

Trade Science Inc.

June 2008

Volume 7 Issue 7

Analytical CHEMISTRY

An Indian Journal

Note

ACAIJ, 7(7) 2008 [551-553]

Identification, isolation, characterization and synthesis of novel impurity in antipsychotic drug: Aripiprazole

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Received: 31st March, 2008 ; Accepted: 5th April, 2008

ABSTRACT

During the process development of aripiprazole (**1**) in our lab, one unknown impurity was detected in HPLC analysis at levels ranging from 0.05 to 2.0%. The unknown impurities have not been reported previously in the preparation of aripiprazole as a bulk drug synthesis. This impurity was isolated, synthesized and characterized as 7-(4(4-(2,3-dichlorophenyl) piperazin-1-yl)butoxy)quinolin-2(1H)-one, by ¹H NMR, IR and mass spectral data.
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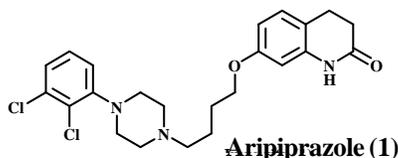
KEYWORDS

Aripiprazole;
Impurities;
Identification;
Isolation;
Charcatization.

INTRODUCTION

Aripiprazole (**1**) is a recently developed anti-psychotic drug used for the treatment of Schizophrenia^[1]. Aripiprazole and its derivatives exhibit a strong activity for influencing the neuro transmission of dopamine receptors^[2]. Furthermore, aripiprazole is metabolized by different biotransformation path ways^[3,4].

The analysis of aripiprazole (**1**) bulk drug revealed the presence of dehydrogenated impurity (**2**), which was up to 2.0%. 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 less than 0.1%. This paper describes the identification, isolation and characterization and synthesis of the unknown impurity present in aripiprazole. Isolation and characterization of this new impurity have not been reported till date to the best of our knowledge.



EXPERIMENTAL

Samples

The investigated samples were obtained from R&D laboratory of Inogen laboratories Pvt Ltd., A GVK Bio Company, 28A, IDA, Nacharam, Hyderabad-500 076, Andhra Pradesh, India. The impurity-(**2**) was synthesized from the same laboratory.

High-performance liquid chromatography

A Waters Model Alliance 2695-separation module equipped with 2996 photo diode array detector with Em- power pro data handling system. The analysis was carried out on Inertsil ODS C18, 250mm long, 4.6mm i.d., and 5-μm particle diameter column. Mobile phase A was phosphate buffer (pH 3.0±0.05) and acetonitrile in the ratio of 80:20 [buffer (pH 6.0)], prepared by dissolving 2.72g of KH₂PO₄ in 1000 ml of water, pH adjusted to 3.0±0.05 with dilute ortho phosphoric acid. Mobile phase B was acetonitrile and methanol in the ratio of 8:2 (v/v). UV detection was at 215 nm and flow rate was kept at 1.0 ml/min. Data acquisition time was 45 min. Pump mode was gradient. and the program was as follows, 0–5 min.: 90% A; 10% B, 5–30 min.: 85% A, 15% B; 30–40 min.: 85% A, 15% B; 30–

Note

40 min.: 35% A, 65% B; 40–45 min.: 35% A, 65% B; 45–50 min.: 90% A; 10% B.

NMR spectroscopy

The ^1H , ^{13}C NMR (proton decoupled) spectra were recorded on Varian 400MHz spectrometer using DMSO-d_6 , CDCl_3 as solvent and tetra methyl silane (TMS) as internal standard.

Mass spectrometry

Mass spectra were recorded on Agilent triple quadrupole mass spectrometer equipped with turboion spray interface at 375°C .

