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High Performance Liquid Chromatographic Method for Simultaneous Quantification of Naloxone and Oxycodone

PALLAPATI SUMAN[1,2,*], TIRUKKOVALLURI SIVA RAO[1] and KALLAM VENKATA SIVA RAMA KRISHNA REDDY[2]

[1]Department of Inorganic & Analytical Chemistry, Andhra University, Visakhapatnam-530 003, India
[2]Laurus Labs Limited, Visakhapatnam-531 021, India

*Corresponding author: E-mail: pallapatisumanphd@gmail.com

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High performance liquid chromatography was applied to the simultaneous determination of naloxone and oxycodone. The method depends on separation and analysis of naloxone and oxycodone using C18 analytical column (250 × 4.6 mm, 5 µm). The mobile phase consists of a mixture of potassium dihydrogen phosphate (pH 5.5; 0.1 M) and acetonitrile (70:30, v/v). The photodiode array detector was set at 210 nm. The retention times of naloxone and oxycodone were 2.893 and 4.946 min, respectively. The total run time was 6 min. The developed method was validated as per the guidelines of International Conference on Harmonization and successfully applied to the simultaneous determination of naloxone and oxycodone in their tablets. All the results of validation parameters were acceptable and confirmed that the method is appropriate for the estimation of naloxone and oxycodone simultaneously.

Keywords: Naloxone, Oxycodone, HPLC, Diode array detector.

INTRODUCTION

Naloxone is a competitive opioid antagonist used for the reversal of post-operative respiratory depression, reversal of respiratory and central nervous system depression from opioid administration during labour and child birth [1,2]. Naloxone reverses the effect of opioid by removing the opioid from the receptors for a short term and thus assists in the restoration of breathing [3]. Naloxone is a derivative of thebaine and chemically described as (4R,4aS,7aR,12bS)-4a,9-dihydroxy-3-prop-2-enyl-2,4,5,6,7a,13-hexahydro-1*H*-4,12-methano benzofuro-[3,2-e]isoquinoline-7-one (Fig. 1).

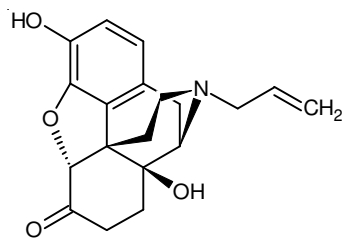

Fig. 1. Chemical structure of naloxone

Oxycodone is an opioid agonist with analgesic activity used in the treatment of moderate to severe pain [4]. Oxycodone exhibits its analgesic activity by binding to the µ-receptors in the central nervous system and thus imitating the effects of endogenous opioids [5]. Oxycodone inhibits the release of dopamine, substance P, γ-aminobutyric acid, acetylcholine and noradrenaline [6]. Oxycodone is a semi-synthetic derivative of codeine and chemically described as (4R,4aS,7aR,12bS)-4a-hydroxy-9-methoxy-3-methyl-2,4,5,6,7a,13-hexahydro-1*H*-4,12-methanobenzofuro[3,2-e]isoquinoline-7-one (Fig. 2).

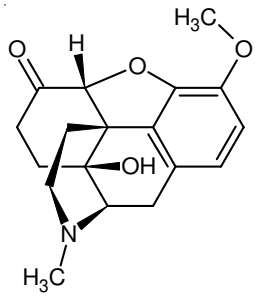

Fig. 2. Chemical structure of oxycodone

The US Food and Drug Administration has approved naloxone and oxycodone combination as prolonged-release tablets for the treatment of severe pain that can be satisfactorily managed only with opioid pain relievers [7]. In the combination tablet, oxycodone exhibits pain killing effect and naloxone is responsible to bring relief from constipation which is a common side effect of treatment with strong pain relievers [8].

