<td></td>
<td>0.4232</td>
<td></td>
</tr>
</tbody>
</table>

Ruggedness
Ruggedness was determined by carrying out analysis by two different analyst and the respective absorbance was noted and the results were indicated as % RSD in Table 4.

Table 4: Ruggedness results of Naproxen

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Absorbance Analyst-1</th>
<th>Absorbance Analyst-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.2538</td>
<td>0.2541</td>
</tr>
<tr>
<td>20</td>
<td>0.2518</td>
<td>0.2521</td>
</tr>
<tr>
<td>20</td>
<td>0.2536</td>
<td>0.2536</td>
</tr>
<tr>
<td>20</td>
<td>0.2528</td>
<td>0.2515</td>
</tr>
<tr>
<td>20</td>
<td>0.2535</td>
<td>0.2519</td>
</tr>
<tr>
<td>20</td>
<td>0.2542</td>
<td>0.2532</td>
</tr>
<tr>
<td>Avg</td>
<td>0.2532</td>
<td>0.2527</td>
</tr>
<tr>
<td>StdDev</td>
<td>0.00085</td>
<td>0.00104</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.3390</td>
<td>0.4132</td>
</tr>
<tr>
<td>Average %RSD</td>
<td></td>
<td>0.3761</td>
</tr>
</tbody>
</table>
Robustness
Analysis was carried out at two different UV spectrometers at room temperature to determine the robustness of the method and the respective absorbance was measured. The results were indicated as %RSD in Table 5.

<table>
<thead>
<tr>
<th>Table 5: Robustness results of Naproxen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (µg/mL)</td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>20</td>
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<tr>
<td>20</td>
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<tr>
<td>20</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>Avg</td>
</tr>
<tr>
<td>StdDev</td>
</tr>
<tr>
<td>% RSD</td>
</tr>
<tr>
<td>Average % RSD</td>
</tr>
</tbody>
</table>

LOQ and LOD
Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected. Limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy. LOQ and LOD were determined using the following equation LOQ-10s/m, LOD-3.3s/m where s is the standard deviation of the response and m is the slope of the related calibration curve. The values of LOQ and LOD were found to be 5.11 and 1.53 µg/mL respectively. The results of various parameters of the developed method are shown in Table 6.

<table>
<thead>
<tr>
<th>Table 6: Summary of developed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>Absorption maxima</td>
</tr>
<tr>
<td>Beer's Law range</td>
</tr>
<tr>
<td>Correlation coefficient</td>
</tr>
<tr>
<td>Regression equation</td>
</tr>
<tr>
<td>Slope</td>
</tr>
<tr>
<td>Intercept</td>
</tr>
<tr>
<td>Accuracy</td>
</tr>
<tr>
<td>Precision (% RSD)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>LOD, µg/mL</td>
</tr>
<tr>
<td>LOQ, µg/mL</td>
</tr>
</tbody>
</table>

Specificity
The specificity of the method was evaluated by recording the spectrum of the blank, placebo, standard, sample solutions (unspiked and spiked) in a concentration of 20 µg/mL between 200 nm to 400 nm on UV Spectrophotometer and taking absorbance measurement at the λmax (Table 7). The results demonstrate that there was no interference from other materials in the pharmaceutical formulations and therefore confirm the specificity of the method.
Quantification of Naproxen in semisolid dosage form
To analyse the concentration of drug in the marketed semisolid formulation (Naprosyn® USP gel), Naproxen gel equivalent to 2 mg was accurately weighed and transferred to a 100mL volumetric flask, dissolved in methanol, sonicated, and finally made up the volume with methanol. The solution was centrifuged for the excipients to settle down and the resulting solution was filtered using 0.45 μ filtration membrane. The solution was suitably diluted so as to obtain a concentration in the linearity range and the absorbance was measured at 331 nm against methanol as blank. The results of analysis are shown in Table 8.

Table 7: Specificity results of naproxen

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluent</td>
<td>0.000</td>
</tr>
<tr>
<td>Placebo solution</td>
<td>0.000</td>
</tr>
<tr>
<td>Reference solution (20 μg/ml)</td>
<td>0.2523</td>
</tr>
<tr>
<td>Test solution (20 μg/ml)</td>
<td>0.2528</td>
</tr>
<tr>
<td>Test solution spiked with impurities (20 μg/ml)</td>
<td>0.2530</td>
</tr>
</tbody>
</table>

Table 8: Quantification of naproxen in semi-solid formulation

<table>
<thead>
<tr>
<th>Semisolid formulation</th>
<th>Label claim</th>
<th>Estimated amount of Naproxen (mg)</th>
<th>% Labeled Claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naprosyn® USP gel</td>
<td>10% w/w</td>
<td>9.89</td>
<td>98.9%</td>
</tr>
</tbody>
</table>

CONCLUSION
The developed method can be concluded to be simple, accurate, reliable and economical. The proposed method is specific without and interference of excipients and hence can be used for the routine analysis of Naproxen bulk and in semisolid formulation as per ICH Guidelines.

REFERENCES


