Trade Science Inc.

January 2008 Volume 7 Issue 4

Analytical CHEMISTRY

An Indian Journal

Note

ACAIJ 7(4) 2008 [270-272]

Isolation, characterization and synthesis of novel impurity in antiviral drug: Valacyclovir hydrochloride

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Received: 7th January, 2007 ; Accepted: 11th January, 2007

ABSTRACT

During the process development of valacyclovir hydrochloride **(1)**, one novel impurity was detected in HPLC analysis. This impurity was isolated, synthesized and characterized as 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]ethyl-N-(acetyl)-L-valinate **(2)**, by ^{1}H NMR, IR and mass spectral data.

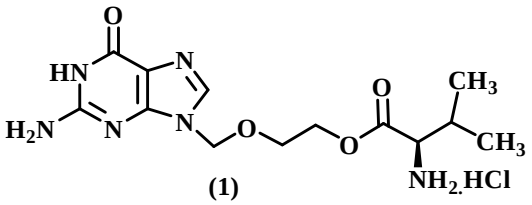
KEYWORDS

Valacyclovir;
Isolation;
Characterization;
Spectroscopy;
HPLC.

INTRODUCTION

Valacyclovir HCl **(1)**, is an active ingredient and it is very important pharmaceutically active substance widely used for the treatment of herpes zoster, genital herpes and cold sores (Herpes Labialis). It is currently available in the market as antiviral drug under the brand name of Valtrex. Although this drug is 15 years old, still it is more prescribed antiviral drug[1-4].

The analysis of Valacylcovir HCl **(1)** bulk drug revealed the presence of novel impurity, which was up to 0.1%. As per the stringent regulatory requirements the impurity profile study has to be carry out for any final product to identify and characterize the unknown impurity that are present at a level of >0.1%. This paper describes the isolation and characterization of impurity present in the bulk drug of Valacyclovir HCl **(1)**.



(1)

EXPERIMENTAL

Samples

The investigated samples were obtained from R&D laboratory of Dr. Reddy's laboratories Ltd., IPDO, C-block, Bachupally, Qutubullapur, Ranga Reddy Dist, Andhra Pradesh, India. The impurity-1 was synthesized from the same laboratory.

High performance liquid chromatography

Note

A Waters Model Alliance 2690-separation module equipped with a Waters 996-photo diode array UV detector was used. The analysis was carried out on Inertsil ODS 3V, 250×4.6×5µ.[GL sciences] with a mobile phase consisting of 3.4 g potassium dihydrogen phosphate dissolved in 1000 ml water and adjusted pH to 6.6 with triethylamine. Mobile phase-A consists buffer and methanol in the ratio of 90: 10 and Mobile phase –B consists buffer, acetonitrile and methanol in the ratio of 50: 20: 30. Program gradient elution (T/%B =0/0, 2.5/0, 15/15, 30/45, 40/90, 55/90, 57/15, 60/0, 65/0) was used with UV detection at 254 nm at a flow rate of 1.0 ml /min. The column temperature was maintained at ambient conditions. The data was recorded using waters millennium software.

NMR spectra scopy

The ^{1}H NMR was recorded on Varian 200 Gemini spectrometer using TMS as internal standard and DMSO-d_6 as solvent.

Mass spectrometry

A mass spectrum was recorded on HP5989A mass spectrometer. The sample was introduced with particle beam interface using LC and reodyne injector. The source manifold and quardrupole temperatures were maintained at 250 and 100^{0}C, respectively.

FT-IR spectroscopy

FT-IR a spectrum was recorded on Perkin-Elmer model spectrum GX series FT-IR as KBr pellet.

