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Research Article

RP-HPLC Method Development and Validation for the Simultaneous Estimation of Oxycodone and Naltrexone in Pharmaceutical Dosage Form

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Abstract
The aim of this study is to develop and validate a new RP-HPLC method for the Simultaneous Estimation of Oxycodone and Naltrexone in Pharmaceutical Dosage Form. The estimation of Oxycodone and Naltrexone was done by RP-HPLC. The assay of Oxycodone and Naltrexone was performed with tablets and the % assay was found to be 100.14 and 99.59 which shows that the method is useful for routine analysis. The linearity of Oxycodone and Naltrexone was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.7 and 0.1 for Oxycodone and Naltrexone which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.2 and 0.2 for Oxycodone and Naltrexone which shows that the method is repeatable when performed in different days also. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions. The developed method used for routine analysis of Oxycodone and Naltrexone in different dosage forms in pharmaceutical industry.

Keywords: Oxycodone and Naltrexone, RP-HPLC, ICH guidelines, LOD, LOQ, Pharmaceutical dosage form.

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1. Introduction

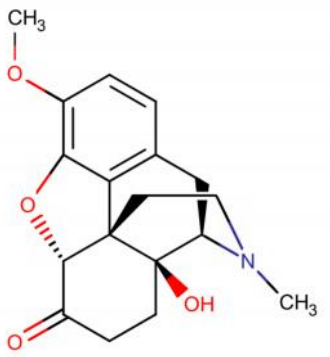

Figure 1. Oxycodone

IUPAC Name: (1S,5R,13R,17S)-17-hydroxy-10-methoxy-4-methyl-12-oxa-4-azapentacyclo[9.6.1.0^{1},^{13}.0^{5},^{17}.0^{7},^{18}]octadeca -7(18),8,10-trien-14-one
Mol Formula : $C_{18}H_{21}NO_4$
Molecular Weight : 315.364 g/mol
Category : narcotic analgesic
Solubility : HCl soluble in water (1 g / 6-7 ml water), slightly soluble in alcohol.
Melting Point : 218-220 °C
pKa : 8.5
Protein Binding : 45%
Metabolism : Hepatic
Half Life : 6 days

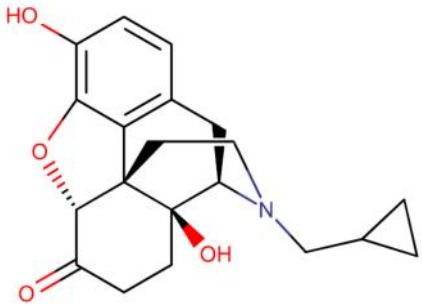

Figure 2. Naltrexone

IUPAC Name : (1S,5R,13R,17S)-4-(cyclopropylmethyl)-10,17-dihydroxy-12-oxa-4-azapentacyclo[9.6.1.0^{1},^{13}.0^{5},^{17}.0^{7},^{18}]octadeca -7(18),8,10-trien-14-one
Mol Formula : $C_{20}H_{23}NO_4$
Molecular Weight : 341.401 g/mol
Category : Narcotic Antagonist
Solubility : Soluble in water
Melting Point : 168-170^{0}C
pKa : 8.20
Protein Binding : 21% bound to plasma proteins over the therapeutic dose range.
Metabolism : Hepatic. When administered orally, naltrexone undergoes extensive biotransformation and is metabolized to 6 beta-naltrexol (which may contribute to the therapeutic effect) and other minor metabolites.
Half Life: 4 hours for naltrexone and 13 hours for the active metabolite 6 beta-naltrexol.

2. Materials and Methods

Table 1: Instruments used

S.No	Instrument	Model
1	HPLC	2695 separation module, uv detector.
2	UV/VIS spectrophotometer	UV 3000^{+}
3	pH meter	AD 1020
4	Weighing machine	ER-200A

Table 2: Chemicals used

S.No	Chemical	Company Name
1	Water	MERCK
2	Mrthanol	MERCK
3	Acetonitrile	MERCK
4	Ortho phosphoric Acid	Fisher scientific
5	Sodium hydroxide	Fisher scientific

Optimized chromatographic conditions:
Instrument used : Waters UPLC with auto sampler and PDA detector.
Temperature : Ambient (25° C)
Mode of separation: Isocratic mode
Column : Inertsil ODS 4.6*250mm, 5µ
Buffer : 0.1% Orthophosphoric acid
pH : 3.0
Mobile phase : 50% buffer 50% Methanol
Flow rate : 1 ml per min
Wavelength : 225 nm
Injection volume : 20 µl
Run time : 10 min.