The combination of naloxone and oxycodone is not official in any pharmacopoeia. No analytical methods are found in the literature for the simultaneous determination of naloxone and oxycodone. But many research reports are available in the literature for the determination of naloxone and oxycodone individually. There are few reports published regarding the determination of naloxone in microparticles, transdermal formulation, plasma and pharmaceutical dosage form. Naloxone has been determined by spectrophotometry [9], HPLC [9-12] and LC-MS [13,14] methods. HPLC [15], HPTLC [16], LC-MS [17-19], GC-MS [20,21] and capillary electrophoresis [22] methods have been adapted to the determination of oxycodone in biological samples like human blood, plasma, urine and in oral pharmaceutical solutions. Therefore, the present study was aimed to develop and validate a HPLC assay method for simultaneous quantification of naloxone and oxycodone in bulk and combined tablet dosage

EXPERIMENTAL

The chromatographic separation followed by photodiode array detection were achieved by employing Waters 2695 alliance HPLC system with Waters 2998 photo diode array detector. Waters Empower2 software was used to control the instrument and process the data. The Waters 2695 alliance HPLC system used in the analysis comprised an autosampler injector, binary pump and column heater.

Naloxone and oxycodone reference standards were obtained from Lara Drugs Private Limited (Telangana, India). Targinact tablets (Napp Pharmaceuticals Limited, Cambridge, UK) labeled to contain 10 mg naloxone and 20 mg oxycodone was employed. Milli-Q water (Millipore, USA) was used all through the experiments. Potassium dihydrogen phosphate and orthophosphoric acid of analytical grade were acquired from Sd. Fine Chemicals Ltd., Mumbai, India. HPLC grade acetonitrile was from Merck India Ltd (Mumbai, India).

Chromatographic conditions: The chromatographic conditions maintained were Thermo C18 (250 mm × 4.5 mm i.d., particle size 5 µm) analytical column as stationary phase, column temperature of 25 ± 2 °C, 0.1 M potassium dihydrogen phosphate: acetonitrile (70:30 v/v, pH 5.5 adjusted with orthophosphoric acid) as mobile phase, flow rate of 1 mL/min, 10 µL of injection volume and wavelength 210 nm as detection analytical wavelength. The mobile phase was filtered through 0.45 µm pore size membrane filter and sonicated for 20 min before analysis.

Stock standard solution: 10 mg of naloxone and 20 mg of oxycodone were weighed accurately, dissolved and diluted with mobile phase in a 100 mL volumetric flask to obtain the final concentration of 100 µg/mL of naloxone and 200 µg/mL of oxycodone. The stock solution was stored at 2-8 °C.

Working standard solutions: The working standard solutions were prepared by diluting the above standard stock solution with mobile phase to reach a concentration range of 5-15 µg/mL for naloxone and 10-30 µg/mL for oxycodone.

Construction of calibration curve for naloxone and oxycodone: 10 µL of working standard solution was injected into the HPLC system three times for each concentration and chromatographed using the chromatographic conditions mentioned above. Using least-squares linear regression analysis, calibration curves were generated by plotting the peak area *vs.* concentration of the analyte.

Tablet sample preparation: Ten tablets were weighed and powdered. An accurate weight of the powder equivalent to 10 mg naloxone and 20 mg oxycodone was transferred into a 100 mL volumetric flask and extracted with 30 mL of mobile phase in an ultrasonic bath for 30 min. The solution thus prepared were diluted to volume with the mobile phase then filtered through a 0.45 µm pore size membrane filter.

Assay of naloxone and oxycodone in tablet dosage form: 1 mL of above prepared tablet sample solution was diluted to 10 mL with mobile phase in a 10 mL volumetric flask to prepare tablet sample solution containing 10 µg/mL of naloxone and 20 µg/mL of oxycodone. Solution prepared was filtered using 0.45 µm pore size membrane filter and then analyzed as mentioned under the construction of calibration curve for naloxone and oxycodone.

