JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Analytical Method Development and Validation for Simultaneous Estimation of Oxycodone Hydrochloride and Naltrexone Hydrochloride in Synthetic Mixture

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Article history:

Received 13 Jun 2017
Revised 02 July 2017
Accepted 24 July 2017
Available online 01 Aug 2017

Citation:
Shah M., Jaiswal-Chaudhary S. V., Sing R. Analytical Method Development and Validation for Simultaneous Estimation of Oxycodone Hydrochloride and Naltrexone Hydrochloride in Synthetic Mixture. **J Pharm Sci Bioscientific Res**. 2017. 7(4):293-302

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ABSTRACT:

A newer, simple, rapid, accurate, precise and sensitive method was developed and validated for estimation of Oxycodone Hydrochloride (OXY) and Naltrexone Hydrochloride (NAL) in Synthetic Mixture. The method employed was First order derivative Concentration range of 10-30 µg/ml for Oxycodone Hydrochloride and 1.2-3.6 µg/ml for Naltrexone Hydrochloride for the proposed method. First order Derivative method, wherein wavelengths selected were 262.93nm (ZCP of Naltrexone Hydrochloride) for Oxycodone Hydrochloride and 238.62 nm (ZCP of Oxycodone Hydrochloride) for Naltrexone Hydrochloride. Area under Curve Method was another method was employed for Simultaneous Estimation of Oxycodone Hydrochloride and Naltrexone Hydrochloride in Synthetic mixture. The Concentration range was selected for estimation of Oxycodone Hydrochloride was 10-30 µg/ml and for Naltrexone Hydrochloride was 1.2-3.6 µg/ml for the AUC Method. For Area under Curve Method, the wavelength ranges selected was 244-264 nm for OXY and NAL at 270-290 nm for estimation of Oxycodone Hydrochloride and Naltrexone Hydrochloride respectively. In HPLC method, Enable C18 (250 x 4.6 mm, 5 µm) column was used as stationary phase and Acetonitrile: Water are in the ratio of 60:40, (v/v) and pH was adjusted to 5.0 with ortho phosphoric acid) as mobile phase was used. The flow rate was 0.8 ml/min and both drugs were detection was carried out at 286.9 nm. The retention time for OXY and NAL was found at 2.136 min and 5.485 min. respectively. Linearity of OXY at 10-30 µg/ml and NAL at 1.2-3.6 µg/ml.

KEY WORDS: Oxycodone Hydrochloride (OXY) and Naltrexone Hydrochloride (NAL), First Order derivative method, ZCP (Zero Cross point), Area under Curve Method (AUC), RP-HPLC.

INTRODUCTION:

Oxycodone Hydrochloride (OXY) 4, 5α-Epoxy-14-hydroxy-3-methoxy-17 methylmorphinan-6-one hydrochloride. It is an Opioid Analgesic used to reduce the pain, whether that pain is from surgery or a result of injury or disease such as cancer.

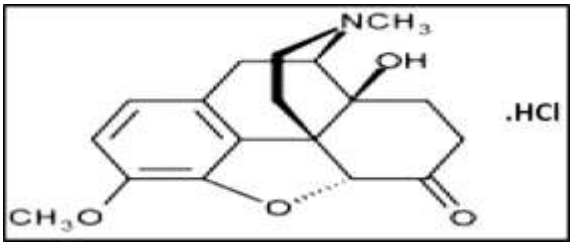


Figure: 1 Structure of Oxycodone Hydrochloride

Naltrexone Hydrochloride (NAL) 17- (cyclopropylmethyl)-4, 5α-epoxy-3, 14-dihydroxymorphinan-6-one hydrochloride. Antagonists that can reverse the actions of opioids are also very important and it is used as an Antidote for opioid Poisoning.

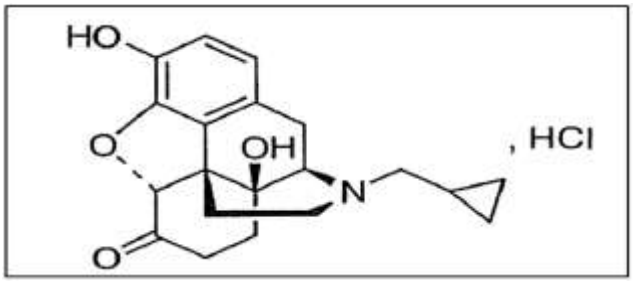


Figure: 2 Structure of Naltrexone Hydrochloride

MATERIALS AND METHODS

Instrument used was an UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800, software – UV probe, version 2.42) with a pair of 1 cm matched quartz cells. All weighing was done on Reptech electronic analytical balance.

