SPECTROPHOTOMETRIC AND FLUORIMETRIC METHODS FOR DETERMINATION OF NALTREXONE IN URINE, SERUM AND TABLETS BY OXIDATION WITH CERIUM (IV)

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ABSTRACT

This paper proposes new methods for simple and sensitive spectrophotometric and fluorimetric determination of naltrexone (NALT) in tablet and biological fluids. The spectrophotometric method involve addition of a known excess of Ce(IV) to NALT in acid medium, followed by determination of residual Ce(IV) by reacting with a fixed amount of methyl orange, and measuring the absorbance at 510 nm. Fluorimetric method was based on the oxidation of NALT with Ce(IV) to produce Ce(III) and its fluorescence was monitored at 350 nm after excitation at 250 nm. In both methods, the amount of Ce(IV) reacted corresponds to the amount of NALT and the measured absorbance or fluorescence were found to increase linearly with the concentration of NALT, which are corroborated by the correlation coefficients of 0.9999 and 0.9996 for spectrophotometric and fluorimetric methods, respectively. Different variables affecting the reaction conditions such as the concentrations of Ce(IV), type and concentration of acid medium, reaction time, temperature, and the diluting solvents were carefully studied and optimized. The accuracy and precision of the methods were evaluated on intra-day and inter-day basis. The proposed methods were successfully applied for the determination of NALT in pharmaceutical formulation and biological samples with good recoveries.

Keywords: Spectrophotometric, fluorimetric, naltrexone, dosage form, Ce(IV).

INTRODUCTION

Opiate addiction is a very serious worldwide problem which has now spread to societies.¹ One of the current treatments is to use narcotic antagonists which generally have chemical structures similar to those of opiates and can preferentially occupy the body's opiate receptors and thus block their euphoric effects. This makes opiate intake delight less and removes the addicts' incentive in opiate using.² Naltrexone (Fig. 1), (17-(cyclopropylmethyl)-4,5a-epoxy-3,14-dihydroxymorphinan-6-one) is a long-acting synthetic opiate antagonist with few side effects that is efficacious when administered orally, either daily or three times a week for a sustained period of time.

Fig. 1. Chemical structure of naltrexone.

Naltrexone has been determined by using a wide variety of analytical techniques, particularly chromatographic, such as thin-layer chromatography,³ high-performance liquid chromatography (HPLC) with electrochemical detection,⁴⁻⁸ gas chromatography,^{9,10} and gas chromatography coupled with mass spectrometry (MS).¹¹ The latest developments use liquid chromatography (LC) with MS detection¹² and LC-MS/MS,^{13,14} primarily for determination of naltrexone in animal plasma. Existing methods use time-consuming off-line sample cleanup^{5-8,13, 14} including liquid-phase or solid-phase extraction steps, which is disadvantageous for a routine therapeutic drug monitoring (TDM) survey aiming to report results within a single day after blood withdraw.¹⁵

In the analytical literature reviewed for naltrexone, no up to date, references were described utilizing spectrophotometric and fluorimetric techniques for the quantitation of naltrexone. Therefore, our goal was to develop a validated spectrophotometric and fluorimetric methods which needs minimum sample preparation and has sufficiently low limit of quantification. The present

paper describes two methods, spectrophotometric and fluorimetric, for the determination of naltrexone after its reaction with Ce(IV) in acid medium.

EXPERIMENTAL

Apparatus

Spectrofluorimetric measurements were carried out on a Shimadzu RF-5301 PC spectrofluorimeter (Kyoto, Japan), equipped with a 150 W xenon lamp and using 1.0 cm quartz cells. The excitation and emission monochromators were fixed with 1.5 mm slits.

Spectroscan 80 D double-beam UV/Visible spectrophotometer (Biotech Engineering Ltd. (UK)), with wavelength range 190 nm ~ 1100 nm, spectral bandwidth 2.0 nm, with 10 mm matched quartz cells was used for all spectrophotometric measurements.