RESULTS AND DISCUSSION

Method development: By using a Thermo C18 column (250 × 4.6 mm, 5 µm) with temperature 25 ± 2 °C, sharp peaks of naloxone and oxycodone with satisfactory separation were achieved. Hence the same column and temperature was used in the present study. To achieve good peak shape and resolution under isocratic conditions, mixtures of acetonitrile and 0.1 M potassium dihydrogen phosphate in different combinations with different flow rates and pH were tried as mobile phase on a Thermo C18 column. Finally, a binary mixture of 0.1 M potassium dihydrogen phosphate (pH 5.5 adjusted with orthophosphoric acid) and acetonitrile in 70:30 v/v proportions with a flow rate of 1 mL/min was found to be the suitable of all combinations. Injection volume as 10 µL was appropriate in separating naloxone and oxycodone. Wavelength of 210 nm was chosen for the simultaneous determination of naloxone and oxycodone with good sensitivity. Under the optimized chromatographic conditions, the retention time of 2.893 min and 4.946 min was obtained for naloxone and oxycodone, respectively (Fig. 3).

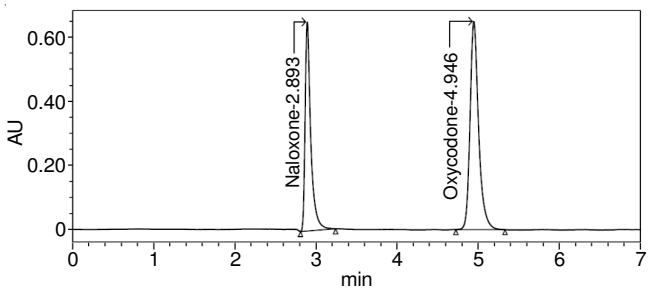

Fig. 3. Typical chromatogram of naloxone (10 µg/mL) and oxycodone (20 µg/mL) using 0.1M KH_2PO_4: acetonitrile of pH 5.5 (70:30, v/v) as a mobile phase

Method validation: The developed HPLC method was validated as stated by the ICH guidelines [23] for system suitability, selectivity, linearity, precision, accuracy, robustness, limit of detection (LOD), limit of quantification (LOQ).

System suitability: The system suitability parameters (% RSD of retention time, % RSD of peak area, USP plate count,

USP resolution and USP tailing factor) were calculated after six replicate injections of standard naloxone and oxycodone solution into the HPLC system. The results are tabulated in Table-1 which is in the acceptable range.

Selectivity: To evaluate the specificity of the proposed method, chromatograms of place blank, mobile phase blank and tablet sample were compared with chromatogram of standard solution of naloxone and oxycodone. The chromatograms are shown in Figs. 4 and 5. No endogenous peak was observed at the retention time of naloxone and oxycodone in the chromatograms of placebo blank and mobile phase blank. The retention time of naloxone and oxycodone in chromatograms of tablet sample solution (Fig. 4) and standard solution (Fig. 5) are same. The common excipients of tablet dosage form and components of mobile phase did not interfere with the assay of selected drug combination and thus indicating the selectivity of the proposed method.

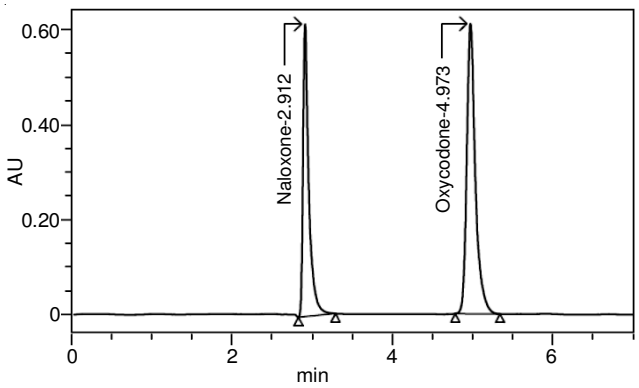

Fig. 4. Chromatogram of tablet sample solution (naloxone - 10 µg/mL and oxycodone - 20 µg/mL)

