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# STABILITY-INDICATING SPECTROFLUORIMETRIC DETERMINATION OF NALBUPHINE HYDROCHLORIDE IN RAW MATERIAL AND PHARMACEUTICAL PREPARATION

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**ABSTRACT:** A Simple, rapid, sensitive, accurate and precise spectrofluorimetric method has been developed for selective determination of nalbuphine hydrochloride (NAL) in presence of its oxidative degradate in bulk powder and in pharmaceutical preparation. The proposed method is based on measuring the native fluorescence of NAL in acetonitrile at 337 nm after excitation at 285 nm. All variables that affect fluorescence intensity such as diluting solvents and pH were studied and optimized .The fluorescence–concentration plot was rectilinear over the range of 1-12 µg/ml with a lower detection limit (LOD) of 0.175 µg/ml and lower quantitation limit (LOQ) of 0.529 µg/ml. The proposed method can selectively analyse the drug in presence of up to 75% of its oxidative degradate with mean recovery of 101.37±0.72. The method was validated and successfully applied for the determination of NAL in its commercial preparation with an average percent recovery ± RSD% of 100.14 ± 0.89. 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.

**INTRODUCTION:** Nalbuphine hydrochloride (**Fig. 1**) is (5α, 6α),-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].

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Few analytical methods have been reported for its analysis including spectrophotometric [3, 4], spectrofluorimetric [4], electrochemical [5] and chromatographic methods [6-12].

Spectrofluorimetry has been widely used in the determination of pharmaceutical compounds [13-18] because it is a highly sensitive, selective, easily operated and economical technique.

The main task of this work is to establish a simple and accurate stability-indicating spectrofluorimetric method for the determination of NAL in presence of its oxidative degradate, which can be used for the routine analysis of the drug in raw material and pharmaceutical preparations.

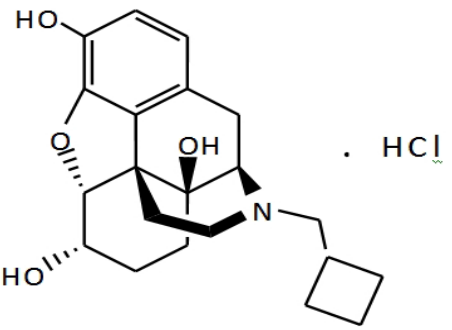



**FIG. 1: STRUCTURAL FORMULA OF NALBUPHINE HYDROCHLORIDE**

## MATERIALS AND METHOD:

### Apparatus:

- Jasco FP-6200 Spectrofluorometer (Japan), equipped with 150 Watt Xenon lamp. Slit widths for both monochromators were set at 10 nm. A 1 cm quartz cell was used. All measurements were done at medium sensitivity.
- Jenway, 3510 pH meter (Jenway, U.S.A.).
- Hot plate, Torrey pines scientific, USA.

**Materials and Reagents:** All chemicals and reagents used throughout the work were of analytical grade.

- Nalbuphine hydrochloride was kindly supplied by Amoun Pharmaceutical Company, Cairo, Egypt. The purity was assigned as 99.25 %.
- 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.
- Water used throughout the procedures was freshly double distilled.
- Acetonitrile, methanol, ethanol, 1-propanol, chloroform, dichloroethane and tetrahydrofuran, all of HPLC grades [Sigma, Germany].
- Sodium hydroxide (El-Nasr Company, Egypt) prepared as 0.1 N aqueous solution.
- Hydrochloric acid (El-Nasr Company, Egypt) prepared as 0.1 N aqueous solution.
- Monobasic potassium phosphate, potassium chloride, boric acid, glacial acetic acid and sodium acetate trihydrate (El-Nasr Company, Egypt).
- Buffers of different pH values prepared as prescribed in US pharmacopeia[19]:
  1. Acetate buffer pH range from 4.1 to 5.5.
  2. Phosphate buffer pH range from 6 to 8.
  3. Alkaline borate buffer pH range from 8 to 10.

