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Stability Indicating Methods for Determination of Nalbuphine- Hydrochloride

M. M. Fouad[1*], S. A. Abdel Razeq[1], Z. A. Elsayed[1] and L. A. Hussin[2]

[1]Department of Analytical Chemistry, Al-Azhar University, Cairo, Egypt.
[2]Department of Analytical Chemistry, Ain-Shams University, Cairo, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Authors SAAR and MMF designed the study and wrote the protocol. Author ZAE performed the statistical analysis. Authors MMF and ZAE managed the analyses of the study and wrote the first draft of the manuscript. Authors ZAE and LAH managed the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

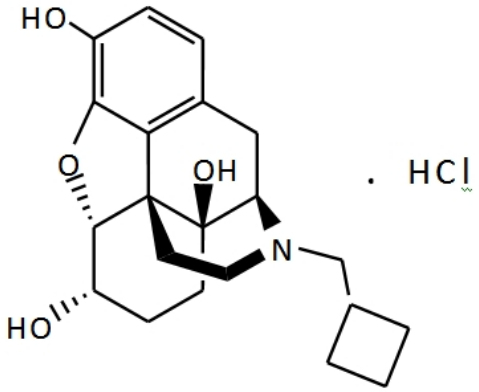
Three simple, sensitive and reproducible spectrometric methods for the selective determination of nalbuphine–HCl in presence of its oxidative degradate were investigated. The first method depended on the quantitative densitometric evaluation of thin layer chromatograms of the drug at 284 nm using chloroform–methanol–acetic acid (7:3:0.05 v/v/v) as a mobile phase, in a concentration range of 10-30 µg/spot. The second one used the pH induced difference absorbance (ΔA) between 0.1M NaOH and 0.1 M HCl drug solutions at 299 nm to determine 20-160 µg mL^{-1} of the drug. The third method was a bivariate calibration algorithm for the determination of nalbuphine–HCl over concentration range of 20-200 µg mL^{-1}. The proposed methods selectively analysed the drug in presence of up to 80% of its oxidative degradate with mean recoveries of 100.63±1.03 for densitometric method and up to 90% with recoveries of 99.97±1.16 and 100.09±1.47% regarding the two other methods, respectively. The three proposed methods were successfully applied to analyse nalbuphine– HCl in its preparations, the results obtained were statistically analysed and found to be in accordance with those given by the compendial method.

**Corresponding author: Email: manalfoad2000@yahoo.com;*

Keywords: Nalbuphine–HCl; stability indicating; densitometric; pH induced difference absorbance (ΔA); bivariate calibration algorithm.

1. INTRODUCTION

Demonstration of the stability of an active pharmaceutical ingredient is an essential component in the development and commercialization of pharmaceutical products. The International Conference on Harmonization (ICH) guideline entitled "stability testing of new drug substances and products requires that stress testing should be carried out to elucidate the inherent stability characteristics of the active substance, in which susceptibility to hydrolysis of analyte under acidic or basic conditions is one of the most required tests" [1].



Nalbuphine-HCl (17-Cyclobutylmethyl-4,5α-epoxy-3,6α,14–triol-HCl) is a semi-synthetic narcotic kappa receptor agonist/ mu receptor antagonist [2]. It can be also used as a supplement to balance anesthesia for pre-operative and post-operative analgesia and for obstetrical analgesics during labor and deliver [3]. Few analytical methods have been reported for its analysis including electrochemical [4], gas chromatography [5-8] and HPLC [8-14].

As there was no previous study concerning the stability of the studied drug, the main task of this work was to establish simple and accurate stability indicating methods for the determination of nalbuphine-HCl in presence of its oxidative degradate, which can be used for the routine analysis of the drug in raw material and pharmaceutical preparations.

2. MATERIALS AND METHODS

2.1 Apparatus

For densitometric method, Camag TLC scanner 3, with WINCATS computer software (Switzerland).Samples were applied on precoated TLC plates, silica gel 60 GF_{254} (20 × 20 cm), (Flukachemie, Switzerland) using Hamilton 10-µL microsyringe (Germany), developed in a chromatographic tank (25 × 25 × 9 cm) and visualized using a UV lamp with short wavelength (254 nm, Desega-Germany). Shimadzu UV-Vis 1601 spectrophotometer

(Tokyo, Japan) was used to perform the other two methods. pH adjustment was carried out using Jenco digital pH/temp meter with Jenway double function glass electrode (UK).

