Forced degradation studies of new formulation containing naltrexone

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Abstract

Naltrexone is one of the classical opioid antagonists. In substantially lower than standard doses, they apply different pharmacodynamics. A daily dose of 1 to 5 mg is considered as low-dose naltrexone (LDN). Clinical reports of LDN have demonstrated its possible benefits in diseases such as fibromyalgia, Crohn's disease, multiple sclerosis, complex-regional pain syndrome, Hailey-Hailey disease, and cancer.

The aim of the present study was to establish the inherent stability of naltrexone and stability indicating assay method for simultaneous determination of naltrexone after being subjected to acidic and basic stress.

A forced degradation study of naltrexone in its tablet form was conducted under the acidic and basic conditions in order to develop a rapid and sensitive stability indicating UV-Visible method for the quantification of naltrexone. Quantification was achieved by UV detection at 286.60 nm, on the basis of peak area. Naltrexone was found to be unstable and degraded in the acidic and basic buffer up to 3 hours.

Keywords

Forced degradation, low dose naltrexone, naltrexone, stress condition.

INTRODUCTION

Addiction is one of the well-known chronic brain diseases (Volkow, 2004). One of the common sources of addiction is opioids, which have been known to cause both medical and social problems. There are currently over half a million people addicted to opioids receiving treatment in Europe and America according to the European Drug Report.

The sixth most common cause of premature death and illness in high-income countries is alcohol (Lee *et al.,* 2016). The term Alcohol Use Disorder (AUD) is used to describe the progression of the addiction and varies in terms of intensity, frequency, and symptoms.

While the most effective treatment for the AUD is abstinence, the majority of patients with alcohol addiction are not able to avoid drinking, thus making it necessary to develop effective alternative therapies.

Nowadays, naltrexone is one of the more common drugs used in the treatment of alcohol and opiate dependence (Modesto-Lowe, 2002). Naltrexone is an oral opioid antagonist which is more closely related to the μ-receptor relative to its affinity for the the δ- and κ-receptors. It is used in the treatment of chronic substance abuse (Brown, 2001).

Naltrexone has been found to effectively block and even reverse the effects of opioids with lower affinity reversible agonists like heroin and methadone and is also used to treat instances of alcohol dependence.

Naltrexone hydrochloride (NLX) is divided into low dose naltrexone (LDN) and full dose naltrexone. In many countries, access to full dose naltrexone is possible by the prescription of either 25 mg or 50 mg oral tablets. 50 mg tablet effectively blocks the effects of heroin for up to 24 hours. Conversely, LDN is a reversible competitive antagonist available in 1.75 mg and 4.5 mg doses that works by temporarily blocking the opioid receptors in the brain and subsequently increasing the production of endorphins through a positive feedback mechanism. The increase in production raises the level of endorphins as well as the level of encephalin in the body.

LDN is an opioid antagonist that blocks the reception of both exogenous opiates and endogenous opioids (endorphins). However, its administration in only small doses results in relatively short lived endorphin-block lasting somewhere between 3-4 hours. In consequence, endorphin deficit in the body causes the hypothalamus to signal for an increase in endorphin production. This is known as "the rebound effect".

Chemical stability of pharmaceutical molecules is a matter of great concern as it affects the safety and efficacy of the drug product. The degree to which a drug maintains its characteristics and properties at the time it was manufactured over the period of storage and use within specific limits is known as drug stability. Overall drug stability is typically divided into different specific kinds which are physical, chemical, therapeutic, microbiological, and toxicological.

Drug stability is further divided into two categories: pre-market stability and commercial stability. Pre-market stability is determined using clinical trials in which the drug is subjected to various conditions as a way to evaluate its safety and efficacy during both the clinical trial and filing period. On the other hand, commercial stability refers to the monitoring of the long-term stability of post-approval batches of the drug. The evaluation of drug stability is usually done by testing the drug product or substance using a stability-indicating method to determine the retest period (in the case of pre-market stability) and shelf life (in the case of commercial stability).

