Research article   **Open Access**

A new analytical method development and validation for the simultaneus estimation of Naltrexone and Oxycodone using RP-HPLC

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ABSTRACT

A simple and selective LC method is described for the determination of NALTREXONE and oxycodone in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 40 volumes of k2hpo4+NaHPO4 and, 60 volumes of Acetonitrile with detection of 212 nm. Linearity was observed in the range 60-140 μg/ml for NALTREXONE (r^2 =0.999) and 30-70 μg /ml for oxycodone (r^2 =0.999) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: Naltrexone and Oxycodone**,** Reverse phase HPLC.

INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books.[1]

Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis – Qualitative and Quantitative analysis.

AIM AND PLAN OF WORK

Aim

To develop new RP HPLC method for the simultaneous estimation of NALTREXONE and oxycodone pharmaceutical dosage form.

Plan of Work

- Solubility determination of NALTREXONE and oxycodone various solvents and buffers.
- Determine the absorption maxima of both the drugs in UV–Visible region in different solvents/buffers and selecting the solvents for HPLC method development.
- Optimize the mobile phase and flow rates for proper resolution and retention times.

- Validate the developed method as per ICH guidelines.

METHODOLOGY

Mobile Phase

A mixture of 80 volumes of Methanol and 20 volumes of Water. The mobile phase was sonicated for 10min to remove gases.

Determination of Working Wavelength (λmax)

In estimation of drug wavelength maxima is used.. So this wavelength is used in estimation to estimate drug accurately.

Preparation of standard stock solution of NALTREXONE

10 mg of NALTREXONE was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 μg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of OXYCODONE

10mg of OXYCODONE was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and

prepare 10 μg /ml of solution by diluting 1ml to 10ml with methanol.

RESULTS AND DISCUSSIONS

Solubility Studies

These studies are carried out at 25 ^{0}C

Naltrexon

Freely soluble in methanol,water and mixed phosphate buffer.

Oxycodone

Freely soluble in ethanol and methanol, and slightly soluble in acetone and very slightly soluble in water.

Wavelength determination

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.
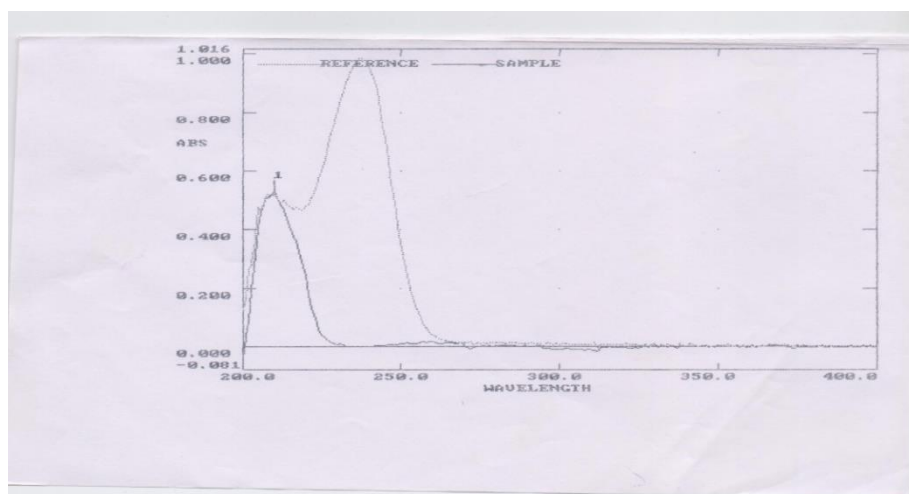
RESULTS

The wavelength of maximum absorption (λ$_{max}$) of the drug, 10 μg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in

the fig. no. 8.1, and The isobestic point was found to be 212 nm for the combination Isobestic point of NALTREXONE and OXYCODONE

METHOD DEVELOPMENT OF NALTREXONE AND OXYCODONE

Trial- 1

Preparation of standard solution

Weigh accurately 10 mg of NALTREXONE and OXYCODONE in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/ml of NALTREXONE and OXYCODONE is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.
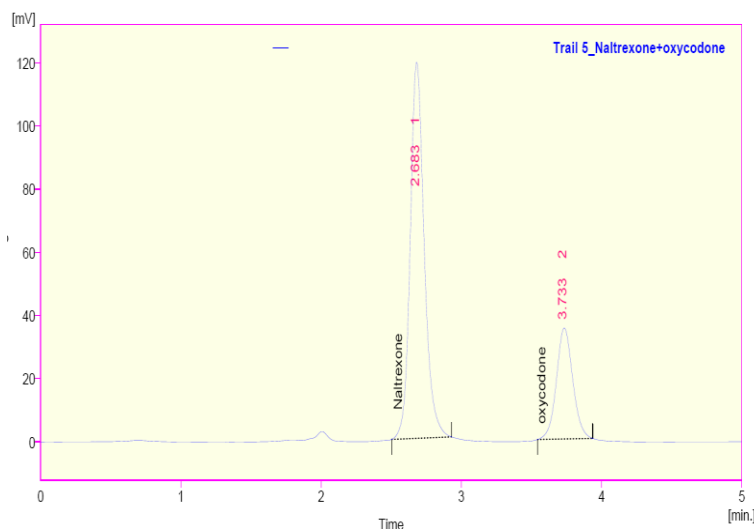

Fig. 3: Chromatogram of OXYCODONE AND NALTREXONE

Observation

- Peak Asymmetry factor for OXYCODONE and NALTREXONE meet the system suitability requirements.
- The run time is very correct.
- Theoretical plates were more than 2000.
- Hence it is taken for optimization.

Table 1: Optimized chromatographic conditions

Mobile phase	k2hpo4+NaHPO4 Buffer:ACN (40:60)
Ph	5.0
Column	Inertsil ODS 3V column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25°C)
Sample temperature	Room temperature(20-25°C)
Wavelength	212
Injection volume	20 µl
Run time	6 min
Retention time	About 2.683min for NALTREXONE and 3.733 min for OXYCODONE.

ASSAY

Preparation of samples for Assay

Preparation of standard solution

Weigh accurately 10mg of NALTREXONE and 5 mg of OXYCODONE in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 15 µg/ml of NALTREXONE and OXYCODONE is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains OXYCODONE-30mg mg NALTREXONE-3.6 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of OXYCODONE and NALTREXONE (15µg/ml) were prepared by dissolving weight equivalent to 15 mg of OXYCODONE and NALTREXONE and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 15µg/ml of OXYCODONE and NALTREXONEwas made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of NALTREXONE and OXYCODONE present in the formulation by using the formula given below, and results shown in above table:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation

