

## "SYNTHESIS, ISOLATION, AND CHARACTERIZATION OF DONEPEZIL OPEN RING IMPURITY"

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## ABSTRACT

**Objective:** The objective of this study is to find out the unwanted impurity from the Donepezil API (Active pharmaceutical Ingredient).

**Method:** Impurities ranging from 0.05% to 0.2% Donepezil were detected by using a simple isocratic reversed phase high performance liquid chromatography (HPLC). These impurities were isolated by using Preparative high performance liquid chromatography.

**Results:** The known impurity of donepezil that is donepezil open ring was synthesised by chemical synthesis and isolated of purity above 95%. The pure impurity was characterized by using IR, NMR, and MS spectral data and confirm its molecular structure of IUPAC name is 4,5-Dimethoxy-2-[2-oxo-3-[1-(phenyl methyl)- 4 piperidinyl] propyl] benzoic acid.

**Conclusion:** The Donepezil open ring impurity was properly synthesised and characterized by using modern machinaries. It is the work for the benefit of the human beings, because impurity in the drugs affect the human body.

**Keywords:** Donepezil, Impurities, Spectroscopy, Identification, Characterization and synthesis.

## INTRODUCTION

Alzheimer's disease is one of the most common causes of mental deterioration of elderly people. It is characterised by degradation of various structures in the brain. Acetylcholinesterase inhibitors are the most frequently prescribed drugs for the treatment of Alzheimer's disease[1,2]. The Food and Drug Administration has approved donepezil for the treatment of the symptoms of Alzheimer's disease. In Alzheimer's disease, some cells in specific regions of the brain die. Because of the cell death, these brain cells lose their ability to transmit nerve impulses. Brain cells normally transmit nerve impulses by secreting various chemicals known as neurotransmitters. Brain cells that make and secrete a neurotransmitter called acetylcholine are affected early in the course of Alzheimer's disease[3]. Donepezil helps prevent the breakdown of acetylcholine in the brain, thus temporarily increasing its concentration. In doing so, donepezil may improve the thinking process by facilitating nerve impulse transmission within the brain[4].

Research leading to the development of donepezil began in 1983 at Eisai, and the first phase I clinical trial took place in 1989. In 1996, Eisai received approval from the United States Food and Drug Administration (USFDA) for donepezil under the brand name Aricept, which it co-marketed with Pfizer. As of 2011, Aricept was the world's best selling Alzheimer's disease treatment.

Aricept (donepezil hydrochloride) is a reversible inhibitor of the enzyme acetylcholinesterase known chemically as (±) 2,3 dihydro -5, 6 dimethoxy 2- [1-(phenylmethyl)- 4- piperidinyl] methyl]- 1H-inden-1-one hydrochloride. Donepezil hydrochloride is commonly referred to in the pharmacological literature as E2020. It has an empirical formula of C<sub>24</sub>H<sub>29</sub>NO<sub>3</sub>HCl and molecular weight is 415.96. Donepezil hydrochloride is a white crystalline powder and is freely soluble in chloroform, soluble in water and glacial acetic acid, slightly soluble in ethanol and in acetonitrile and practically insoluble in ethyl acetate and hexane. The molecular structure is given in figure-1:

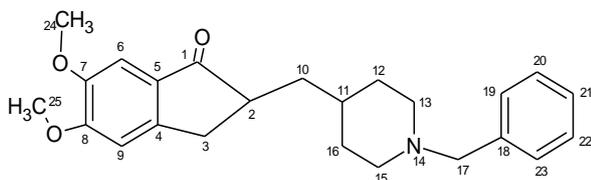


Fig. 1: Structure of Donepezil

Donepezil has been tested in other cognitive disorders including Lewy body dementia [5] and vascular dementia[6] but it is not currently approved for these indications. Donepezil has been found to improve sleep apnea in Alzheimer's patients[7]. The studies found that speech of autistic children who were mild to moderately affected appeared to improve from the use of Donepezil [8], medication [9]

A literature search revealed that only analytical procedure is available but nobody has reported synthesis, isolation, and characterization of impurity in the purified form, from donepezil API[10]. The present communication involves the isolation, preparation of impurity and characterization by chromatographic and spectroscopic technique.

## MATERIALS AND METHODS

The raw material of Donepezil was received from Sitec labs Mumbai, India. The HPLC grade acetonitrile and methanol solvents were obtained from Merck co, Mumbai, India. The HPLC grade Sodium decane sulphate, Perchloric acid, and Phosphoric acid were obtained from Sigma Aldrich, Mumbai, India. The synthesis grade Hydrogen peroxide was obtained from Merck co, Mumbai, India.

