Naproxen (NAP) \[(+)-(\text{S})-2-(6\text{-methoxynaphthalen}-2\text{-yl})\text{ propanoic acid}]\), is a non-steroidal anti-inflammatory drug (NSAID). Due to an aryl acetic structure, naproxen exhibits analgesic and antipyretic properties (Scheme 1). Only the (+) enantiomer possesses anti-inflammatory properties. Naproxen is used for the reduction of moderate to severe pain, fever, inflammation, rheumatoid arthritis, musculoskeletal disorders and gout.

Anti-inflammatory effects of naproxen are generally thought to be related to its inhibition of cyclooxygenase and consequent decrease in prostaglandin concentrations in various fluids and tissues (1, 2). Naproxen is generally well tolerated and the most common side effects that have been reported are: gastrointestinal complaints, headache, vomiting, diarrhea, constipation, decreased appetite, rash, dizziness and drowsiness. In some cases, gastrointestinal and also bleeding ulcers could be produced and in a few cases renal failure, hepatic injury, urticaria, ecchymosis and vasculitis have been reported.

Naproxen is rapidly and completely absorbed after oral administration and it is the predominant species in serum, with a therapeutic range of 30 to 90 \(\mu\text{g/mL}\) (3). NSAIDs may cause an increased risk of serious cardiovascular thrombotic events, myocardial infarction, and stroke, which can be fatal. This risk may increase with duration of use. Due to its extortionary use as non-prescription drug, naproxen has been detected in surface water, groundwater, wastewater and even in drinking water in the range from ng/L to several g/L (4), therefore, it is required to develop a simple, effective, rapid and accurate method that can be used in routine quality control.

Naproxen has been determined by several analytical methods like: HPLC (5-8), HPTLC (9), TLC/UV (10), LC-MS/MS (11, 12), capillary electrophoresis (13, 14), fluorimetric methods (15, 16), chemiluminescence (17-19), voltametry (20, 21), liquid phos-
phorimetry (22), solid-phase microextraction coupled to liquid chromatography (23), synchronous spectrometry (24), and spectrophotometry (25-30). Several chromatographic methods have been reported for single and simultaneous determinations of naproxen in tablets (31), human blood plasma (32) and urine (33). Although HPLC methods are highly sensitive and specific, they are considered expensive.

Spectrophotometry is considered as the most convenient analytical technique in pharmaceutical analysis because of its inherent simplicity and availability in most quality control and clinical laboratories (34-37). In this work, we developed two simple, rapid, accurate, precise, sensitive and less time consuming spectrophotometric methods for quantitative determination of naproxen in pure, pharmaceutical preparation and human serum samples. These methods are based on the formation of ion pair complex between naproxen and sulfone phthalein acid dyes, namely bromocresol green (BCG) and bromothymol blue (BTB).

**MATERIALS AND METHODS**

**Stock standard solutions of naproxen**

A stock standard solution (200 µg/mL) was prepared by dissolving accurately weighed 20 mg of pure naproxen in methanol and diluting to the mark with the same solvent in a 100 mL calibrated flask. This stock solution was diluted appropriately with methanol to obtain suitable working solutions. Freshly prepared solutions were always employed.

**Stock standard solutions of reagents**

Stock solution of BCG and BTB (100 µg/mL), were prepared by dissolving 10 mg of reagents in methanol and diluting to the mark with double distilled water in a 100 mL calibrated flask. The acid dye reagents were stable for one week.

**Apparatus**

All absorbance measurements and spectral runs were made on a RAYLEIGH UV-1800 single beam spectrophotometer (BRAIC, Beijing, China) with 1

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![Figure 1. Calibration curve of naproxen with BCG](image1.png)

![Figure 2. Calibration curve of naproxen with BTB](image2.png)
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...cm matched quartz cells. The pH measurements were carried out with a Metrohm 827 pH lab pH meter.

