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Reverse Phase-HPLC method for the analysis of Naltrexone hydrochloride in bulk drug and its pharmaceutical formulations

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Abstract

A simple, precise, rapid and reproducible reversed phase high performance liquid chromatographic method has been developed for the quantitative estimation of Naltrexone Hydrochloride (NAL) in bulk drug and its formulation dosage forms using column oyster (250 x 4.6mm, 5µm). Mobile phase consist of combination of ammonium acetate buffer (pH 5.8) and acetonitrile in the ratio 60: 40 v/v respectively and was pumped at 1.0 mL/min and the injection volume was 10 µL. The detection was carried out at 220nm and calibration curve was linear in the range of 12-36 µg/mL. The method was validated statistically for its linearity, precision, accuracy. The intra- and inter-day variation was found to be less than 1% showing high precision of the assay method. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining NAL in bulk drug sample and its pharmaceutical formulations.

Key words: NAL, RP-HPLC and Naltrexone.

INTRODUCTION

Naltrexone Hydrochloride [1-4] (NAL) is chemically (5α)-17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-one hydrochloride. NAL is used in the treatment of alcoholism and as narcotic antagonist [5].

Literature survey reveals that a very few physico-chemical methods appeared in the literature for the determination of NAL in pharmaceutical formulations. The methods so far reported include. A LC electro spray Tandem MS method for the analysis of NAL in canine plasma employing a molecular model to demonstrate the absence of internal standard deuterium isotope effects [6].

Nano level detection of NAL in its pharmaceutical preparation at Au microelectrode in flowing solutions by fast fourier transforms continuous cyclic voltammetry as a novel detector [7].

The aim of this study to develop a simple, rapid, precise, accurate RP-HPLC method for the determination of NAL in bulk drug and its pharmaceutical dosage forms.

MATERIAL AND METHODS

Equipment

Quantitative HPLC determination performed on Shimadzu prominence isocratic HPLC system with LC-20 AT pump, SPD-20A detector, chromatogram were analyzed using CFR software.

Chemical and Regents

All the chemicals and reagents used were of HPLC grade and analytical grade.
1) Acetonitrile (Ranbaxy fine chemicals Ltd, New Delhi)
2) Ammonium acetate buffer (SD Fine Mumbai)

Chromatographic Conditions

The contents of the mobile phase were ammonium acetate buffer and acetonitrile in the ratio of 60: 40 v/v respectively. The content of mobile phase and buffer solution were filtered before use through 0.45 µm membrane filter degassed with Helium, purge for 15mins and pumped from the respective solvents reservoirs to the column at a flow rate of 1 mL/min. The run time was set at 10 min and column temperature was maintained at 30^0C. The volume of the injection loop was 10 µL. Prior to injection of the drug solution, the column was equilibrated for at least 30 min with mobile phase flowing through the systems. The elements were monitored at 220 nm and the data were acquired by using CFR software.

Procedure

About 100 mg of NAL was accurately weighed and dissolved in acetonitrile so as to give 1 mg/mL solution. Subsequent dilutions of this solution were made with acetonitrile to get concentrations of 12-36 µg/mL of NAL. The standard solutions prepared as above were injected five times into the column at flow rate of 1.0 mL/min. The peak areas of the drug concentration were calculated. The regression of the drug concentration over the peak areas was obtained. This regression equation was used to estimate the amount of NAL in tablet dosage forms.
NAL solutions containing 18 and 36 µg/mL were subjected to the proposed HPLC analysis for finding out intra and inter day variations. The recovery studies were carried out by adding known amount of NAL to the pre-analyzed and subjecting them to the proposed HPLC method.

Assay

Twenty tablets each containing 50 mg were weighed and powdered. An accurately weighed portion of the powder equivalent to 100 mg of NAL was transferred to a 100 mL volumetric flask containing 50 mL of acetonitrile. The contents of the flask were sonicated for 30 mins to dissolved NAL and made upto volume with acetonitrile and the resulting mixture was filtered through 0.45 µm filter so as to get the stock solution of 1 mg/mL. Subsequent dilutions of these solutions were made with acetonitrile to get concentration of 12-36 µg/mL. This solution (10µL) was injected five times into the column. The mean values of the peak areas of six such determinations were calculated and the drug content in the tablets was quantified using the

regression equation obtained above. The same procedure was followed for the estimation of NAL in other commercially available tablet dosage forms.

