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RESEARCH ARTICLE

Validation of Novel RP-HPLC Method for the Estimation of Naloxegol in Pharmaceutical Dosage Forms

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ABSTRACT

Background: Naloxegol is a peripherally acting μ-opioid antagonist**. Purpose:** The aim of the present research was to develop and validate a ReversePhase High Performance Liquid Chromatography for quantitative determination of Naloxegol in pharmaceutical dosage forms. **Methodology:** HPLC system used was Shimadzu coupled to a Photodiode Array Detector and was operated in an isocratic mode. Separation was achieved using Inertsil-C18 ODS columnhaving dimensions 250 mm × 4.6 mm, 5 μm and the mobile phase composed of 90 volumes of methanol and 10 volumes of acetonitrile mixture. The flow rate of the mobile phase was 1 mL min^{-1}. Detection wavelength was 250 nm and temperature was 25°C. **Findings:** The method was validated with regard to linearity, accuracy, precision, selectivity, and robustness in accordance with ICH guidelines. **Conclusion:** From this study it was concluded that the proposed method is accurate, reproducible and precise. **Application:** The method was applied successfully for the estimation of Naloxegol in marketed tablet dosage form.

Keywords: High Performance Liquid Chromatography; Naloxegol

INTRODUCTION

Naloxegol[1] is chemically a PEGylated (polyethylene glycol-modified) derivative of α-naloxone under the category of analgesics, opioid narcotics. It is used in the treatment of Opioid-induced constipation and has a significant effect on cardiac repolarization. It is a μ-opioid receptor antagonist with inherently low abuse potential.[2–4] Morphine has widespread effect on the central nervous system and on smooth muscles. Naloxegol is freely soluble in ethanol, methanol, practically insoluble in water. Metabolism of Naloxegol is primarily hepatic (90%), converted to Naloxegol-3-glucuronide (M3G) and Naloxegol-6-glucuronide. Virtually all morphine is converted to glucuronide metabolites; only a small fraction (less than 5%) of absorbedmorphine is demethylated**.** A small amount of Naloxegol isexcreted in bile asglucuronide conjugates, with minor enterohepatic recycling. About 7 to 10% of administered morphine sulfate is excreted in the feces.

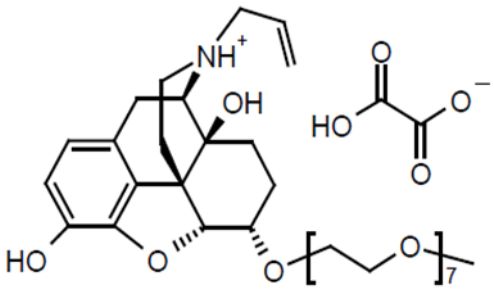

Fig. 1: Structure of Naloxegol Oxalate

Mode of action of Naloxegol and the precise mechanism of the analgesic action of morphine is unknown. However, specific CNS opiate receptors have been identified and likely play a role in the expression of analgesic effects. Morphine first acts on the μ-opioid receptors. The mechanism of respiratory depression involves a reduction in the responsiveness of the brain stem respiratory centers to increases in carbon dioxide tension and to electrical stimulation.

It has been shown that morphine binds to and inhibits GABA inhibitory inter-neurons. These inter-neurons normally inhibit descending pain inhibition pathway. So,

without the inhibitory signals, pain modulation proceeds downstream.[5–7] The review of literature revealed that there is no HPLC method reported so far hence the present work was aimed at developing a simple, reliable and cost effective RP-HPLC method for quantitative estimation of Naloxegol in pharmaceutical dosage form.

MATERIALS

Naloxegol oxalate was obtained from Nektar Therapeutics Pvt. Ltd. Commercial brand name of Naloxegol is Movantik and manufactured by Astra Zeneca Pharmaceuticals LP. It is a white crystalline powder slightly soluble in water. All other chemicals and solvents were obtained from Merck Specialties, Mumbai. High quality pure water was prepared using the Millipore purification system (Millipore, Molsheim, France, model Exil SA 67120).