Reagents

All the chemicals and reagents used were of analytical grade (Merck) and used without further purification. Naproxen was supplied as a gift sample from Sobhan Pharmaceutical (Rasht, Iran). Tablet formulation of naproxen was procured from a local pharmacy for analysis.

General procedures

Procedure for calibration curves

BCG method: Aliquots equivalent to 5-105 µg/mL naproxen were transferred into a series of 5 mL volumetric flasks. To each flask, 1 mL of 50 µg/mL of BCG solution and 1 mL of phosphate buffer solution (pH = 3.5) were added and made up to mark with methanol and distilled water, then, left to stand for 10 min at room temperature (20 ± 5°C). The absorbance of the yellow colored complex were measured at 424 nm against a blank reagent prepared in the same way without addition of the naproxen. To obtain the standard calibration graph, plot of the values of absorbance against the drug concentration (Fig. 1) was used.

BTB method: Aliquots equivalent to 5-85 µg/mL naproxen were transferred into a series of 5 mL volumetric flasks. To each flask, 1 mL of 50 µg/mL of BTB solution and 1 mL of phosphate buffer solution (pH = 3.0) were added and made up to mark with methanol and distilled water, then, left to stand for 10 min at room temperature (20 ± 5°C). The absorbance of the yellow colored complexes were measured at 422 nm against a blank reagent prepared in the same way without addition of naproxen. To obtain the standard calibration graph, plot of the
values of absorbance against the drug concentration (Fig. 2) was used.

**Procedure for the assay of tablets**

For the analysis of naproxen in tablets by the proposed methods, ten tablets of naproxen were weighed and pulverized into a fine powder. An accurately weighed portion of the powdered tablets equivalent to 250 mg of naproxen was transferred into 100 mL beaker and was dissolved in the least amount of methanol, filtered through a Whatmann No. 41 filter paper, washed with methanol into a 100 mL calibrated flask and diluted to volume with methanol. Solutions of working range concentration were prepared by proper dilution of this stock solution with methanol and followed the above procedure for the analysis. The drug content of the tablets formulation was then calculated (Table 1). For further confirmation, the standard addition technique was applied to test the reliability and recovery of the proposed methods, in which variable amounts of the drug were added to the previously analyzed portion of pharmaceutical (Table 2).

**Procedure for spiked serum**

The proposed methods have been successfully applied for the determination of naproxen in human blood serum samples. The results were obtained from four replicate measurements of serum samples containing naproxen and indicated that the proposed methods were effective for the determination of naproxen in human blood serum samples (Table 3).

**Stoichiometric relationship**

The composition of ion-pair complexes were established by applying Job’s method of continuous variations; a $1 \times 10^{-4}$ M standard solution of naproxen and $1 \times 10^{-4}$ M solution of reagents (BTB, BCG) were used. A series of solutions were prepared in which the total volume of drug and reagent was kept at 1.0 mL. The reagents were mixed in various proportions and diluted to volume in a 5 mL calibrated flask with the appropriate solvent following the

<p>| Table 1. Application of the proposed methods for the determination of naproxen in tablet dosage form. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th><strong>Method</strong></th>
<th><strong>Naproxen labeled amount (mg)</strong></th>
<th><strong>Found (mg)</strong></th>
<th><strong>Recovery (%)</strong></th>
<th><strong>RSD (%)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>BCG</td>
<td>250</td>
<td>251.41</td>
<td>100.56</td>
<td>0.81</td>
</tr>
<tr>
<td>BTB</td>
<td>250</td>
<td>249.18</td>
<td>99.67</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Mean of four determinations.

<p>| Table 2. Determination of naproxen in tablet dosage form using the standard addition technique. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th><strong>Sample</strong></th>
<th><strong>Taken (µg/mL)</strong></th>
<th><strong>Added (µg/mL)</strong></th>
<th><strong>BTB</strong></th>
<th><strong>BCG</strong></th>
<th><strong>BTB</strong></th>
<th><strong>BCG</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Naproxen (250 mg)</td>
<td>10</td>
<td>0</td>
<td>10.14</td>
<td>101.40</td>
<td>9.84</td>
<td>98.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>12.38</td>
<td>102.17</td>
<td>11.91</td>
<td>99.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>13.76</td>
<td>98.28</td>
<td>13.69</td>
<td>97.78</td>
</tr>
</tbody>
</table>

*Mean of four determinations.