Instrumentation and Chromatographic conditions

The analysis was performed by using Chromosil C-18 column, 250 X 4.6mm internal diameter with 5 micron particle size column and UV detector set at 286.9 nm, in conjunction with a mobile phase of Acetonitrile and Water in the ratio of 60:40 % v/v (pH 5 adjusted with OPA) at a flow rate of 0.8 ml/min. The retention time of Oxycodone Hydrochloride and Naltrexone Hydrochloride was found to be 2.136 and 5.485 minute. The injection volume was 10µl.

Mobile Phase Preparation

Prepared a mixture of Acetonitrile (HPLC Grade) and Water (HPLC Grade) in the ratio of 60:40 % v/v (pH 5 adjusted with OPA) mixed and sonicated.

PREPARATION OF STANDARD AND WORKING SOLUTION IN WATER

OXYCODONE HYDROCHLORIDE standard stock solution (1000 µg/ml)

A 10 mg of OXY standard was weighed and transferred to a 10 ml volumetric flask OXY was dissolved in distilled Water. The flask was shaken and volume was made up to the mark with distilled Water to give a solution containing 1000 µg/ml OXY.

Preparation of working standard solution of OXY (100 µg/ml)

From Stock solution of OXY (1000 µg/ml) 2.5 ml of solution was pipette out and transferred to 25 ml volumetric flask. The volume was adjusted to the mark with distilled Water to give a solution containing 100µg/ml OXY.

NALTREXONE HYDROCHLORIDE standard stock solution (1000 µg/ml)

A 10 mg of NAL standard was weighed and transferred to a 10 ml volumetric flask NAL was dissolved in distilled Water. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 1000 µg/ml NAL.

Preparation of 2nd standard solution of NAL (100 µg/ml)

From Stock solution of NAL (1000 µg/ml) 2.5 ml of solution was pipette out and transferred to 25 ml volumetric flask. The volume was adjusted to the mark with distilled Water to give a solution containing 100µg/ml NAL.

Preparation of working standard solution of NAL (10 µg/ml)

From Working Standard solution of NAL (100 µg/ml) 2.5 ml of solution was pipette out and transferred to 25 ml volumetric flask. The volume was adjusted to the mark with distilled Water to give a solution containing 10µg/ml NAL.

Preparation of Synthetic Mixture of OXY and NAL

Synthetic Mixture was prepared by mixing Both API and Excipients in 3:1 ratio. For that we weighed 670 mg of Oxycodone Hydrochloride & 80 mg of Naltrexone Hydrochloride and total 250 mg of all excipients (Sucrose, Magnesium Stearate, Ascorbic Acid, Gelatin, Titanium Dioxide, Talc and SLS) and mixed them properly.

Sample Stock Preparation (Synthetic Mixture) of OXY and NAL

Synthetic Mixture powder equivalent to 20 mg OXY and 2.4 mg NAL was accurately weighed and transferred to volumetric flask of 100ml capacity. Powder was dissolved in distilled Water in volumetric flask. The flask was shaken and volume was made up to the mark with distilled Water. The solution was filtered through whatmann filter

paper (0.45µ) and first few drops of filtrate were discarded. 1 ml of aliquot was taken and transferred to volumetric flask of 10 ml capacity which give a solution containing 20µg/ml OXY and 2.4 µg/ml of NAL in synthetic mixture.

Method I: First Order Derivative Method

The overlain spectra were converted to first order derivative spectra and from these overlain Derivative Spectra (Fig 1) Zero Crossing Point was found at 262.93nm for OXY and 238.62nm for NAL selected for the First Order Derivative method of two drugs. The absorbance at 262.93nm for OXY and 238.62nm for NAL was measured