AT: Peak area due to assay preparation

WS: Weight of NALTREXONE /OXYCODONEin mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation
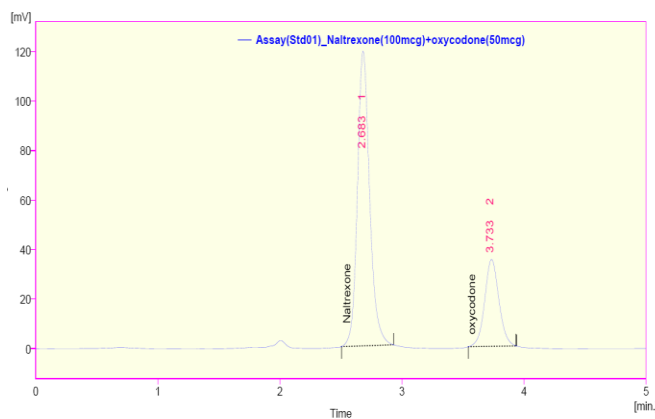
Fig: Chromatogram of Assay standard preparation-1
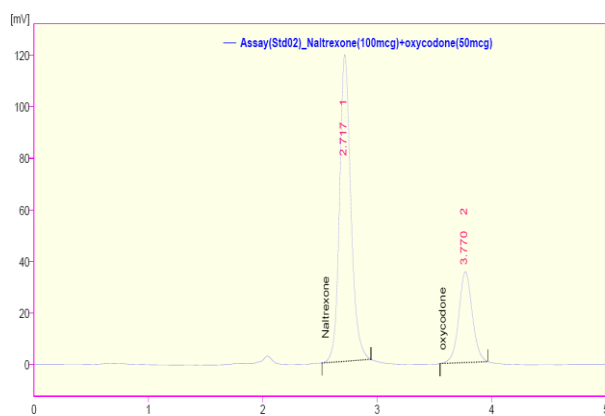
Fig: Chromatogram of Assay standard preparation-2
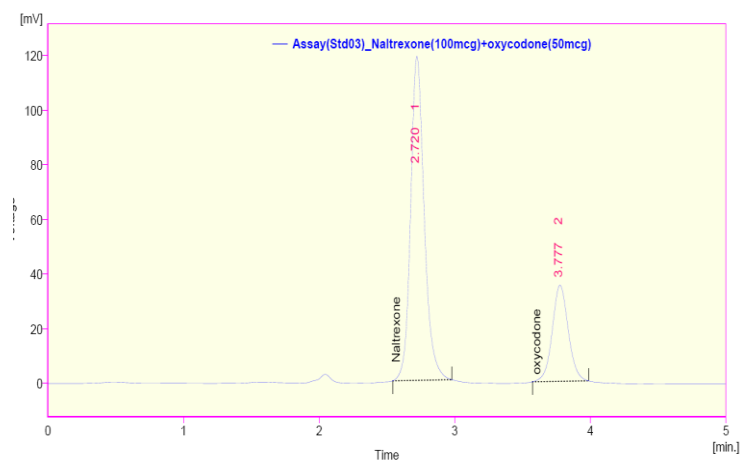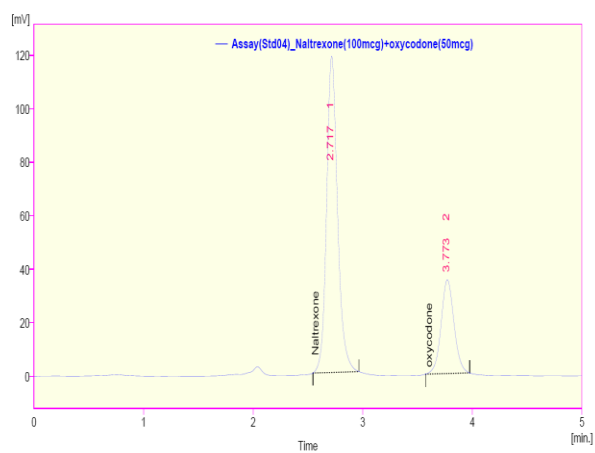
Fig: Chromatogram of Assay standard preparation-3
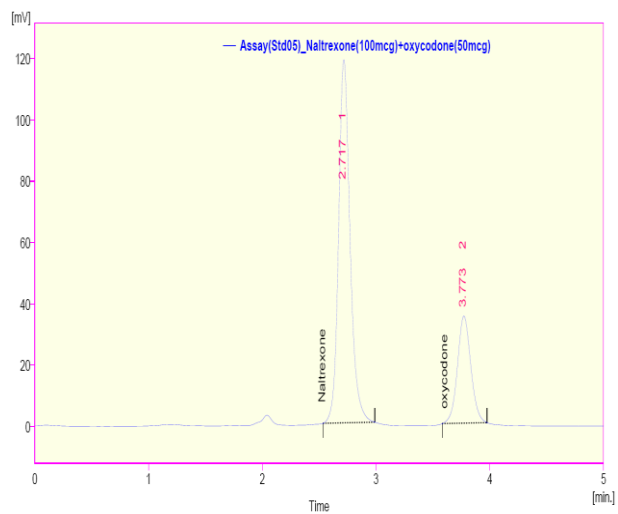

Fig: Chromatogram of Assay standard preparation-4

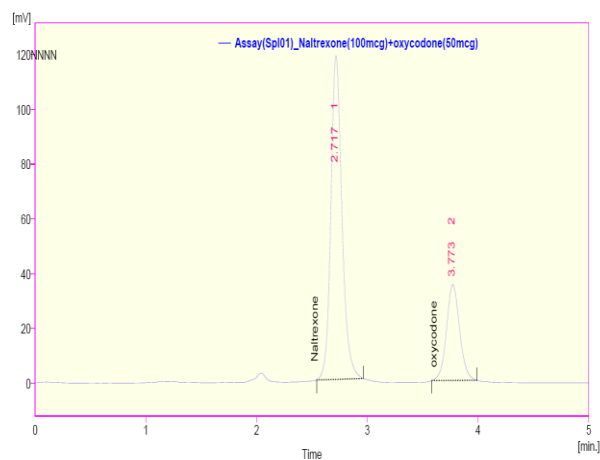
Fig: Chromatogram of Assay standard preparation-5

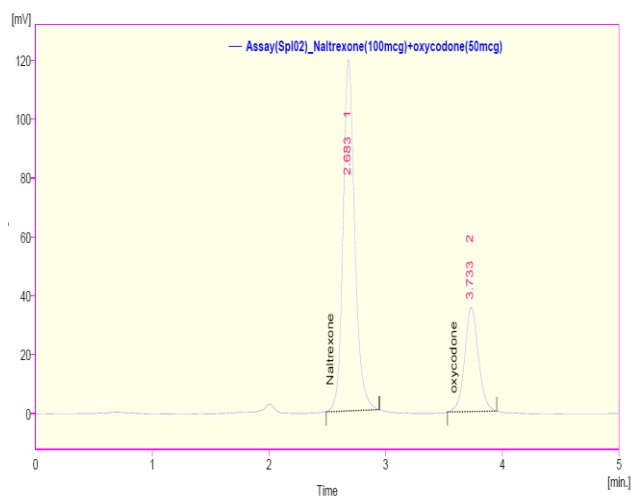**Fig:** Chromatogram of Assay sample preparation-1
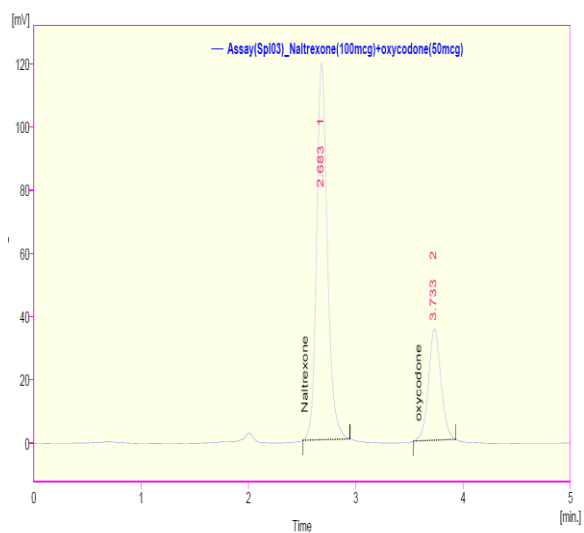

Fig: Chromatogram of Assay sample preparation-2
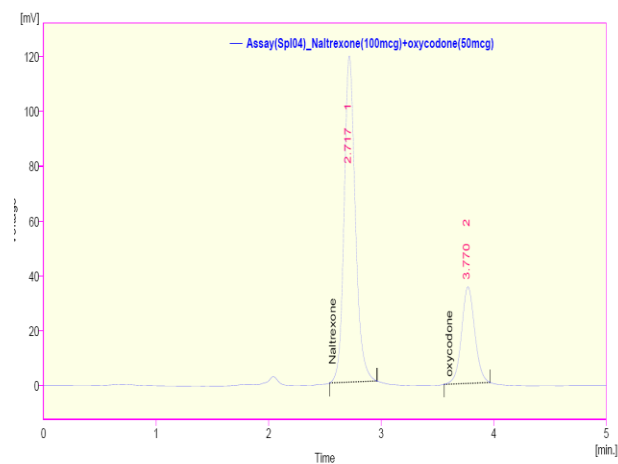