<p>| Table 3. Application of the proposed methods for the determination of naproxen in human blood serum. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th><strong>Method</strong></th>
<th><strong>Added (µg/mL)</strong></th>
<th><strong>Recovery a (%)</strong></th>
<th><strong>RSD (%)</strong></th>
<th><strong>Difference (%)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>BCG</td>
<td>5</td>
<td>101.45</td>
<td>0.70</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>102.00</td>
<td>0.67</td>
<td>2.00</td>
</tr>
<tr>
<td>BTB</td>
<td>5</td>
<td>98.82</td>
<td>0.67</td>
<td>-1.18</td>
</tr>
</tbody>
</table>

*Mean of four determinations.
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Figure 5. Absorption spectrum of (a) pure naproxen and (b) ion-pair complex of naproxen with BTB against reagent blank.

Figure 6. Effect of different solvents on the formation of colored product (BCG method).

Figure 7. Effect of different solvents on the formation of colored product (BTB method).
above mentioned procedure. The plot reached a maximum value at a mole fraction of 0.5, which indicated that a 1:1 (drug : dye) ion-pairs are formed through the electrostatic attraction between positive protonated drug and BTB or BCG anions.

RESULTS AND DISCUSSION

Absorption spectra
The absorption spectra of the ion-pair complexes, formed between naproxen and each of BPB and BCP were measured at 200-800 nm against the blank solution prepared under the same conditions (Figs. 4 and 5). The complexes showed maximum absorbance at 424 and 422 nm for naproxen-BCG and naproxen-BTB complex, respectively. The measurements were made at these wavelengths for tablets and human serum samples. The absorption band of the reagent showed $\lambda_{\text{max}}$ at 612 and 592 nm for BCG and BTB, respectively.

Optimum conditions for complex formation
In order to establish the optimum conditions necessary for a rapid and quantitative formation of the colored product with maximum stability and sensitivity, the absorbance of a series of solutions was measured by varying one parameter while keeping the others constant.

Selection of solvent
The effect of several solvents such as ethanol, methanol, acetone, acetonitrile, dichloromethane and chloroform were investigated on the absorbance of the yellow color complexes. It is found that the reaction mixture is becoming turbid when diluted with distilled water. The difference in absorbance values with other solvents are shown in Figures 6 and 7. It is apparent from the figures that the highest absorbance was obtained in methanol medium. Therefore, methanol was selected as the best solvent.

Effect of reagent concentration
The effect of the dye concentration on the intensity of the color developed at selected wavelengths was tested using different volumes of the reagents. The results shown that 1 mL of BCG and BTB were found to be optimum for these proposed methods and excess of these dyes do not affect the color of the complexes or the absorbances (Fig. 8).

Effect of pH
The influence of pH of buffer solution on the development and stability of the color using phosphate-HCl buffer solution was studied over the pH range 2.0-7.0. The maximum color intensity was observed in the pH of 3.0 and 3.5 for naproxen-BTB and naproxen-BCG complex, respectively (Fig. 9). Moreover, the optimum volume of buffer solution added to 5 mL to give constant absorbance value was also studied and found to be 1 mL.

Effect of reaction time
The effect of time on the formation and stability of the ion-pair complexes was studied by measuring the absorbances of these complexes at increasing time intervals. At the beginning, the absorbance
increased gradually along with the time. After 10 min, it achieved stability and remained basically unaltered (Fig. 10).

**Effect of sequence of additions**

The order of reagent addition was very important; changing the order produced low result. The most favorable sequence is ‘drug-reagent-buffer-solvent’ for the complete color development and the highest absorbance at the recommended wavelength. Other sequences needed longer time and produced lower absorbance values.