2.2 Samples

2.2.1 Pure and market samples

Nalbuphine-HCl (white or almost white crystalline powder, freely soluble in water) was kindly supplied by Amoun Pharmaceutical Company, Cairo, Egypt. Its purity was found to be 101.8% as stated by the supplier. Nalufin ampoules, B.N. 320, each ampoule (1 mL) claimed to contain 20 mg nalbuphine-HCl (equivalent to 20.959 mg nalbuphine), the product of Amoun Pharmaceutical Company, purchased from local market.

2.2.2 Degraded samples

0.25 g of nalbuphine-HCl were accurately weighed, 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 25-mL volumetric flask and diluted to volume with ethanol to obtain a stock solution labeled to contain degradate derived from 10 mg mL^{-1} nalbuphine-HCl for densitometric method. 5 mL of the later solution was diluted to 50 mL with ethanol to obtain a solution containing degradate derived from 1mg mL^{-1} intact nalbuphine-HCl which was used for ΔA and bivariate methods.

2.3 Chemicals and Reagents

All reagents used were of analytical grade and solvents were of spectroscopic grade.
Chloroform and methanol were obtained from Sigma – Aldrich (USA). Ethanol absolute was from Riedell-detlean (Germany). Hydrogen peroxide (50%), glacial acetic acid and NaOH were purchased from El Nasr Co. (Egypt). HCl was from Prolabo (France).

2.4 Standard Solutions

Stock ethanolic solution of nalbuphine-HCl (10 mg mL^{-1}) was prepared to be used for densitometric method. Dilution was then made with ethanol to obtain a solution of 1 mg mL^{-1} used for ΔA and bivariate methods. This solution was stable for one month at 4ºC.

2.5 Procedures

2.5.1 Densitometric method

2.5.1.1 Linearity

In a series of 10-mL volumetric flasks, aliquots of standard nalbuphine -HCl solution (10 mg mL^{-1}) equivalent to 10-30 mg drug were transferred and diluted to volume with ethanol. 10 µL of each solution was applied to precoated 20 × 20 cm TLC aluminum silica gel 60 GF_{254} plates. Plates were spotted 2 cm apart from each other and 2 cm apart from the bottom edge. The chromatographic tank was pre-saturated with the mobile phase for 20 min, then developed by ascending chromatography using chloroform-methanol-acetic acid (7:3:0.05 v/v/v) as a mobile phase. The plates were air dried, detected under UV-

lamp (254 nm) and then scanned at 284 nm. Calibration curve was constructed by plotting area under the peak versus corresponding drug concentration in µg/spot.

2.5.1.2 Assay of laboratory prepared mixtures

Into a set of 10-mL volumetric flaks, different volumes (2.70-1.00 mL) of intact nalbuphine-HCl solution (10 mg mL^{-1}) were transferred and mixed with (0.30-4.00 mL) of its oxidative degradate solution. Volumes were completed to the mark with ethanol. 10 µL of each mixture was applied on to TLC plate and then proceed as mentioned under Linearity staring from" spotted 2 cm…..". Peak areas of the obtained chromatograms were measured and the concentration of the drug was calculated from its corresponding regression equation.

2.5.2 ∆A Spectrophotometric method

2.5.2.1 Linearity

Aliquots of standard drug solution (1 mg mL^{-1}) equivalent to 0.20-1.60 mg of nalbuphine-HCl were transferred into two sets of 10-mL volumetric flasks. The first one was diluted to volume with 0.1 M HCl and the other set with 0.1 M NaOH. ∆A of each concentration was measured at 299 nm by placing the acidic solution in the reference beam and the alkaline one in the sample beam. Calibration curve was constructed relating ∆A values to drug concentrations in µg mL^{-1}.