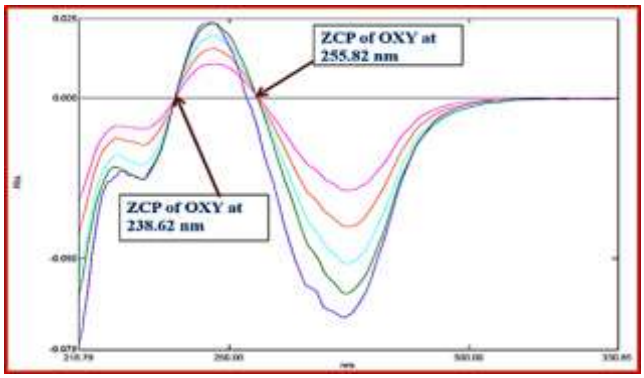


Figure 3: Derivative Spectra of Oxycodone Hydrochloride

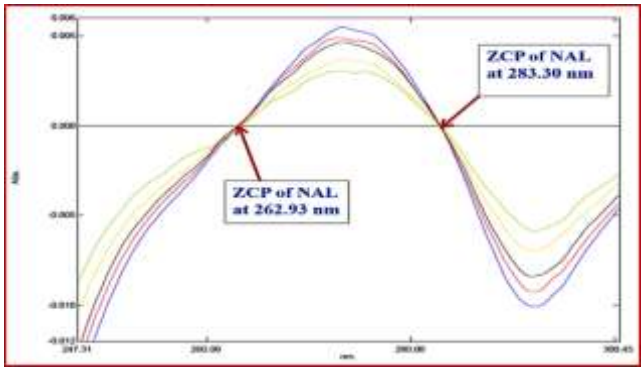


Figure 4: Derivative Spectra of Naltrexone Hydrochloride

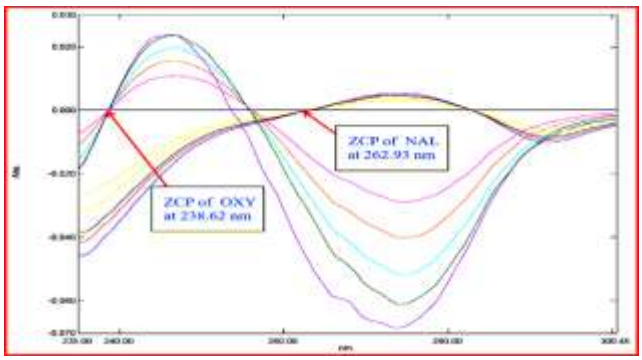


Figure 5: Overlain Derivative spectra of Oxycodone Hydrochloride (OXY) and Naltrexone Hydrochloride (NAL)

RESULT AND DISCCUSION

The proposed method was validated as per ICH guideline. Method discussed in the present work provide a convenient and accurate way for analysis of OXY and NAL.

In First Order Derivative method, wavelengths selected were 262.93nm for OXY (ZCP of NAL) and 238.62nm for NAL (ZCP of OXY). The plot of absorbance versus respective concentrations of OXY and NAL were found to be linear in the concentration range of 10-30 µg/ml for OXY and 1.2-3.6 µg/ml for NAL with correlation coefficient 0.9961 at 262.93 nm for OXY and 0.9977 at 238.62nm for NAL as shown in table. and figures 3, 4 & 5.

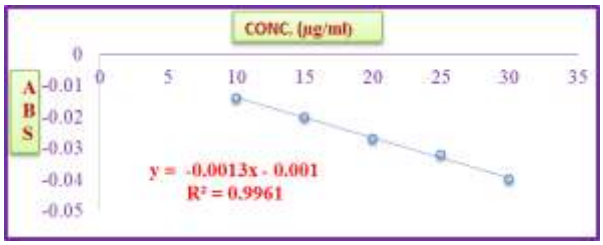


Figure 6: Calibration curve of standard OXY at 262.93 nm

Table 1: Linearity data of OXY at 262.93nm

Concentration (µg/ml)	Absorbance at 262.93nm (Mean ± SD) (n=5)	% RSD
10	-0.014 ± 0.000114	0.8120
15	-0.020 ± 0.000158	0.7906
20	-0.027 ± 0.000187	0.6929
25	-0.032 ± 0.000187	0.5846
30	-0.040 ± 0.000339	0.8477

Figure: 7 Calibration curve of standard NAL at 238.62nm

Table 2: Linearity data of NAL at 238.62nm

Concentration (µg/ml)	Absorbance at 238.62nm (Mean ± SD) (n=5)	% RSD
1.2	-0.023 ± 0.000158	0.6874
1.8	-0.028 ± 0.000212	0.7576
2.4	-0.032 ± 0.000273	0.8478
3	-0.036 ± 0.000187	0.5196
3.6	-0.040 ± 0.000219	0.5469

