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DEVELOPMENT AND VALIDATION OF REVERSED-PHASE HPLC ISOCRATIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF OXYCODONE AND NALTREXONE

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ABSTRACT

An isocratic reversed-phase liquid chromatograpic assay method was developed for the quantitative determination of oxycodone and naltrexone. A Inetsil C-18, 5 µm column with a mobile phase containing Acetonitrile: Methanol: 0.1%Triethylamine buffer (pH-adjusted to 3 using o-phosphoric acid) 25:35:40(v/v). The flow rate was 1.0 mL/min and effluents were monitored at 250 nm. The retention times of oxycodone and naltrexone were 5.57 min and 2.60min, respectively. The proposed method was validated with respect to linearity, accuracy, precision, and robustness.

Keywords – RP-HPLC, Oxycodone and Naltrexone, Method validation

1. INTRODUCTION

The chemical name of oxycodone HCl (OXD) **(**Fig.1.0A**)** is 4,5α-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one hydrochloride. The empirical formula is $C_{18}H_{21}NO_4.HCl$ and its molecular weight is 351.82. Oxycodone HCl is a white to off-white, fine powder. It has a solubility of 0.20 g/mL at pH 6.

Oxycodone is a semi-synthetic opioid with an agonist activity on mu, kappa and delta receptors. Equivalence with regard to morphine is 1:2. Its effect commences one hour after administration and lasts for 1-2 h in the controlled-release formulation. Plasma halflife is 3–5 h (half that of morphine) and stable plasma levels are reached within 24 h (2–7 days for morphine). Oral bioavailability ranges from 60 to 87%. Oxycodone metabolism is more predictable than that of morphine, and therefore titration is easier. Side effects are those common to opioids, but it causes somewhat less nausea, hallucinations and pruritus than morphine. Above all, oxycodone is an effective analgesic; it has been used in many ways, such as in the terminal stages of cancer, in the postoperative phase and so on. [1-3]

The chemical name of naltrexone HCl (NAT) (Fig.1.0B) is (5α)-17-(Cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-one hydrochloride. The empirical formula is $C_{20}H_{23}NO_4.HCl$ and its molecular weight is 377.86. Naltrexone HCl is a white to slightly off-white powder that is soluble in water.

Naltrexone is an opioid receptor antagonist used primarily in the management of alcohol and opioid dependence. It is marketed in generic form as its hydrochloride salt, naltrexone hydrochloride, under the trade names Revia and Depade. Once-monthly extended-release injectable formulation is also marketed under the trade name Vivitrol, in some countries including the United States.

Naltrexone undergoes rapid and almost complete absorption after oral administration. Approximately 96% of the dose is absorbed from the gastrointestinal (GI) tract.

Naltrexone undergoes extensive first-pass metabolism, and oral bioavailability ranges from 5% to 40%. Peak plasma levels (C_{max}) of naltrexone, as well as those of the active metabolite 6-β-naltrexol, occur within one hour after oral administration. The mean elimination half-life of naltrexone is four hours, and the mean elimination half-life of 6-β-naltrexol is 13 hours [3]. Protein binding of naltrexone is only 21%. [1-3]

In the present investigation, an attempt has been made to estimate Oxycodone HCl and naltrexone HCl by reverse phase high performance liquid chromatography (RP HPLC). Although LC-MS/MS is a versatile tool, the development of HPLC based estimation method makes it more economical and simpler both in terms of maintenance and data interpretation. In the current study, an effort has been made to identify a common mobile phase to come up with the isocratic elution of both drugs in combination.

Literature survey revealed various HPLC [4-8] methods have been reported for estimation of AML individually or in combination with other drugs. Whereas, different GC-MS[9], HPLC[10–12, 15,16], FTIR [13] and spectrophotometric [14] methods have been reported for estimation of IND individually or in combination with other drugs.