Fig: Chromatogram of Assay sample preparation-3
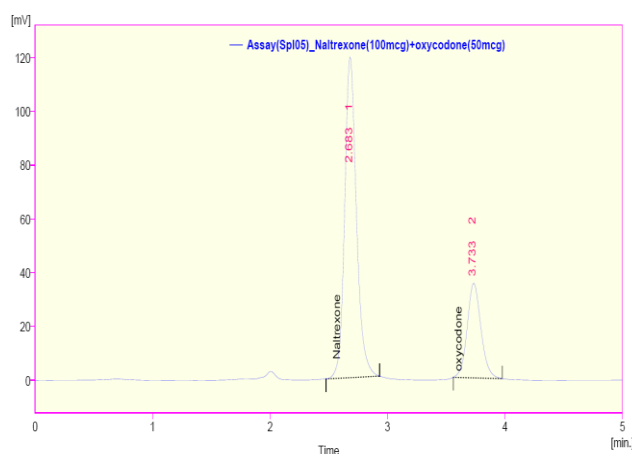
Fig: Chromatogram of Assay sample preparation-4

Fig: Chromatogram of Assay sample preparation-5

NALTREXONE			OXYCODONE	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	825.949	824.612	284.554	287.747
Injection-2	824.058	831.231	288.051	289.831
Injection-3	829.293	827.465	288.444	283.577
Injection-4	823.414	825.068	287.123	287.13
Injection-5	830.957	829.984	285.368	286.687
Average Area	826.734	827.672	286.708	286.9944
Assay(%purity)	100.113434		100.099893	

Observation

The amount of OXYCODONE and NALTREXONE present in the taken dosage form was found to be 100.11% and 100.09 % respectively.

VALIDATION

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of mixed standard solution

Weigh accurately 60 mg of NALTREXONE and 40 mg of OXYCODONE in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 60 µg/ml of NALTREXONE and 40 µg/ml of OXYCODONE is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains OXYCODONE - 400 mg NALTREXONE -600 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of OXYCODONE and NALTREXONE (µg/ml) were prepared by dissolving weight equivalent to 400 mg of OXYCODONE and 600 mg of NALTREXONE and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 40 µg/ml of OXYCODONE and 60µg/ml of NALTREXONE was made by adding 1 ml of stock solution to 10 ml of mobile phase.
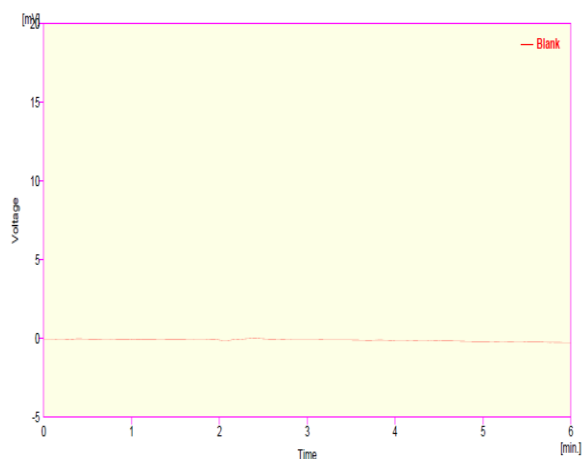
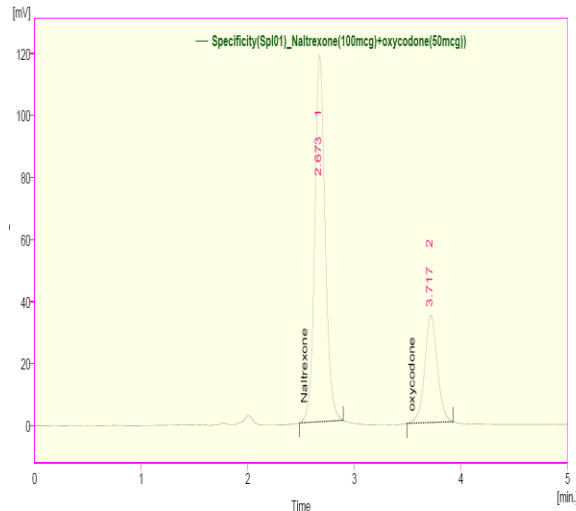. **Fig:** Blank chromatogram for specificity by using mobile phase

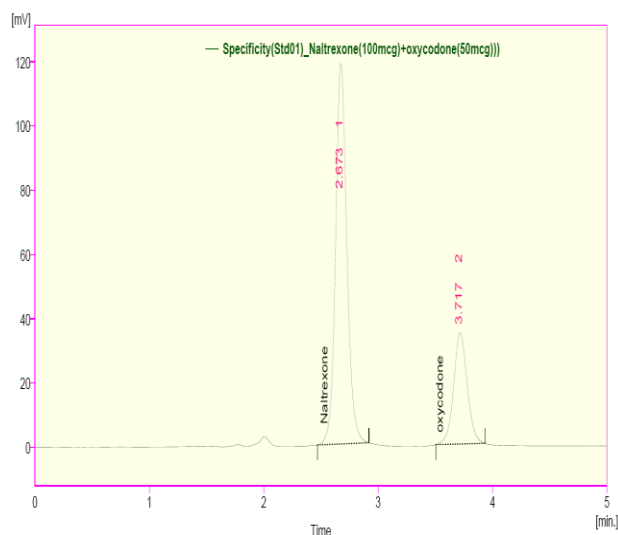**Fig:** Chromatogram for specificity of OXYCODONE and NALTREXONE sample

Fig: Chromatogram for Specificity of OXYCODONE and NALTREXONE standard

Observation

It is observed from the above data; diluents or excipients peaks are not interfering with the OXYCODONE and NALTREXONE peaks.

Linearity and range

Preparation of standard stock solution

Standard stock solutions of NALTREXONE and OXYCODONE (microgram/ml) were prepared by dissolving 60 mg of NALTREXONE and 40 mg of OXYCODONE dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase.

Further dilutions were given in the table

Table 9.3 .1: Linearity Preparations

Preparations	Volume from standard stock transferred in ml		Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)	
				NALTREXONE	OXYCODONE
Preparation 1	0.6	0.3	10	60	30
Preparation 2	0.8	0.4	10	80	40
Preparation 3	1.0	0.5	10	100	50
Preparation 4	1.2	0.6	10	120	60
Preparation 5	1.4	0.7	10	140	70
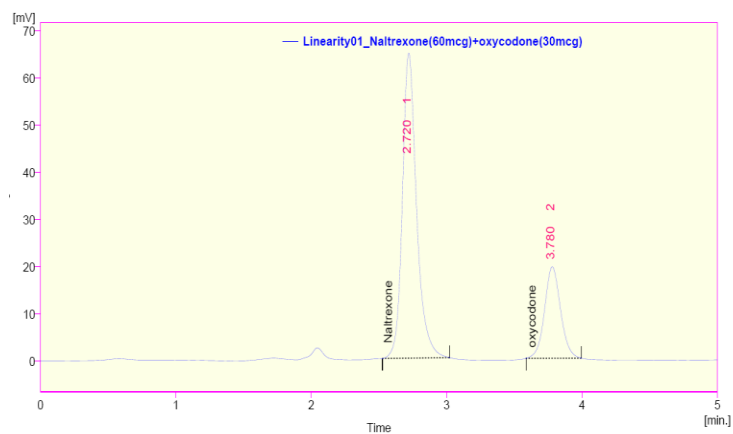
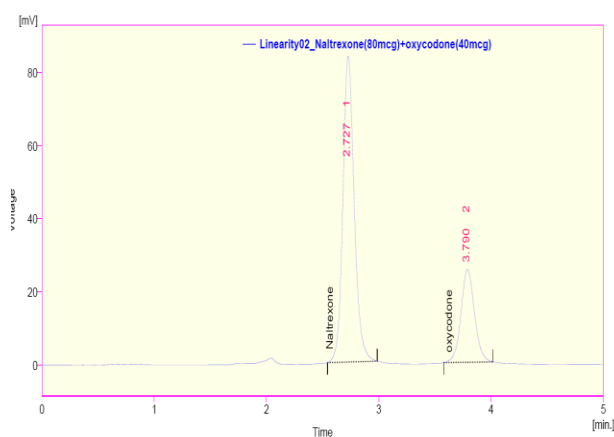**Fig:** Chromatogram of OXYCODONE and NALTREXONE preparation-1