Table 3: Precision Study result for OXY and NAL

Parameters	Conc.		Absorbance (Mean ± S.D) (n=3)		% RSD	
	OXY (µg/ml)	NAL (µg/ml)	OXY	NAL	OXY	NAL
Intra-day precision	10	1.2	-0.0140± 0.000100	-0.0235 ± 0.000200	0.7142	0.8510
	15	1.8	-0.0201± 0.000153	-0.0281 ± 0.000200	0.7587	0.7117
	20	2.4	-0.0273± 0.000153	-0.0332 ± 0.000306	0.5581	0.9183
Inter-day precision	10	1.2	-0.0138± 0.000153	-0.0231± 0.000252	1.1015	1.087
	15	1.8	-0.0198± 0.000208	-0.0278 ± 0.000252	1.0478	0.9030
	20	2.4	-0.0264± 0.000252	-0.0329± 0.000351	0.9520	1.0663
Repeatability (n=6)	20	2.4	-0.02697±0.00024	-0.03202± 0.00023	0.8981	0.7235

Table 4: Accuracy Study result for OXY and NAL

Drug	Accuracy Level%	Amount of Drug Taken (µg/ml)	Amount of Drug Spiked (µg/ml)	Absorbance (Mean ± S.D) (n=3)	Total amount Found (µg/ml) (n=3)	% Recovery (n=3)
OXY	80%	10	8	-0.0245 ± 0.00025	18.13	100.73
	100%	10	10	-0.0245 ± 0.00025	20.02	100.11
	120%	10	12	-0.0298 ± 0.00020	22.15	100.69
NAL	80%	1.2	0.96	-0.0301 ± 0.00030	2.157	99.86
	100%	1.2	1.2	-0.0319 ± 0.00030	2.424	101.01
	120%	1.2	1.44	-0.0336 ± 0.00029	2.657	100.64

Table 5: Analysis of Synthetic Mixture of Oxycodone HCl and Naltrexone HCl by Proposed Method.

OXY			NAL		
Labelled amount (mg)	Amount found (mg) Mean ± S.D. (n=3)	%Assay Mean ±S.D. (n=3)	Labelled amount (mg)	Amount found (mg) Mean ± S.D. (n=3)	% Assay Mean ±S.D. (n=3)
20	**19.853 ± 0.0907**	**99.26 ± 0.4536**	**2.4**	**2.363 ± 0.02516**	**98.47 ± 1.0485**

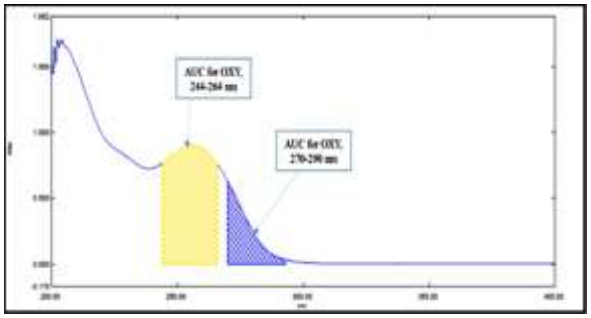


Figure 8: Spectra of OXY for AUC between 244-264 nm

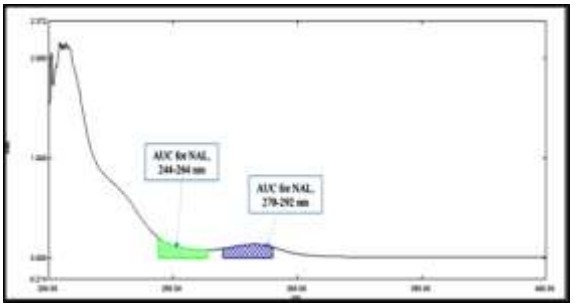


Figure 9: Spectra of NAL for AUC between 270-290 nm

CONCLUSION

The lower value of relative standard deviation for repeated measurement indicates that the method is precise.

The value of % recovery is approximately 100%, which indicates that these methods can be used for estimation of these two drugs in combined dosage form without any interference due to the other components present in the formulations. Hence this study presents simple, accurate, precise and rapid spectroscopic analytical method for the estimation of these two drugs in Synthetic Mixture.