2.5.2.2 Assay of laboratory prepared mixtures

Into two sets of 10-mL volumetric flasks, different volumes (0.90-0.20 mL) of intact nalbuphine-HCl solution (1 mg mL^{-1}) were introduced and mixed with (0.10-1.80 mL) of its degradate solution and diluted to 10 mL with 0.1 M HCl in one set and 0.1 M NaOH in the second set. The absorbance of the alkaline solution was measured against the acidic one at 299 nm and the intact drug concentration was calculated from the corresponding regression equation.

2.5.3 Bivariate method

2.5.3.1 Linearity

Aliquots of standard ethanolic nalbuphine-HCl solution or its oxidative degradate equivalent to 0.20-2.00 mg or 0.20-1.20 mg, respectively were transferred separately into two sets of 10- mL volumetric flasks then diluted to volume with ethanol. Calibration curves at different wavelengths 240, 247, 250, 260, 267, 270, 280, 285, 290, 300 and 310 nm were constructed and the regression equation at each wavelength was calculated. From both sets of regression equations, the sensitivity matrices K was calculated, the optimum pair of wavelengths were chosen (247 and 270 nm) to carry out the determination and the regression equations used in the bivariate algorithm were deduced.

2.5.3.2 Assay of laboratory prepared mixtures

Different volumes (0.90-0.20 mL) of intact nalbuphine-HCl solution (1 mg mL^{-1}) were transferred and mixed with (0.10-1.80 mL) of its degraded solution in a set of 10-mL volumetric flaks. The volume was completed to mark with ethanol, and the absorbance of

each mixture was recorded at 247 and 270 nm. The concentrations of the intact drug and its degradate were calculated using Kaiser method [15].

2.6 Application to Pharmaceutical Preparations

Contents of 10 Nalufin® ampoules (each containing 20 mg aqueous nalbuphine- HCl) were mixed. A volume equivalent to 100 mg drug was transferred into 10-mL volumetric flask and completed to volume with ethanol to obtain a solution labeled to contain 10 mg mL^{-1} nalbuphine-HCl which was analysed by densitometric method. The later solution was appropriately diluted with ethanol to obtain a solution labelled to contain 1mg mL^{-1} nalbuphine-HCl analysed by ΔA and bivariate methods as detailed above under "Linearity".

3. RESULTS AND DISCUSSION

Three different analytical methods were developed; densiotometric, ΔA and bivariate method aiming for the selective quantitation of nalbuphine-HCl in presence of its oxidative degradate.

3.1 Degradation of Nalbuphine-HCl

Stressed degradation of nalbuphine-HCl 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 5% H_2O_2 for 6 hours. This was confirmed by appearance of two spots on the TLC at two different R_f one corresponding to the intact and the other for its oxidative degradate; Fig. 1.

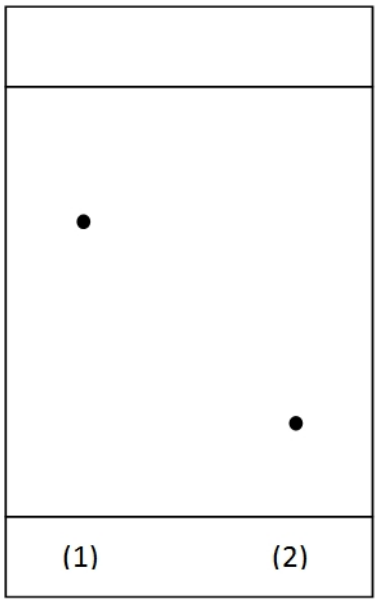


Fig. 1. Thin layer chromatogram of: (1) Intact nalbuphine-HCl. (2) Nalbuphine-HCl oxidative degradate

Mobile phase: Chloroform-methanol-acetic acid (7:3:0.05 v/v/v)

IR spectrum of the pure drug exhibits a broad band between 2900 - 3500 cm^{-1}, characteristic to the – OH groups. Appearance of new band at 1750 cm^{-1} in the IR spectrum of the degradate confirms the oxidation of the –OH group (at position no.3) after refluxing the drug with 5% H_2O_2; Fig. 2.