### Standard Solutions:

**Standard Solution of Intact NAL:** A standard solution of NAL (100 µg/ml) was prepared by dissolving 10 mg of NAL in 50 ml of water and complete to 100 ml with water. This solution was stable for one month when kept in the refrigerator [3].

**Standard Solution of Degraded Sample:** 100 mg of pure NAL powder were dissolved in 45 ml distilled water and transferred to a 100-ml round bottomed flask to which 5 ml of 50% $H_2O_2$ was added. The solution was heated under reflux for 6 hours and evaporated to dryness under vacuum. The obtained residue was extracted with ethanol (2 × 10 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 NAL[3].

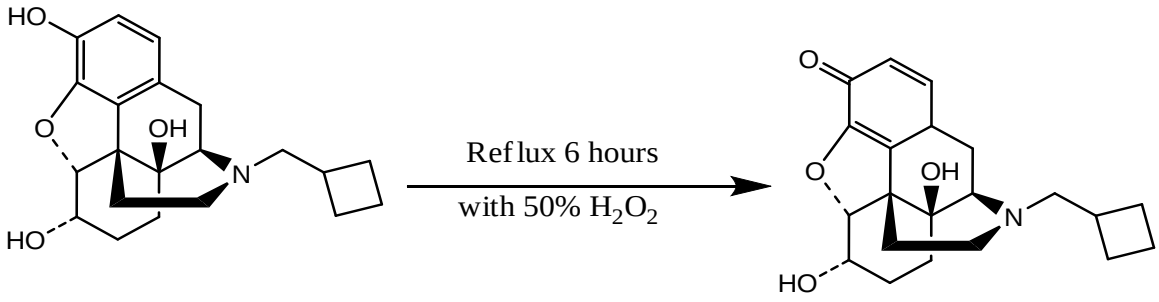
### Procedure:

**Construction of the Calibration Curve (General Procedure):** Different aliquots of NAL standard solution ranging from (10–120) µg were transferred to a 10-ml volumetric flasks and 1 ml of 0.1 N HCl was added. The solutions were diluted with acetonitrile to 10 ml and mixed well. The fluorescence intensity was measured at 337 nm ($\lambda_{excitation}$= 285 nm).The measured fluorescence intensity vs the final concentration in µg/ml were plotted to get the calibration graph. Alternatively, the regression equation was derived.

**Analysis of Pharmaceutical Preparation:** Contents of 10 Nalufin® ampoules (each containing 20 mg NAL) were mixed well. A volume equivalent to 100 mg of NAL was transferred into 100-mL volumetric flask and completed to volume with water to obtain a solution labeled to contain 1 mg/ml of NAL. Transfer aliquots covering the working concentration range into 10 ml volumetric flasks. Proceed as described under "General Procedure". Determine the content of the ampoules either from the calibration curve or using the corresponding regression equation.

## RESULTS AND DISCUSSION:

**Degradation of NAL:** Stressed degradation of NAL was studied by refluxing the drug using different media; aqueous, 1M NaOH, 1M HCl and 50% $H_2O_2$ 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% $H_2O_2$ for 6 hours[3].




Nalbuphine

Oxidative degradate

**FIGURE 2: PROPOSED DEGRADATION PATHWAY OF NAL**

**Spectral Characteristics:** NAL exhibits a native fluorescence in acetonitrile and its emission can be measured at 337 nm ($\lambda_{emission}$) after excitation at 285 nm ($\lambda_{excitation}$). The emission and excitation spectra of NAL in acetonitrile are shown in **figure 3.**

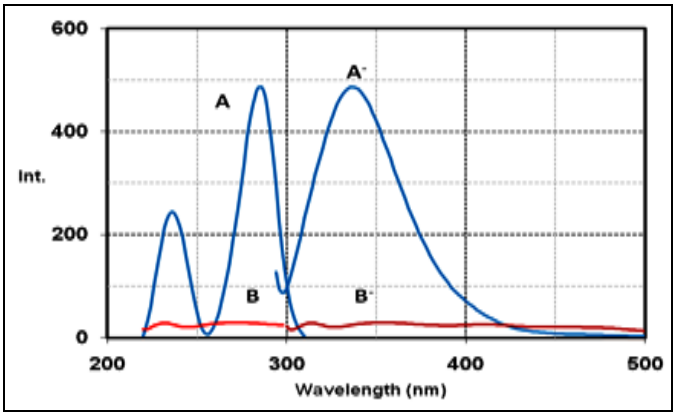



**FIGURE (3): EXCITATION (A,B) AND EMISSION (A', B') SPECTRA OF NAL (6 µg/ml ) AND ITS OXIDATIVE DEGRADATE (6 µg/ml ) RESPECTIVELY, IN ACETONITRILE USING 1 mL OF 0.1 N HCl**