Solutions

i. Standard drug solution was prepared by dissolving 20 mg of naltrexone hydrochloride (NALT) in 100 mL of distilled water. The analytical standard solutions of NALT were prepared daily by appropriate dilution of the stock standard solution in water. Pharmaceutical grade naltrexone hydrochloride was received from the Egyptian International Pharmaceutical Industries Company (EIPICO), Cairo, which was reported to be 99.8% purity, as gift and was used as received. NALT is soluble in distilled water; therefore distilled water may be used as solvent.

ii. Ce(IV) solution at concentration of 5.0×10^{-3} M was prepared from ceric ammonium sulphate in 1.0M sulphuric acid and was kept in the refrigerator at 4°C for two weeks.

iii. A 1.0×10^{-3} M of methyl orange was prepared by dissolving the accurate weighed amount of 0.0327 g in 100 mL water.

iv. A 1.0 M sulphuric acid was prepared.

All other reagents were of analytical reagents grade and all solutions were prepared in distilled water.

Procedure for Calibration Curves

Spectrophotometric method

Different aliquots (0.2 – 1.4 mL) of ($200 \mu\text{g mL}^{-1}$) NALT were transferred into a series of 25-mL calibrated flasks. Then, 2.0 mL of 1.0 M H_2SO_4 was added followed by 0.8 mL of 5×10^{-3} M Ce(VI). The contents were shaken well and were set aside for 10 min with occasional shaking. Finally, 1.4 mL of 1.0×10^{-3} M methyl orange was added to each flask, the volume was adjusted up to the mark with distilled water and mixed well. The absorbance of each solution was measured at 510 nm against the corresponding reagent blank.

Fluorimetric method

To a set of 10-mL test tubes, solutions were added in the following order:

1.0 mL of 5×10^{-3} M Ce(VI), 2.0 mL of 1.0 M H_2SO_4 and (0.1–0.7 mL aliquots of $100 \mu\text{g mL}^{-1}$ NALT standard working solution. The mixtures were heated in a thermostated water-bath at 100°C for 25 min, cooled to room temperature and transferred quantitatively into a set of 25-mL measuring flasks and diluted to the mark with bidistilled water. The fluorescence intensity was measured for the reaction solution at emission wavelength of 350 nm with the excitation wavelength at 250 nm and plotted against the concentration of NALT to obtain the standard calibration graph.

Procedure for tablets

Ten tablets of dextrexone (Manufactured by Delta Pharmaceuticals Co., Egypt) each containing 50 mg of NALT were completely powdered. An accurately weighed portion, equivalent to 20 mg was dissolved in about 10 mL of bidistilled water and any remaining residue was removed by filtration. The filtered solution was then transferred into a 100 mL calibrated flask and diluted to 100 mL with water. Suitable dilution was made to fit the applicable concentration range and the above described procedures were followed. The nominal content of the tablet was assayed from the calibration curves.

Procedure for spiked biological fluids

1.0 mL serum sample was deproteinized by adding 5 mL acetonitrile, in a centrifuge tube, which was then centrifuged for 5 min at 2500 rpm. The supernatant was diluted with distilled water to suitable concentration. Spiked urine was 50-fold diluted with distilled water. The treated serum and urine samples were spiked with convenient amounts of NALT stock solutions. Human serum and urine were kindly provided by healthy volunteers.

RESULTS AND DISCUSSION

Optimization of Reaction Conditions

Investigations were carried out to establish the most favorable conditions for the oxidation. The parameters optimized included the effect of Ce(IV) concentration, heating temperature and time were investigated.

Spectrophotometric Method

Effect of Ce(IV) concentration

The influence of Ce(IV) concentration on the color intensity of the reaction product was studied using increasing volumes of 5×10^{-3} M Ce(IV) solution. It was found that maximum and constant absorbance was attained using 0.8 mL of 5×10^{-3} M Ce(IV) solution (Fig. 2).