**Stability of ion-pair complexes**

The stability of the ion-pair complexes formed between drug and acidic dye was evaluated. The formation of the ion-pair complexes was rapid and the yellow color products were stable for 3 days for naproxen-BTB and naproxen-BCG without any change in color intensity in dark and room temperature.

**Effect of interferences**

The effects of common excipients and additives were studied for their possible interferences in the assay of naproxen. The results revealed the fact that no significant interference was observed from the excipients, such as glucose, fructose, sucrose, lactose and starch commonly present in pharmaceutical formulations. This shows that the methods are applicable in the case of pharmaceutical preparations of the naproxen.

**Method validation**

The proposed methods have been extensively validated in terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ) (Table 4). The accuracy was expressed in terms of percent recovery of the known amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. In order to determine the accuracy and precision of the methods, solutions containing two different concentrations of the studied drug were prepared and four replicates determinations, covering the usable concentration range, were carried out for pure form and the pharmaceutical preparation of naproxen. The analytical results obtained for this investigation are summarized in Table 5. The low values of RSD % indicate good precision and reproducibility of the proposed methods. The average percent recoveries obtained were quantitative, indicating good accuracy of the meth-

### Table 4. Analytical parameters for the determination of naproxen by the proposed methods.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Naproxen - BCG</th>
<th>Naproxen - BTB</th>
</tr>
</thead>
<tbody>
<tr>
<td>λₘₐₓ (nm)</td>
<td>424</td>
<td>422</td>
</tr>
<tr>
<td>Beer’s law limits (µg/mL)</td>
<td>10-105</td>
<td>5-85</td>
</tr>
<tr>
<td>ε (L/mol cm)</td>
<td>2.59 x 10⁶</td>
<td>2.969 x 10⁶</td>
</tr>
<tr>
<td>pH</td>
<td>3.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Regression equation (y)</td>
<td>y = 0.005x + 0.177</td>
<td>y = 0.007x + 0.194</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.9969</td>
<td>0.9965</td>
</tr>
<tr>
<td>RSD %</td>
<td>0.76</td>
<td>0.54</td>
</tr>
<tr>
<td>LOD (µg/mL)</td>
<td>0.347</td>
<td>0.312</td>
</tr>
<tr>
<td>LOQ (µg/mL)</td>
<td>1.158</td>
<td>1.02</td>
</tr>
</tbody>
</table>

* y = a + bx, where x is the concentration in µg/mL.

### Table 5. Evaluation of the accuracy and precision of the proposed methods for naproxen determination.

<table>
<thead>
<tr>
<th>Method</th>
<th>Added (µg/mL)</th>
<th>Recovery a (%)</th>
<th>RSD (%)</th>
<th>Er (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCG</td>
<td>10</td>
<td>102</td>
<td>0.99</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>99.94</td>
<td>99.94</td>
<td>-0.06</td>
</tr>
<tr>
<td>BTB</td>
<td>5</td>
<td>98.4</td>
<td>1.07</td>
<td>-1.6</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>101.1</td>
<td>0.67</td>
<td>1.10</td>
</tr>
</tbody>
</table>

* Mean of four determinations.
The performance of the proposed method was compared with that of other existing UV-visible spectrophotometric methods (Table 6).

### CONCLUSION

 Unlike HPLC method, the spectrophotometric procedure is simple and is not high cost. The main purpose of this study was to establish two simple, economic and rapid UV/Visible spectrophotometric methods for determination of naproxen in pure, tablet dosage form and human serum samples. The reagents utilized in this work are cheap and available and the proposed methods do not include any crucial reaction conditions. Also these methods were applied directly to the analysis of pharmaceutical dosage forms and serum samples without the need for separation or extraction steps prior to drug analysis. The high recovery percentage and low relative standard deviation reflect the high accuracy and precision of these proposed methods. Therefore, the proposed methods can be successfully applied for the routine analysis of naproxen.

### Acknowledgments

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