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Identification, isolation and characterization of potential process-related impurity and its degradation product in vildagliptin

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ABSTRACT

Vildagliptin is a member of a new class of oral anti-diabetic drug. One unknown impurity was identified in the range of 0.01–0.06% in different laboratory batches of vildagliptin along with known impurities by HPLC analysis. The structure of unknown impurity was proposed as (2*S*)-1-[2-[(3-hydroxyadamantan-1-yl)imino]acetyl]pyrrolidine-2-carbonitrile (Impurity-E) using LC/ESI–MS^n study. The unknown impurity was found to be unstable in diluent (H_2O:CH_3CN) and degrading into another stable impurity. The degraded stable impurity was isolated from enriched reaction crude sample by semi preparative liquid chromatography. The structure of stable impurity was established using FT-IR, NMR (^1H, ^{13}C and DEPT), 2D NMR (HSQC, HMBC and COSY) and mass spectral data as (8a*S*)-3-hydroxy-octahydropyrrolo[1,2-*a*]piperazine-1,4-dione (Impurity-F). Impurity identification, abnormal behaviour of impurity-E, isolation of impurity-F, fragmentation mechanism and structural elucidation were also discussed.

1. Introduction

In the world, the influenced people by Type 2 diabetes mellitus (T2DM) are intensely rising on day to day [1]. According to the International Diabetes Federation (IDF), 382 million people were affected by diabetes in 2013 and this will reach to 592 million by 2035 [2]. The population almost ages years or older 10% in USA [3], 8.5% in Europe [4] and 4–5% in France [5] has T2DM. Vildagliptin (LAF237, (2*S*)-1-[2-[(3-hydroxy-1-adamantyl) amino]acetyl]pyrrolidine-2-carbonitrile) **(1)** is a member of a new class of oral anti-diabetic drug developed by Novartis Pharmaceuticals Corporation. The drug was approved by the Food and Drug Administration (FDA) as a selective reversible inhibitor of dipeptidyl peptidase 4 (DPP-4) under the trade name, Galvus, on February, 2007. The drug inactivates incretin hormones such as glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), and has shown enriched postprandial glycemic control in T2DM patients [6].

Quantitative methods for the estimation of vildagliptin in pharmaceutical dosage forms [7,8], in plasma sample [9] and assay of vildagliptin using reverse phase high performance liquid chromatography (RP-HPLC) [10] have been reported. Four process related impurities (Impurity-A to -D) (Fig. 1) were disclosed in vildagliptin process [11], however, synthesis and characterization including their pharmacology evaluation has been done by Hughes et al. [12]. To date, liquid chromatographic methods for identification of impurities in vildagliptin are not found in the literature. It is well-known that process related impurities formed in API are making critical issues in pharmaceutical industry and can demonstrate promising impact on quality and safety of the drug product. Therefore, impurity profiling study, even the level of impurities below to 0.05% in API, has attained central attention [13]. The knowledge for guiding impurity profiling has been growing rapidly using chromatographic, spectroscopic and spectrometric techniques [14–16].

Our group recently has developed process for the production of vildagliptin by following Thippannachar et al. route with slight modifications [11]. The key steps (The last two steps of our route): step-(i) the reaction of (2*S*)-1-(chloroacetyl) pyrrolidine-2-carbonitrile with 3-amino-1-adamantanol was directly related to the formation of reported impurities-A to -D and step-(ii) purification of crude vildagliptin with methyl ethyl ketone was the cause for unknown process related impurity (Fig. 1). In addition, unknown impurity demonstrated abnormal behavior in diluent (ACN:H_2O) and degraded into another new product, which is formed by hydrol-

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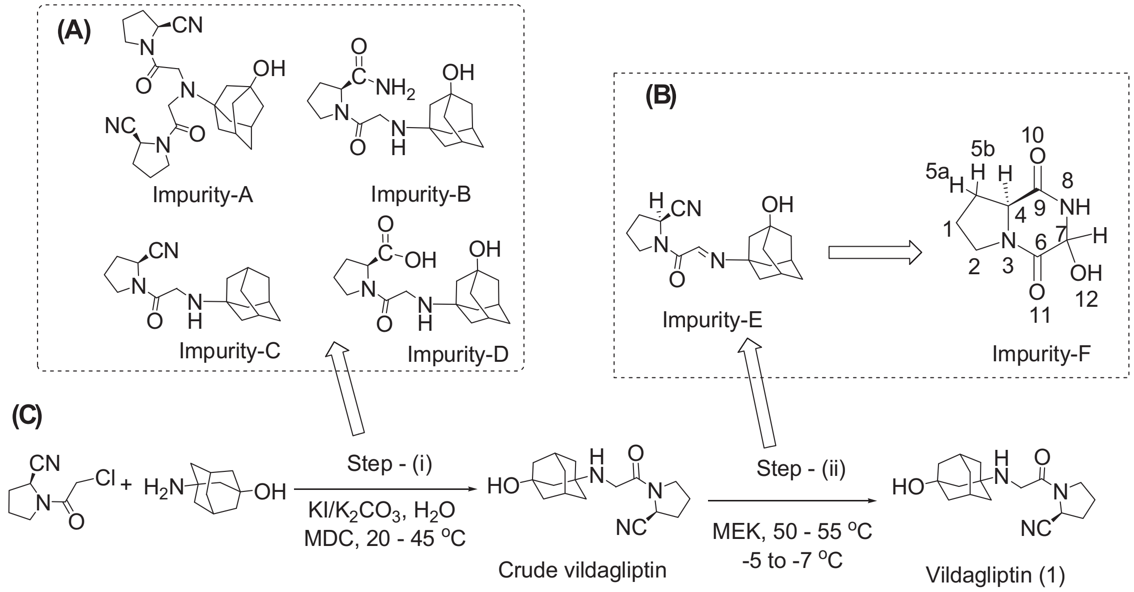