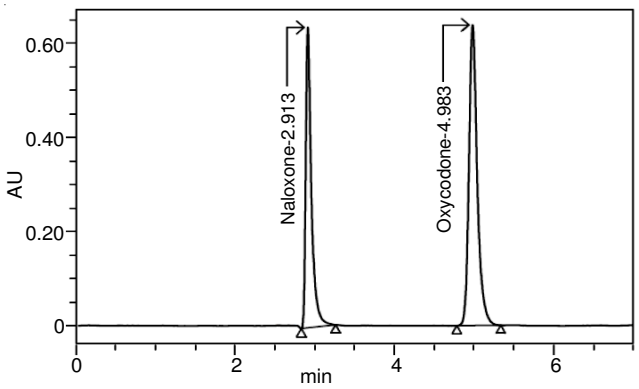

Fig. 5. Chromatogram of standard solution of (naloxone - 10 µg/mL and oxycodone - 20 µg/mL)

Linearity: A linear relationship was established by plotting the peak area against the drug concentration (µg/mL). The concentration range was found to be 5-15 µg/mL and 10-30 µg/mL for naloxone and oxycodone, respectively. Linear regression analysis of the data gave the following equations:

$Y = 29571\ c - 1248$ ($R^2 = 0.9999$) for naloxone

$Y = 21742\ c + 47.5$ ($R^2 = 0.9999$) for oxycodone

where Y is the peak area, c is the concentration of the drug (µg/mL) and R^2 is the regression coefficient. The high values of regression coefficients (> 0.999) with small intercept indicate the good linearity of the calibration curves for naloxone and oxycodone.

Limit of detection (LOD) and limit of quantification (LOQ): The smallest amount of drug (LOD) that could be detected was 3.750 µg/mL for naloxone and 3.860 µg/mL for oxycodone. The LOQs were 10.550 µg/mL and 10.410 µg/mL for naloxone and oxycodone, respectively. The low values of LOD and LOQ point out the adequate sensitivity of the proposed method for the quantification of naloxone and oxycodone.

Precision and accuracy: The proposed method was assessed by studying the accuracy as per cent recovery and precision as per cent relative standard deviation (% RSD). Precision and accuracy was determined by analyzing standard solution of naloxone (10 µg/mL) and oxycodone (20 µg/mL) six times. The peak areas obtained were used to calculate mean recovery and % RSD. The results are shown in Table-2. The mean recovery and % RSD values indicate that the developed method could estimate naloxone and oxycodone with good accuracy and precision, respectively.

TABLE-2
PRECISION AND ACCURACY DATA FOR THE DETERMINATION OF NALOXONE AND OXYCODONE BY THE PROPOSED METHOD

Sample No.	Naloxone (10 µg/mL)		Oxycodone (20 µg/mL)	
	Peak area	Recovery (%)	Peak area	Recovery (%)
1	2954197	99.43	4342803	99.19
2	2958653	99.58	4341482	99.16
3	2959513	99.61	4347175	99.29
4	2950997	99.33	4342453	99.18
5	2953886	99.42	4340411	99.14
6	2954007	99.43	4345039	99.24
Mean	2955411	99.474	4343312	99.202
RSD	0.1210	0.1182	0.0635	0.0623

Recovery studies: In recovery studies, known amount of standard naloxone and oxycodone was spiked into preanalyzed