Method II: Area Under Curve Method

In this method the area was measured at two wavelengths, that is the wavelength of maximum area of the components. For this measurement, the solutions of OXY and NAL were prepared separately in Distilled Water at a concentration of 20 µg/ml and 2.4 µg/ml respectively.

They were scanned in the wavelength range of 200-400 nm. Area of the resulting solution was measured at selected wavelength 244 to 264 nm and 270-290 nm. The concentration of OXY and NAL can be obtained.

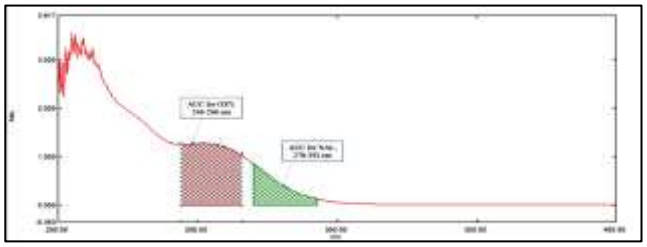


Figure 10: Combination Spectra of OXY (20 µg/ml) and NAL (2.4 µg/ml) at 244-264 nm to 270-292 nm

RESULT AND DISCCUSION

The proposed method was validated as per ICH guideline. Method discussed in the present work provide a convenient and accurate way for analysis of OXY and NAL.

In Area Under Curve method, wavelengths selected were 244-264 nm for OXY and 270-290nm for NAL.The plot of absorbance versus respective concentrations of OXY and NAL were found to be linear in the concentration range of 10-30 µg/ml for OXY and 1.2-3.6 µg/ml for NAL with correlation coefficient 0.9915 at 244-264 nm for OXY and 0.9947 at 270-290nm for NAL as shown in table.

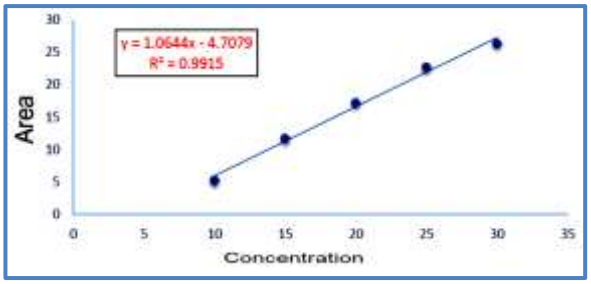


Figure 11: Calibration curve of standard OXY at 244-264nm

Table 6: Linearity data of OXY at 244-264nm

Concentration (µg/ml)	Area at 244-264 nm (Mean ± SD) (n=5)	% RSD
10	5.169 ± 0.0024	0.0480
15	11.660 ± 0.1343	1.1522
20	17.153 ± 0.0028	0.0167
25	22.164 ± 0.2707	1.2215
30	26.302 ± 0.0047	0.0182

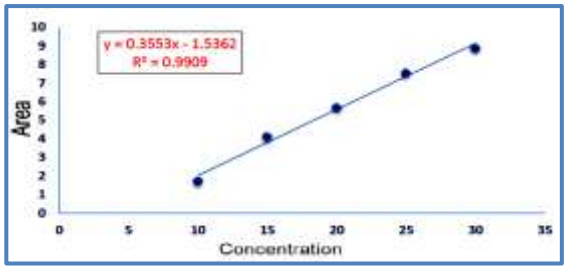


Figure: 12 Calibration curve of standard OXY at 270-290nm

Table 7: Linearity data of OXY at 270-290nm

Concentration (µg/ml)	Area at 270-290nm (Mean ± SD) (n=5)	% RSD
10	1.707 ± 0.0043	0.2519
15	4.086 ± 0.0037	0.0926
20	5.656 ± 0.0042	0.0756
25	7.534 ± 0.0044	0.0588
30	8.865 ± 0.0034	0.0384