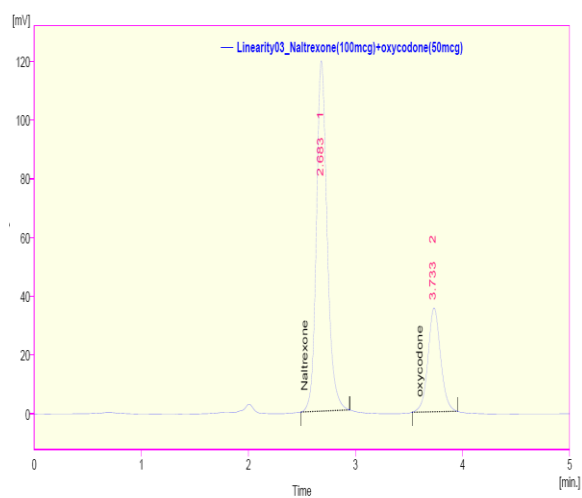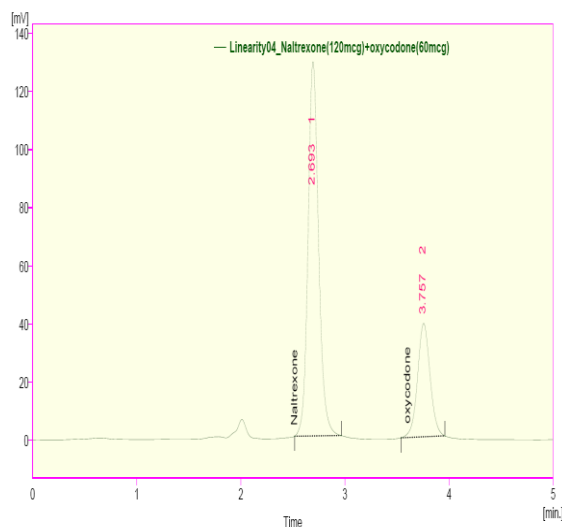**Fig:** Chromatogram of OXYCODONE and NALTREXONE preparation-2

Fig: Chromatogram of OXYCODONE and NALTREXONE preparation-3

Fig: Chromatogram of OXYCODONE and NALTREXONE preparation-4
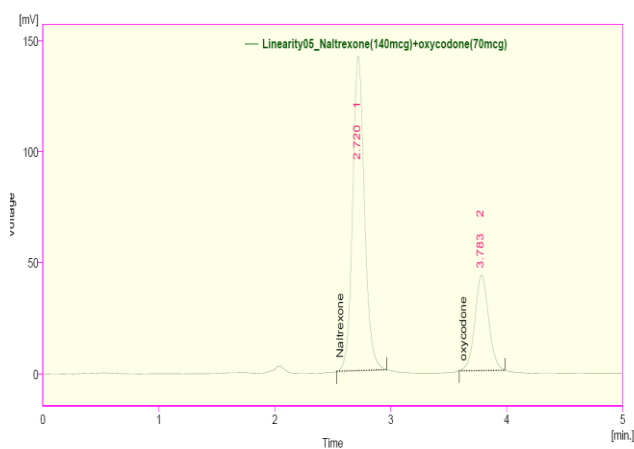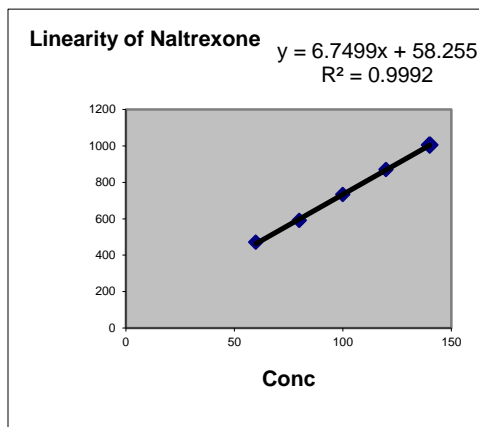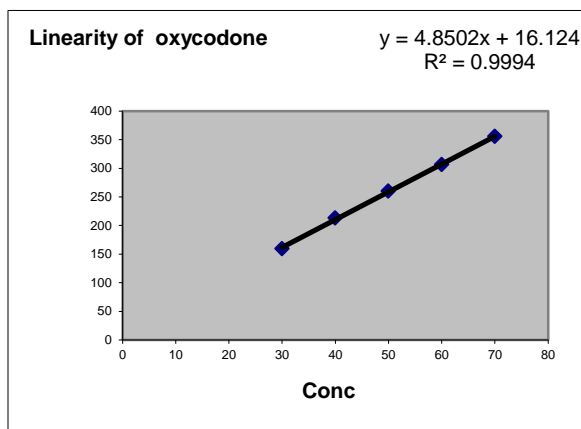

Fig: Chromatogram of OXYCODONE and NALTREXONE for preparation-5

Linearity graph of NALTREXONE

Linearity graph of OXYCODONE

The relationship between the concentration of NALTREXONE and OXYCODONE and area of NALTREXONE and OXYCODONE should be linear in the specified range and the correlation should not be less than 0.99

Observation

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of NALTREXONE and OXYCODONE is 0.996 and 0.997. The relationship between the concentration of NALTREXONE and OXYCODONE and area of NALTREXONE and OXYCODONE is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limit.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100%, 150%
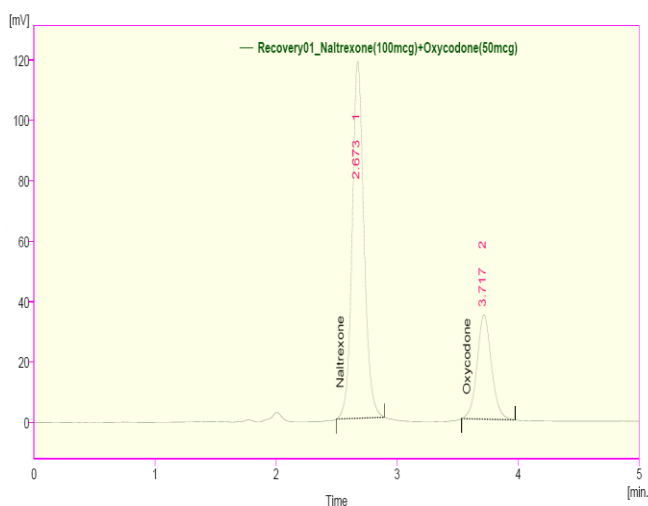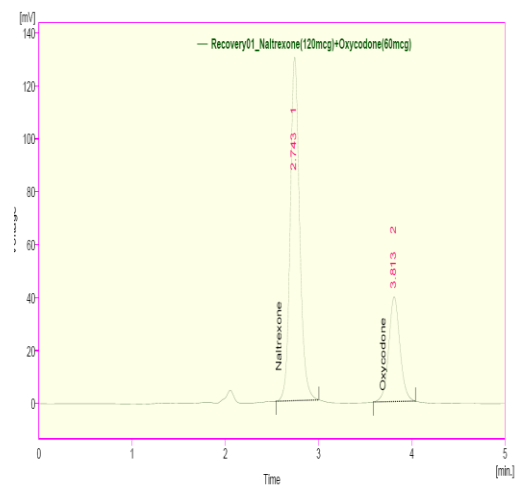