Preparation of buffer and mobile phase:
Preparation of 0.1% OPA buffer:
1 ml of OPA is taken in 1000ml of HPLC water pH was adjusted with 0.1M NAOH up to 3.0.final solution was filtered through 0.45 µm Membrane filter and sonicate it for 10 mins.
Preparation of mobile phase:
Accurately measured 500 ml (50%) of above buffer and 500 ml of Methanol HPLC (50%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.
Diluent Preparation:
The Mobile phase was used as the diluent.
Validation parameters:
Assay:
Standard Solution Preparation:
Accurately weigh and transfer 80 mg of Oxycodone and 50 mg of Naltrexone working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5

ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 80 mg of Oxycodone and 50 mg of Naltrexone working standard into a 10 ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the Oxycodone & Naltrexone peaks and calculate the %Assay by using the formulae.

Linearity:

Preparation of stock solution:

Accurately weigh and transfer 80 mg of Oxycodone and 50 mg of Naltrexone working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Procedure:

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision:

Preparation of stock Solution:

Accurately weigh and transfer 80 mg of Oxycodone and 50 mg of Naltrexone working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Procedure:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate precision/ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day within the laboratory.

Preparation of stock solution:

Accurately weigh and transfer 80 mg of Oxycodone and 50 mg of Naltrexone working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Procedure:

The standard solutions prepared in the precision was injected on the other day, for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy: For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Preparation of Standard stock solution:

Accurately weigh and transfer 80 mg of Oxycodone and 50 mg of Naltrexone working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 40 mg of Oxycodone and 25 mg of Naltrexone working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Limit of detection:

Limit of Detection: (For Oxycodone)

Preparation of 120µg/ml solution:

Accurately weigh and transfer 80 mg of Oxycodone working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of 0.56 µg/ml solution:

Further pipette 0.045ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Limit of Detection: (for Naltrexone)

Preparation of 75 µg/ml solution:

Accurately weigh and transfer 50 mg of Naltrexone working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of 1.39 µg/ml solution:

Further pipette 0.186 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Limit of quantification: (for Oxycodone)

Preparation of 120 µg/ml solution:

Accurately weigh and transfer 80mg of Oxycodone working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and

make volume up to the mark with the same solvent. (Stock solution). Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of 1.87 µg/ml solution: Further pipette 0.156 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Limit of Quantification: (for Naltrexone)

Preparation of 75 µg/ml solution:

Accurately weigh and transfer 50 mg of Naltrexone working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of 4.67 µg/ml solution:

Further pipette 0.625ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

The flow rate was varied at 0.9 ml/min to 1.1ml/min.

Standard solution 160 ppm of Oxycodone & 75 ppm of Naltrexone was prepared and analysed using the varied flow rates along with method flow rate.

The Organic composition in the Mobile phase was varied from ±10%: Standard solution 160 ppm of Oxycodone & 75 ppm of Naltrexone was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

