Stability-Indicating HPLC Method for Determination of Nalbuphine Hydrochloride

Khalid A. Attia, Mohammed W. Nassar, Ahmed El-Olemy*

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt
*ahmed_olemy2000@yahoo.com

Abstract: A simple, sensitive, stability-indicating HPLC method was developed and validated for the quantitative determination of nalbuphine hydrochloride in presence of its degradation product. The analysis was carried out on a BDS Hypersil C18 (250 x 4.6 x 5μm particle size) using a mobile phase consisting of 5 mM sodium acetate buffer; pH 5.5: acetonitrile (40:60, v/v). The analysis was performed at ambient temperature with a flow rate of 1 ml/min and UV detection at 210 nm. The method showed good linearity over the concentration range of 1-15 μg/ml with a lower detection limit of 0.243 and quantification limit of 0.737 μg/ml. The proposed method can selectively analyse the drug in presence of up to 87% of its oxidative degrade with mean recovery± RSD% of 100.08±0.678. The method was validated and successfully applied for determination of nalbuphine in its commercial preparation and the obtained results were statistically compared with those of the reported method by applying t-test and F-test at 95% confidence level and no significant difference was observed regarding accuracy and precision.

Keywords: Stability-indicating, nalbuphine, HPLC, internal standard, validation.

1. INTRODUCTION

Nalbuphine hydrochloride (Figure 1) is (5R,6S) 17-(Cyclobutylmethyl)-4,5-epoxymorphinan-3,6,14-triol hydrochloride [1]. It is a phenanthrene derivative opioid analgesic. It has mixed opioid agonist and antagonist activity. It is used for the relief of moderate to severe pain, including that associated with myocardial infarction, and as an adjunct to anaesthesia [2].

Figure 1. Structural formula of nalbuphine hydrochloride

Few analytical methods have been reported for its analysis including spectrophotometric[3-5], spectrofluorimetric [5,6], electrochemical [7] and chromatographic methods [8-14].

High performance liquid chromatography (HPLC) is an important qualitative and quantitative technique, generally used to separate, identify, and quantify the active compounds in pharmaceutical and biological samples [15]. It is the most versatile, safest, dependable and fastest chromatographic technique for the quality control of drug components [16].

The main purpose of this work is to establish a sensitive, accurate and precise stability-indicating reversed phase HPLC procedure for the determination of nalbuphine in bulk powders and in pharmaceutical preparation in the presence of its degradation product.
2. EXPERIMENTAL

2.1. Pure Samples

Pure nalbuphine hydrochloride (99.25%) was kindly supplied by Amoun Pharmaceutical Company, Cairo, Egypt.

Pure guaifenesin (99.20%) was kindly supplied by Medical Union Pharmaceuticals, Abu-Sultan, Ismailia, Egypt.

2.2. Pharmaceutical Preparation

Nalufin ® ampoules: each ampoule (1 ml) claimed to contain 20 mg nalbuphine hydrochloride (B.No. 369, manufactured by Amoun Pharmaceutical Company), purchased from local market.

2.3. Chemicals and Reagents

All reagents used were of analytical grade, solvents were of HPLC grade, water used throughout the procedure was freshly distilled.

- Acetonitrile, HPLC grade (Sigma-Aldrich, Germany).
- Ethanol, HPLC grade (Sigma-Aldrich, Germany).
- Analytical grade acetic acid, hydrogen peroxide (50%) and sodium acetate (El-Nasr Company, Egypt).

2.4. Standard Solutions

2.4.1. Standard Solution of Intact Nalbuphine Hydrochloride

Standard solution of nalbuphine hydrochloride (100 μg/ml) was prepared by dissolving 10 mg of the drug powder in 50 ml of the mobile phase [5 mM sodium acetate buffer; pH 5.5: acetonitrile (40:60, v/v)] and complete to 100 ml with the mobile phase.

2.4.2. Standard Solution of Guaifenesin (Internal Standard)

Standard solution of guaifenesin as internal standard (100 μg/ml) was prepared by dissolving 10 mg of the drug powder in 50 ml of the mobile phase and complete to 100 ml with the mobile phase.

2.4.3. Standard Solution of Degraded Sample

100 mg of pure nalbuphine hydrochloride powder were dissolved in 45 ml of distilled water and transferred to a 100-ml round bottomed flask to which 5 ml of 50% H₂O₂ were added. The solution was heated under reflux for 6 hours and evaporated to dryness under vacuum. The obtained residue was extracted with ethanol (2x10 ml), filtered into a 100-ml volumetric flask and diluted to volume with ethanol to obtain a stock solution labeled to contain degradate derived from 1 mg/ml of nalbuphine hydrochloride[4]. Working solution of degradate (100 μg/ml) was obtained by further dilution of the stock solution with the mobile phase.