Fig: Chromatogram of 125% recovery (injection 1)
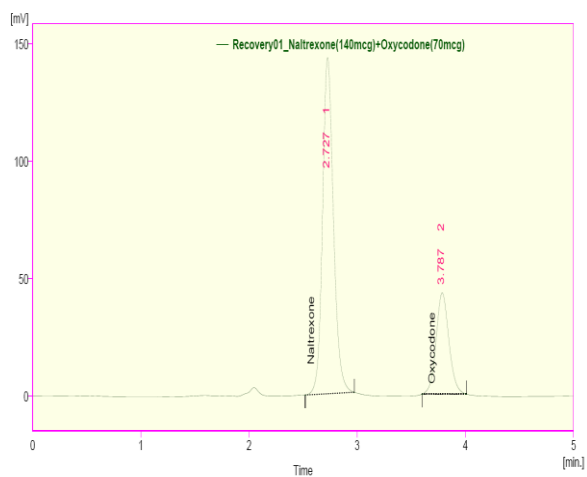
Fig: Chromatogram of 150% recovery (injection 2)

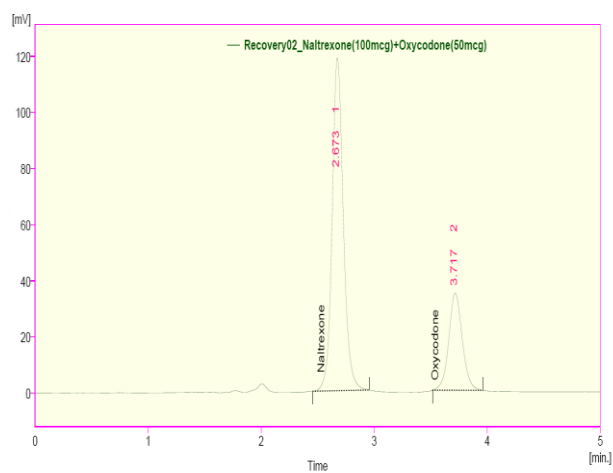**Fig:** Chromatogram of 175% recovery (injection 3)

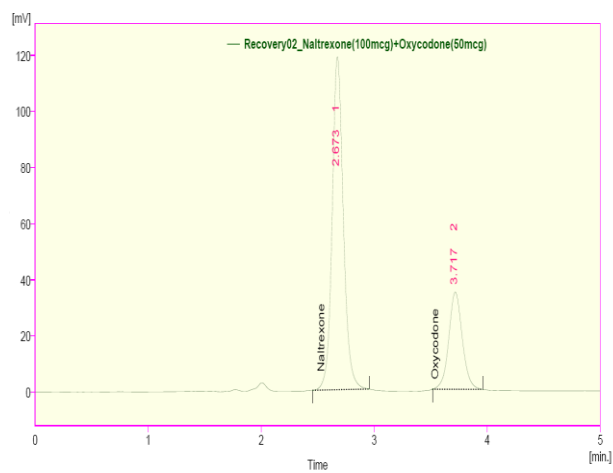
Fig: Chromatogram of 125% recovery (injection 1)

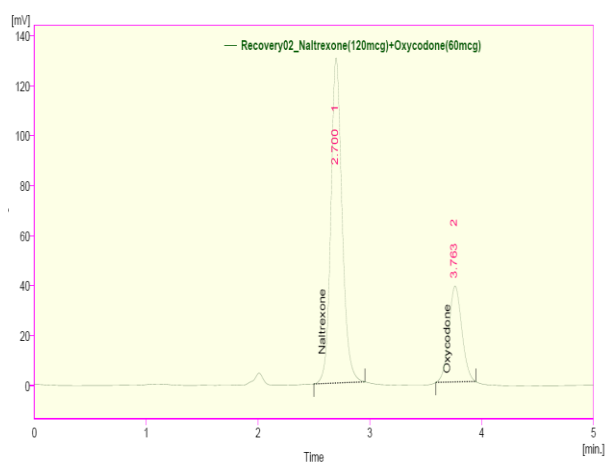**Fig:** Chromatogram of 150% recovery (injection 2)

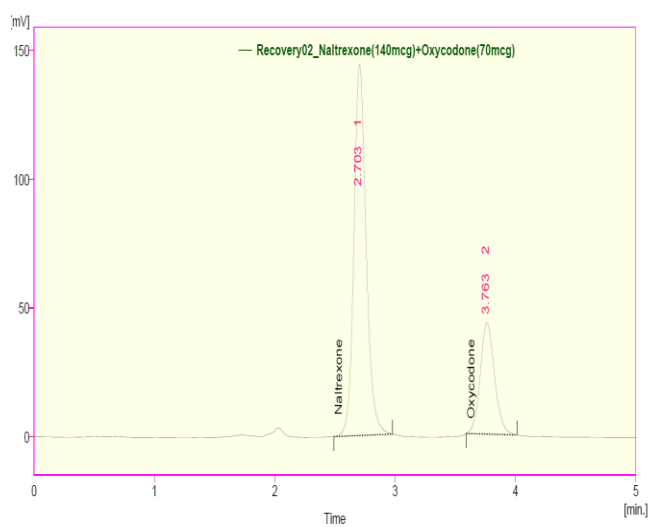**Fig:** Chromatogram of 175% recovery (injection 3)

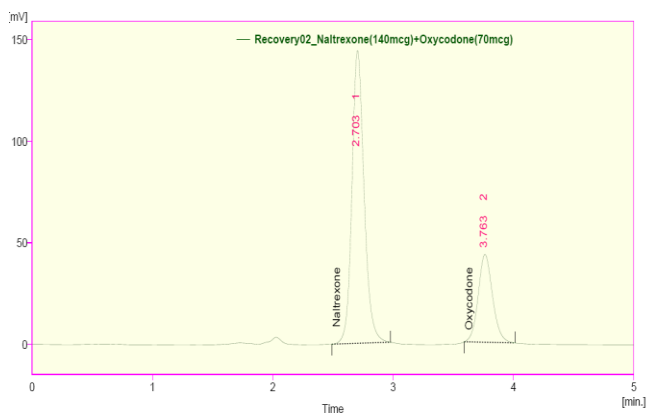**Fig:** Chromatogram of 125% recovery (injection 1)

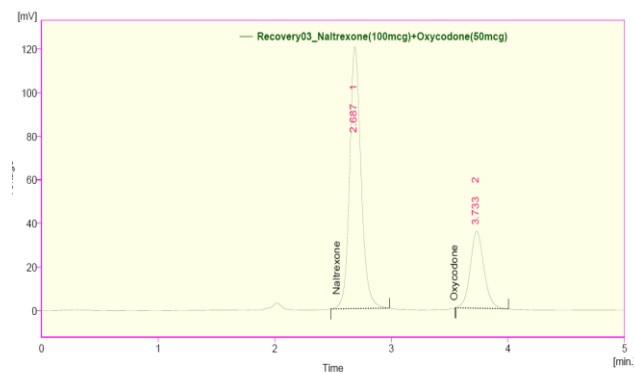**Fig:** Chromatogram of 150% recovery (injection 2)

Fig: Chromatogram of 175% recovery (injection 3)

Acceptance criteria

The % recovery of OXYCODONE and NALTREXONE should lie between 98% and 110%.