Forced degradation studies are an integral step in designing a stability program for drug products and substances that comply with regulatory standards. Such studies involve forcefully degrading the drug product/substance using conditions that are

severe and regularly accelerated, thus resulting in degradation products best suited for the study of molecular stability. As such, forced degradation is an essential stability testing that use more stringent conditions than those found in speeding up testing (Klick, 2005). Forced degradation studies are useful for the development and regulation of drug products as they aid the identification of potential degradation products. Moreover, they provide data for prediction of the degradation pathway, the efficacy of stability-indicating methods, conditions not suitable for the stability of the drug, the selection of storage conditions and packaging material(s) (Alsante, 2007). There are a number of similarities between forced degradation and preformulation degradation studies, both of which are useful in supporting the development of a stability-indicating method. International Conference on Harmonization (ICH) guidelines state that the purpose of stress testing is to determine the potential degradation of drug products as part of the effort to determine the molecule's intrinsic stability and validate its stability-indicating pathway. ICH guidelines contain many stipulations regarding the management of forced degradation, although these fail to outline the process of stress testing through a detailed practical approach. It is widely accepted that stress tests can be useful for the secondary analysis of drug products

before they are entered into the registration dossier, despite the fact that they are not considered essential in formal stability testing studies. Stress testing is an integral part of the preformulation assessment of the stability of a drug candidate in which stress is intentionally applied to induce degradation by adding other reactants (eg., acids, bases, and peroxides), exposing materials to compressive or sheer forces (eg., solid-state physical stability), increasing humidity when relevant (eg., solid-state chemical and physical stability), raising temperature, subjecting the test materials to various pH conditions or intense ultraviolet (UV) and visible light (eg., photo stability). By making the drug more susceptible to degradation, forced degradation provides a valuable guide for the selection of storage conditions.

Furthermore, it also helps to determine the degradation pathway of the drug, distinguish between drug-related and non-drug related degradation in the formulation, identify the degradation product structure, and evaluate the intrinsic stability of the drug. Forced degradation studies are also useful for generating degradants in a short span of time (typically a few weeks). The samples they generate are useful for the development of a stability-indicating method to be utilized in later analyses of samples generated by studies on accelerated and long-term stability.

The aim of the study was to establish the inherent stability and stability indicating assay method for simultaneous determination of naltrexone after being subjected to stress conditions, such as acidic and basic stress.

MATERIALS AND METHODS

The materials used in this study include: Naltrexone HCl reference standard, distilled water, sodium hydroxide (NaOH), hydrochloric acid (HCl). Naltrexone HCl reference standard was obtained from United States Pharmacopeia (USP) (Lot: 120449). NaOH and HCl were purchased from Merck.

Preparation of standard solution

Standard solution was prepared by dissolving approximately 6.0 mg of NLX reference standard in 25 mL of distilled water to get a concentration of 0.24 mg/mL.

Preparation of sample stock solution of capsules containing 3 mg NLX (stock-1)

Contents of 4 capsules were unlocked and weighted accurately. The content was dispersed in distilled water using a 25 mL

volumetric flask. The dispersion was kept in the ultrasonic bath (Selecta Ultrasound H-D) for 30 minutes at 25 ^{0}C. The volume was completed to 25 mL by using distilled water and then filtered through a quantitative cellulose filter (Millipore Millex- HN, Nylon 0.45 µm). Finally, the sample was placed in a UV-Vis spectrophotometer. All absorbance measurements were obtained in a quartz cuvette (1 cm optical path length), from 200-700 nm even though the wavelength of naltrexone was 280.65 nm.

Preparation of sample solution of capsules containing 3 mg NLX (sample-1)

Sample solution was prepared using sample stock solution (stock-1). 2.5 mL stock-1 solution was taken into 5 mL volumetric flask and diluted to 5 mL with distilled water to obtain a concentration of 0.24 mg/mL.