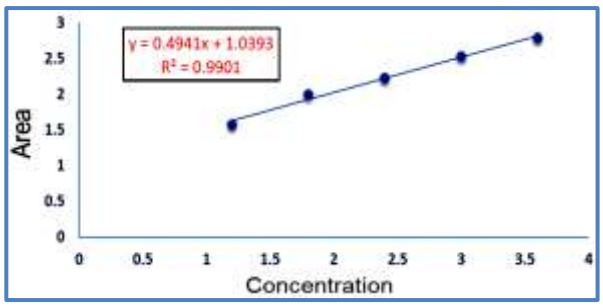


Figure 13: Calibration curve of standard NAL at 244-264nm

Table 8: Linearity data of NAL at 244-264nm

Concentration (µg/ml)	Area at 244-264nm (Mean ± SD) (n=5)	% RSD
1.2	1.576 ± 0.0043	0.2729
1.8	1.998 ± 0.0038	0.1922
2.4	2.228 ± 0.0051	0.2322
3.0	2.531 ± 0.0028	0.1131
3.6	2.791 ± 0.0033	0.1204

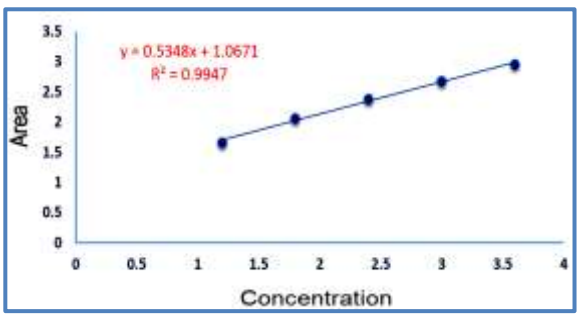


Figure: 14 Calibration curve of standard NAL at 270-290nm

Table 9: Linearity data of NAL at 270-290nm

Concentration (µg/ml)	Area at 270-290nm (Mean ± SD) (n=5)	% RSD
1.2	1.664 ± 0.0032	0.1928
1.8	2.068 ± 0.0044	0.2162
2.4	2.392 ± 0.0063	0.2666
3	2.682 ± 0.0052	0.1947
3.6	2.959 ± 0.0038	0.1286

Table 12: Analysis of Synthetic Mixture of Oxycodone HCl (244-264nm) and Naltrexone HCl (270-290nm) by Proposed Method.

OXY			NAL		
Labelled amount (mg)	**Amount found (mg) Mean ± S.D. (n=3)**	**%Assay Mean ±S.D. (n=3)**	**Labelled amount (mg)**	**Amount found (mg) Mean ± S.D. (n=3)**	**% Assay Mean ±S.D. (n=3)**
20	**19.993 ± 0.1137**	**99.96 ± 0.5686**	**2.4**	**2.37 ± 0.0207**	**98.75 ± 0.8333**

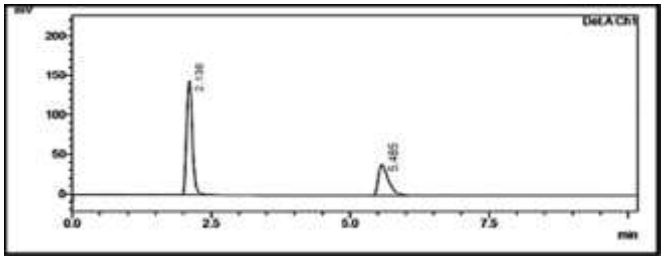
Table 10: Precision Study result for OXY (244-264nm) and NAL (270-290nm)

Parameters	Conc.		Area (Mean ± S.D) (n=3)		% RSD	
	OXY (µg/ml)	NAL (µg/ml)	OXY	NAL	OXY	NAL
Intra-day precision	10	1.2	5.168±0.0030	1.662± 0.0015	0.0580	0.0918
	15	1.8	11.253±0.0040	2.065± 0.0041	0.0359	0.2015
	20	2.4	17.155±0.0025	2.393± 0.0030	0.0146	0.1276
Inter-day precision	10	1.2	5.174±0.0079	1.665± 0.0040	0.1534	0.2817
	15	1.8	11.309±0.0985	2.068± 0.0070	0.8709	0.3395
	20	2.4	17.161±0.0109	2.396± 0.0041	0.0639	0.1737
Repeatability (n=6)	20	2.4	17.153±0.00292	2.395 ± 0.00892	0.0170	0.3725

Table 11: Accuracy Study result for OXY (244-264nm) and NAL (270-290nm)

Drug	Accuracy Level%	Amount of Drug Taken (µg/ml)	Amount of Drug Spiked (µg/ml)	Area (Mean ± S.D) (n=3)	Total amount Found (µg/ml) (n=3)	% Recovery (n=3)
OXY	80%	10	8	14.59 ± 0.0225	18.15	100.83
	100%	10	10	16.56 ± 0.0415	20.04	100.20
	120%	10	12	18.74 ± 0.0419	22.1	100.45
NAL	80%	1.2	0.96	2.260 ± 0.0507	2.15	99.54
	100%	1.2	1.2	2.361 ± 0.0152	2.39	99.58
	120%	1.2	1.44	2.449 ± 0.0338	2.63	99.62

CONCLUSION



The lower value of relative standard deviation for repeated measurement indicates that the method is precise.

