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A NEW RP HPLC METHOD DVELOPMENT AND VALIDATION FOR THE SIMULTANIOUS ESTIMATION OF NALETREXONE AND OXYCODONE USING BULK DOSAGE FORMS

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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 30 volumes of ammonium acetate buffer,40 volumes of acetonitrile and 30 volumes of Methanol with detection of 212 nm. 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.

KEY WORDS: Naltrexone, oxycodone, bulk dosage form

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INTRODUCTION

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Often a time lag exists from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs (1, 2).Analytical methods should be used within good manufacturing practice (GMP) and good laboratory practice (GLP) environments, and must be developed using the protocols set out in the International Conference on Harmonization (ICH) guidelines (Q2A and Q2B) (3, 4).

Method development is a continuous process that progresses in parallel with the evolution of the drug product. The goal and purpose of the method should reflect the phase of drug development. During early drug development, the methods may focus on API behavior. They should be suitable to support preclinical safety evaluations, pre-formulation studies, and prototype product stability studies. As drug development progresses, the analytical methods are

refined and expanded, based on increased API and drug product knowledge. The methods should be robust and uncomplicated, while still meeting the appropriate regulatory guidelines. Scouting experiments are frequently performed during method development to establish the performance limits of the method, prior to formal validation experiments. These may include forced degradation studies, which are an integral part of development of a stability-indicating method. API is typically subjected to degradation by acid, base, peroxide, heat, and light. This allows for a determination of the capability of the method to separate and quantify degradation products, while providing insight into the main mechanisms of degradation. Once a stability-indicating method is in place, the formulated drug product can then be subjected to heat and light in order to evaluate potential degradation of the API in the presence of formulation excipients (5).

Method validation is the process by which it is established, through laboratory studies, that the performance characteristics of the method meet the requirements for its intended purpose. It is a part of the overall validation process that also includes software validation, instrument qualification and system suitability. Typical analytical characteristics used in method validation are highlighted below. Although all analytical procedures or methods used in a regulated laboratory must be validated, this chart focuses specifically on liquid chromatography.

To generate reproducible and reliable results, the samples, standards, and reagents used for the HPLC method must be stable for a reasonable time (e.g., one day, one week, and one month, depending on need). For example, the analysis of even a single sample may require 10 or more Chromatographic runs to determine system suitability, including standard concentrations to create a working analytical curve and duplicate or triplicate injections of the sample to be assayed. Therefore, a few hours of standard and sample solution stability can be required even for short (10-min) separation. When more than one sample is analyzed automated, overnight runs often are performed for better lab efficiency. Such practices add requirements for greater solution stability.

Stability is defined as the capacity of a drug substance or drug product to remain within the established specifications to maintain its identify, strength, quality and Purity throughout the retest or expiration dating period

The purpose of stability testing is to provide evidence on how the quality of active substances or pharmaceutical products varies with time under the influence of a variety of environmental factors such as temperature, humidity & light. In addition, product – related factors influence the stability.

Naltrexone-Derivative of noroxymorphone that is the N-cyclopropylmethyl congener of naloxone. It is a narcotic antagonist that is effective orally, longer lasting and more potent than naloxone, and has been proposed for the treatment of heroin addiction. The FDA has approved naltrexone for the treatment of alcohol dependence. Naltrexone is a pure opiate antagonist and has little or no agonist activity. The mechanism of action of naltrexone in alcoholism is not understood; however, involvement of the endogenous opioid system is suggested by preclinical data. Naltrexone is thought to act as a competitive antagonist at mc, κ, and δ receptors in the CNS, with the highest affintiy for the μ receptor. Naltrexone competitively binds to such receptors and may block the effects of endogenous opioids. This leads to the antagonization of most of the subjective and objective effects of opiates, including respiratory depression, miosis, euphoria, and drug craving. The major metabolite of naltrexone, 6-β-naltrexol, is also an opiate antagonist and may contribute to the antagonistic activity of the drug. Naltrexone, a pure opioid antagonist, is a synthetic congener of oxymorphone with no opioid agonist properties. Naltrexone is indicated in the treatment of alcohol dependence and for the blockade of the effects of exogenously administered opioids. It markedly attenuates or completely blocks, reversibly, the subjective effects of intravenously administered opioids. When co-administered with morphine, on a chronic basis, naltrexone blocks the physical dependence to morphine, heroin and other opioids. In subjects physically dependent on opioids, naltrexone will precipitate withdrawal symptomatology.

