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ORIGINAL ARTICLE

LC and LC–MS study for simultaneous determination of tramadol hydrochloride and ketorolac tromethamine in bulk and formulation with their major degradation products

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Abstract The present work was aimed to separate, identify and characterize the major degradation products of tramadol hydrochloride and ketorolac tromethamine. A rapid, specific and accurate stability indicating reversed phase liquid chromatographic method has been developed for simultaneous determination of tramadol hydrochloride and ketorolac tromethamine in bulk and formulation. The drugs were subjected to hydrolysis (acidic, alkaline and neutral), oxidation, photolytic and thermal stress, as per ICH guidelines. The separation, identification and characterization of major stressed degradation products were performed using high performance liquid chromatography combined with quadrupole electrospray ionization mass spectroscopy (LC/ESI-MS) on a C-18 column. Tramadol hydrochloride was found to degrade in acidic and oxidative conditions while ketorolac tromethamine undergoes extensive degradation under oxidative, UV and acid hydrolysis stress. From the mass spectral data, probable structures were assigned to the degradation products. The identified major degradation product for tramadol under acid stress may be 1-(3′-methoxy-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)-N,N-dimethylmethanamine. Ketorolac tromethamine was also found to convert in to numerous degradation products under oxidative stress.

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1. Introduction

Tramadol hydrochloride (TRH) is (1*R*, 2R)-*rel*-2-[(dimethylamino)-methyl]-1-(3-methoxyphenyl) cyclohexanol.[1] It is a μ-opioid receptor agonist and centrally acting analgesic. Ketorolac tromethamine (KTM) is (±)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid, 2-amino-2-(hydroxymethyl)-1,3-propanediol[2] (Fig. 1). It is an analgesic and non-steroidal anti-inflammatory drug (NSAID). It acts by the inhibition of both cylooxygenase-1 (COX-1) and cylooxygenase-2 (COX-2) enzymes responsible for inflammation.

The combination formulation containing TRH and KTM is used as an analgesic for the short-term treatment of moderate to severe pain. It has higher analgesic efficacy than each of its components. Also, it has a faster onset of action and greater duration of effect.

KTM has been investigated either alone or in combination with other drugs by various methods like LC–MS/MS,[3] HPTLC,[4] LC–MS[5] and HPLC.[6–8]

Devarajan et al. have studied acid and base degradation of KTM alone using 0.5 N HCl and 0.5 N NaOH respectively with a reaction time of 10 min. HPTLC was used to separate one degradation product formed under both conditions.[4] Structure of this product was not elucidated. Salaris et al. have carried out alkali and acid degradation study under similar reaction conditions as reported by Devarajan et al.[5] They have used LC–MS for the identification of degradation products. No degradation product was reported under the experimental conditions probably due to very short reaction time.

Various techniques have been reported for the investigation of TRH which includes UV[9] and HPLC.[9–15] Only two reports exist involving the study of compatibility and stability of binary mixtures of TRH and KTM injection concentrate and diluted infusion using HPLC.[16,17] LC–MS is a sensitive technique used for the identification of other analytes in the presence of their degradation products.[18–21]

No study so far has been reported on characterization of degradation products of this combination under stress conditions prescribed by ICH Q1A(R2).[22] It is difficult to investigate multicomponent formulation along with degradation products. The challenge is to separate the drugs from the number of degradation products generated. This paper describes HPLC–UV and HPLC–MS method to determine TRH and KTM simultaneously in the presence of their degradation products. Relative stability of both drugs under various stress conditions has been assessed and identification of major degradation products has been performed with the help mass spectroscopy.

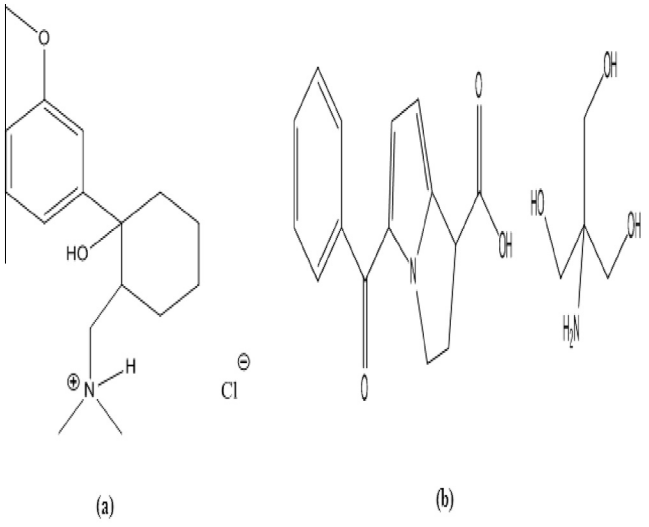

Figure 1 Structures of (a) TRH, (b) KTM.

2. Experimental

2.1. Chemicals and reagents

Pure TRH was obtained as a gift sample from Alkem Laboratories, Gujarat. KTM was provided as a gift sample by Dr. Reddy's Laboratory, Hyderabad. Methanol, acetonitrile and water were of HPLC grade (Merck, India). Formic acid (AR) was used for pH adjustment. Analytical reagent grade hydrochloric acid, sodium hydroxide and hydrogen peroxide used in the present study were purchased from S.D. Fine Chemicals (Mumbai, India). Voydol-C capsules manufactured by RAAM Laboratories were used for analysis. Each capsule contains 25 mg of TRH and 10 mg of KTM.

2.2. Instrumentation

2.2.1. HPLC–UV specifications

Dionex Ultimate 3000 HPLC (UV detection simultaneous determination at four wavelengths) with chromeleon software version 6.8 SR 10 build 2818 equipped with column oven and autosampler was used. Detection wavelength was set at 270 nm.

2.2.2. HPLC–MS specifications and chromatographic conditions

Dionex Ultimate 3000 HPLC (UV detection simultaneous determination at four wavelengths) with chromeleon software version 6.8 SR 10 build 2818 equipped with column oven, online degasser and autosampler was used. Applied biosystems MS (MDS SCIEX, 4000 Q TRAP) equipped with Turbo V electrospray ionization source (ESI) and analyst version 1.4.2 software were used for data acquisition and processing. The samples were infused into the mass spectrophotometer from HPLC system through ESI interface. The optimized parameters are given in Table 1. The samples were separated on a Neosphere C-18 column (4.6 internal diameter, 250 mm length, 5 μm particle size, Hexon Laboratories Pvt. Ltd.). The mobile phase composition was water: methanol: acetonitrile (53:23:24, v/v/v) with 0.5% of formic acid. The mobile phase flow rate was 1.0 ml/min and the detection wavelength was 270 nm. Injection volume was 25 μl.

Other equipments used were hot air oven (Dolphin, PPI unix96), pH meter (HANNA, HI 2211) and analytical balance (Shimadzu AUX 220, Japan).

2.3. Selection of analytical wavelength

Spectra of TRH (20 μg mL^{-1}) and KTM solution (10 μg mL^{-1}) were obtained separately using double beam UV Visible spectrophotometer (Shimadzu UV-1800).