## High performance liquid chromatography (HPLC)

An Agilent HPLC system equipped with 1100 series low pressure quaternary gradient pump along with pulse dampener, Photo diode array detector with auto liquid sampler handling system has been used for the analysis of the sample. An Inertsil ODS-2, 4.6mm x 15cm x5μ column was employed for the testing of reaction mass of Donepezil open ring impurity. The column eluent was monitored at detection wavelength 271nm. The mobile phase was 2.5 gm of Sodium decane sulfonate in 650 ml of purified water with 1 ml of Perchloric acid, in this added 350 ml of hplc grade Acetonitrile and made its PH-1.8 with Phosphoric acid. Chromatography was performed at 35 °C with the flow rate of 1.4 ml/min. Data was recorded by using Chem station software.

## High performance liquid chromatography (Preparative HPLC)

Preparative HPLC is the technique of choice for compound isolation and purification within the pharmaceutical and life science industries. Agilent technologies purification solution from nano gram to gram sample quantities. Agilent 1200 Series purification system with low delay volumes optimized for high recovery and purity, with PDA detector and flow rate is 0.001 to 100 ml/min with max. Pressure 400 bar. A ODS-C18 250mm x 21.2mm x 10μ reverse phase silica column was employed for the separation of open ring

impurity of Donepezil. Solvent used for the separation was Water: ACN with flow rate of 20 ml/min, with the detection of 271nm.

### Flash chromatography

Flash chromatography known as medium pressure chromatography, was differed from the conventional technique. Open ring impurity of Donepezil separated by using Teledyne ISCO combi-flash. To increase the purity of the open ring from the reaction mass, here used reverse phase column with reverse phase mobile phase.

A C18 silica column of 24 gm x 30 $\mu$  used for the separation of open ring impurity using reverse phase mobile phase of Water and Acetonitrile. The flow rate used 20ml min<sup>-1</sup> with the detection wavelength 271 nm.

### Microwave

The use of microwave irradiation in organic synthesis has become increasingly popular within the pharmaceutical and academic arenas, because it is a new enabling technology for drug discovery and development. For the synthesis of the open ring of Donepezil used CEM Discover microwave system. Its a system of ISO 9001-2000 approved. This system perform atmospheric (up to 70ml working volume ) and pressurized (up to 50 ml working volume) reaction. Use a wide range of vessels as well as standard condensers, addition funnels and stirring options with refluxing capability. CEM reaction tubes are pressure rated to 500 psi and use septa that tolerate multiple piercing for reagent addition or sample withdrawal. Microwave system gave faster reactions with increased yields, improved selectivity, and superior reproducibility. Optimization of the reaction very quickly and in fewer steps gave more time to use creativity to explore the available chemical diversity. The instrument specification, overall dimension is (36.2 cm x 43.7 cm x 22.1 cm), weight = 30lbs, Magnetic frequency= 2450 MHz, Power output= 300 Watts, temperature = -90°C to 300°C.

### Mass spectrometry (LC-MS/MS)

The LC-mass spectrometry (MS) and MS-MS studies were carried out on an Ion trap 6320 Series electron spray ion trap spectrometer (Agilent Technologies).The source voltage was kept at 3.0 kV. Parameters: nebulizer gas = 30psi; dry gas = 3 L/min; dry temperature= 150 °C; capillary voltage =24500 to 21500 V. Nitrogen was used as both a sheath and auxiliary gas. Mass range was kept at m/z 50-600.The chromatography conditions and mobile phase are column = Inertsil ODS-2(15 cmx4.6 mm x5  $\mu$ ), temperature= 35 °C, Wavelength = 271nm, Mobile phase = Water : ACN = 650 : 350, make PH = 1.8 with formic acid.The flow rate was maintained at 1.4 mL/min.

### Nuclear magnetic resonance

The <sup>1</sup>H, and <sup>13</sup>C nuclear magnetic resonance(NMR) spectroscopy experiment of the impurity was carried out at a frequency of 500 MHz at 25 °C on an NMR spectrometer (Varian, Palo Alto, California). <sup>1</sup>H chemical shifts are reported on the  $\delta$  scale in ppm relative to tetramethylsilane 0.00 and CDCl<sub>3</sub> ( $\delta$ 77.00 ppm) and DMSO, D<sub>6</sub> ( $\delta$ =39.50) respectively. <sup>1</sup>H experiments were run using a mixing time of 1000ns.

### FT-IR Spectroscopy

The IR spectra were recorded in the solid state as KBr dispersion medium using Perkin Elmer spectrum 100 FT-IR spectrophotometer.

### Synthesis of impurity

Synthesis of donepezil open ring impurity i.e 4,5- dimethoxy-2-[ 2-oxo-3-[ 1- (phenyl methyl)-4- piperidinyl] propyl] benzoic acid, it's molecular formula is C<sub>24</sub>H<sub>29</sub>NO<sub>5</sub> with the CAS No- 197010-25-6. The synthesis procedure is as below,

In a Microwave glass tube, donepezil API was charged with Acetonitrile solvent. In that mixture added hydrogen peroxide of 40% very slowly at 15 °C - 20 °C and stirred for 15-20 mins. After that expose the reaction mass at 80 °C at 40-to- 50 watt power in the CEM Discover Microwave for twice in 5 mins interval. Reaction mass checked in HPLC and got the required impurity of 30% purity, and checked the reaction mass in LCMS for the confirmation of the required impurity mass. To improve the percentage of the required impurity used flash chromatography and preparative chromatography, for the characterization of the impurity.