TABLE-1
RESULTS OF SYSTEM SUITABILITY STUDIES

Sample No.	Naloxone (10 µg/mL)					Oxycodone (20 µg/mL)				
	Retention time	Peak area	Plate count	Tailing factor	Resolution	Retention time	Peak area	Plate count	Tailing factor	Resolution
1	2.913	2945586	9960	1.71	–	4.983	4335011	11879	1.31	13.16
2	2.92	2956314	9910	1.73	–	4.984	4334221	11891	1.31	13.18
3	2.914	2979716	9922	1.73	–	4.977	4357864	11708	1.33	13.11
4	2.92	2976090	9819	1.76	–	4.985	4335418	11789	1.33	13.1
5	2.916	2923042	9920	1.74	–	4.980	4375040	11843	1.33	13.2
Mean	2.917	2956149	9906	1.734	–	4.982	4347510	11822	1.322	13.150
RSD	0.1127	0.7862	0.5282	1.0476	–	0.0657	0.4217	0.6349	0.8286	0.3315
Recommended limit	RSD ≤ 2	RSD ≤ 2	> 2000	≤ 2	–	RSD ≤ 2	RSD ≤ 2	> 2000	≤ 2	> 1.5

TABLE-3
RECOVERY RESULTS FOR THE DETERMINATION OF NALOXONE AND OXYCODONE BY THE PROPOSED METHOD

Spiked level (%)	Naloxone				Oxycodone			
	Added (µg/mL)	Found (µg/mL)	Recovery (%)	Mean (%)	Added (µg/mL)	Found (µg/mL)	Recovery (%)	Mean (%)
50	4.975	4.980	100.107	99.833	9.930	9.925	99.951	100.060
	4.975	4.969	99.877		9.930	9.946	100.165	
	4.975	4.951	99.515		9.930	9.936	100.064	
100	9.950	9.957	100.069	100.097	19.860	19.847	99.932	99.905
	9.950	9.962	100.117		19.860	19.838	99.890	
	9.950	9.960	100.104		19.860	19.839	99.892	
150	14.925	14.928	100.023	100.032	29.790	29.784	99.981	100.018
	14.925	14.928	100.021		29.790	29.804	100.046	
	14.925	14.933	100.052		29.790	29.798	100.028	

TABLE-4
EFFECT OF FLOW RATE AND COLUMN TEMPERATURE ON THE SYSTEM SUITABILITY PARAMETERS

Parameter	Naloxone (10 µg/mL)			Oxycodone (20 µg/mL)		
	USP tailing	USP plate count	USP resolution	USP tailing	USP plate count	USP resolution
Flow rate 1.0 + 0.1 mL/min	1.79	11340	–	1.43	13542	13.96
Flow rate 1.0 – 0.1 mL/min	1.76	8491	–	1.36	10699	12.49
Temperature 25 + *5* °C	1.80	11779	–	1.44	13369	13.96
Temperature 25 – *5* °C	1.76	8317	–	1.35	10580	12.38

tablet sample solution at three concentration levels. The per cent recovery of naloxone and oxycodone was once again determined by the proposed method. The results are tabulated in Table-3. Recovery values (close to 100 %) indicate that the developed method was appropriate for accurate quantification of naloxone and oxycodone. Tablet excipients did not interference in the assay of naloxone and oxycodone.

Robustness: The effects of small changes in the flow rate of mobile phase (1.0 ± 0.1 mL/min) and column temperature (25 ± *5* °C) on the system suitability parameters (USP plate count, USP resolution and USP tailing factor) were examined. The robustness of the method was determined at concentration of 10 µg/mL for naloxone and 20 µg/mL for oxycodone. From the results (Table-4), it was found that the slight changes in flow rate and column temperature had no significant influence on the system suitability parameters, confirming the method robustness.

Conclusion

Naloxone and oxycodone in combined tablet dosage form has been determined using high performance liquid chromatographic method. The HPLC-PDA detector system with C18 analytical column (250 mm × 4.6 mm, 5 mm, particle size) was used in this study. Potassium dihydrogen phosphate (pH 5.5) and acetonitrile in the ratio of 70:30 (v/v) was chosen as the mobile phase with a flow rate of 1.0 mL/min and a detection wavelength of 210 nm. The HPLC method for the simultaneous quantification of naloxone and oxycodone was successfully developed and validated. The method was validated in terms of system suitability, linearity, sensitivity, accuracy, precision, selectivity and robustness. The results of the validation parameters were found to be within the acceptance limits.

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