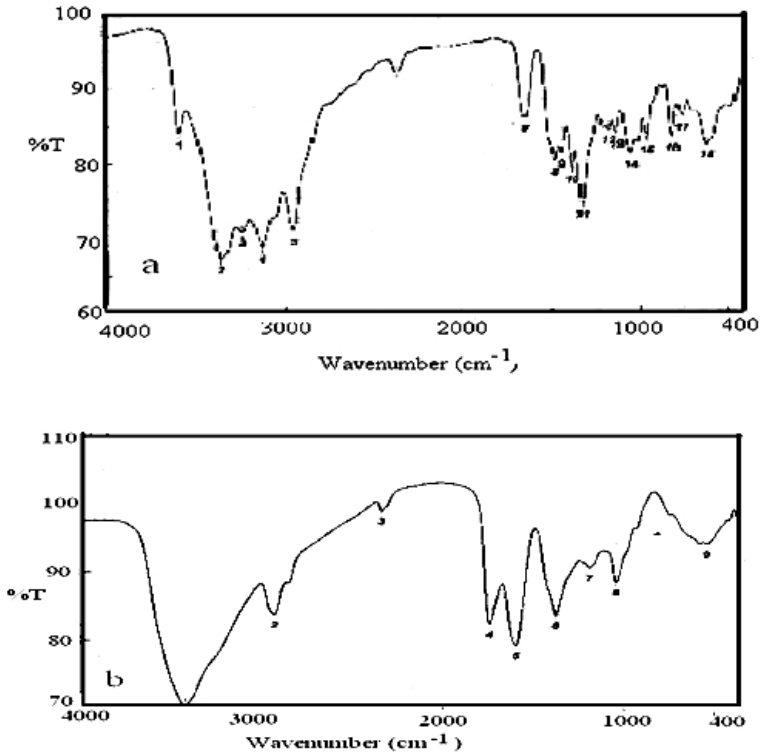


Fig. 2. IR Spectra of: a) Intact nalbuphine-HCl on KBr disc b) Nalbuphine-HCl oxidativedegradate on KBr disc

Thus a degradation pathway was suggested as shown in Fig. 3.

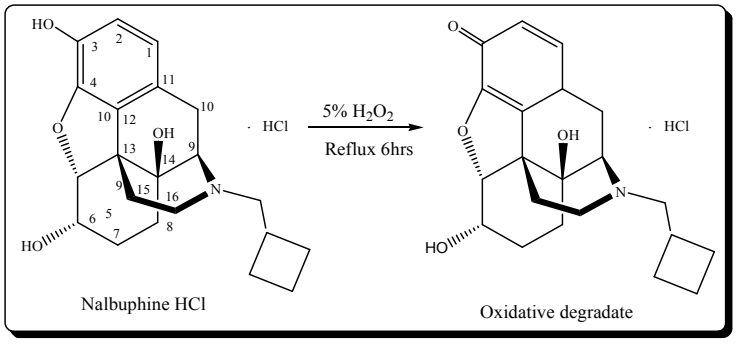


Fig. 3. Proposed degradation pathway of nalbuphine-HCl

3.2 Densitometric Method

TLC is known to be one of the simplest chromatographic techniques used for the separation and identification of drugs due to its advantages of low operating costs and the need of minimum sample preparation. Complete separation of nalbuphine-HCl and its oxidative degradate was obtained at R_f values of 0.68 and 0.20 using chloroform–methanol–acetic acid (7:3:0.05 v/v/v) as a developing mobile phase. Quantitatively the separated spots of nalbuphone-HCl were scanned densitometrically at 284 nm without any interference of degradation product, Fig. 4.