Preparation of sample stock solution of capsules containing 4.5 mg NLX (stock-2)

Contents of 3 capsules were unlocked and weighted accurately. The content was dispersed in distilled water using a 25 mL volumetric flask. The dispersion was kept in the ultrasonic bath (Selecta Ultrasound H-D) for 30 minutes at 25 ^{0}C. The volume was completed to 25 mL by using distilled water and then filtered through a quantitative cellulose filter (Millipore Millex- HN, Nylon 0.45 µm). Finally, the sample was

placed in a UV-Vis spectrophotometer. All absorbance measurements were obtained in a quartz cuvette (1 cm optical path length), from 200-700 nm even though the wavelength of naltrexone was stipulated to about 280.65 nm.

Preparation of sample solution of capsules containing 4.5 mg NLX (sample-2)

Sample solution was prepared using sample stock solution (stock-2). 2.5 mL stock-2 solution was taken into 5 mL volumetric flask and diluted to 5 mL with distilled water to obtain a concentration of 0.24 mg/mL.

The absorbances of the standard, sample-1 and sample-2 solutions were measured by UV-spectrophotometer. The assay amounts of these solutions were calculated according to the UV absorbance results.

Force Degradation Study

Forced degradation of NLX solutions was carried out under acid/base hydrolysis. 0.1 N HCl and 0.1 N NaOH solutions were prepared for the force degradation method.

Preparation of sample solution of capsules containing 3 mg and 4.5 mg NLX via acid induced hydrolysis (sample-3 and sample-4)

For the preparation of capsule solutions containing 3 mg and 4.5 mg NLX, 2.5 mL of the sample stock of each NLX (stock-1 and stock-2) were taken into 5 mL volumetric flask separately, and 0.5 mL

acid was added. The solutions were stirred for 3 hours on the magnetic stirrer by protecting from light. After 3 hours, the solutions were neutralized with 0.5 mL base. The volume was completed to 5 mL by using distilled water and then filtered through a quantitative cellulose filter (Millipore Millex- HN, Nylon 0.45 µm). Finally, the absorbance was measured by UV-Vis spectrophotometer.

Preparation of sample solution of capsules containing 3 mg and 4.5 mg NLX via base induced hydrolysis (sample-5 and sample-6)

For the preparation of capsule solutions containing 3 mg and 4.5 mg NLX, 2.5 mL of the sample stock of each NLX (stock-1 and stock-2) were taken into 5 mL volumetric flask separately, and 0.5 mL base was added. The solutions were stirred for 3 hours on the magnetic stirrer by protecting from light. After 3 hours, the solutions were neutralized with 0.5 mL acid. The volume was completed to 5 mL by using distilled water and then filtered through a quantitative cellulose filter (Millipore Millex- HN, Nylon 0.45 µm). Finally, the absorbance was measured by UV-Vis spectrophotometer.

The absorbances of the sample-3, sample-4, sample-5 and sample-6 solutions were measured by UV-spectrophotometer. The assay amounts of these solutions were calculated according to the UV absorbance results.

RESULTS AND DISCUSSION

The UV absorbance values of NLX standard, capsules containing 3 mg and 4.5 mg NLX are shown in Table 1. The naltrexone peak appeared at 280.65 nm.

The aborbance values of the standard, capsules containing 3 mg and 4.5 mg NLX solutions were 0.85139, 0.83068 and 0.83173, respectively.

Table 1. The UV absorbance results of NLX standard and drugs containing 3 mg and 4.5 mg NLX.

Type of the solution	Concentration (mg/mL)	Wavelength (nm)	Absorbance
Standard of naltrexone	0.24	280.65	0.85139
Capsule containing 3 mg naltrexone	0.23	280.65	0.83068
Capsule containing 4.5 mg naltrexone	0.23	280.65	0.83173

The UV absorbances of the sample-3, sample-4, sample-5 and sample-6 solutions are shown in Table 2. The assay amounts of the solutions calculated according to the UV absorbance results are given in Table-3.

Table 2. The absorbances of the solutions 3 mg and 4.5 mg naltrexone with acid and base.