LC-MS/MS analysis was carried out using Agilent triple quadrupole mass spectrometer coupled with a 6310LC trap HPLC equipped with SPD 10A VP UV-visible detector and LC 10 AT VP pumps. Analyst software was used for data acquisition and data processing. The turboion spray voltage was maintained at 5.5 kV and temperature was set at 375°C . High pure nitrogen gas was used as auxiliary gas and curtain gas. Zero air was used as nebulizer gas. LC-MS spectra were acquired from m/z 100 to 1000 in 0.1 amu steps with 2.0 s dwell time. Aripiprazole sample was subjected to LC-MS/MS analysis. The analysis was carried out using Zorbax SB, C18, $50\text{mm} \times 4.6\text{mm}$ column with 1.8 μm particle diameter with mobile phase consisting of a mixture of TFA in water 0.05 % as A and 0.05 % TFA in acetonitrile as B. Flow rate was 1.0 ml/min. New impurity was detected in laboratory batch sample. The masses of detected peaks were identical to the values of the known compounds. Additionally, one impurity corresponding to mass of 445.0 was also observed in sample of Aripiprazole.

FT-IR spectroscopy

FT-IR spectra were recorded as KBr pellet on NICOLET 380 FT-IR Instrument model thermo electron corporation-spectrum one.

RESULTS AND DISCUSSION

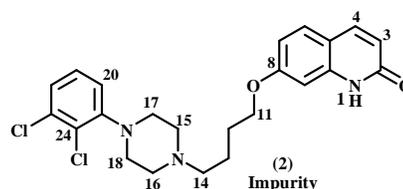
An isocratic reverse phased solvent system was used for the isolation of impurity. All fractions of impurity were isolated, concentrated and extracted with chloroform, the isolated solid obtained from the concentrated fractions was used to generate spectral data. The details of the elucidation of structure of this impurity are presented as following sections

Detection of impurities

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

TABLE 1

S. no.	Retention time	Compound	Nature
1.	14.14	Impurity (2)	Process related



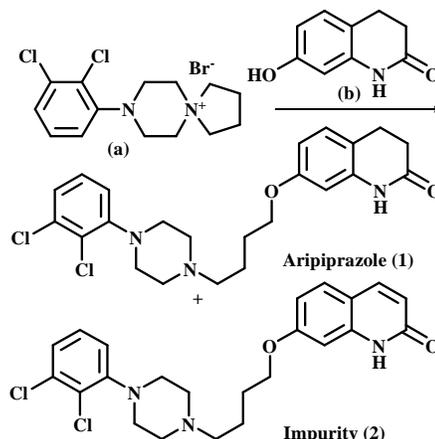
Isolation of impurity by preparative HPLC

A water preparative liquid chromatograph equipped with PDA detector (Waters Corporation, MILFORD, MA01 757, USA) was used. Gemini C18 ($100\text{mm} \times 30\text{mm}$ i.d.) preparative column packed with 5 μm particle size was employed for isolation of impurity. The mobile phase consisted of (A) 0.1M ammonium acetate solution and (B) acetonitrile. Flow rate was kept at 40 ml/min and detection was carried out at 254 nm.

Origin of impurity

The quaternary spiro ammonium salt (a) reacts with 7-hydroxy-4,5-dihydroquinoline-2(1H)-one (b) gives aripiprazole (SCHEME 1). But under basic conditions at high temperatures this spiro quaternary ammonium salt may act as a phase transfer catalyst and the product aripiprazole undergoes dehydrogenation and this yields to the impurity^[5,6].

This impurity was isolated and detected in gradient HPLC method. The same was enriched from mother liquor



SCHEME 1: Synthetic scheme of aripiprazole

TABLE 2: Comparative ^1H , ^{13}C (proton decoupled) and DEPT NMR assignments for Aripiprazole and impurity 7-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butoxy) quinolin-2(1H)-one