RESULTS AND DISCUSSION

The present study was carried out to develop a sensitive, precise and accurate HPLC method for the analysis of NAL in bulk samples and its pharmaceutical dosage form. The retention time for NAL in bulk and its formulation were 5.198 and 5.202 min respectively for a run period of 10 mins. Each of the samples was injected five times and the same retention times were observed in all cases. The peak area of different concentration set up as above was calculated. The peak area for the solution was reproducible as indicated by low coefficient of variation (0.769). A good linear relationship (r = 0.9999) was observed between the concentrations of NAL and respective peak areas. The calibration graph was found to be Y = 10543.281 + 86.858X where Y is the peak area and X is the concentration of NAL in the range of 12-36 µg/mL when NAL solution containing 18 µg/mL, 36 µg/mL, were analyzed by the proposed RP-HPLC method for finding out intra and inter-day variations. A low coefficient of variation was observed (Table 2). This shows that the present HPLC method is highly precise. The drug content in the tablet was quantized using the proposed analytical method. The mean content of NAL in two different brands of capsule dosage forms is shown in (Table 3). The amount of NAL from the pre-analyzed sample containing known amounts of the drug is shown in (Table 4). About 98.31 and 99.34 NAL could be recovered from the pre-analyzed sample indicating the high accuracy of the proposed HPLC method.

The absence of additional peaks indicates no interference of the excipients used in the tablets. The tablets were found to contain 92.66 to 99.45 of the labeled amount. Less than 1% CV indicates reproducibility of the assay of NAL in the capsule dosage form. The proposed method validated according to ICH guidelines [8]. The proposed RP-HPLC method was found to be simple, precise, highly accurate, specific and less time consuming.