Fig. 2. Effect of mL added of 5.0×10^{-3} M Ce(IV) on the reaction product of NALT by spectrophotometric method.

Effect of time and temperature

The reaction between NALT and Ce(IV) in the presence of H_2SO_4 was found to be completely after 5.0 min of mixing, but 10 min was sufficient to get maximum absorbance. Raising the temperature does not accelerate the oxidation process and does not give reproducible results, so the optimum temperature is the ambient. The color produced from the reaction between the remaining unreacted Ce(IV) and methyl orange develops immediately.

Effect of dye concentration

The effect of dye concentration on the intensity of the color developed was tested using different volumes (0.5–1.6 mL) of 1.0×10^{-3} M methyl orange (MO). It was observed that 1.4 mL of MO was necessary for maximum color intensity. After this volume, the absorbance remains constant by increasing the volume of the reagent. The color was found to be stable for at least 3 h at $25 \pm 2^\circ\text{C}$.

Fluorimetric Method

In general, naltrexone is not fluorescent compound and at the same time it is easily oxidisable species. Ce(IV) is a non-fluorescent metal ion from the lanthanide series. In sulfuric acid medium, Ce(III) shows inherent fluorescence. Because Ce(IV) is easily reduced to Ce(III), it seemed probable that this system would offer good methods for the fluorimetric determination of naltrexone.

Fluorescence spectra

Oxidation of NALT with Ce(IV) in an acid medium yields a highly fluorescent Ce(III) which exhibits maximum fluorescence at 350 nm after excitation at 250 nm (Fig. 3). The oxidation product was found not to be fluorescent. This confirmed the fluorescence induced in the oxidation of the investigated drug with Ce(IV) was not attributed to its oxidation product; however, it was mainly due to the formation of Ce(III).

Fig. 3. (a) Excitation and (b) emission spectra for the fluorescent Ce(III) produced by oxidation of $2.4 \mu\text{g mL}^{-1}$ NALT with 2.0×10^{-4} M Ce(IV).

Effect of acid concentration

The oxidation reaction was conducted in acid medium to avoid the precipitation of hydrated ceric oxide, $\text{CeO}_2 \cdot \text{H}_2\text{O}$. To select the most appropriate acid for optimum reaction development, acids as HNO_3 , HCl and H_2SO_4 were tested to determine the most suitable for optimum reaction development. Either hydrochloric or sulphuric acid could be used as the fluorescence of Ce(III) is high; however sulphuric acid was selected because blank reading was less than in hydrochloric acid medium. Nitric acid could not be used due to the inhibitory effect of nitrate ions on the fluorescence of Ce(III).¹⁶ The effect of the concentration of H_2SO_4 was studied. The results showed that the fluorescence intensity of system reached the maximum and remained constant when the concentration of H_2SO_4 was 0.15–0.3 M. So 0.2 M was chosen for further experiments.

Effect of temperature and heating time

The effect of temperature and heating time on the oxidation of NALT with Ce(IV) was studied by carrying out the reaction at ambient temperature ($25 \pm 2^\circ\text{C}$) and in a water bath adjusted at elevated temperature ranging from 40 to 100°C for different periods of time ranging from 5 to 30 min. The results revealed that the optimum temperature was 100°C (Fig. 4a). Complete reaction was attained after 10 min and longer heating time had neither enhancement nor negative effect on the reaction (Fig. 4b). For precisions considerations, 15 min was selected for subsequent experiments.

Effect of cerium (IV) concentration

The effect of Ce(IV) concentration on the fluorescence intensities was optimized as shown in Fig. 5. It was found that maximum relative fluorescence intensity was obtained with a Ce(IV) concentration of 2.0×10^{-4} M. At concentrations lower than this range the fluorescence intensity decreases due to insufficient concentration of Ce(IV) for oxidation, whereas higher amounts of Ce(IV) may probably quench the fluorescence and decrease the fluorescence intensity. A volume of 1.0 mL from this reagent (final concentration of 2.0×10^{-4} M) was used in the rest of work.