Preparation of working standard solutions

The stock solution equivalent to 20 ppm to 80 ppm were prepared, sonicated and filtered through 0.45μ membrane.

Preparation of sample solution

Twenty tablets ofNalaxegol were weighed and powdered. The powder equivalent to 500 mg of the active ingredient was accurately weighed and transferred into a 100 mL volumetric flask containing 50 mL mobile phase and sonicated for 15 minutes and the volume was made up to 100 mL with mobile phase and filtered through 0.45micron membrane.

Instrumentation

The analytical system consisted of a Binary gradient pump, a manual injector of 20μL capacity, and a diode array detector. As the stationary phase, Inertsil-C18 ODS column,5 μm particle size, 250 mm × 4 mm (Merck, Darmstadt, Germany) was used. The mobile phase contained methanol: acetonitrile; 90:10 v/v. The flow rate of the mobile phase was 1 mL min^{-1}. The wavelength of the detector was set at 250 nm. Separation was performed at 25 °C.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

In HPLC method,mobile phase contained methanol: acetonitrile; 90:10 v/v. The detection was carried out at 250 nm. The mobile phase flow rate was 1 mL min^{-1}. Typical retention times of Naloxegol were about 4.23 min (Fig. 1). In pure drug solution, purity peak of Naloxegol was 100 % and peak asymmetry was 1.2.

Method Validation

HPLC method was validated according to the International Conference on Harmonization Guidelines, (ICH Q2B, validation of analytical procedures methodology). The method was validated for parameters such as system suitability, linearity, precision, accuracy, and robustness.[8–11]

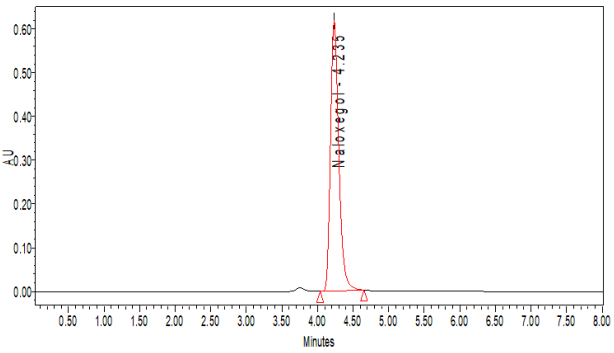

Fig. 2: The Standard chromatogram of Naloxegol

System suitability

A Standard solution was prepared by using Naloxegol working standard as per test method and injected five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD of retention times and peak areas for Naloxegol. The system suitability parameters are given in Table 1.

Table 1: Results for system suitability

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	4.235	7037.5151	10168	1.106
2	4.260	7039.6279	10214	1.109
3	4.240	7037.5151	10200	1.110
4	4.203	7041.1612	10198	1.107
5	4.201	7041.5928	10210	1.108
Mean	4.2278	7039.482	10198	1.108
SD	0.025352	1.93867	——-	——-
%RSD	0.599639	0.02754	——-	——-

Linearity

Linearity was evaluated in the concentration range 40–120 mg L^{-1}. The samples of each solution were injected three times and each series comprised of five experimental points.

The calibration plots were linear in the concentration range of 40–120 mg L^{-1} (n = 5, r = 0.9992). The calibration curve was described by standard straight line equation; y = (5491604 ± 239226)c.The b value, calculated from equation y = ac + b, was insignificant because it was lower than the critical value $t_b = b/S_b$. Statistical analysis using Mandel's fitting test confirmed linearity of the calibration curve (Figure 2).