Several HPLC methods have been described for the determination of oxycodone and naltrexone by RP-HPLC but no validated HPLC-UV methods were reported so far for the simultaneous estimation of oxycodone and naltrexone. Hence, the main objective of the present investigation was to develop a validated RP HPLC method for the simultaneous analysis of oxycodone and naltrexone accordance with International Conference on Harmonization (ICH) guidelines. [17-25]

2. MATERIALS AND METHODS

The Liquid chromatographic system consisted of Shimadzu- Model LC20AT, Spin chrome soft ware HPLC with variable wavelength programmable UV/VIS detector and Rheodyne injector with 20 µL fixed loop. The analytes were monitored at 250nm. Chromatographic analysis was performed on Inertsil C-18 column having 250 × 4.6 mm i.d. and 5 µm particle size. All drugs and chemicals were weighed on Shimadzu electronic balance (AX 200, Shimadzu Corp., Japan).

2.1. Chemicals and Reagents

Analytically pure samples of OXD and NAT were obtained as a gift samples from Alembic Pharmaceuticals Ltd (Baroda, India) HPLC grade methanol obtained from E. Merck Ltd., Mumbai, India while analytical reagent grade acetontrile, methanol, triethyl amine (pH-adjusted to 3 using o-phosphoric acid) obtained from Astron Chemicals, India.

2.2. Chromatographic conditions

A Inertsil C-18 (250×4.6 mm i.d) chromatographic column equilibrated with mobile phase Acetonitrile: Methanol: 0.1%Triethylamine buffer (pH-adjusted to 3 using o-phosphoric acid) 25:35:40(v/v) was used. Mobile phase flow rate was maintained at 1 mL min-1 and effluents were monitored at 250 nm. The sample was injected using a 20 µL fixed loop, and the total run time was 10 min.

2.3. Preparation of standard stock solutions

OXD and NAT were weighed (25 mg each) and transferred to two separate 25 ml volumetric flasks and dissolved in methanol. Volumes were made up to the mark with methanol to yield a solution containing 1000 µg mL^{-1} of OXD and NAT, respectively. Aliquots from the stock solutions of OXD and NAT were appropriately diluted with mobile phase to obtain working standard of 100 µg mL^{-1} .

2.4. Method Validation

The developed method was validated for various parameters like linearity and range, accuracy, precision, robustness, system suitability, specificity, LOQ and LOD.

2.4.1. Linearity and Range

Appropriate aliquots of OXD and NAT working standard solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentration range of 5-25 µg/ml for OXD and 2-10 µg/ml for NAT. The solutions were injected using a 20 µL fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average peak area versus concentrations and regression equations were computed for both drugs.

2.4.2. Precision

The intra-day and inter-day precision studies were carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for three different concentrations and the results were reported in terms of relative standard deviation. The instrumental precision studies were carried out by 3 different concentrations and results are reported in terms of relative standard deviation.

2.4.3. Accuracy

The accuracy of the method was determined by calculating recoveries of OXD and NAT by method of standard additions. Known amount of OXD and NAT were added to a pre quantified sample solution and the amount of OXD and NAT were estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

2.4.4. Detection limit and Quantitation limit

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using the following equation as per ICH guidelines.

LOD = 3.3 ×s /S; LOQ = 10 ×s /S; Where s is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

2.4.5. Solution stability

Stability of sample and standard solution were stable up to 48 h at room temperature.

2.4.6. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products. Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into a pre weighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

2.4.7. Robustness

Robustness of the method was studied by deliberately changing the experimental conditions such as flow rate and percentage of organic phase.

3. RESULTS AND DISCUSSION

3.1. Optimization of mobile phase

Various mixtures containing methanol, water, ACN and aqueous buffer were tried as mobile phases in the initial stage of method development. Finally, the system containing mixture of Acetonitrile: Methanol: 0.1%Triethylamine buffer (pH-adjusted to 3 using ortho-phosphoric acid) 25:35:40(v/v) was found to be satisfactory and gave two well-resolved peaks for OXD and NAT. The retention time for OXD and NAT were 5.57 min and 2.60 min, respectively (Fig.2). The resolution between OXD and NAT was found to be 2.0, which indicates good separation of both the compounds. Column efficiency parameters were reported in table 1. The mobile phase flow rate was maintained at 1 mL/min. Overlaid UV spectra of both the drugs showed that OXD and NAT absorbed appreciably at 250 nm, so detection was carried out at 250 nm. Blank and standard chromatograms were depicted in Figure.2 &3.