Type of the solution	Wavelength (nm)	Absorbance
Capsule containing 3 mg naltrexone with acid (sample-3)	280.65	0.70930
Capsule containing 3 mg naltrexone with base (sample-4)	280.65	0.75815
Capsule containing 4.5 mg naltrexone with acid (sample-5)	280.65	0.72241
Capsule containing 4.5 mg naltrexone with base (sample-6)	280.65	0.73349

**Table 3**. Assay analysis before and after forced degradation.

Type of the solution	Assay (%)
Capsule containing 3 mg naltrexone	101.81
Capsule containing 4.5 mg naltrexone	101.93
Capsule containing 3 mg naltrexone with acid	86.89
Capsule containing 3 mg naltrexone with base	92.92
Capsule containing 4.5 mg naltrexone with acid	88.54
Capsule containing 4.5 mg naltrexone with base	89.90

It is obviously clear that the treatment of NLX drugs with acid or base decreases the assay amounts of the NLX capsules. The degraded amounts are shown in Table 4.

Table 4. Summary of forced degradation study of naltrexone drug.

Stress condition	Time (h)	Assay for the capsule containing 3 mg NLX (%)	Assay for the capsule containing 4.5 mg NLX (%)	Remark
Acid hydrolysis	3	86.89	88.54	Degradation observed
Base hydrolysis	3	92.92	89.90	Degradation observed

UV spectra of the standard and all samples of naltrexone are given in Figure 1.
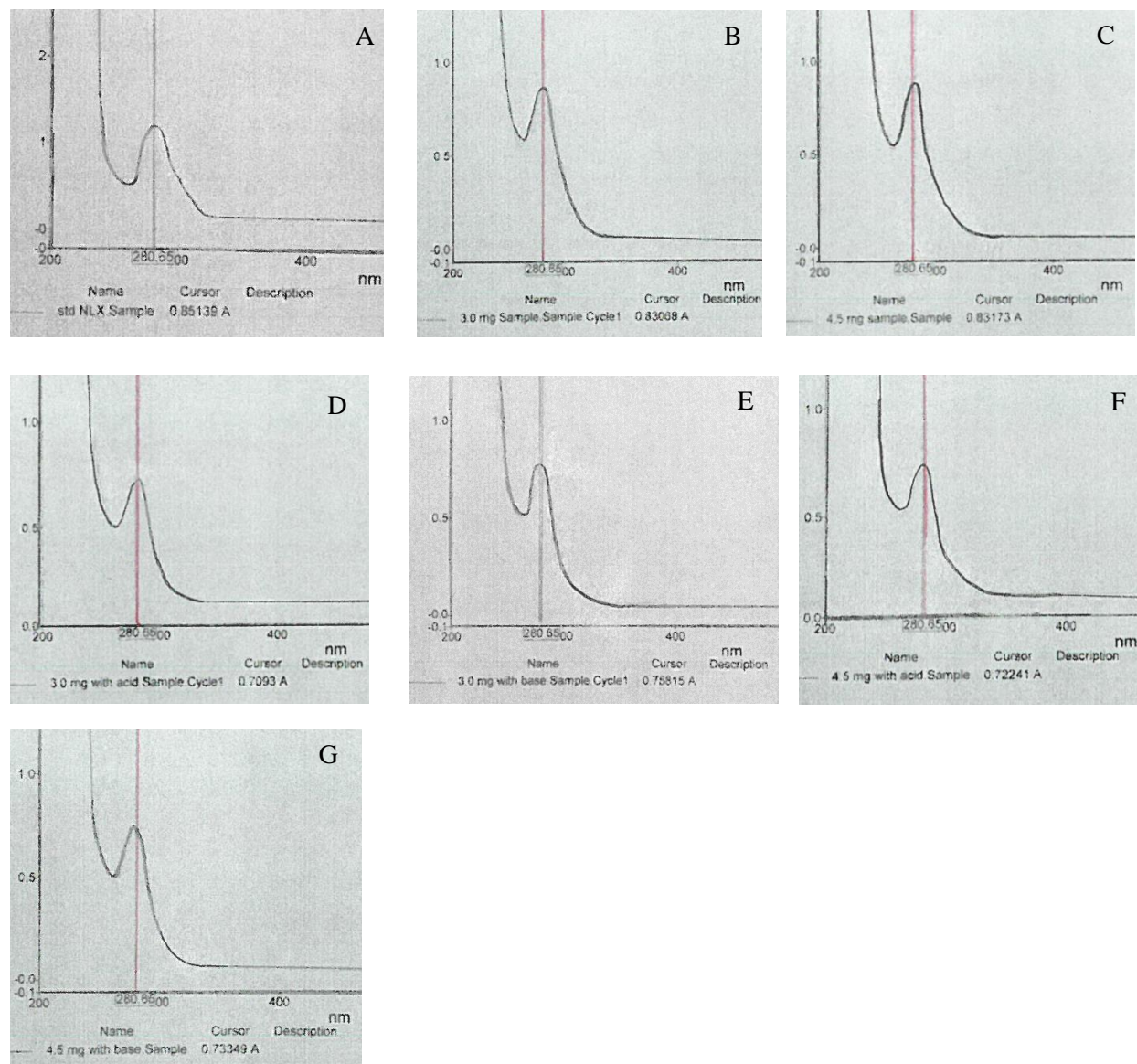
Figure 1. The UV spectra of NLX standard and NLX samples. **A)** The peak of standard of naltrexone **B)** The peak of capsule containing 3 mg naltrexone **C)** The peak of capsule containing 4.5 mg naltrexone **D)** The peak of capsule containing 3 mg naltrexone with acid **E)** The peak of capsule containing 3 mg naltrexone with base **F)** The peak of capsule containing 4.5 mg naltrexone with acid **G)** The peak of capsule containing 4.5 mg naltrexone with base.