2.5. Apparatus

- HPLC, LDC Analytical (Milton Roy, USA), equipped with Diode - array UV-Visible detector and auto sampler injector. The chromatographic analysis was carried out using (EZ Chrom Elit) data analysis program.
- Hot plate (Torrey pines Scientific, USA).
- Jenway, 3510 pH meter (Jenway, USA).
- UV lamp with short wavelength (254 nm) (Vilber Lourmat, France).
- Precoated TLC plates, silica gel 60 GF254, (20 x 20 cm), 0.22 mm thickness (Fluka, Chemie, Switzerland).
Stability-Indicating HPLC Method for Determination of Nalbuphine Hydrochloride

2.6. Procedures

2.6.1. Chromatographic Conditions

At ambient temperature, isocratic separation was carried out on BDS Hypersil C18 (250 X 4.6 X 5μm particle size) using mobile phase consists of 5 mM sodium acetate buffer; pH 5.5: acetonitrile (40:60, v/v). The mobile phase was degassed by a degasser before pumped at flow rate 1 ml/min. The injected volume of the standard solution was 20 µl and UV detection at 210 nm.

2.6.2. Linearity (Construction of the Calibration Graph)

Aliquots of standard drug solution (100 µg/ml) containing (10–150 µg) of intact nalbuphine were transferred into a series of 10 ml volumetric flasks containing (150 µg) of guaifenesin (internal standard) and adjusted to volume with mobile phase. Into HPLC column, 20 µl were injected form each concentration and chromatographed under the conditions described. Calibration graph was constructed by plotting the peak area ratio against the corresponding drug concentration in µg/ml.

2.6.3. Procedure for Synthetic Mixture (Specificity)

Aliquots of intact nalbuphine containing (130–20 µg) were transferred into a series of 10 ml volumetric flasks containing 150 µg of internal standard and nalbuphine degradate (20–130 µg) then diluted to the volume with the mobile phase. 20 µl of each solution were injected into the HPLC column and the corresponding chromatograms were monitored at 210 nm. The intact drug concentrations were calculated from the corresponding regression equation.

2.6.4. Procedure for Pharmaceutical Preparation

Contents of 10 Nalufin® ampoules (20 mg/ml) were mixed well. A volume equivalent to 10 mg of nalbuphine hydrochloride was transferred into 100-ml volumetric flask and completed to volume with the mobile phase to obtain a solution labeled to contain 100 µg/ml of nalbuphine hydrochloride. Transfer aliquots covering the working concentration range into 10 ml volumetric flasks. Proceed as described under “linearity”. Determine the content of the ampoules from the corresponding regression equation.

3. Results & Discussions

In the present study, a simple and sensitive reversed phase HPLC procedure was suggested for the selective quantitative determination of nalbuphine in presence of its oxidative degradation product.

3.1. Degradation of Nalbuphine

Stressed degradation of nalbuphine was studied by refluxing the drug using different media; aqueous, 1M NaOH, 1M HCl and 50% H2O2 for different time intervals. No degradation took place using aqueous, acidic or basic conditions, whereas complete degradation was attained when the drug was refluxed with 50% H2O2 for 6 hours[4], as shown in Figure 2.

![Figure 2. Proposed degradation pathway of nalbuphine](image)

3.2. Optimization of Experimental Conditions

Different chromatographic conditions affecting the chromatographic separation were optimized after taking in consideration the resolution between the drug, its degradation product and the internal standard. Several mobile phases were tried in order to separate the intact drug from its degradeate and
the internal standard including 5 mM sodium acetate buffer; pH 5.5: acetonitrile in different ratios. Good separation was carried out on BDS Hypersil C18 (250 X 4.6 X 5μm particle size) column using a mobile phase consists of 5 mM sodium acetate buffer; pH 5.5: acetonitrile (40:60, v/v) at flow rate 1 ml min⁻¹ and UV detection at 210 nm.

In HPLC chromatogram, showed in Figure 3, the peak of intact nalbuphine, its degradation product and the internal standard were clearly separated and their corresponding peaks were sharply developed at reasonable retention times of 1.9 + 0.03, 2.8 + 0.05 and 4.7 + 0.02 minutes for degradation product, guaifenesin (internal standard) and intact nalbuphine respectively.

![HPLC chromatogram](image)

**Figure 3.** HPLC chromatogram of mixture of intact nalbuphine(5 μg/ml), degraded nalbuphine (40 μg/ml) and guaifenesin as internal standard(15 μg/ml).