Table: Recovery results for OXYCODONE

Recovery level	Accuracy NALTREXONE			Average % Recovery
	Amount taken(mcg/ml)	Area	%Recovery	
50	80	801.032		99.42802786
	80	816.586	97.98288895	
	80	820.921		
			99.88546694	
			100.4157277	100.5398588
100	100	911.538		
	100	911.492	100.3500659	
	100	916.756	100.3450018	
				99.62110719
			100.9245089	
150	120	1180.092		
	120	1164.339	101.0448726	
	120	1145.961		
			99.69602869	
			98.1224203	

Table : Recovery results for NALTREXONE

Recovery level	Accuracy OXYCODONE			Average % Recovery
	Amount taken(mcg/ml)	Area	%Recovery	
100	40	284.882		
	40	287.502	199.4130628	102.83
	40	287.785	201.2470229	
			201.4451186	99.36
120	50	331.682		
	50	311.036	116.0861751	99.103
	50	334.897	108.8602323	
140	60	356.491	117.2114007	
	60	357.47	74.86148176	
	60	355.771	75.06706729	
			74.71028505	

Observation

The percentage mean recovery of NALTREXONE and OXYCODONE is 100.43 % and 99.85 % respectively.
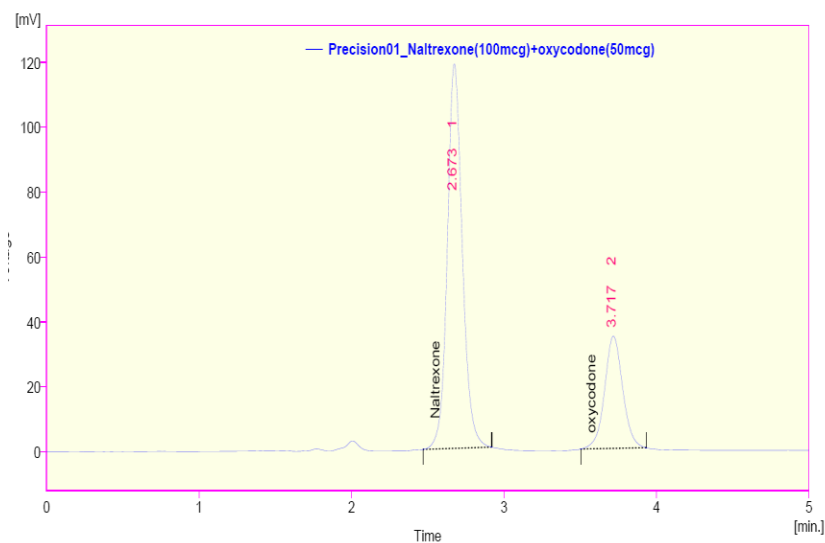
Precision

Method precision

Method precision

Prepared sample preparations of OXYCODONE and NALTREXONE as per test method and injected 6 times in to the column.

Acceptance criteria

The % Relative standard deviation of Assay preparations of OXYCODONE and NALTREXONE should be not more than 2.0%.
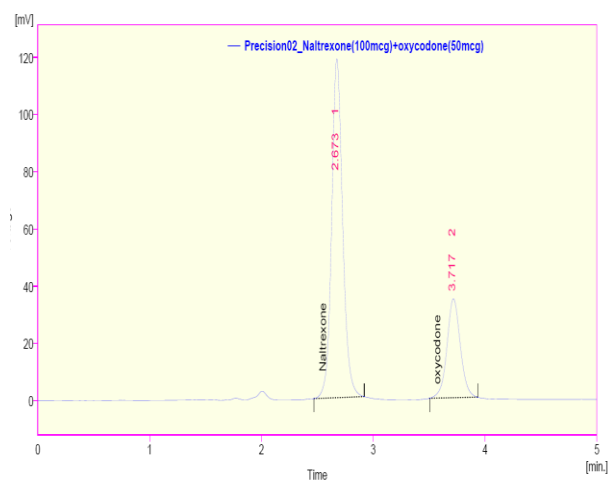

Fig: Chromatogram of precision injection 1
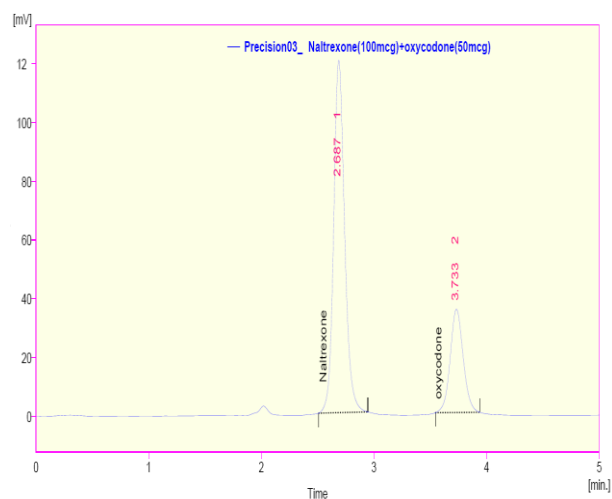
Fig: Chromatogram of precision injection 2

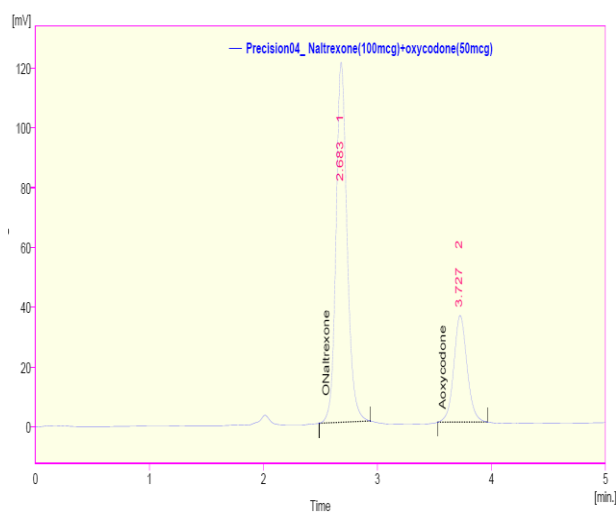
Fig: Chromatogram of precision injection 3

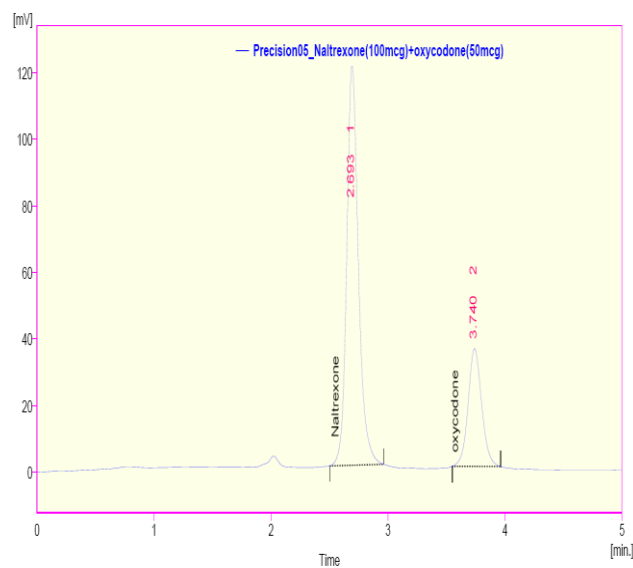**Fig:** Chromatogram of precision injection 4