Oxycodone-Oxycodone is a semisynthetic derivative of codeine that acts as a narcotic analgesic more potent and addicting than codeine. An extended-release (ER) form of oxycodone (Xtampza ER) was approved for the management of daily, around-the-clock pain management in April, 2016. Oxycodone acts as a weak agonist at mu, kappa, and delta opioid receptors within the central nervous system (CNS). Oxycodone primarily affects mu-type opioid receptors, which are coupled with G-protein receptors and function as modulators, both positive and negative, of synaptic transmission via G-proteins that activate effector proteins. Binding of the opiate stimulates the exchange of GTP for GDP on the G-protein complex. As the effector system is adenylate cyclase and cAMP located at the inner surface of the plasma membrane, opioids decrease intracellular cAMP by inhibiting adenylate cyclase. Subsequently, the release of nociceptive neurotransmitters such as substance P, GABA, dopamine, acetylcholine, and noradrenaline is inhibited. Opioids such as oxycodone also inhibit the release of vasopressin, somatostatin, insulin, and glucagon. Opioids close N-type voltage-operated calcium channels (kappa-receptor agonist) and open calcium-dependent inwardly rectifying potassium channels (mu and delta receptor agonist). This results in hyperpolarization and reduced neuronal excitability. Oxycodone, a semisynthetic opiate agonist derived from the opioid alkaloid, thebaine, is similar to other phenanthrene derivatives such as hydrocodone and morphine. Oxycodone is available in combination with aspirin or acetaminophen to control pain and restless leg and Tourette syndromes (6-8).

MATERIALS AND METHODS (9-11)

Determination Of Working Wavelength (λmax)

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.

Preparation of standard stock solution of naltrexone

10 mg of NALTREXONEwas weighed and transferred in to 100ml volumetric flask and dissolved in water and then make up to the mark with water and prepare 100µg /ml of solution by diluting 1.0ml to 10ml with water.

Preparation of standard stock solution of oxycodone

5 mg of OXYCODONE was weighed in to 100ml volumetric flask and dissolved in water and then dilute up to the mark with water and prepare 50 µg /ml of solution by diluting 0.5ml to 10ml with water.

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 10mg of naltrexone and 10 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 10 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 (µg/ml) were prepared by dissolving weight equivalent to 10 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 10µg/ml of oxycodone and Naltrexonewas made by adding 1 ml of stock solution to 10 ml of mobile phase.

RESULTS AND DISCUSSION

The amount of naltrexone and oxycodone present in the taken dosage form was found to be 100.11 % and 100.09 % respectively (Table-1 and fig-1)

Table-1 Assay Results

	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	

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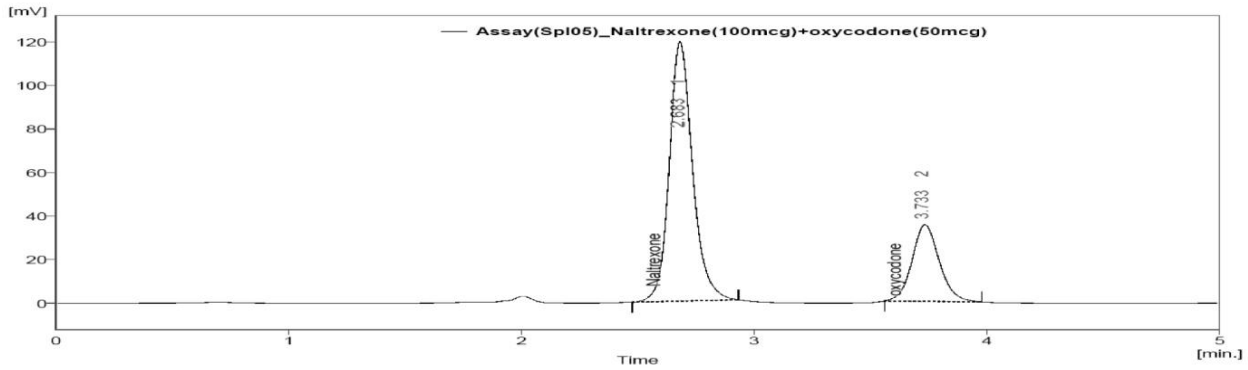

Fig-1 Chromatogram of Assay sample

The % RSD for the retention times and peak area of naltrexone and oxycodone were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit (Table-2 and 3).