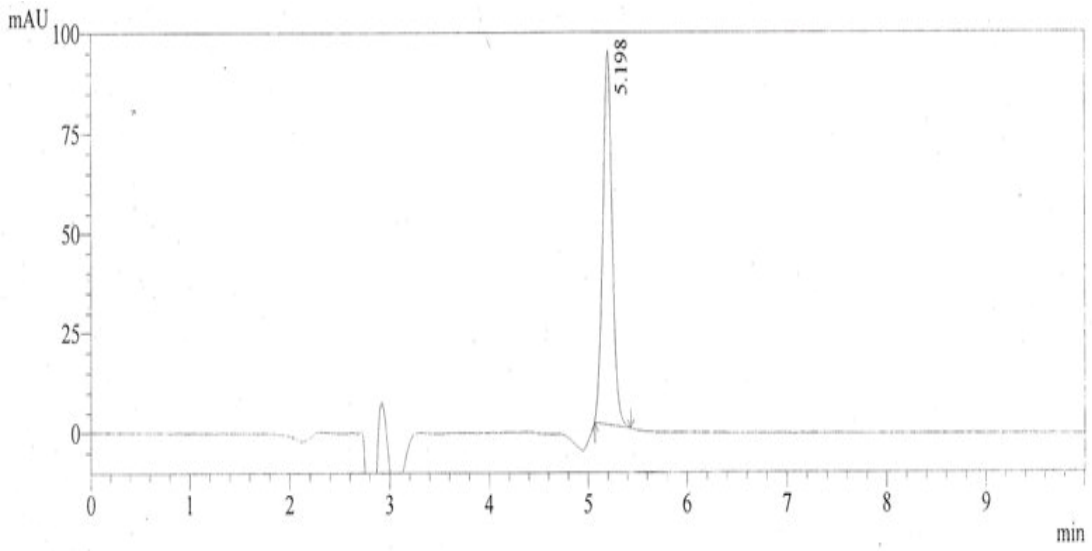

Fig 1: Model chromatogram for NAL in bulk drug sample

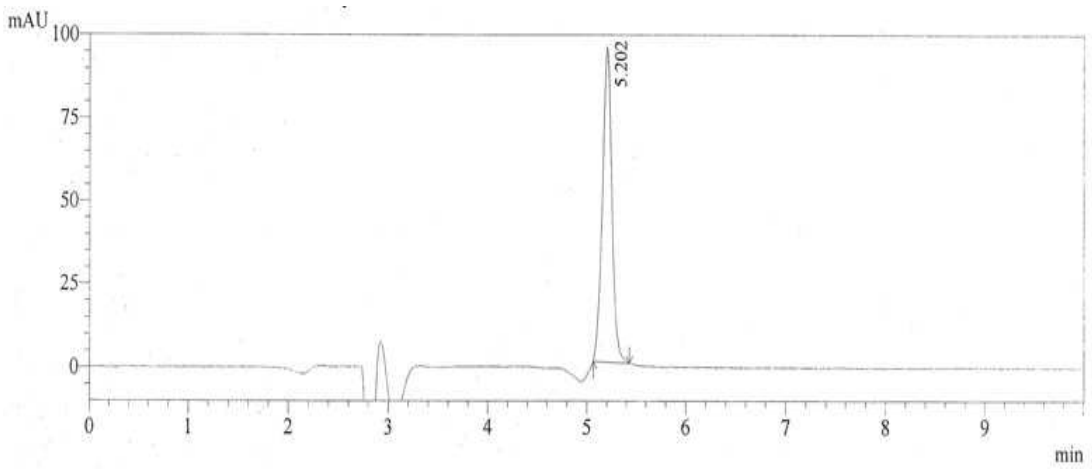

Fig.-2: Model chromatogram for NAL in Pharmaceutical formulation

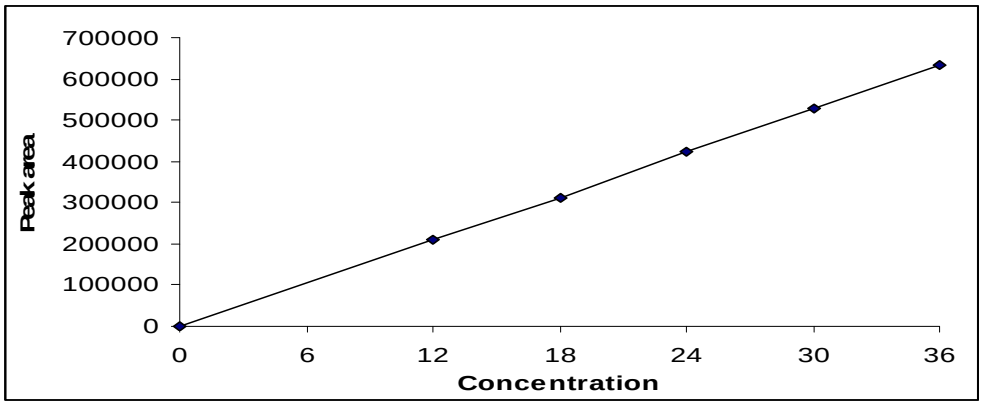

Fig.-3: Standard calibration graph of NAL

Table 1: Optical Characteristics and Precision

Parameter	**RP-HPLC NAL**
Retention time (t) (min)	5.198
Theoretical plates (n)	11426.74
Plates per meter (N)	76173.830
Height equivalent to theoretical plate (HETP) (mtr)	13.128
Linearity range (µg mL^{-1})	12-36
LOD (µg mL^{-1})	0.6
LOQ (µg mL^{-1})	20.0
Regression equation (y=a+bc) Slope (b)	 86.858
Intercept (a)	10543.281
Correlation coefficient (r)	0.9999
Relative standard deviation (%)*	0.769
Range At upper level % RSD	 0.891
At lower level % RSD	0.506
% error in bulk samples**	0.560

**Average of five determinations; **Average of three determinations*

Table 2: Inter and intraday precision for NAL assay in pharmaceutical dosage form by proposed method

Concentration of NAL (µg/mL)	Observed concentration of NAL (µg/mL)			
	Intra day		Interday	
	Mean	% CV	Mean	% CV
18	319784.4	1.99	320927	1.92
36	628514.4	0.956	629064	1.040

*Average of five determinations

Table 3: Assay results of NAL in pharmaceutical formulations

Sample	Labelled amount (mg)	Amount found*±SD	Reference method (UV)	% recovery± RSD
Tablet T_1	50	49.72 ± 0.32	48.76	99.45
Tablet T_2	50	46.33 ± 0.48	49.21	92.66

T_1=Naltima 50 mg (Intas Pharma); T_2=Nodict 50 mg (Sun pharma) ;
**Average ± standard deviation of five determinations*

Table 4: Recovery studies of NAL in pharmaceutical formulations

Pharmaceutical formulations	Amount (µg/mL)		% Recovery ± R.S.D.
	Taken + added	Found* ± SD	
Tablet I	25 + 25	49.15 ± 0.02	98.31 ± 0.14
Tablet II	50 + 50	99.25 ± 0.04	99.24 ± 0.09

*Average five determinations

CONCLUSION

The proposed RP-HPLC method for the estimation of Naltrexone Hydrochloride in pharmaceutical dosage form is accurate, precise, linear, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the bulk drug and its formulations.

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