Study of Forced Degradation Behavior of Idrocilamide and Development of Stability Indicating LC Method

O. M. El-Houssini
National Organization for Drug Control and Research, Cairo, Egypt 51 Wezaret EL-Zerah st, Agouza, Giza, P.O. box 12553 Egypt
e-mail: olamha23@hotmail.com
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Abstract—The objective of this study was to report the stability profile of novel muscle relaxant drug idrocilamide (Idr) based on information obtained from forced degradation studies. The drug was subjected to acidic (1 M HCl) and alkaline (1 M NaOH) hydrolysis and oxidative decomposition (50% H2O2). The products formed under different stress conditions were investigated by LC. The LC method was fine tuned using the samples generated from forced degradation studies. Satisfactory resolution between peaks with the shortest possible analysis time was achieved on C18 5 µm column (Luna, Phenomenex, USA), with mobile phase methanol–acetonitrile–water–glacial acetic acid (25 : 30 : 44 : 1, v : v : v : v), pumped at 1 mL/min flow rate. Quantification was achieved at 280 nm based on peak area, using DAD detector. The proposed LC method was utilized to investigate the accelerated oxidative degradation of Idr. Besides, Idr’s degradants were identified using IR and MS, and the possible degradation pathway was outlined. The proposed method was validated, and the forced degradation studies proved the stability indicating power of the method. The method was also applied to analyze commercial samples.

Keywords: idrocilamide, forced degradation studies, accelerated oxidation, stability indicating method
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Idrocilamide, N-(2-hydroxyethyl)-3-phenyl-2-propanamide, N-(2-hydroxyethyl)cinnamamide [1] is centrally acting muscle relaxant. It is reported to have local muscle relaxant and anti-inflammatory effects and is now mainly used topically [2]. Few methods have been reported for Idr determination: HPLC determination in rabbit plasma with pharmacokinetic study [3] and percutaneous penetration capacity and HPLC determination in human plasma [4], HPLC and TLC determination of Idr in presence of its degradants [5].

The International conference on harmonization guideline entitled stability testing of new drug substance and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance [6]. So hydrolytic and oxidative stability are required. The integral aim of the study was to postulate possible degradation pathway of the drug and to identify and determine the degradants formed. Only one method was reported to determine the drug in presence of its degradants [5].

The proposed method had the advantage over the reported method in using mild conditions in degradation process, which resulted in complete degradation of the drug. Besides the chosen chromatographic conditions gave the best resolution with minimal elution time for the drug and its degradants, which made the method the best to be used in routine work and quality control laboratories for the purity evaluation and quantitative determination of the drug and its degradants.

EXPERIMENTAL

Instrumentation. HPLC consist of Hewlett Packard series 1100 equipped with quaternary pump, diode array detector and a manual injector 20-µL loop was used. Column used was Phenomenex Luna C18 (250 × 4.6 mm, 5 µm particle size).

Reagents and samples. All solvents used were LC grade and all reagents were analytical grade. Methanol, acetonitrile and glacial acetic acid were obtained from Merck (Darmstadt, Germany). Sodium hydroxide and hydrogen peroxide (50%) were obtained from ADWIC (Egypt). Hydrochloric acid was obtained from Riedel-de Haën (Germany). High pure water was prepared by using Millipore 0.45 µm white nylon HNWP 47 mm filter. Idrocilamide of pharmaceutical grade was kindly supplied from the Egyptian Group of Pharmaceutical Industries, Cairo, Egypt (EGPI). (Its purity was found to be 100.4 ± 1.1%). Ciladro cream was manufactured by EGPI (Batch No. 200610), claimed to contain 5 g Idr per 100 g cream.

1 The article is published in the original.
Chromatography. The mobile phase was prepared by mixing methanol, acetonitrile, water, glacial acetic acid in ratio 25 : 30 : 44 : 1 (v : v : v : v). The flow rate was 1 mL/min. All determinations were performed at ambient temperature. The injection volume was 20 µL.

Preparation of acid and alkali induced degradants. Ten milligrams of Idr was accurately weighed and dissolved in 3 mL of methanol in two rounded bottle flasks. To the first flask, 20 mL of 1 M HCl and to the second 20 mL 1 M NaOH was added. The solutions were refluxed for 3.5 and 2.5 h for the acid and alkali, respectively and then cooled. Each solution was then neutralized and evaporated to dryness. The residues were dissolved in 7 mL methanol and filtered. Each solution was transferred quantitively to a 10-mL volumetric flask and the volume of each was completed with the same solvent.

Preparation of oxidative degradant. Accelerated oxidation study. Ten milligrams of Idr was accurately weighed and dissolved in 3 mL of methanol and then transferred to a 10-mL volumetric flask; 2 mL of 50% (w : v) H₂O₂ was added and the volume was completed with methanol : water (1 : 1). The solution was kept in a cold and dark place for 90 days. The degradation was monitored periodically at one week interval.

Stressed oxidation study. Ten milligrams of Idr was accurately weighed and dissolved in 3 mL of methanol in a rounded bottle flask; 2 mL of 50% (w : v) H₂O₂ was added. The solution was refluxed for 2 h and then evaporated and the residue was dissolved in 7 mL of methanol—water (1 : 1). The solution was then transferred to a 10-mL volumetric flask and the volume was completed with the same solvent.

Standard solutions and calibration. Stock standard solutions were prepared by dissolving 25 mg of Idr in 100 mL of methanol, 10 mg of acid and alkali degradants separately in 10 mL methanol and 10 mg of oxidative degradant in 10 mL of methanol—water (1 : 1). Standard solutions were prepared by diluting the stock standard solution with methanol to reach concentration ranges of 6.25—11.25, 12.5—125, 12.5—125 and 30—175 µg/mL for Idr, acid, alkali and oxidative degradants, respectively.

Triplicate 20 µL injections were made for each concentration and chromatographed under the conditions described above. The peak area of each concentration was plotted against the corresponding concentration to obtain the calibration graph.

Sample preparation. An amount of cream equivalent to 25 mg Idr, was accurately weighed and diluted to 100 mL with methanol. The sample solution was filtered. Further dilutions of the sample solution were carried out with the mobile phase to reach the linearity range specified for Idr. The general procedure described under calibration was followed and the concentration of Idr was calculated.

RESULTS AND DISCUSSION

Oxidation study investigation. For the stressed oxidation study, it was found that the drug was degraded when refluxed for 2 h with 2 mL of 50% H₂O₂. The disappearance of the drug peak (Fig. 1) in the HPLC chromatogram and the appearance of another peak at different retention time indicated the decomposition of the drug (Fig. 2).

Also the drug was left to undergo accelerated oxidation, and the degradation process was monitored periodically. The appearance of intact peak with the same retention time and shape of the peak appeared with stressed oxidation was observed. However the peak of the drug did not disappear. From this comparison it is clear that stressed oxidation conditions fastened the oxidation of the drug giving the same degradant.

Identification of degradants. When Idr was refluxed with 1 M HCl and 1 M NaOH for 3.5 and 2.5 h, dg1 was found to be the major degradation product (Figs. 3, 4). However when Idr was refluxed with 50% H₂O₂ for 2 h dg2 was found to be the degradation prod-