Table-2 Results for system suitability of naltrexone

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.673	820.419	3708	1.259
2	2.673	830.419	3708	1.259
3	2.687	821.688	3745	1.308
4	2.683	832.647	3736	1.259
5	2.693	843.524	3532	1.333
6	2.707	838.437	3801	1.370
Mean	2.6860	831.189	-	-
SD	0.0129	9.096	-	-
%RSD	**0.48**	**1.09**	-	-

Table-3 Results for system suitability of oxycodone

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	3.717	296.026	1.143	4770
2	3.717	298.026	1.143	4770
3	3.733	292.016	1.118	5076
4	3.727	298.483	1.147	5058
5	3.740	294.746	1.147	4830
6	3.757	289.026	1.147	5140
Mean	3.732	294.721	-	-
SD	0.015	3.648	-	-
%RSD	**0.41**	**1.24**	-	-

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of naltrexone and oxycodone is 0.999 and 0.996. 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 limits (Fig-2 and 3).

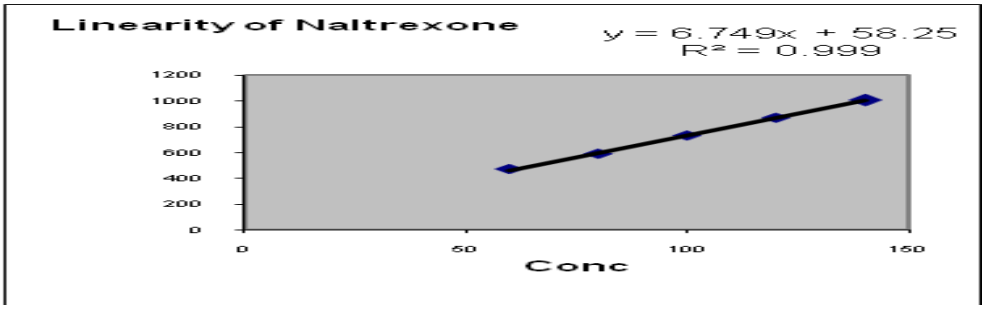

Figure-2 Linearity graph of naltrexone

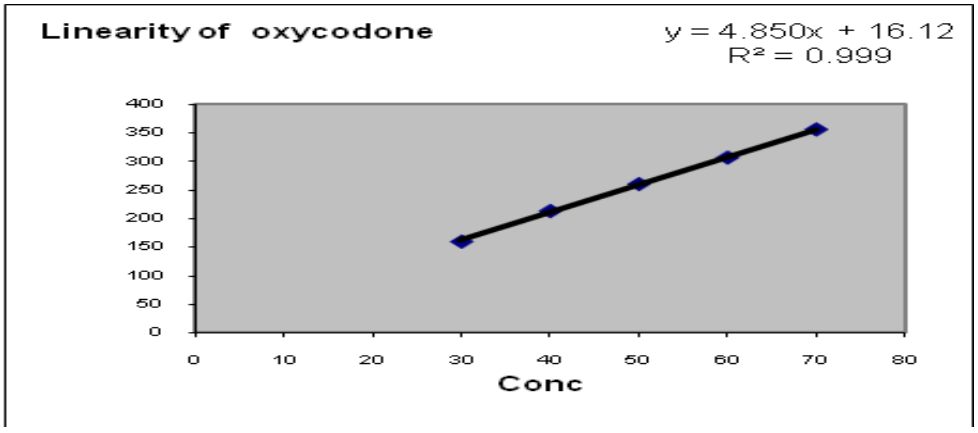

Figure-3 Linearity graph of oxycodone

The percentage mean recovery of naltrexone and oxycodone is 100.43% and 99.85% respectively.
From the observation it was found that the system suitability parameters were within limit at all variable conditions (Table-4).

Table-4 Result of Robustness study

Parameter		Naltrexone		Oxycodone	
		Retention time(min)	**Tailing factor**	**Retention time(min)**	**Tailing factor**
Flow Rate	**0.8 ml/min**	3.363	1.333	4.660	1.171
	1.2 ml/min	2.597	1.391	3.490	1.167
Wavelength	**210nm**	2.710	1.241	3.770	1.111
	214nm	2.727	1.286	3.787	1.111

From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged (Table-5).

Table-5 Results for Ruggedness

Naltrexone	%Assay	Oxycodone	%Assay
Analyst 01	100.1	**Analyst 01**	98.9
Anaylst 02	99.5	**Analyst 02**	100.6

CONCLUSIONFrom the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation Naltrexone and Oxycodone was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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