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A NOVEL VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATIONOF NALOXEGOL

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ABSTRACT

A novel RP-HPLC method was developed and validated for quantitative determination of Naloxegol in pharmaceutical dosage forms. An isocratic RP-HPLC method was developed with Inertsil-C18 ODS column (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. The method was validated with regard to linearity, accuracy, precision, selectivity and robustness. Linearity was evaluated in the concentration range 40–120 mg L^{-1}. The coefficient of correlation was found to be 0.9999. The intra-day and inter-day precision values of measured concentration of naloxegol was calculated and the %RSD for intra-day and inter-day were found to be 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. The limit of detection and limit of quantification for Naloxegol was found to be 2.4 ug/mL and 7.3ug/mL, respectively. The method was applied successfully for the determination of Naloxegol during kinetic studies and routine quality control analysis.

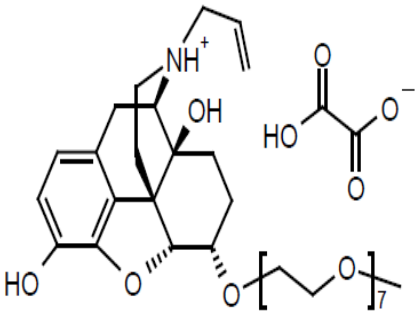
KEYWORDS: High performance liquid chromatography, Naloxegol.

INTRODUCTION[1,4]

Naloxegol is chemically named as (3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one under the category of analgesics, opioid narcotics. Naloxegol is a principal alkaloid in opium and the prototype opiate analgesic and narcotic. Morphine has widespread effects in the central nervous system and on smooth

muscle and stored at 25 °C (77 °F), excursions permitted between 15 and 30°C (59 and 86 °F).[1,2] Naloxegol is freely soluble in ethanol, methanol, practically insoluble in water. Metabolism of Naloxegol is Primarily hepatic (90%), converted to Naloxegolol and also 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 absorbed morphine is de methylated.[3] Excretion of Naloxegol is a small amount of glucuronide conjugates are excreted in bile, with minor entero hepatic recycling. 7 to 10% of administered morphine sulfate is excreted in the feces and mode of action of Naloxegol is 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 mu-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 interneurons. These interneurons normally inhibit the descending pain inhibition pathway. So, without the inhibitory signals, pain modulation can proceed downstream.

***Structure of Naloxegol*[5,6]**



Experimental standards and reagents

Naloxegol was obtained from Nektar Therapeutics Pvt. Ltd. Commercial brand name for Naloxegol is Astra zeneca Pharmaceuticals and manufactured by *Movantik.* It is a white crystalline powder slightly soluble in water.

All other chemicals and solvents were obtained from *Merck*, High quality pure water was prepared using the Millipore purification system.

Chromatographic system and conditions

The analytical system consisted of a quaternary pump (L-7100), an auto sampler (L-7200), a column oven (L-7360) and a diode array detector (L-7455). As the stationary phase, Inertsil-C18 ODS column, 5 µm particle size, 250 mm × 4 mm) was used.

In HPLC method with mobile phase composed of 90 volumes of methanol and 10 volumes of acetonitrile (retention time 4.2 min) 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 In blank sample, purity of peak of Naloxegol was 100% and peak asymmetry was 1.2.

METHOD VALIDATION

RESULTS AND DISCUSSION

Method development and optimization

Some important parameters like pH of the mobile phase, concentration of the acid or buffer solution, etc., were tested for a good chromatographic separation. Trials showed that mobile phase with reverse phase C18 column gives symmetric and sharp peaks.

After the optimization of chromatographic conditions, estimation of Naloxegolwas carried out by the developed RP-HPLC method. Standard solution of drug was injected separately and chromatogram of Naloxegol was recorded.**(Fig:1)** Now the sample solution was injected separately and chromatogram was recorded until the reproducibility of the peak areas were satisfactory **(Fig:2).**

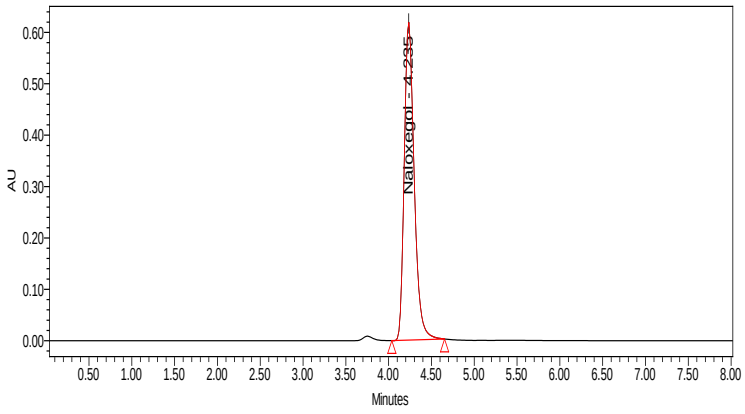


Fig. 1: Chromatogram of standard solution.