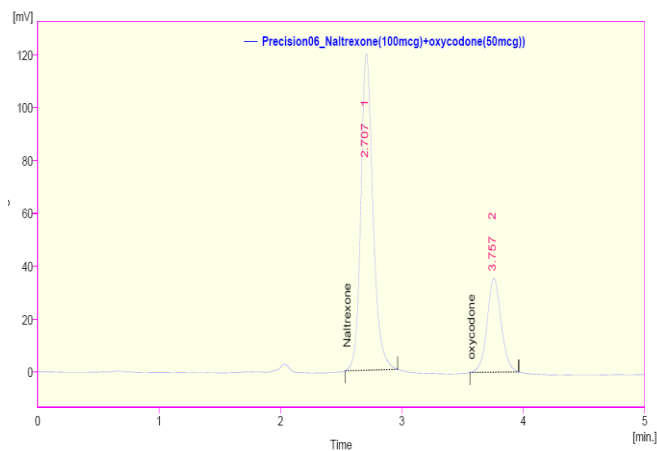**Fig:** Chromatogram of precision injection 5

Fig: Chromatogram of precision injection 6

Table: Results for precision of OXYCODONE and NALTREXONE Observation:

NALTREXONE			OXYCODONE		
S.No.	Rt	Area	S.No.	Rt	Area
1	2.673	810.419	1	3.717	286.026
2	2.673	810.419	2	3.717	286.026
3	2.687	811.688	3	3.733	282.016
4	2.683	812.647	4	3.727	288.483
5	2.693	831.524	5	3.740	285.746
6	2.707	828.437	6	3.757	286.026
Avg	2.6860	817.522	avg	3.732	285.721
Stdev	0.0129	9.735	stdev	0.015	2.080
%RSD	**0.48**	**1.19**	**%RSD**	**0.41**	**0.73**

Test results for NALTREXONE and OXYCODONE are showing that the %RSD of Assay results are within limits.

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision.

Acceptance criteria

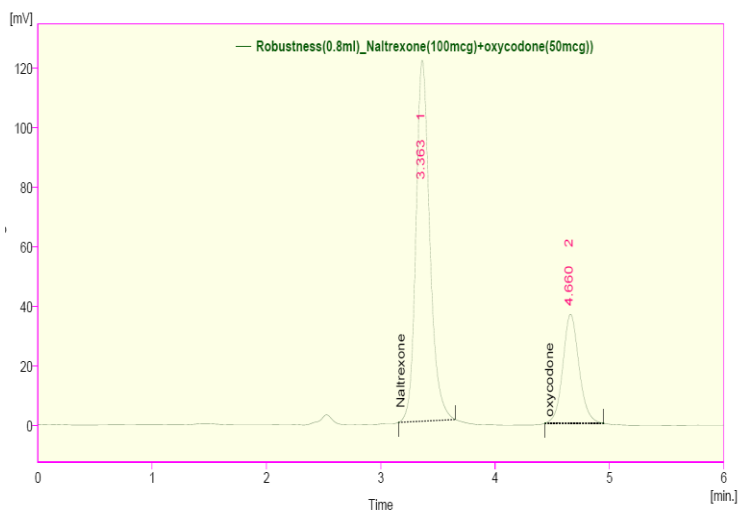The system suitability should pass as per the test method at variable conditions.

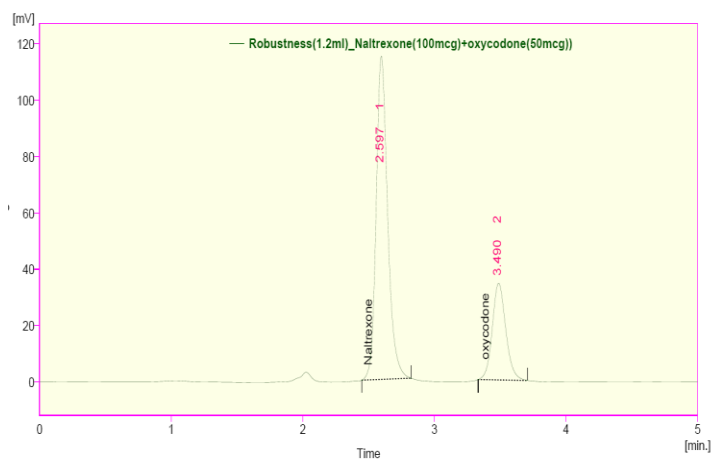**Fig:** Chromatogram of OXYCODONE and NALTREXONE Robustness (0.8 ml/min)

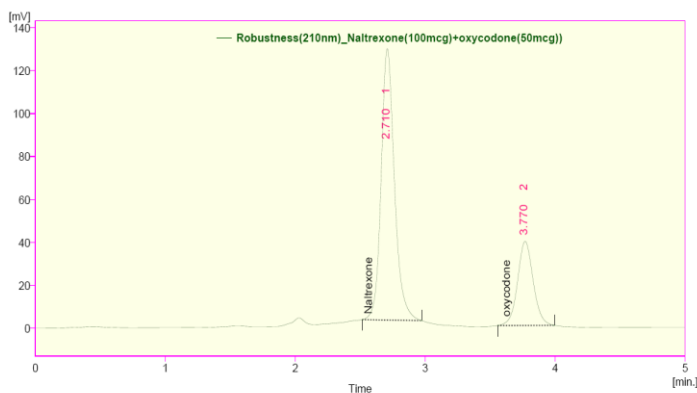**Fig:** Chromatogram of OXYCODONE and NALTREXONE for Robustness (1.2 ml/min)

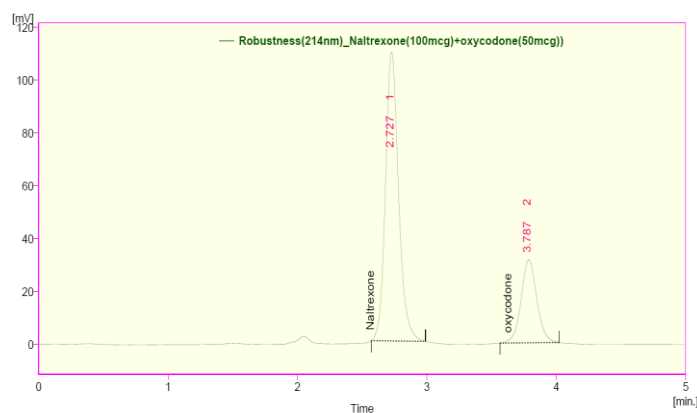**Fig:** Chromatogram of OXYCODONE and NALTREXONE for Robustness (210)

Fig: Chromatogram of OXYCODONE and NALTREXONE for Robustness (214)

Observation

From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.
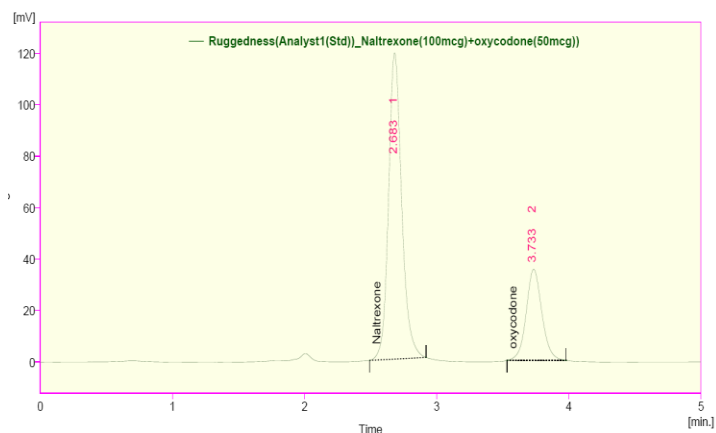