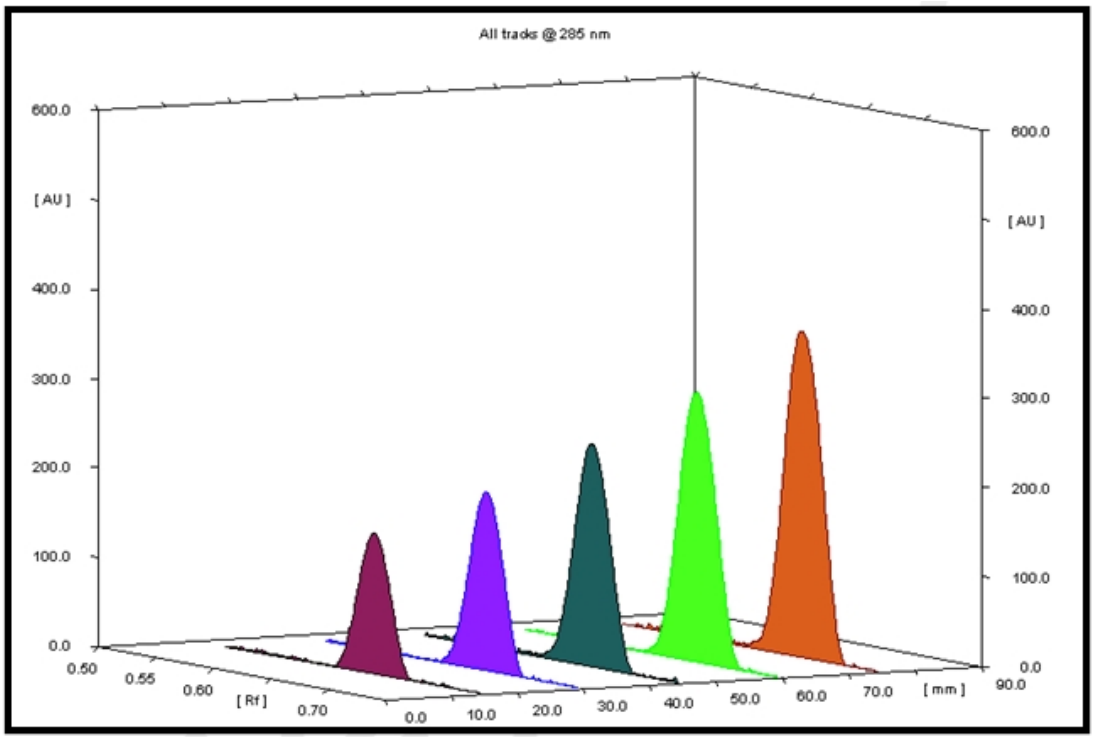


Fig. 4. Densitometric chromatogram of nalbuphine-HCl (10-30 µg/spot) at 284 nm

3.3 ΔA Spectrophotometric Method

ΔA technique was found to be the solving key for interference due to co-formulates additives and degradation products. Nalbuphine-HCl was found to be pH sensitive, showing a bathochromic shift in its λ_{max} from 285 nm in HCl to 298 nm in NaOH. (ΔA) measurements of both intact drug and its oxidative degradate between their acidic and alkaline solutions at 299 nm indicated no interference from drug degradate; Fig. 5.