### 3.3. Method Validation [17-19]

#### 3.3.1. Linearity and Range

Under the described experimental conditions, the calibration graph for the method was constructed by plotting the peak area ratio versus drug concentration in μg/ml. The regression plot was found to be linear over the range of 1-15 μg/ml. The linear regression equation for the graph is:

\[
y = 0.2307 x - 0.0052 \quad (r^2 = 0.9997).
\]

Where y is the peak area ratio, x is the drug concentration in μg/ml and \(r^2\) is the squared correlation coefficient.

Linearity range, regression equation, intercept, slope and squared correlation coefficient for the calibration data were presented in Table 1.

#### 3.3.2. Limits of Detection and Quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to ICH guidelines from the following equations (2, 3):

\[
LOD = 3.3 \text{Sa} / \text{slope}
\]

\[
LOQ = 10 \text{Sa} / \text{slope}
\]

Where Sa is the standard deviation of y-intercepts of regression lines.

LOD was found to be 0.243 μg/ml, while LOQ was found to be 0.737 μg/ml, as shown in Table 1.
Table 1. Spectral Data for Determination of Nalbuphine by the Proposed HPLC Procedure:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Proposed HPLC Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>210</td>
</tr>
<tr>
<td>Linearity Range (μg/ml)</td>
<td>1 – 15</td>
</tr>
<tr>
<td>LOD (μg/ml)</td>
<td>0.243</td>
</tr>
<tr>
<td>LOQ (μg/ml)</td>
<td>0.737</td>
</tr>
<tr>
<td>- Regression Equation</td>
<td></td>
</tr>
<tr>
<td>- Slope ± S.D.</td>
<td>$y^* = b x^{**} + a$</td>
</tr>
<tr>
<td>- Intercept ± S.D.</td>
<td>$0.2307 \pm 1.9 \times 10^{-3}$</td>
</tr>
<tr>
<td>Correlation Coefficient ($r^2$)</td>
<td>0.9997</td>
</tr>
</tbody>
</table>

$y^*$ is peak area ratio.

$x^{**}$ is concentration in μg/ml

3.3.3. Accuracy and Precision

Accuracy and precision of the method were determined by applying the proposed procedure for determination of three different concentrations, each in triplicate, of nalbuphine in pure form within linearity range in the same day (intraday) and in three successive days (interday). Accuracy as percent recovery (R%) and precision as percent relative standard deviation (RSD%) were calculated and results are listed in Table 2.

Table 2. Intraday and Interday Accuracy and Precision for Determination of Nalbuphine by the Proposed HPLC Procedure:

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>Intraday</th>
<th>Interday</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found Conc.± SD</td>
<td>Accuracy (R %)</td>
<td>Precision (RSD %)</td>
</tr>
<tr>
<td>2</td>
<td>1.99±0.015</td>
<td>99.25</td>
</tr>
<tr>
<td>8</td>
<td>8.01±0.061</td>
<td>100.14</td>
</tr>
<tr>
<td>12</td>
<td>12.08±0.109</td>
<td>100.67</td>
</tr>
</tbody>
</table>

3.3.4. Specificity

The specificity of the proposed procedure was assured by applying it to laboratory prepared mixtures of the intact drug together with its degradation product. The proposed procedure was adopted for the selective determination of intact nalbuphine in presence of up to 87% of its degradation product. The percentage recovery ±RSD % was 100.08±0.678, as shown in Table 3.

Table 3. Determination of Nalbuphine in Mixtures with its Degradation Product by the Proposed Procedure

<table>
<thead>
<tr>
<th>Intact (μg/ml)</th>
<th>Degradate (μg/ml)</th>
<th>Degradate %</th>
<th>Intact Found (μg/ml)</th>
<th>Recovery % of Intact</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>2</td>
<td>13</td>
<td>13.03</td>
<td>100.23</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>33</td>
<td>10.08</td>
<td>100.80</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>53</td>
<td>7.031</td>
<td>100.44</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>73</td>
<td>3.9968</td>
<td>99.92</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>87</td>
<td>1.9802</td>
<td>99.01</td>
</tr>
<tr>
<td>Mean±RSD%</td>
<td></td>
<td></td>
<td>100.08±0.678</td>
<td></td>
</tr>
</tbody>
</table>

3.3.5. System Suitability:

System suitability test was applied to a representative chromatogram to check various parameters such as the number of theoretical plates (N), resolution factor (R), capacity factor (k’), tailing factor (T) and selectivity factor ($\alpha$). The results obtained shown in Table 4, revealed that the chromatographic conditions described here allow complete base line separation between drug, it’s degrade and the internal standard peaks with minimum tailing.
3.3.6. Robustness:

The robustness of the proposed procedure was evaluated by slight changes in the chromatographic conditions such as flow rate (± 0.1 ml/min.), pH of the mobile phase (±0.2) and mobile phase contents ratio (±3%). In each case only one parameter was changed while other conditions were kept constant. These minor changes did not affect the separation and resolution of nalbuphine from its degradation product and internal standard, confirming robustness of the procedure, as shown in Table 4.