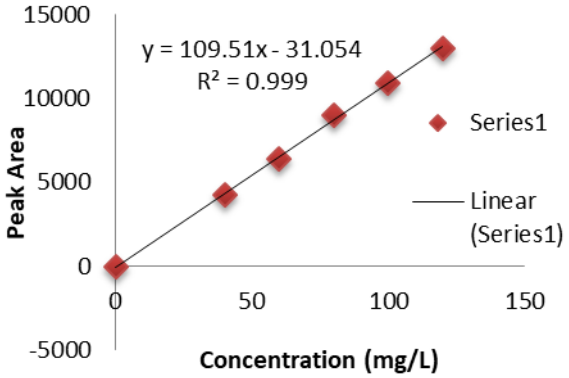

Fig. 3: Calibration curve of Naloxegol

Precision

Precision of the assay was determined in relation to repeatability (intra-day) and intermediate precision (inter-day). In order to evaluate the repeatability of the method, six samples were determined during the same day for three concentrations of Naloxegol. Intermediate precision was studied by comparing the assays performed on two different days.The intra-day andinter-day precision values of measured concentration of Naloxegol were calculated and the results are given in Table 2. The RSD values were 0.05 and 0.02, respectively, demonstrating that the method was precise. Good recoveries were obtained for each concentration, confirming that the method was accurate.

Table 2: Results forprecision

	System Precision		Method Precision	
S.No	Area of Naloxegol	Rt(min)	Area of Naloxegol	Rt (min)
1	7035.56	4.223	7037.5151	4.201
2	7037.58	4.218	7039.6279	4.237
3	7035.56	4.240	7037.5151	4.247
4	7040.15	4.203	7040.1612	4.221
5	7045.13	4.231	7046.1712	4.223
Mean	7038.796	4.223	7039.482	4.225
S.D	4.011699		1.734005	
%RSD	0.056994	0.09	0.024633	0.08

Limits of Detection (LOD) and Quantification (LOQ)

The LOD and LOQ parameters were determined from the regression equation of Naloxegol LOD = 3.3 S_y/m, LOQ = 10 S_y/m, where S_y is a standard error and m is the slope of the corresponding calibration curve. Under applied chromatographic conditions, the LOD of Naloxegol was 2.4 mg L^{-1} and LOQ of Naloxegol was 7.3 mg L^{-1}.

Robustness

The robustness study was performed by slight modification in flow rate of themobile phase. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 0.8 mL/min and1.2 mL/min.The system suitability parameters were evaluated and found to be within the limits for 0.8 mL/min and 1.2 mL/min flow. Naloxegol was resolved from all other peaksand the retention times were comparable with those obtained for mobile phase havingflow rates 1.0 mL/min. The results of robustness study were shown in Table 3.

DISCUSSION

The method was also found to be quite robust with no significant changes in the operating conditions. %RSD for validation parameters such as accuracy and precision were within acceptable limits of ± 2.0%. This indicates that the proposed method is highly precise and accurate. This confirmed that the method is quite suitable for analysis of Naloxegol in tablet.

CONCLUSIONS

The RP-HPLC method developed for the analysis of Naloxegol in Pharmaceutical preparations enabled simple and rapid separation and quantification in a single run. Moreover, the method was found to conform to the acceptance criteria of validation parameters as per ICH guidelines. Thus, the method can be applied for routine quality control of Naloxegol in pharmaceutical dosage form.

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Table 3: Results for Robustness

	Std Area	Tailing Factor		Std Area	Tailing Factor		Std Area	Tailing Factor
Flow rate 0.8 mL/min	6079.40	1.106	Flow rate 1.0 mL/min	7037.51	1.110	Flow rate 1.2 mL/min	7035.56	1.123
	5895.63	1.110		7039.62	1.112		7037.58	1.125
	5935.37	1.112		7037.51	1.110		7035.56	1.124
	6056.36	1.118		7041.16	1.111		7040.15	1.124
	6059.63	1.117		7041.59	1.112		7045.13	1.123
Avg	6005.278	1.112	Avg	7039.48	1.111	Avg	7038.79	1.1238
SD	83.617	0.0044	SD	1.734005	0.00089	SD	4.011699	0.0007
%RSD	1.39	0.4003	%RSD	0.024633	0.0804	%RSD	0.056994	0.0065

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