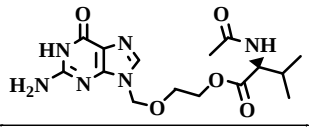
RESULTS AND DISCUSSION

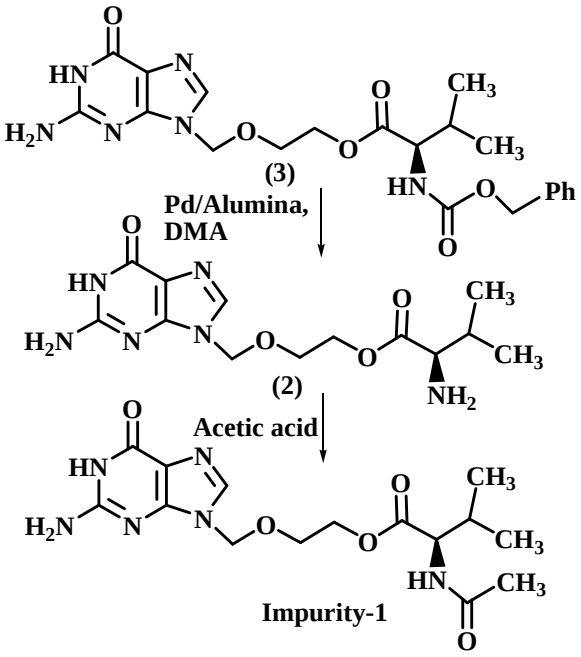
An isocratic reverse phased solvent system was used for the isolation of Impurity-1. All fractions of Impurity-1 was isolated, concentrated and extracted with chloroform, the isolated solid obtained from the concentrated fractions was used to generate spectral data. The detail of the elucidation of structure of this Impurity-1 is presented in the following sections.

Detection of impurity

A typical LC-chromatogram of Valacyclovir HCl bulk drug was recorded using the LC–method. The target impurity was marked as Impurity-1 and retention time and structure are shown in TABLE 1.

TABLE 1

S. no.	Retention time	Compound	Structure	Nature
1.	1.2	Impurity-1		Process related



SCHEME 1: Synthetic scheme of N-acetyl impurity

Isolation of impurity by preparative HPLC

A Waters delta 4000 preparative chromatography system equipped with Waters 2487 UV-Vis detector, Fraction collector model Waters FCM-II and Rheodyne Injector Model 7725I with 1.0 ml loop was used. A 250×20 mm i.d column packed with 5µ Inertsil ODS (GL sciences Inc. Japan) was employed for separation. The mobile phase consisted of 0.01 M CH_3COONH_4 (pH =3.5 with CH_3COOH): CH_3CN in the ration of 80:20 (v/v). The flow rate was set at 10.0 mL/min. Detection was carried out at 254 nm.

Origin of impurity-1

During the deprotection of **(3)** with Pd/Alumina in dimethylacetamide (DMA) as a solvent medium afforded **(2)** and DMA is hydrolyzed during the pH adjustment of **(2)** with aqueous HCl and further condensation of acetic acid with **(2)** yields impurity-1. This impurity-1 was isolated detected in gradient HPLC method. The same enriched from mother liquor and was character-

Note

ized as Impurity-1 by using of ^{1}H-NMR, IR and Mass spectral data.

Structure elucidation of impurity-1

The APCI (+ve) mass spectra further confirmed this with the presence of protonated molecular ion peak as base peak at m/z 367.1, ^{1}H-NMR (400MHz, DMSO-d_6) δ= 0.8-0.9 (d, 6H); 1.7(m, 1H); 4.1(d, 1H); 9.8-11.6(s, 2H); 2.5(s, 3H); 3.7(t, 2H); 2.5(t, 2H); 5.3(s, 2H); 7.8(s, 1H); 3.4 - 4.6(s, 2H). IR (KBr, cm^{-1}) 3470, 3324, 2966, 1728, 1698, 1629, 1610, 1575, 1541, 1183, 1103, 780, 762.

ACKNOWLEDGMENTS

The authors wish to thank the management of Dr. Reddy's laboratories Ltd., IPDOC-block actives for supporting this work. Co-operation from colleagues of Dr. Reddy's Laboratories Ltd., IPDO C-block, is higly appriciated.

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