Table 4. Robustness Results for Determination of Nalbuphine by the Proposed HPLC Procedure

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Retention Time (t_R)</th>
<th>Capacity Factor (K')</th>
<th>Theoretical Plates (N)</th>
<th>Resolution (R)</th>
<th>Selectivity Factor (α)</th>
<th>Tailing Factor (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Rate (ml/min.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td>5.5</td>
<td>6.9</td>
<td>2830</td>
<td>6.11</td>
<td>1.88</td>
<td>1.38</td>
</tr>
<tr>
<td>1</td>
<td>4.7</td>
<td>5.7</td>
<td>2880</td>
<td>6.33</td>
<td>1.90</td>
<td>1.34</td>
</tr>
<tr>
<td>1.1</td>
<td>4.1</td>
<td>4.85</td>
<td>3010</td>
<td>6.48</td>
<td>1.96</td>
<td>1.30</td>
</tr>
<tr>
<td>pH of Mobile Phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.7</td>
<td>4.7</td>
<td>5.7</td>
<td>2875</td>
<td>6.30</td>
<td>1.92</td>
<td>1.33</td>
</tr>
<tr>
<td>5.5</td>
<td>4.7</td>
<td>5.7</td>
<td>2880</td>
<td>6.33</td>
<td>1.90</td>
<td>1.34</td>
</tr>
<tr>
<td>5.3</td>
<td>4.6</td>
<td>5.6</td>
<td>2895</td>
<td>6.12</td>
<td>1.87</td>
<td>1.32</td>
</tr>
<tr>
<td>Mobile Phase Ratio (Buffer : Acetonitrile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37:63</td>
<td>4.5</td>
<td>5.4</td>
<td>3000</td>
<td>6.42</td>
<td>1.99</td>
<td>1.28</td>
</tr>
<tr>
<td>40:60</td>
<td>4.7</td>
<td>5.7</td>
<td>2880</td>
<td>6.33</td>
<td>1.90</td>
<td>1.34</td>
</tr>
<tr>
<td>43:57</td>
<td>4.9</td>
<td>5.9</td>
<td>2850</td>
<td>6.08</td>
<td>1.84</td>
<td>1.38</td>
</tr>
</tbody>
</table>

3.3.7. Stability of Standard Solutions:

The stability of standard solutions of nalbuphine and the internal standard (guaifenesin) were determined by repeated analysis of these solutions that stored either at room temperature or in refrigerator at different time intervals and comparing the responses (peak areas) with those of freshly prepared standard solutions. From the results it was found that, both nalbuphine and guaifenesin standard solutions were stable for at least 4 and 7 days when stored at room temperature and in refrigerator respectively.

3.4. Pharmaceutical Applications:

The proposed HPLC procedure was applied to the determination of nalbuphine in Nalufin® ampoules. Satisfactory results were obtained in good agreement with the label claim, indicating no interference from excipients and additives. The obtained results were statistically compared to those obtained by the reported method [4]. No significant differences were found by applying t-test and F-test at 95% confidence level [20], indicating good accuracy and precision of the proposed method for the analysis of the studied drug in its pharmaceutical dosage form, as shown in Table 5.

Table 5. Determination of Nalbuphine in Nalufin® Ampoules by the Proposed and Reported Methods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Proposed Method</th>
<th>Reported Method [4]</th>
</tr>
</thead>
<tbody>
<tr>
<td>N*</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>X **</td>
<td>100.22</td>
<td>99.61</td>
</tr>
<tr>
<td>SD</td>
<td>0.686</td>
<td>1.030</td>
</tr>
<tr>
<td>RSD%</td>
<td>0.685</td>
<td>1.034</td>
</tr>
<tr>
<td>t***</td>
<td>1.09</td>
<td>(2.31)</td>
</tr>
<tr>
<td>F***</td>
<td>2.26</td>
<td>(6.39)</td>
</tr>
</tbody>
</table>

* Number of experiments.

** The mean of percent recovery of pharmaceutical preparation.

*** The values in parenthesis are tabulated values of “t” and “F” at (P = 0.05)
4. CONCLUSION

The proposed method is simple, rapid, accurate and precise and can be used for the analysis of nalbuphine in pure form and in pharmaceutical dosage form (either alone or in the presence of its degradation product).

REFERENCES

Ahmed El-Olemy et al.


AUTHOR’S BIOGRAPHY

Name : Ahmed El-Olemy
Affiliation: Assistant Lecturer of Analytical Chemistry
Address : Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, 11751, Nasr City, Cairo, Egypt