3.2. Validation of the Proposed Methods

The developed method was validated for various parameters including linearity and range, accuracy, precision, robustness, system suitability, specificity, LOQ and LOD.

3.2.1. Linearity and Range

The calibration curve for OXD was found to be linear in the range of 5–25 µg/ml with a correlation coefficient of 0.9998. The calibration curve for NAT was found to be linear in the range of 2-10 µg/ml with a correlation coefficient of 0.9997. The regression analysis of calibration curves are reported in table 2.

3.2.2. Precision

Instrument precision was determined by performing injection repeatability test and the RSD values for OXD and NAT were found to be 0.64% and 0.11%, respectively. The intra-day and inter-day precision studies were carried out and the low RSD values indicate that the method is precise.

3.2.3. Accuracy

The accuracy of the method was determined by calculating recoveries of OXD and NAT by method of standard addition. The recoveries were found to be 97.85–100.83% and 99.38–100.37% for OXD and NAT, respectively (table 3). The high values indicate that the method is accurate.

3.2.4. Limit of detection and limit of quantification

The LOD and LOQ were measured by using an equation. The detection limits for OXD and NAT were 0.316 µg/ml and 0.022µg/ml, respectively, while quantitation limits were 0.959 µg/ml and 0.065 µg/ml, respectively. The above data shows that a nano gram quantity of both drugs can be accurately and precisely determined.

3.2.5. Stability of standard and sample solutions

Stability of standard and sample solution of OXD and NAT were evaluated at room temperature for 48 h. The relative standard deviation was found to be below 2.0%. It showed that both standard and sample solution were stable up to 48 h at room temperature.

3.2.6. Specificity

The specificity study was carried out to check the interference from the excipients used in the formulations by preparing synthetic mixture containing both the drugs and excipients. The chromatogram showed peaks for both the drugs without any interfering peak and the recoveries of both the drugs were above 98%.

Table.1.0: System suitability results of OXD and NAT

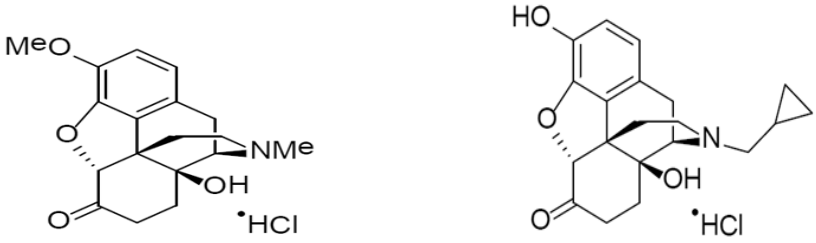
Retention Time	Oxycodone (OXD)	5.57 min
	Naltrexone (NAT)	2.60 min
Peak Area	Oxycodone (OXD)	1081625
	Naltrexone (NAT)	891011
Theoretical plates	Oxycodone (OXD)	85697
	Naltrexone (NAT)	95771
Tailing Factor	Oxycodone (OXD)	1.07
	Naltrexone (NAT)	1.02
Resolution	Oxycodone (OXD)	-
	Naltrexone (NAT)	3.6

Table: 2.0. Calibration curve data

Parameters	Oxycodone (OXD)	Naltrexone (NAT)
Range	5-25 (µg/mL)	2-10 (µg/mL)
Slope	72737	150167
Correlation coefficient	0.9998	0.9997

Table: 3.0. Recovery data

Level of % recovery	Mean %recovery		%RSD	
	Oxycodone (OXD)	Naltrexone (NAT)	Oxycodone (OXD)	Naltrexone (NAT)
80	99.98	100.37	1.45	0.29
100	100.93	99.38	1.77	1.41
120	97.85	99.92	1.79	0.84