### Optimization of Experimental Conditions:

**(i) Effect of Diluting Solvents:** The general procedure for the method was repeated using a fixed amount of NAL (60 µg) and different diluting solvents and found that; acetonitrile is the best diluting solvent as shown in **figure 4**.

**(ii) Effect of pH and Buffer:** The general procedure for the method was repeated using a fixed amount of NAL (60 µg) and different buffers with different pH and found that; 0.1 N HCl gives the best result as shown in **figure 5**.

**(iii) Effect of HCl Volume:** The general procedure for the method was repeated using a fixed amount of NAL (60 µg) and different volumes of 0.1 N HCl and found that; 1 ml gives the best result as shown in **figure 6**.

**(iv) Effect of Time:** The general procedure for the method was repeated using a fixed amount of NAL (60 µg) at different time interval and found that; it is stable at least for one hour as shown in **figure 7**.

**(v) Effect of Temperature:** The effect of temperature was studied in the range of 40–100°C using a thermostatically controlled water bath. It was found that, increasing the temperature causes decrease of fluorescence

intensity. It may be due to the collision between the excited singlet state and the solvent molecules which causes loss of energy. So, the fluorescence intensity of NAL was measured at room temperature (25 °C).

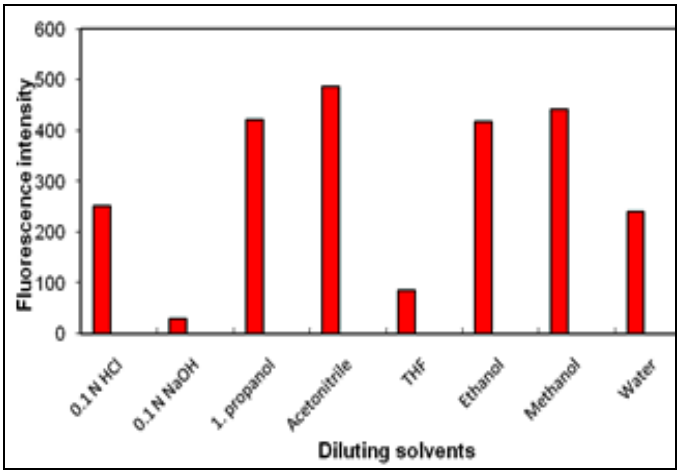



**FIGURE (4): EFFECT OF DIFFERENT DILUTING SOLVENTS ON FLUORESCENCE INTENSITY OF NAL (6 µg/ ml)**

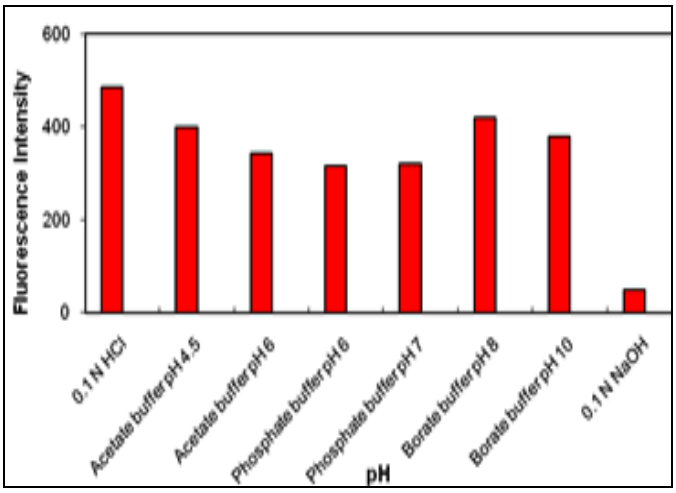



**FIGURE (5): EFFECT OF pH ON FLUORESCENCE INTENSITY OF NAL (6 µg/ ml)**

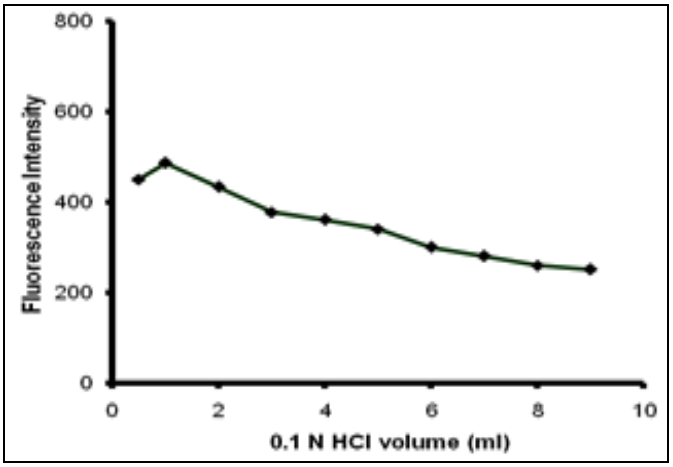