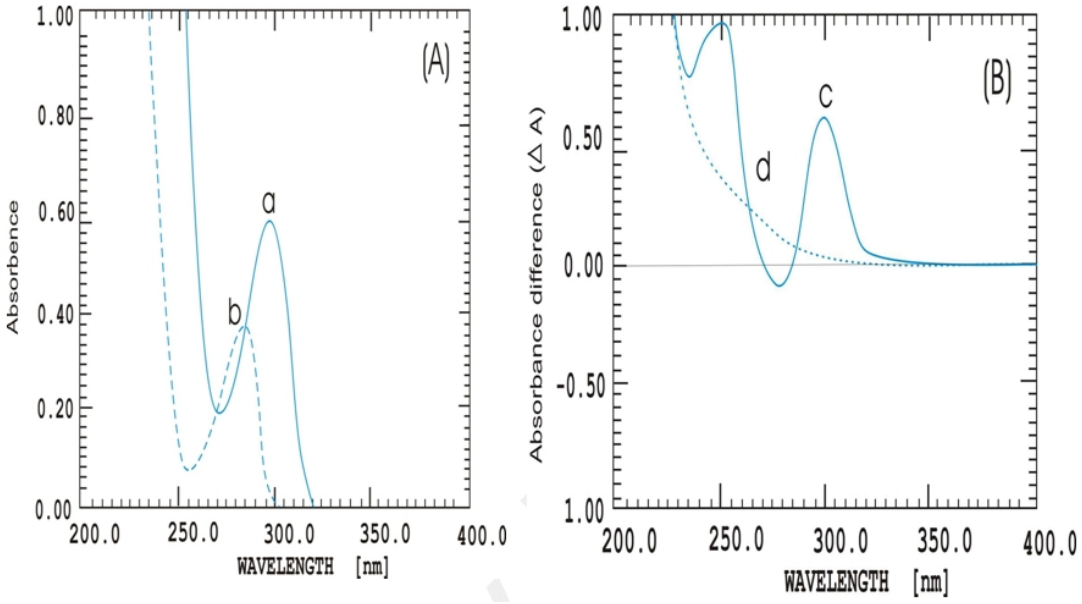


Fig. 5. Absorption spectra of: a) Nalbuphine-HCl (100µg mL^{-1}) in 0.1 M NaOH b) Nalbuphine-HCl (100µg mL^{-1}) in 0.1 M HCl. c) ∆A spectrum of intact nalbuphine -HCl between NaOH and HCl solutions. d) ∆A spectrum of nalbuphine oxidative degradate between NaOH and HCl solutions

3.4 Bivariate Method

Bivariate calibration spectrophotometric method is a direct method which has been proposed for the resolution of mixtures [15-19]. The principle of bivariate calibration is the measurement of two components (A and B) at two selected wavelengths (λ_1 and λ_2) to obtain two equations [15]:

$$A_{AB1} = m_{A1}C_AC_B + e_{AB1} \qquad A_{AB2} = m_{A2}C_AC_B + e_{AB2}$$

The resolution of each equation set allows the evaluation of C_A and C_B values:

$$C_A = (A_{AB1} - e_{AB1} - m_{B1}C_B)/m_{A1} \qquad C_B = [m_{A2}(A_{AB1} - e_{AB1}) + m_{A1}(e_{AB2} - A_{AB2})/m_{A2}m_{B1} - m_{A1}m_{B2}$$

Where C_A, C_B are the concentration of component A (drug), component B (degradate); m_{A1}, m_{A2} are the slope values of the drug at λ_1, λ_2; m_{B1}, m_{B2} are the slope values of the degradate at λ_1, λ_2; A_{AB1}, A_{AB2} are the absorbance of the binary mixture at λ_1, λ_2; e_{AB1}, e_{AB2} are the sum of the intercepts of drug and degradate at λ_1, λ_2, respectively.

This simple mathematic algorithm allows the resolution of the two components by measuring the absorbance of their mixture at the two selected wavelengths and using the parameters of the linear regression functions evaluated individually for each component at the same wavelengths.

In order to apply the bivariate method in the resolution of nalbuphine- HCl and its oxidative degradate, the absorbance of the two components at eleven different selected wavelengths

was recorded in the region of overlapping; 240, 247, 250, 260, 267, 270, 280, 285, 290, 300, 310 nm; Fig. 6. The calibration curve equations and their respective linear regression coefficients were obtained directly with the aim of ensuring that there was a linear relationship between the absorbance and the corresponding concentration. All of the calibration curves at the selected wavelengths showed a satisfactory linear regression coefficient ($r \geq 0.9985$).

According to Kaiser method [15], the slope values of the linear regression equations for both intact drug and its oxidative degradate at the selected wavelengths were used to calculate the sensitivity matrices K to find out the optimum pair of wavelength at which the binary mixtures were recorded; Table 1.

$$K = \begin{bmatrix} m_{A_1} & m_{B_1} \\ m_{A_2} & m_{B_2} \end{bmatrix}$$

For the bivariate determination of nalbuphine-HCl and its oxidative degradate, 247 and 270 nm were found to give the maximum value of K and thus can be used for the analysis; Table 1.