3. Results and Discussion

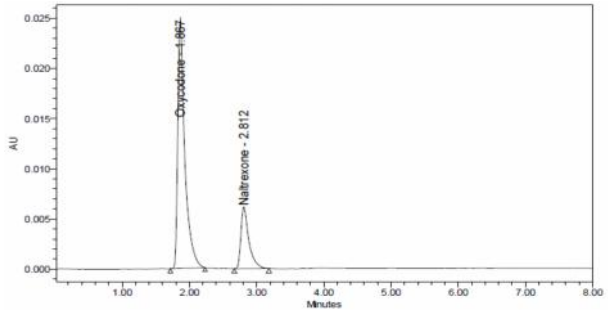

Figure 3. Chromatogram for system suitability

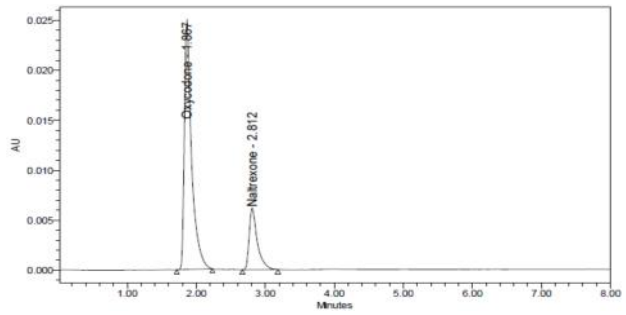

Figure 4: Chromatogram for Standard

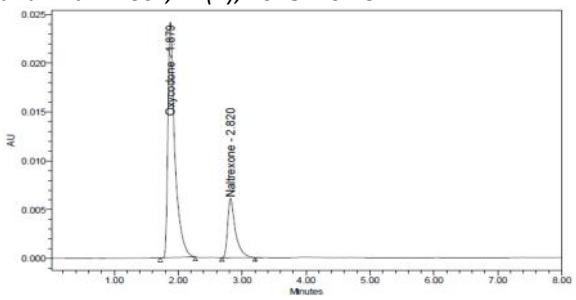

Figure 5: Chromatogram for Sample

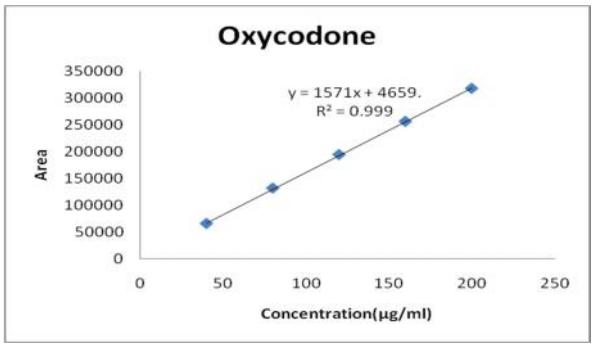

Figure 6: Calibration graph for Oxycodone

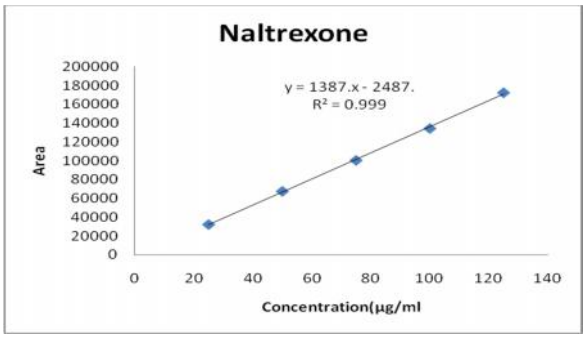

Figure 7: Calibration graph for Naltrexone

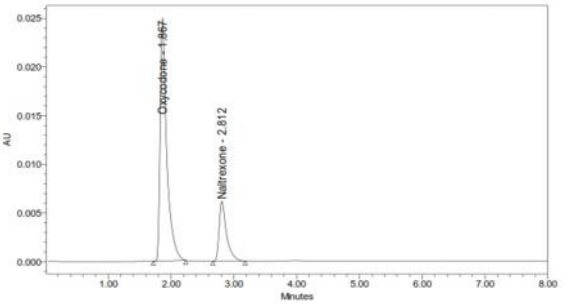

Figure 8: Chromatogram for Precision -6

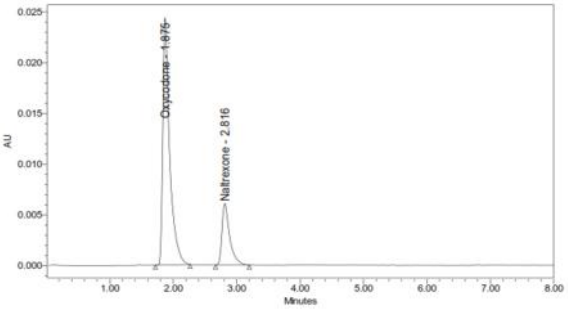

Figure 9: Chromatogram for ID Precision -6

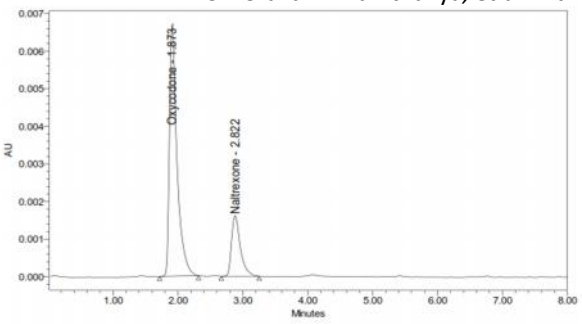

Figure 10: Chromatogram for Accuracy 50

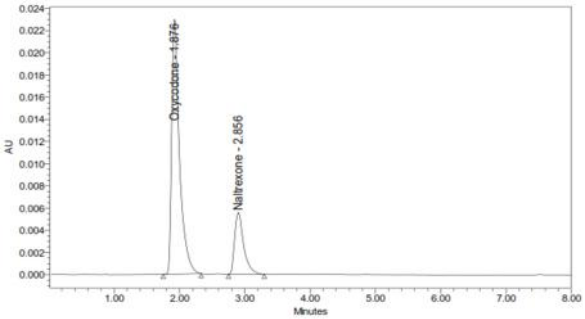

Figure 11: Chromatogram for Accuracy 100%-3

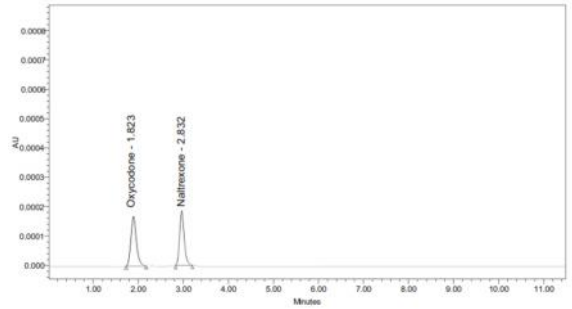

Figure 12: Chromatogram for LOD

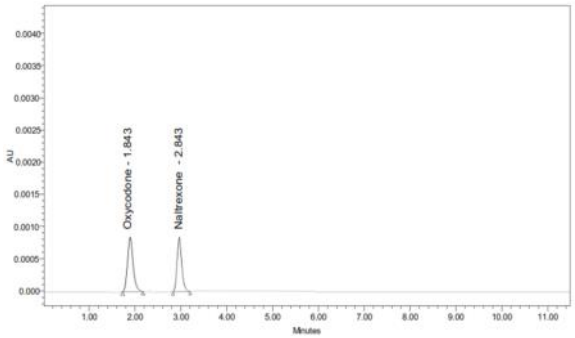

Figure 13: Chromatogram for LOQ

Table 3: Results for variation in flow for Oxycodone

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	3828.18	1.21
2	1	3828.18	1.18
3	1.1	3115.92	1.21

Table 4: Results for variation in flow for Naltrexone

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Plate Count	USP Tailing	USP Resolution
1	0.9	3213.92	1.21	4.96
2	1	3115.92	1.12	4.96
3	1.1	3415.92	1.21	4.96

Table 5: Degradation results for Naltrexone and Oxycodone

Sample Name	Naltrexone		Oxycodone	
	Area	% Degraded	Area	% Degraded
Standard	107223	100	191642	100
Acid	98959	7.71	183252	4.38
Base	98921	7.74	183532	4.23
Peroxide	98978	7.69	183253	4.38
Thermal	98851	7.81	187552	2.13
Photo	98789	7.87	186452	2.71

4. Conclusion

The estimation of Oxycodone and Naltrexone was done by RP-HPLC. The assay of Oxycodone and Naltrexone was performed with tablets and the % assay was found to be 100.14 and 99.59 which shows that the method is useful for routine analysis. The linearity of Oxycodone and Naltrexone was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.7 and 0.1 for Oxycodone and Naltrexone which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.2 and 0.2 for Oxycodone and Naltrexone which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 98.0% - 102.0%. The total recovery was found to be 99.40% and 99.74% for Oxycodone and Naltrexone. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQ for Oxycodone was found to be 3.00 and 9.98 and LOD and LOQ for Naltrexone was found to be 2.98 and 10.00. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

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