Position	^1H , δ (ppm) Multiplicity	Aripiprazole ^{13}C , δ (ppm)	DEPT	^1H , δ (ppm) multiplicity	Impurity ^{13}C , δ (ppm)	DEPT
1	10.0 (br, 1H)	-	-	11.8 (br, 1H)	-	-
2	-	170.1	-	-	162.3	-
3	2.4 (t, 2H)	29.3	CH ₂	6.8 (d, 1H)	121.5	CH
4	2.7 (t, 2H)	26.3	CH ₂	7.7 (d, 1H)	139.5	CH
5	-	128.3	-	-	110.7	-
6	7.2(d, 1H)	128.5	CH	7.5 (d, 1H)	127.2	CH
7	6.5(d, 1H)	109.9	CH	7.2 (d, 1H)	109.9	CH
8	-	157.7	-	-	159.7	-
9	7.3(s, 1H)	106.2	CH	7.15 (d, 1H)	117.4	CH
10	-	136.6	-	-	137.4	-
11	4.3(t, 2H)	68.4	CH ₂	4.2(t, 2H)	68.4	CH ₂
12	1.7(m, 2H)	27.3	CH ₂	1.9(m, 2H)	27.3	CH ₂
13	1.5(m, 2H)	24.6	CH ₂	1.7(m, 2H)	24.6	CH ₂
14	3.9(t, 2H)	54.1	CH ₂	2.5(t, 2H)	54.1	CH ₂
15	2.9(Brad, 2H)	48.8	CH ₂	3.1(Brad, 2H)	48.8	CH ₂
16	2.9(Brad, 2H)	48.8	CH ₂	3.1(Brad, 2H)	48.8	CH ₂
17	2.4(Brad, 2H)	49.3	CH ₂	2.7(Brad, 2H)	49.3	CH ₂
18	2.4(Brad, 2H)	49.3	CH ₂	2.7(Brad, 2H)	49.3	CH ₂
19	-	150.0	-	-	150.0	-
20	6.4(d, 1H)	117.6	CH	6.5(d, 1H)	117.6	CH
21	7.15 (t, 1H)	129.1	CH	6.8 (t, 1H)	129.1	CH
22	7.05(d, 1H)	123.9	CH	6.9(d, 1H)	123.9	CH
23	-	133.3	-	-	133.3	-
24	-	127.2	-	-	127.2	-

s, singlet; d, doublet; dd, doublet of a doublet; m, multiplet

and was characterized as impurity by using of ^1H -NMR, IR, Mass spectral data.

Structural elucidation of impurity: 7-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butoxy) quinolin-2(1H)-one

The molecular ion peak at m/z ;446.17 [(MH)+] in positive ion mode by LC-MS analysis indicated a molecular weight of 445.2, which is 2 amu less than that of aripiprazole(1). Being less polar than aripiprazole, it was suggested that the loss of two hydrogens happened to the molecule. To confirm the retention time, pure impurity was co-injected with aripiprazole crude sample in HPLC. It was observed that the retention time was matching with the retention time of impurity. IR spectrum of the isolated impurity exhibits lower value of carbonyl characteristic absorption band (1659cm^{-1}) than the aripiprazole carbonyl (1678cm^{-1}) due to double bond stretching. In ^1H NMR spectrum, a triplet at 2.49 ppm and another triplet at 2.76 corresponds to CH_2 - CH_2 in 2,3 dihydro-2 (1H)-quinonone of aripiprazol was disappeared and an additional two doublet were observed at aromatic region at 6.87 ppm and 7.72 ppm and In ^{13}C NMR spectrum, signal at 29.3 ppm and 26.3 ppm corresponds to CH_2 - CH_2 carbon adjacent to the keto group in 2,3-dihydro-2(1H)-quinonone was disappeared and the additional signal was observed at 121.6

ppm and 139.5 ppm confirmed as $\text{CH}=\text{CH}$ carbon by DEPT experiment. The structure of the isolated new impurity was also confirmed by the following spectral data. IR (KBr, cm^{-1}) 3375 (NH), 1659 (C=O), ^1H NMR, ^{13}C NMR and DEPT data for aripiprazole and impurity are given in TABLE 2.

ACKNOWLEDGMENT

The authors gratefully acknowledge the management of Inogen laboratories Pvt Limited for providing necessary facilities.

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