Effect of diluting solvents

Dilution effect with different solvents on the relative fluorescence intensity revealed that best solvents were water, MeOH, and EtOH as they were all more or less comparable to each other. However water was the solvent of choice for

economic and environmental safety purposes. Other solvents as DMSO and acetonitrile showed a distinct sharp decrease in the fluorescence intensity. The induced fluorescence intensity was found stable for more than 3 h.

Fig. 4. Effect of temperature (a) and heating time (b) on the fluorescence intensity induced from the oxidation of $2.4 \mu\text{g mL}^{-1}$ NALT with 2.0×10^{-4} M Ce(IV).

Fig. 5. Effect of mL added of 5.0×10^{-3} M of Ce(IV) on the reaction product of NALT by fluorimetric method.

Validation of the Proposed Methods

Linearity and sensitivity

The calibration curves were constructed for the proposed fluorimetric and spectrophotometric methods over the concentration ranges cited in Table 1. The sensitivity of the proposed methods was evaluated by determining the detection limits; Table 1 presents the results of the statistical analysis of the experimental data: regression equations calculated from calibration graphs along with the standard deviation of the slope, and intercept on the ordinate and the standard deviation of residuals. Small values obtained by these parameters indicate the negligible scatter of the experimental points around the fitted regression lines.

Limits of detection (LOD) and quantitation (LOQ) were calculated as $3.3 \sigma/S$ and $10 \sigma/S$, respectively; where σ = standard deviation of the blank and S = slope of the calibration curve.¹⁷ The data compiled in Table 1 summarize the characteristics of the calibration curves (Fig. 6).

Fig. 6. Calibration graphs for determination of the oxidation product of NALT with 2.0×10^{-4} M Ce(IV) by: (a) fluorimetric and (b) spectrophotometric methods.

Table 1. Optical characteristics and statistical analysis of calibration graphs for the determination of NALT by the proposed methods.

Parameter	Method	
	Spectrophotometry	Fluorimetry
λ_{max} , nm	510	
λ_{ex} , nm		250
λ_{em} , nm		350
Temp. °C	25	100
Linear range, $\mu\text{g mL}^{-1}$	1.6-11.2	0.4-2.8
Molar absorptivity, $\text{L.mol}^{-1} \text{cm}^{-1}$	4.73×10^4	
Sandell's sensitivity, $\mu\text{g cm}^{-2}$	0.0079	
Correlation coefficient (r)	0.9999	0.9996
$S_{y/x}$	0.0002	3.0495
Intercept (a)	0.0552	-81.7
Slope (b)	0.1189	153.25
SD of slope (S_b)	3×10^{-5}	2.411
SD of intercept (S_a)	4×10^{-4}	7.781
LOD, $\mu\text{g mL}^{-1}$	0.1831	0.0142
LOQ, $\mu\text{g mL}^{-1}$	0.6042	0.0469

Accuracy and precision

In order to determine the accuracy and precision of the proposed method, the assay described under "Procedure for calibration curves" was repeated seven times on the same day to determine the repeatability (intra-day accuracy and precision) and five times on different days to determine the intermediate precision (inter-day accuracy and precision). These assays were performed for three levels of analyte. The results of this study are summarized in Table 2. The percentage relative standard deviation (%RSD) values were $\leq 1.57\%$

(intra-day) and $\leq 1.63\%$ (inter-day) indicating high precision of the method. The accuracy of the method was determined by the percent mean deviation from known concentration, by using the formula:

The obtained results are presented in Table 2. The percentage relative error (Er %) and the percentage relative standard deviation (RSD %) are considered very satisfactory. This level of precision of the proposed methods was adequate for the quality control analysis of naltrexone.