**FIGURE 6: EFFECT OF VOLUME OF 0.1 N HCl ON FLOURESCENCE INTENSITY OF NAL (6 µg/ ml)**

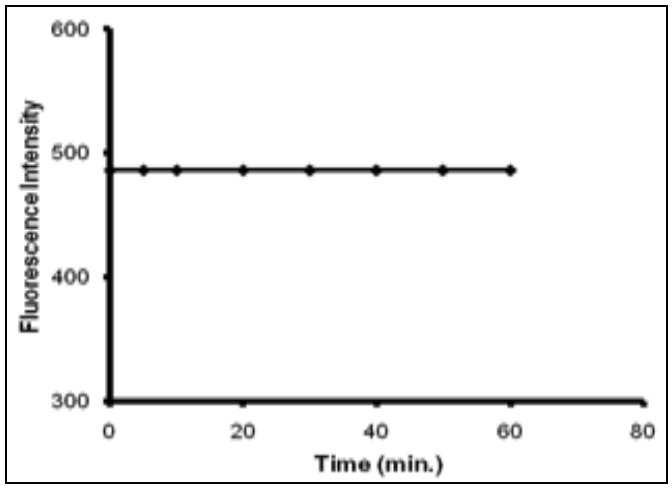



**FIGURE (7): EFFECT OF TIME ON FLUORESCENCE INTENSITY OF NAL (6 µg/ ml)**

## Validation of the Method:

**(i) Linearity and Range:** Under the described experimental conditions, the calibration graph for the method was constructed by plotting fluorescence intensity versus concentration in µg/ml. The regression plot was found to be linear over the range of 1-12 µg/ml. The linear regression equation for the graph is:

$$\mathbf{FI = 75.830\ C + 24.057 (r^2 = 0.9997)}$$

Where **FI** is the fluorescence intensity, **C** is the drug concentration in µg $ml^{-1}$ and $\mathbf{r^2}$ is the correlation coefficient.

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

**(ii) Limits of Detection and Quantitation :** The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to ICH $Q_2$ Recommendation[20] from the following equations:

$LOD = 3.3\ S_a / slope$

$LOQ = 10\ S_a / slope$

Where $S_a$ is the standard deviation of the intercept of regression line.

LOD was found to be 0.175 µg/ml, while LOQ was found to be 0.529 µg/ml. The small values of LOD and LOQ indicate good sensitivity.

**(iii) Accuracy and Precision:** Three replicate determinations of three different concentrations of NAL in pure form within linearity range were performed in the same day (intra-day) and in three successive days (inter-day).Accuracy as recovery percent (R%) and precision as percentage relative standard deviation (RSD%) were calculated and results are listed in **table 2**. The small values of RSD% indicate high precision of the method. Moreover, the good R% confirms excellent accuracy.

