Characterization of Impurities in Acarbose Hydrate by using LCMS/MS and NMR

Maruthi R*, Chandan R S, Anand Kumar Tengli

Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru-570015, Karnataka, India

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INTRODUCTION

Acarbose is an anti-diabetic medicine used in some countries in the treatment of type II diabetes mellitus, as well as prediabetes (Ramirez and Borja, 2008; Steele et al., 1993; Okayama et al., 2002). This gradually produces certain enzymes that break food down into sugars. The chemical substances for Acarbose [Figure 1] (3R,4R,5S,6R)-5-[(2R,3R,4R,5S,5S,6R)-6-(hydroxymethyl)-oxy-3,4-dihydroxy-6-(hydroxy-2-yl)oxy-6-(hydroxy-methyl)-oxide-6-(hydroxymethyl)-3,4-try] oxy-2,3,4-trio. It inhibits the antihyperglycemic activity of alpha-glucosidase as well as pancreatic alpha-amylase. Acarbose binds and inhibits enteric incisions found on the edge of the small intestine brushing with alpha-glucosidase. Hydrated in glucose and other monosaccharides, it prevents large carbohydrates from breaking down into glucose and reduces the postprandial blood levels of blood glucose. AC also hydrolyzes complex starch to oligosaccharides in the small intestine and inhibits alpha-amylase. The most common side effects are stomach pain, diarrhea and flatulence and Acarbose is available in oral tablet form (Clissold and Edwards, 1988; Wang et al., 2011).

Acarbose is a pseudotetrasaccharide and inhibitor of alpha-glucosidase and pancreatic alpha-amylase with antihyperglycemic activity. Acarbose binds to and inhibits alpha-glucosidase, an enteric enzyme found in the brush border of the small intestines that hydrolyzes oligosaccharides and disaccharides into glucose and other monosaccharides. This prevents the breakdown of larger carbohydrates into glucose...
and decreases the rise in postprandial blood glucose levels. In addition, Acarbose inhibits pancreatic alpha-amylase, which hydrolyzes complex starches to oligosaccharides in the small intestines (PubChem, 2021). The antihyperglycemic action of acarbose results from competitive, reversible inhibition of pancreatic alpha-amylase and membrane-bound intestinal alpha-glucoside hydrolase enzymes. Pancreatic alpha-amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine, while the membrane-bound intestinal alpha-glucosidase hydrolyze oligosaccharides, trisaccharides, and disaccharides to glucose and other monosaccharides in the brush border of the small intestine. In diabetic patients, this enzyme inhibition results in delayed glucose absorption and a lowering of postprandial hyperglycemia.

**Figure 1: Structure of Acarbose**

**Figure 2: HPLC Spectrum of Acarbose**

Because its mechanism of action is different, the effect of Acarbose to enhance glycemic control is additive to that of sulfonylureas, insulin or metformin when used in combination. In addition, Acarbose diminishes the insulinotropic and weight-increasing effects of sulfonylureas. Acarbose has no inhibitory activity against lactase and consequently would not be expected to induce lactose intolerance.

**MATERIALS AND METHODS**

**Experimental**

**Figure 3: LCMS/MS Spectra of Acarbose**

**Figure 4: LCMS/MS spectra of IMP B**

**Figure 5: Structure of IMP B**

**Figure 6: LCMS/MS spectra of IMP D**
Chemicals and Reagents

TCI Chemicals Pvt Ltd offers Acarbose (99 percent pure) samples. Acetonitrile grade HPLC from Merck was acquired. Ammonium Acetate was purchased from Loba Chemie from analytical reagent grade. Merck has obtained DMSO.

Instrumentation

Using Shimadzu UFLC 20 AD with Photodiode array detector, chromatographic separations have been obtained. LC systems software is used for data analysis. LC solutions. Shimadzu electronic balance was used for weighing the samples, and Mark ultra sonicator was used for sonication of the samples and mobile phases. LC-MS/MS was performed by using a Shimadzu LC-MS/MS 8030 system with triple Quadra pole mass spectrometry. NMR was performed on FT NMR spectrometer system at 400MHz (Make: Agilent USA model: 400MRDD2).

HPLC Experiment

Chromatography was performed using Shimadzu LC-20AD on a Phenomenex C18 (5μ, 230* 4.6 mm), with an ambient temperature. The flow rate was 1.0 ml/min and the injection volume was 10μL. Binary gradient schematics. The mobile stage consists of an 80:20 to a pH4 ratio of acetonitrile and ammonium (Cherkaoui et al., 1998; Chaudhari and Maheshwari, 2014; Dai et al., 2010).
LCMS and LCMS/MS Experiment

LC-MS/MS was performed by using a Shimadzu LC-MS/MS 8030 system with triple Quadra pole mass spectrometry.

NMR Experiment

NMR was performed on FT NMR spectrometer system – 400MHz (Make: Agilent USA model: 400 MRDD2).

RESULTS AND DISCUSSION

At RT 39.07min, the LCMS / MS Figures 2 and 3 Spectrum of main impurities displayed peaks of m / c = 646, which is consistent with the molecular formulation C25H43NO18. And Fragmentation of Acarbose Hydrate shown in Figure 10 (Novak et al., 2005; Nirogi et al., 2013).
CONCLUSIONS

A rapid and precise LC-MS/MS and NMR method was developed for identifying impurities in Acarbose hydrate. Combination of these methods enabled a complete structural prediction of three major impurities which are present in very low levels before isolation and purification. After isolation, these impurities were subjected to NMR and structure was elucidated.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.
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