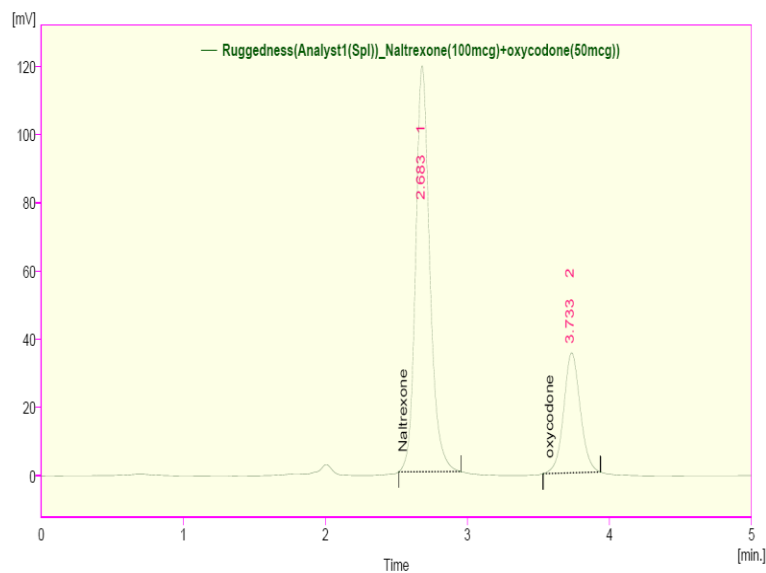**Fig:** Chromatogram of Analyst 01 standard preparation

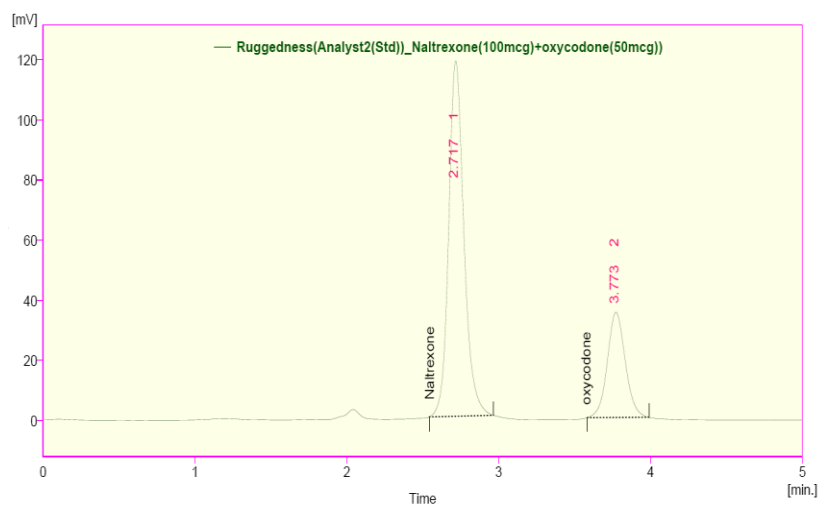**Fig:** Chromatogram of Analyst 01 sample preparation

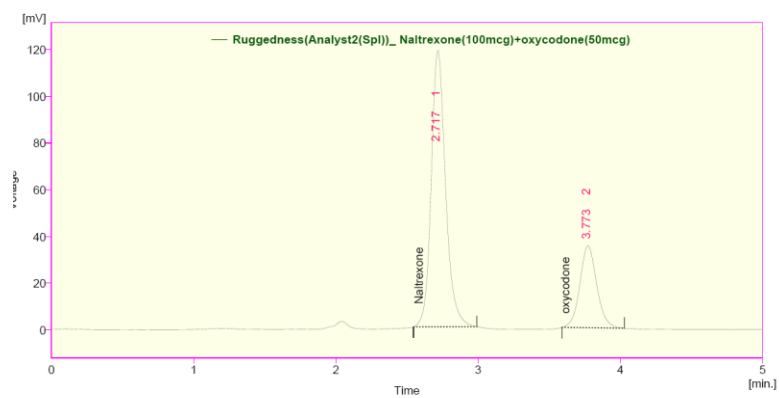**Fig:** Chromatogram of Analyst 02 standard preparation

Fig: Chromatogram of Analyst 02 sample preparation

Table 9.9.5: Results for Ruggedness

NALTREXONE	%Assay	OXYCODONE	%Assay
Analyst 01	100.1	**Analyst 01**	98.9
Anaylst 02	99.5	**Anaylst 02**	100.6

Observation

From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

BIBLIOGRAPHY

[1]. C.R. Ganellin; David J. Triggle. Dictionary of Pharmacological Agents. CRC Press. 1996, 1396–. ISBN 978-0-412-46630-4.

[2]. A, b "NADAC as of 2016-10-12 | Data.Medicaid.gov". Centers for Medicare and Medicaid Services. Retrieved 12 October 2016.

[3]. "Naltrexone". ATC/DDD Index. WHO Collaborating Centre for Drug Statistics Methodology. Retrieved 2016. DDD ... 50 mg

[4]. Srisurapanont, Manit; Jarusuraisin, Ngamwong. "Opioid antagonists for alcohol dependence". Cochrane Database of Systematic Reviews (2): CD001867. doi:10.1002/14651858.CD001867.pub2. 2005. PMID 12076425.

[5]. Donoghue, Kim; Elzerbi, Catherine; Saunders, Rob; Whittington, Craig; Pilling, Stephen; Drummond, Colin. "The efficacy of acamprosate and naltrexone in the treatment of alcohol dependence, Europe versus the rest of the world: A meta-analysis". Addiction. 110 (6), 2015, 920–30. doi:10.1111/add.12875. PMID 25664494.

[6]. C. Garbutt, James. "Efficacy and Tolerability of Naltrexone in the Management of Alcohol Dependence". Current Pharmaceutical Design. 16 (19), 2010, 2091 doi: 10.2174/138161210791516459. PMID 20482515.

[7]. Maisel, Natalya C.; Blodgett, Janet C.; Wilbourne, Paula L.; Humphreys, Keith; Finney, John W. "Meta-analysis of naltrexone and acamprosate for treating alcohol use disorders: When are these medications most helpful?". Addiction. 108 (2), 2013, 275–93. doi:10.1111/j.1360-0443.2012.04054.x. PMC 3970823 . PMID 23075288.

[8]. Anderson, Kenneth. "Drink Your Way Sober with Naltrexone". Psychology Today. Retrieved 2016.

[9]. A b Sinclair, J. D. "Evidence about the use of naltrexone and for different ways of using it in the treatment of alcoholism". Alcohol and Alcoholism. 36 (1), 2001, 2–10. doi:10.1093/alcalc/36.1.2. PMID 11139409.

[10]. Dijkstra, Boukje A. G.; De Jong, Cor A. J.; Bluschke, Sarah M.; Krabbe, Paul F. M.; Van Der Staak, Cees P. F. "Does naltrexone affect craving in abstinent opioid-dependent patients?". Addiction Biology. 12 (2), 2007, 176–82. doi:10.1111/j.1369-1600.2007.00067.x. PMID 17508990.

[11]. A b Galanter, Marc; Kleber, Herbert. The American Psychiatric Publishing Textbook of Substance Abuse Treatment, 4. ISBN 1585622761[page needed]

[12]. Minozzi, Silvia; Amato, Laura; Vecchi, Simona; Davoli, Marina; Kirchmayer, Ursula; Verster, Annette. "Oral naltrexone maintenance treatment for opioid dependence". Cochrane Database of Systematic Reviews (4), 2011, CD001333. doi:10.1002/14651858.CD001333.pub4. PMID 21491383.

[13]. Johansson, Björn Axel; Berglund, Mats; Lindgren, Anna. "Efficacy of maintenance treatment with naltrexone for opioid dependence: A meta-analytical review". Addiction. 101 (4), 2006, 491–503. doi:10.1111/j.1360-0443.2006.01369.x. PMID 16548929.

[14]. Comer, Sandra D.; Sullivan, Maria A.; Yu, Elmer; Rothenberg, Jami L.; Kleber, Herbert D.; Kampman, Kyle; Dackis, Charles; o'Brien, Charles P. "Injectable, Sustained-Release Naltrexone for the Treatment of Opioid Dependence". Archives of General Psychiatry. 63 (2), 2006, 210, doi:10.1001/archpsyc.63.2.210. PMC 4200530. PMID 16461865.