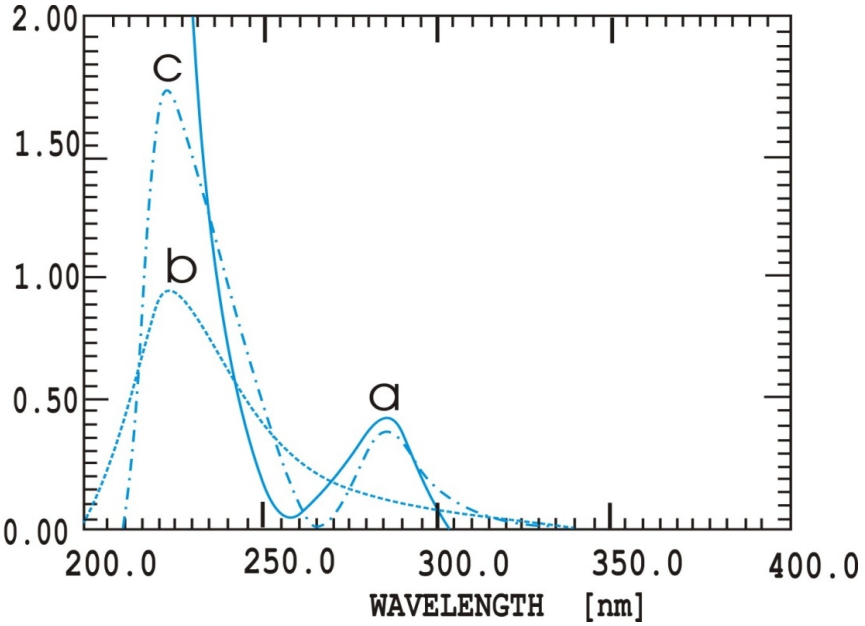


Fig. 6. Absorption spectra of: a) Intact nalbuphine-HCl (100µg mL^{-1}) in ethanol. b) Nalbuphine-HCl oxidative degradate (100µg mL^{-1}) in ethanol. c) Mixture of intact nalbuphine (50µg mL^{-1}) and its oxidative degradate (50 µg mL^{-1}) in ethanol

3.5 Method Validation

3.5.1 Linearity

For the densitometric method, linear relationship was found to exist between peak areas of the separated spots and the corresponding drug concentration over the range of

10–30 µg/spot. While linearity between absorbance and drug concentration cover the range of 20-160 or 20-200 µg mL^{-1} using ΔA or bivariate spectrophotometric methods, respectively. Regression parameters were computed and presented in Table 2, where the densitometric method was found to be the most sensitive as indicated by the highest slope of the curve (slope= 817.63).

3.5.2 Accuracy and precision

Triplicate analysis at four concentration levels within the linearity range were assessed to study the intraday accuracy and precision of the proposed method. The interday accuracy and precision were analysed by repeating the procedure for 3 successive days within 3 months, using the same drug concentrations. Accuracies(R%) ranged between 98.95-102.50 % for nalbuphine-HCl by the proposed methods, While precision was evaluated by calculating the intraday RSD% which was found to be 0.05-1.04 and interday RSD% were 0.20-1.41; Table 2.

3.5.3 Selectivity

It was assured by applying the proposed methods to laboratory prepared mixtures of the intact drug together with its degradation product. Good mean recoveries of intact nalbuphine-HCl were obtained (100.35, 99.97 and 100.09% for the three proposed methods, respectively) in presence of 10-80% of its oxidative degradate by the denistometric method or 10-90% by ΔA and bivariate methods; Table 3.

3.5.4 Ruggedness

The ruggedness of the proposed methods was checked by studying the effect of different sources of chloroform, NaOH and ethanol in densitometric, ΔA and bivariate methods, respectively. It was found that RSD% was less than 1.65%, whereas peak area remains acceptable throughout the assay for densitometric method. RSD% was less than 1.01% and 1.00 % for ΔA and bivariate methods, respectively proving the ruggedness of the three methods.

3.6 Application to Pharmaceutical Preparations

The proposed methods were applied to the determination of nalbuphine-HCl in Nalufin® ampoules, where no interferences from excipients and additives was observed as indicated by mean recoveries ranging from 99.67 to 101.96|%; Table 4. Statistical analysis of the results obtained by the suggested methods compared with a compendial method of nalbuphine-HCl [2] revealed no significant difference with respect to accuracy and precision within a probability of 95%; Table 4. However, the proposed densitometric method was more sensitive and more selective being stability-indicative for nalbuphine-HCl.