The value of % recovery is approximately 100%, which indicates that these methods can be used for estimation of these two drugs in combined dosage form without any interference due to the other components present in the formulations. Hence this study presents simple, accurate, precise and rapid spectroscopic analytical method for the estimation of these two drugs in Synthetic Mixture.

Method III: RP- HPLC Method

An RP-HPLC method was developed and validated for the estimation of Oxycodone Hydrochloride (OXY) and Naltrexone Hydrochloride (NAL) in Synthetic Mixture.

RESULT AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of Acetonitrile and Water (60:40% v/v). Adjusted to pH 5 with orthophosphoric acid and 0.8 ml/min flow rate proved to be better than the other mixtures in terms of resolution and peak shape. The optimum wavelength for detection was set at 286.9 nm at which much better detector responses for both drugs were obtained. As it was shown in Fig. the retention times were 2.136 min for OXY and 5.485 min for NAL.

The percentage Assay was found to be 99.63 % for OXY and 99.86 % for NAL. The proposed method was validated as per ICH parameter. Linearity of the method was found to be in the range of 10-30 µg/ml for OXY and 1.2-3.6 µg/ml for NAL. The correlation co-efficient was found to be 0.9996 with slope 9136.5 for OXY and 0.9997 with slope 35950 for NAL. LOD was found to be 0.00469 µg/ml and 0.00018 µg/ml for OXY and NAL respectively. LOQ was found to be 0.01422 µg/ml and 0.000547 µg/ml for OXY and NAL respectively. The average recovery of OXY and NAL was found to be 100.05-100.53 % and 99.96-100.62 %. Results obtained by applying the RP-HPLC method showed that the concentrations of OXY and NAL can be simultaneously determined in prepared mixtures.

Figure 15: Chromatogram of Synthetic Mixture

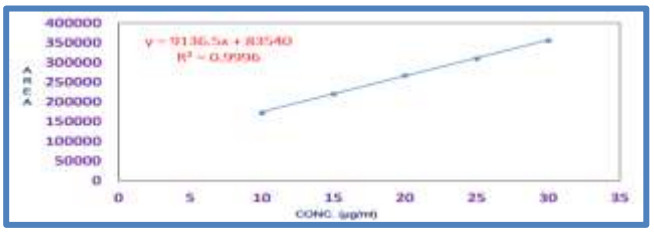


Figure 16: Linearity of OXY

Table 13: Data for Linearity Study of OXY

Concentration (µg/ml)	PEAK AREA (Mean ± SD) (n=3)	% RSD
10	173474.1 ± 13.938	0.0080
15	221344.0 ± 13.536	0.0061
20	268238.2 ± 20.195	0.0075
25	311477.9 ± 16.066	0.0051
30	356820.8 ± 15.535	0.0043

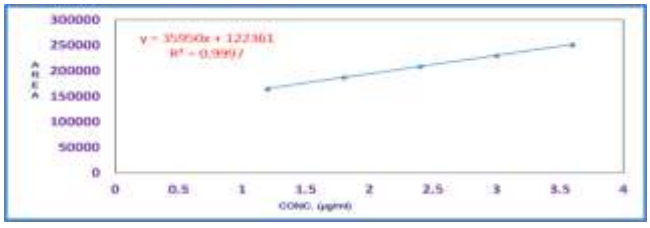


Figure 17: Linearity of NAL

Table 14: Data for Linearity Study of NAL

Concentration (µg/ml)	**PEAK AREA (Mean ± SD) (n=3)**	**% RSD**
1.2	164818.9 ± 8.0562	0.0048
1.8	187561.1 ± 6.7668	0.0036
2.4	209204.1 ± 7.1547	0.0034
3.0	230339.5 ± 4.3863	0.0019
3.6	251278.4 ± 7.8500	0.0031