(A) **(B)**

Fig. 1.0 : Chemical Structures of A) Oxycodone (OXD) and B) Naltrexone (NAT)

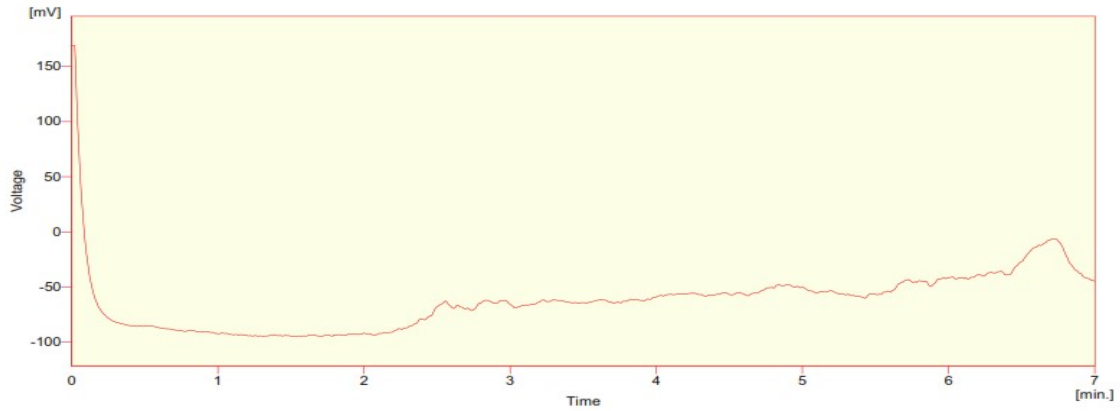


Fig.2.0: Blank chromatogram of Oxycodone (OXD) and Naltrexone (NAT)

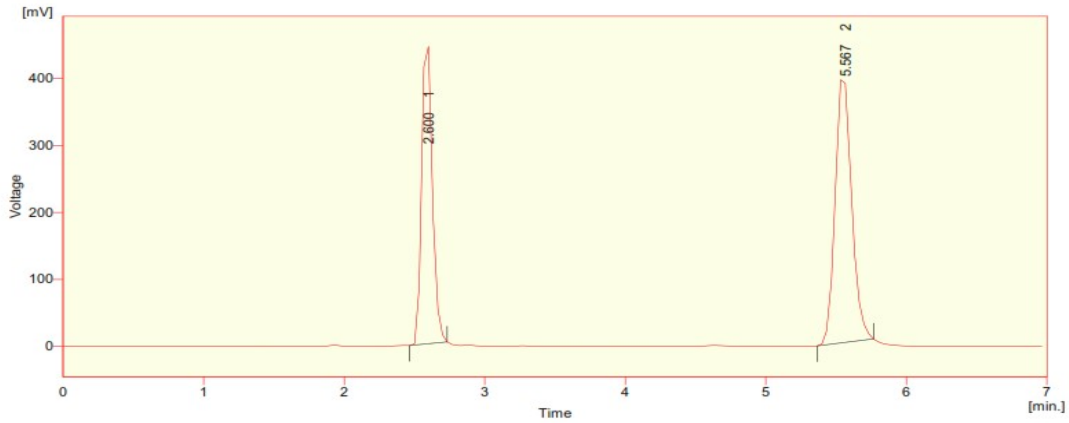


Fig. 3.0: RP-HPLC chromatogram of OXD (RT 2.60 min) and NAT (RT 5.57 min)

4. CONCLUSION

This developed and validated method for simultaneous analysis of oxycodone and naltrexone in pharmaceutical preparations is very simple, rapid, accurate and precise. Moreover, it has advantages of short run time and the possibility of analysis of a large number of samples, both of which significantly reduce the analysis time per sample. Hence, this method can be conveniently used for routine quality control analysis of OXD and NAT in their pharmaceutical formulations.

5. ACKNOWLEDGMENT

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6. CONFLICT OF INTEREST

Authors declare that, there is no conflict of interest.

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