Table 1. Values of the sensitivity matrix determinates calculated according to Kaiser's method (k x 10^{-9}) for the mixture of nalbuphine-HCl and its oxidative degradate by the proposed bivariate method

λ/ λ	240	247	250	260	267	270	280	285	290	300	310
240	0	288	300.7	277.3	139.1	91.4	-111.6	-194.6	-214.5	44.3	55.2
247		0	95	87.8	7.8	**310**	-183.6	-240.8	-245.2	80	22.8
250			0	20.4	-28.6	-60.5	-181.8	-225.4	-225.1	-12.9	11.4
260				0	-28.6	-47.3	-117.0	-141.14	-13.99	-11.7	4.2
267					0	28.6	-70.2	-91.0	-92.3	-1.3	7.8
270						0	-56.7	-79.8	-82.7	3.7	10.8
280							0	-25.2	3.4	23.4	21.6
285								0	11.2	30.8	25.2
290									0	31.4	24.6
300										0	3.0
310											0

Table 2. Validation and regression parameters for the determination of nalbuphine- HCl by the proposed densitometric, ∆A and bivariate methods

Parameter	Densitometric method	∆A method	Bivariate method	
λ_{max} (nm)	284	299	247	270
Linearity range	10-30 µg/spot	20-160 µg mL^{-1}	20-200 µgmL^{-1}	20-200 µg mL^{-1}
$A_{1cm}^{1\%}$	—	57.55 µg mL^{-1}	41.03 µg mL^{-1}	18.31 µg mL^{-1}
Regression parameters slope	817.63	0.0057	0.0040	0.0018
Intercept	457.06	0.0144	0.0060	0.0009
Correlation coefficient (r^2)	0.9990	0.9991	0.9990	0.9992
Accuracy (R%)*				
Intraday	99.33-101.20	99.15-101.63	98.95-100.54	
Interday	101.70-102.00	99.99-102.50	98.95-101.65	
Precision (RSD %)*				
Intraday	0.20-0.82	0.17-1.04	0.05-0.43	
Interday	0.79-1.23	0.34-1.41	0.20-0.68	

** calculated according to mean of total samples*

Table 3. Determination of nalbuphine-HCl in mixtures with its oxidative degradate by the proposed densitometric, ∆A and bivariate methods

Densitometric method				∆A method				Bivariate method			
Intact µg/spot	Degradate µg/spot	% degraded	Recovery % of intact	Intact µg mL^{-1}	Degradate µg mL^{-1}	% degraded	Recovery % of intact	Intact µg mL^{-1}	Degradate µg mL^{-1}	% degraded	Recovery % of intact
27	3	10	99.88	90	10	10	98.36	90	10	10	101.88
24	6	20	100.71	85	15	15	99.81	80	20	20	100.81
21	9	30	99.33	80	20	20	99.47	40	60	60	100.50
18	12	40	101.84	75	25	25	99.08	20	80	80	98.15
15	15	50	100.63	70	30	30	98.89	20	180	90	99.10
12	18	60	99.76	60	40	40	99.88				
10	40	80	100.58	50	50	50	100.91				
				20	80	80	102.28				
				20	180	90	101.05				
Mean±SD			100.35±0.90				99.97±1.23				100.09±1.47

Table 4. Results obtained by the proposed methods compared with compendial method [2] for the determination of nalbuphine-HCl in its pharmaceutical preparations

Parameters	Densitometric method	∆ A method	Bivariate method	Compendial method [2]
Linearity range	10-30 µg/spot	20-160 µg mL^{-1}	20-200 µg mL^{-1}	20-250 µg mL^{-1}
N	5	5	5	5
Mean %	100.75	101.96	99.67	101.30
SD	0.90	1.17	1.35	1.40
Variance	0.81	1.37	1.82	1.96
t _	0.74	0.81	1.88	-
F -	2.42	1.43	1.08	-

-The theoretical t- and F- values at p = 0.05 were 2.31 and 6.39, respectively.
-Ref (2) involved UV-measurement of nalbuphine-HCl in ethanol at 284 nm.

4. CONCLUSION

The suggested methods are simple, accurate and selective. Densitometric method is the most sensitive one. They permit the determination of nalbuphine-HCl in its pure form and pharmaceutical preparations. The three methods proved their ability to be used for stability-indication of the drug. Therefore, they can be used for purity testing, stability studies, quality control and routine analysis of the drug. Moreover, these methods would be useful for laboratories with limited equipments, as only a TLC scanner or a UV-Vis dual beam spectrophotometer would be needed.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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