Table 2. Intra-day and inter-day precision and accuracy of the reaction of NALT by the proposed methods.

Frequency of analysis	Method	Taken, $\mu\text{g mL}^{-1}$	Found, $\mu\text{g mL}^{-1}$	Recovery, %	RSD, %	Er, %
Intra-day (n=7)	Spectrophotometry	3.2	3.19	99.98	1.0772	-0.012
		6.4	6.40	100.03	1.4950	0.03
		9.6	9.59	99.99	0.2736	-0.004
	Fluorimetry	0.8	0.79	99.99	1.5724	-0.002
		2	1.99	99.99	0.8534	-0.006
		2.8	2.79	99.99	0.3700	-0.004
Inter-day (n=5)	Spectrophotometry	3.2	3.19	99.98	1.6335	-0.014
		6.4	6.39	99.99	0.8736	-0.002
		9.6	9.59	99.99	0.4349	-0.001
	Fluorimetry	0.8	0.81	101.25	2.5003	0.014
		2	2.01	100.50	0.7186	0.018
		2.8	2.81	100.36	1.7738	0.01

Table 3. Determination of NALT in tablet and biological fluids using the proposed methods.

Sample	Spectrophotometry				Fluorimetry			
	Taken, $\mu\text{g mL}^{-1}$	Found, $\mu\text{g mL}^{-1}$	Recovery, %	RSD ^a %	Taken $\mu\text{g mL}^{-1}$	Found, $\mu\text{g mL}^{-1}$	Recovery, %	RSD ^a %
Deltrexone 50 mg/tablet	4.8	4.75	99.01	1.4503	2.0	2.06	103.05	2.1763
	8.0	7.92	99.09	1.1327	2.8	2.84	101.42	1.9994
Serum	4.8	4.81	100.21	2.0484	2.0	2.05	102.97	1.8474
	8.0	8.16	102.06	2.6129	2.8	2.81	100.63	0.8694
Urine	4.8	4.79	99.97	0.3143	2.0	2.02	101.30	1.7526
	8.0	8.18	102.30	2.1844	2.8	2.79	99.93	1.0204

Interference studies

The effects of common excipients and additives were tested for their possible interferences in the assay of NALT and the results are shown in Table 3. It is evident that the excipients and additives did not interfere in the determination of NALT.

Robustness and ruggedness.

Robustness was examined by evaluating the influence of small variation of method variables, including concentration of analytical reagents and reaction time on the performance of the proposed methods. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation of method variables did not significantly affect the procedures. This provided

an indication for the reliability of the proposed methods during its routine application for the analysis of NALT. Ruggedness was also tested by applying the proposed methods to the assay of NALT using the same operational conditions but using two different instruments at two different laboratories and different elapsed time. Results obtained from lab-to-lab and day-to-day variations were found to be reproducible, the full range of recovery values was 99.98–101.25% for the proposed methods.

Analysis of tablets

The proposed methods were applied to the determination of NALT in dextrexone tablets. The results obtained are satisfactorily accurate and precise as indicated by the excellent % recovery and RSD% less than 2.2% (Table 3).

Analysis of spiked serum and urine samples

The high sensitivity of the proposed methods allowed the determination of NALT in spiked urine and serum samples. The recovery studies were carried out on the sample, where known amounts of NALT were added and the results of spiked urine and serum samples are given in Table 3. Recovery was from 99.93 to 102.30 for urine and 100.21 to 102.97 for serum. This indicates good level of precision and accuracy.

CONCLUSION

The suggested spectrophotometric and fluorimetric methods have the advantage of being simple, accurate, sensitive and suitable for routine quality control of pure drug and in pharmaceutical formulation without risk of interference used by excipients expected to present in formulation and could be applied for the assay of NALT in biological fluids. The fluorimetric method exhibits the highest sensitivity that makes it possible to measure concentrations down to $0.4 \mu\text{g mL}^{-1}$. This study is the first report for the determination of NALT.

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