**(iv) Specificity:** The specificity of the proposed method was assured by applying the laboratory prepared mixtures of the intact drug together with its degradation product. The proposed method was adopted for the specific determination of intact NAL in presence of up to 75% of its corresponding degradates. The percentage recovery ± RSD % was 101.37 ± 0.72 (**table 3)**.

**Pharmaceutical Applications:** The proposed method was applied to the determination of the studied drug in **Nalufin® ampoules**. The results were validated by comparison to a previously reported method[4]. No significant difference was found by applying t-test and F-test at 95% confidence level, indicating good accuracy and precision of the proposed method for the analysis of the studied drug in its pharmaceutical dosage form (**table 4**).

**TABLE 1: SPECTRAL DATA FOR DETERMINATION OF NAL BY THE PROPOSED METHOD:**

| Parameters | Proposed Method |
|---|---|
| $\lambda_{emission}$ (nm) | 337 |
| $\lambda_{excitation}$ (nm) | 285 |
| Linearity range (µgml$^{-1}$) | 1—12 |
| LOD (µgml$^{-1}$) | 0.175 |
| LOQ (µgml$^{-1}$) | 0.529 |
| Regression equation* | |
| Slope (*b*) | 75.830 |
| Intercept (*a*) | 24.057 |
| Correlation Coefficient ($r^2$) | 0.9997 |

* $y = a + bx$ where $y$ is the fluorescence intensity and $x$ is the concentration.

**TABLE (2): INTRADAY AND INTERDAY ACCURACY AND PRECISION FOR THE DETERMINATION OF NAL BY THE PROPOSED METHOD:**

| | Intra-day | | | Inter-day | | |
|---|---|---|---|---|---|---|
| Conc. µg.ml$^{-1}$ | Found Conc. ± SD | Accuracy (R%) | Precision (RSD%) | Found Conc. ± SD | Accuracy (R%) | Precision (RSD%) |
| 2 | 2.01 ± 0.032 | 100.45 | 1.598 | 1.99 ± 0.006 | 99.35 | 0.314 |
| 6 | 6.01 ± 0.089 | 100.17 | 1.475 | 6.08 ± 0.056 | 101.27 | 0.920 |
| 10 | 10.07 ± 0.125 | 100.68 | 1.241 | 9.99 ± 0.076 | 99.89 | 0.760 |

**TABLE 3: DETERMINATION OF INTACT NAL IN MIXTURES WITH ITS OXIDATIVE DEGRADATE BY THE PROPOSED METHOD:**

| Intact (µg ml$^{-1}$) | Degradate (µg ml$^{-1}$) | Degradate % | Intact found (µg ml$^{-1}$) | Recovery % of Intact |
|---|---|---|---|---|
| 9 | 3 | 25 | 9.03 | 100.33 |
| 7 | 5 | 42 | 7.01 | 101.43 |
| 5 | 7 | 58 | 5.01 | 102.00 |
| 3 | 9 | 75 | 3.05 | 101.70 |
| Mean ± RSD% | | | | 101.37±0.72 |

**TABLE 4: DETERMINATION OF NAL IN NALUFIN® AMPOULES (20 mg/ml) BY THE PROPOSED AND REPORTED METHODS**

| Parameters | Proposed method | Reported method[4] |
|---|---|---|
| ***N**** | 5 | 5 |
| $\bar{X}$ | 100.14 | 99.61 |
| ***SD*** | 0.89 | 1.03 |
| ***RSD%*** | 0.89 | 1.03 |
| ***t*** ** | 0.86 (2.31) | —— |
| **F** ** | 1.34 (6.39) | —— |

* No. of experimental. ** The values in the parenthesis are tabulated values of t and F at (p= 0.05).

**CONCLUSION:** The proposed method is simple, rapid, accurate, selective and inexpensive. It permits the determination of nalbuphine hydrochloride in its pure form and pharmaceutical preparations. The method proved its ability to be used for stability-indication of the drug. Therefore, it can be used for purity testing, stability studies, quality control and routine analysis of the drug.

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