Forced degradation studies can help to understand the degradation pathways. Moreover, it determines the active ingredients in the drug and it helps to clarify the structure of the degradants. The information gained from the stability analysis can be helpful for improving the formulation, manufacturing, and storage of the drug. In order to have adequate time to get more information about the stability of the molecule, it is better to start degradation studies earlier in the drug development process.

The aim of this experiment was to compare the assay analysis before and after acidic and basic forced degradation reaction. As it was shown, degradation was observed for naltrexone sample during acidic and basic stress conditions. Naltrexone was degraded in acidic and basic buffer. For acidic degradation of 3 mg naltrexone, the amount decreased from 101.81% to 86.89% and for 4.5 mg naltrexone, the amount decreased from 101.93% to 88.54%. On the other hand, for basic degradation of 3 mg naltrexone, the amount decreased from 101.81% to 92.92% and for 4.5 mg naltrexone, the amount decreased from 101.93% to 89.90%.

REFERENCES

Alsante M, Ando A, Brown R, Ensing J, Hatajik TD, Kong W, Tsuda Y (2007). The role of degradant profiling in active pharmaceutical ingredients and drug products. *Adv Drug Deliv Rev* **59**(1): 29-37.

Brown SA, D'Amico EJ, McCarthy DM, Tapert SF (2001). Four year outcomes from adolescent alcohol and drug treatment. *J Stud Alcohol* **62**: 381–388.

Klick S, Muijselaar PG, Waterval JCM, Eichinger T, Korn C, Gerding KT, Debets AJ, Griend CS, Beld C, Somsen GW, De Jong GJ (2005). Toward a generic approach for : Stress testing of drug substances and drug products. *Pharm Technol* **29**(2): 48-66.

Lee JD, Friedmann PD, Kinlock TW, Nunes EV, Boney TY, Hoskinson RA, Wilson D, McDonald R, Rotrosen J, Gourevitch MN, Gordon M, Fishman M, Chen DT, Bonnie RJ, Cornish JW, Murphy SM, O'Brien CP (2016). Extended-Release Naltrexone to Prevent Opioid Relapse in Criminal Justice Offenders. *N Engl J Med* **374**(13): 1232–1242.

Modesto-Lowe V, Van Kirk J (2002). Clinical uses of naltrexone: A review of the evidence. *Exp Clin Psychopharmacol* **10**(3): 213-227.

Volkow ND, Li TK (2004). Drug addiction: the neurobiology of behaviour gone awry. *Nat Rev Neurosci* **5**(12): 963-970.