Fig. 1. (A) Chemical structures of reported impurities-A to -D, (B) Chemical structures of new impurity-E and its degradation impurity-F, (C) Last two synthetic key steps in our process and cause for formation of process-related impurities.

ysis and intramolecular cyclization simultaneously. In this context, a comprehensive study has been under taken to identify, postulate the structure of unknown impurity present in different laboratory batches, isolation and characterization of degradation product using suitable spectrometric and spectroscopic analysis.

2. Experimental part

2.1. Samples, reagents and chemicals

The sample of different vildagliptin batches (Key steps) and vildagliptin (Reference standard purity 99.92%) were obtained from Chemical Research Division, Micro Labs Ltd. (Bangalore, India). HPLC grade acetonitrile, methanol and acetic acid were purchased from Spectrochem Pvt Ltd. (Bangalore, India), sodium dihydrogen phosphate monohydrate from Sigma–Aldrich (Bangalore, India) and analytical reagent grade sodium hydroxide were procured from S D Fine-Chem Limited (Chennai, India). The commercial grade methyl ethyl ketone (MEK) was purchased from Taiwan Fieldrich Corporation (Taipei, Taiwan). Purified water by Milli Q plus purification system from Millipore (Bradford, PA, USA) was used during the course of experimental studies. DMSO-d_6 and D_2O were purchased from Cambridge isotope laboratories, Inc. (Andover, MA, USA).

2.2. Preparations of standards and sample solutions

The standard vildagliptin solution (Reference standard purity >99.3%) was prepared by dissolved in diluent, a mixture of acetonitrile-water (10:90 v/v) to obtain standard stock solution (0.2 mg/ml). The system stock solution of the standard was prepared by dissolving weighted impurities (3.0 mg) using diluent (10 ml). The system suitability test solution was made by mixing vildagliptin (30.0 mg) and 1.0 ml of system suitability stock solution in diluent (10 ml). The vildagliptin test samples were prepared by accurately weighted (150.0 mg) into a 10 ml volumetric flask, charged diluent (6.0 ml), attained the homogeneous solution with ultrasonic bath by agitation for 2–4 min. Later, filled the volumetric flask up to mark by diluent and cooled to RT.

2.3. High performance liquid chromatography instrumentation method

A Shimadzu, Nexera-X2 model ultra high performance liquid chromatography technique (UHPLC) (Shimadzu Corporation, Kyoto, Japan) equipped with a photodiode array detector (SPD 20A) and SPD M30 dual wavelength absorbance detector was used. The Empower software was utilized for monitoring the processing, data acquisition and system control. The test sample solutions were made with diluent. The sample solutions for analysis were injected on to a reverse phase Purospher® STAR RP-18e column with 150 mm × 4.6 mm i.d. dimension 3.0 μm particle size (Merck Millipore, Darmstadt, Germany) by maintaining temperature at 45 °C. The gradient elution employed solution A and B as mobile phase components. The solvent-A was 10 mM sodium dihydrogen phosphate monohydrate in water; pH was adjusted to 7.0 using 2.0 M sodium hydroxide, while solvent-B was a mixture of solvent-A and acetonitrile (7:3, v/v). The mobile phase inflated through the column with a flow rate of 1.0 ml/min and injection volume was 5 μL. The liner gradient programme was set for separation of components as follows: time/solvent-*A* (%): 0.0/95, 3.0/95, 6.0/90, 10.0/70, 12.0/55, 20.0/25, 22.0/00, 25.0/00, 26.0/95, 35.0/95 with an equilibration flow rate 0.6 ml/min. The analytes were monitored at a wavelength of 210 nm.

2.4. Liquid chromatography–mass spectrophotometric method

The ESI/MS and MS/MS experiments were performed on a Velos Pro ion trap mass spectrophotometer from Thermo Scientific (San Jose, CA, USA). Thermo X-Caliber, version 2.2, software was used for analysis of data. The instrument was operated using electrospray ionization source (positive ion mode) and the source voltage was maintained at 4.0 kV, spray current at 100.0 μA, the disolvation temperature at 200 °C and capillary temperature at 350 °C. Nitrogen gas was used for disolvation and as tube lens gas. MS/MS studies were carried out by maintaining normalized collision energy 35% with the mass range *m/z* 50-1200. The LC part consisted of Shimadzu, Nexera-X_2 (Shimadzu Corporation, Japan) HPLC equipped with photodiode array detector. LC separation was performed on Waters XBridge C-18, 250 mm × 4.6 mm, 5.0 μm (Milford, MA, USA) column and maintained the temperature at 45 °C. The mobile