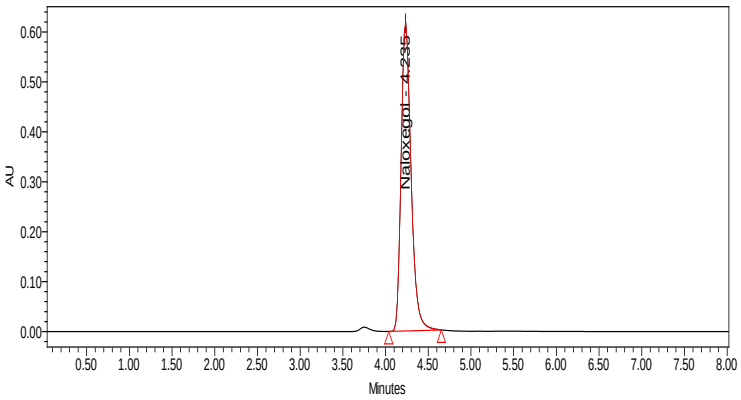


Fig:2: Chromatogram of sample solution.

After the development of RP-HPLC method for the estimation of Naloxegol injection form, validation of the method was also carried out by checking Linearity, Accuracy, Precision, LOD, LOQ, Specificity, Robustness & System suitability parameters.

Validation

The described method has been validated for the estimation naloxegol.

Linearity

Linearity was performed by preparing standard solutions of Naloxegol at different concentration levels ranging from 40-120µg/ml.20µ*l* ml of each solution was injected into the HPLC system. The peak responses were measured at about 250nm and the corresponding Calibration graph was plotted by using the values mentioned under **Table 01** with a correlation coefficient value 0.999 shown.

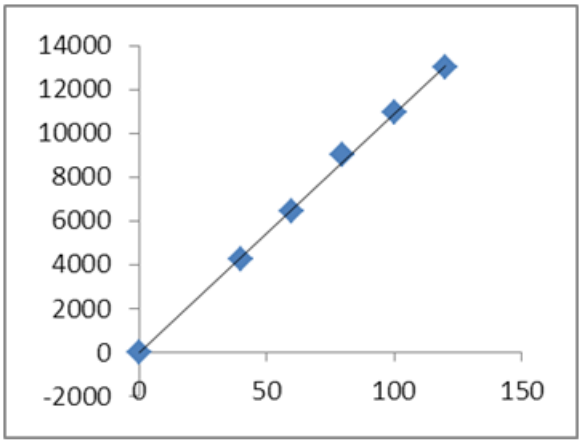


Fig. 3: Calibration Plot for Naloxegol.

Precision

Precision was performed by injecting six replicates of standard and sample solutions which were prepared and analyzed on same day & on different days by using the proposed method. The resulting chromatogram were recorded and shown. The percent relative standard deviation (% RSD) for peak responses was calculated & the results are presented in Table 02.

Accuracy

Accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed sample solution. The standard addition method was performed at 50%, 100% and 150% level of sample solution. The resulting solutions were analyzed in triplicate at each level as per the ICH guidelines. The percent recovery was calculated and results are presented in Table 03.

Limit of Detection

Limit of Detection (LOD) is defined as lowest concentration of analyte that can be detected, but not necessarily quantified, by the analytical method. Limit of detection is determined by the analysis of sample with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected and it was found to be 2.4µg/ml of naloxegol.

Limit of quantification

Limit of quantification (LOQ) is the concentration that can be quantitated reliably with a specified level of accuracy and precision. LOQ was found to be 7.3 µg/ml of naloxegol.

Robustness

Robustness of the developed method was demonstrated by purposely altering the experimental conditions. Robustness of method was carried out with variation of mobile phase, flow rate ±0.1 ml/min. The results were incorporated in Table 04. It indicates that there was no effect on the results, hence the developed method is said to be more robust.

Specificity

Specificity is the ability of the analytical method to measure the analyte free from interference due to other components. Specificity was determined by comparing test results obtained from analyses of sample solution containing ingredients with that of test results

those obtained from standard drug. Chromatograms for standard & samples were recorded and they represent no interference.

System suitability

System suitability tests were carried out on freshly prepared standard stock solution of Naloxegol and it was calculated by determining the standard deviation of Naloxegol by injecting standard solutions in six replicates at frequent time interval and the values were recorded in Table 05.

Table 01: Linearity of Naloxegol by RP-HPLC.

Concentration of Naloxegol (µg/mL)	Peak area
0	0.0000
40	4245.32
60	6424.35
80	8998.56
100	10956.24
120	12992.24

Table 02: System precision of Naloxegol.

S. No	Area of Naloxegol	R_t (min)
1.	7035.56	4.223
2.	7037.58	4.218
3.	7035.56	4.240
4.	7040.15	4.203
5.	7045.13	4.231
Mean	7038.796	4.223
S.D	4.011699	
%RSD	0.056994	0.09

Table 03: Data of Accuracy.

Concentration Level	Spiked Concentration	Obtained Concentration	% recovery	Mean
50% Naloxegol	30	29.68	99.82	99.82
	30	28.92	98.89	
	30	28.99	98.98	
100% Naloxegol	60	59.96	100.92	100.3
	60	58.92	100.83	
	60	59.84	100.56	
120% Naloxegol	80	79.94	101.38	101.5
	80	79.52	101.50	
	80	79.79	101.42	

Table 04: 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	7037.51	1.110	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	7039.48	1.111	7038.79	1.1238
SD	83.617	0.0044	1.734005	0.00089	4.011699	0.0007
%RSD	1.39	0.4003	0.024633	0.0804	0.056994	0.0065

Table 05: Data of 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	-------	-------

CONCLUSION

The RP-LC method developed for the analysis of Naloxegol in their pharmaceutical preparations is simple, precise and accurate. The method is useful for routine analysis due to short run time.

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