Table 15: Precision Study result for OXY and NAL

Parameters	**Conc.**		**Peak Area (Mean ± S.D) (n=3)**		**% RSD**	
	OXY (µg/ml)	**NAL (µg/ml)**	**OXY**	**NAL**	**OXY**	**NAL**
Intra-day precision	10	1.2	173473.5±12.130	164821.9 ± 12.6183	0.0069	0.0007
	15	1.8	221347.9 ± 6.6252	187555.5 ± 12.3314	0.0029	0.0065
	20	2.4	268243.1 ± 9.8534	209196.8 ± 8.3072	0.0036	0.0039
Inter-day precision	10	1.2	173462.0 ± 8.0865	164802.8 ± 16.7035	0.0104	0.0101
	15	1.8	221332.9 ± 2.5540	187535.0 ± 16.5784	0.0056	0.0088
	20	2.4	268220.7 ± 3.1386	209162.7 ± 11.9176	0.0048	0.0056
Repeatability (n=6)	20	2.4	268233.0 ± 2.8062	209202.5 ± 8.4793	0.0047	0.0040

Table 16: Accuracy Study result for OXY and NAL

Drug	Level	Amount of Synthetic Mixture (µg/ml)	Amount of Std. Spiked (µg/ml)	Total amount (µg/ml)	Peak Area Mean ± S.D. (n=3)	Amount of sample found (µg/ml)	%Recovery
OXY	0	10	0	10	173470.4 ± 8.5564	9.84	-
	80	10	8	18	248092.9 ± 11.4293	18.01	100.05
	100	10	10	20	268238.1 ± 10.8445	20.1	100.53
	120	10	12	22	285100.1 ± 7.2507	22.06	100.27
NAL	0	1.2	0	1..2	164819.7 ± 10.8408	1.181	-
	80	1.2	0.96	2.16	200108.8 ± 6.8039	2.163	100.12
	100	1.2	1.2	2.4	209210.5 ± 7.9525	2.415	100.66
	120	1.2	1.44	2.64	217251.5 ± 7.1337	2.639	99.98

Table 17: Analysis of Synthetic Mixture of OXY and NAL by RP-HPLC Method.

Brand Name	Actual Concentration (µg/ml)		Amount Obtained Mean ± S.D. (n=3) (µg/ml)		% Assay OXY ± S.D. (n=3)	% Assay OXY ± S.D. (n=3)
Synthetic Mixture	OXY 20	NAL 2.4	OXY 19.926 ± 0.0680	NAL 2.396 ± 0.0208	99.63 ± 0.3403	99.86 ± 0.8673

Table 18: Summary of validation parameters of proposed RP-HPLC method

Sr.No.	Parameters		Value founds	
			OXY	**NAL**
1	Linearity and Range (µg/ml)		10 – 30	1.2 – 3.6
2	Correlation coefficient		0.9996	0.9997
3	Accuracy (% Recovery)		100.05 – 100.53	99.96 – 100.62
Precision (% RSD)				
4	Intra-Day		0.0029 – 0.0069	0.0039 – 0.0076
5	Inter-Day		0.0048 – 0.0104	0.0056 – 0.0101
Robustness (% RSD)				
6	Changes in Mobile Phase Ratio	**58:42**	0.0077	0.4474
	(60:40 **±** 2 % v/v)	**62:38**	0.0031	0.4451
	ACN: Water (pH5 by OPA)			
7	Changes in Flow Rate	**0.7**	0.0054	0.7865
	(0.8 **±** 0.1 ml/min.)	**0.9**	0.0048	0.8972
8	LOD µg/ml		0.0046	0.0001
9	LOQ µg/ml		0.0142	0.0005
10	Assay %		99.63 **±** 0.3403	99.86 **±** 0.8673

CONCLUSION

Proposed study describes a new and simple RP-HPLC method for the estimation of Oxycodone Hydrochloride and Naltrexone Hydrochloride in Synthetic Mixture. The method validated was according to ICH guidelines, it is found to be simple, sensitive, accurate and precise.

Therefore the proposed method was used for the routine analysis of the pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The authors are thankful to ROFEL, Shri G.M Bilakhia College of Pharmacy, Namdha Campus, Vapi (Gujarat) for providing necessary equipment, facility & chemicals to complete research work.

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