### RESULTS AND DISCUSSION

#### Detection of impurity by HPLC

Typical analytical HPLC chromatogram of donepezil bulk drug and it's open ring impurity obtained by using the HPLC method discussed under the heading "High performance Liquid Chromatography (analytical)". The targeted impurity under study are marked as open ring impurity eluted at retention time of about 6.872 mins.

#### Isolation of the impurity by flash chromatography

A simple reverse phase chromatographic system, discussed under the heading "Flash Chromatography" was used for increasing the percentage of the impurity, it does not give the pure material for characterization. Mobile phase used for the flash chromatography was 0.1% Formic acid in water and Acetonitrile and the gradient was 0-5 mins = 100 : 00, 5- 18 mins = 70 : 30, 18 - 22 mins = 70 : 30, 22 -25 mins = 00 : 100. Reaction mass separated and got 77.34% purity of open ring impurity from 30.0%. The flash chromatography helped to increase the purity and loading of the reaction mass and minimised the time period too. The purity checked by using the HPLC method discussed under the heading "HighPerformanceLiquid Chromatography" (Analytical) and its purity obtained 77.34%.

#### Isolation of the impurity by PREP-HPLC

A simple reverse phase chromatographic system, discussed under the heading, "High performance Liquid Chromatography (preparative) was used for isolation of the impurity. In this chromatographic system, the open ring impurity eluted at about 41.020 mins . The donepezil open ring impurity fraction was collected between 39.7min to 42.6min, and it is exhibited in figure no-2 The impurity fraction was concentrated at room temperature under high vacuum on a Buchii Rotavapour Model R124. The concentrated volume take in acetonitrile solvent and put it in the Lyophilizer for getting solid open ring impurity. Purity of the impurity was tested in analytical method discussed under the heading, "High Performance Liquid Chromatography" (HPLC). The purity was found to be 99.119 % and it is exhibited in figure no.3, before carrying out spectroscopic experiments.

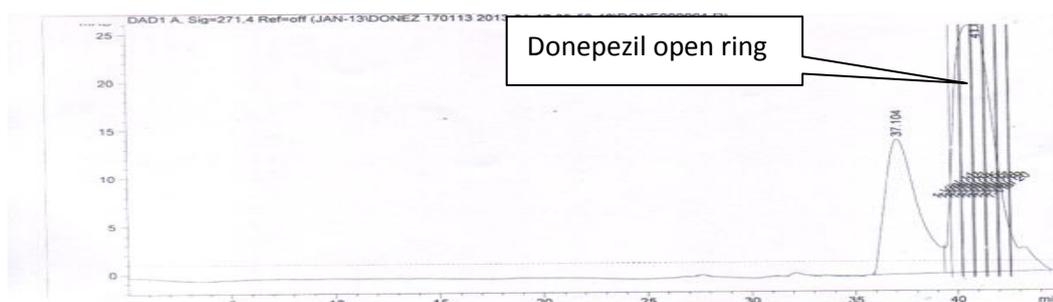


Fig. 2: Chromatograph of preparative hplc.



Fig. 3: Chromatographic purity of impurity after prep hplc.

#### LC-MS/MS Analysis

LC-MS/MS analysis of donepezil bulk drug sample and open ring impurity of donepezil was performed using the chromatographic system as described under the heading "Mass Spectrometry (LC-MS/MS)". Result of LC-MS/MS analysis revealed that impurity exhibited molecular ion at  $m/z(M+1)=412.2$ amu. and its MS/MS shown 394.2,368.2,amu. donepezil API exhibited molecular ion at

$m/z(M+1)=380.2$ amu, and its MS/MS shown 362.2,288.1,243.1amu.

#### Structure elucidation

The Molecular Structure of Open Ring impurity of Donepezil is as Figure-4. The Molecular Formula is  $C_{24}H_{29}NO_5$  and its Molecular weight is 411.5.

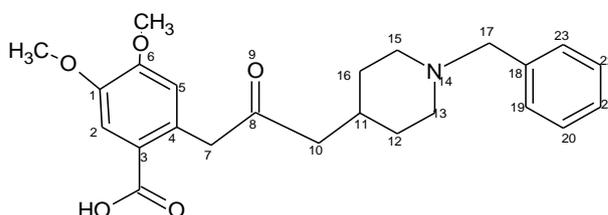


Fig. 4: Structure of Donepezil open ring impurity

The IR spectra recorded in the solid state as KBr dispersion. FT-IR and mass spectral data of donepezil and its open ring impurity exhibited in the table no-1.

Table 1: FT-IR and mass spectral data of donepezil and its open ring impurity

S. No.	Compound	IR	MS, MS/MS data
1	Donepezil	2926(aromatic C-H stretching), 1694(C=O stretching)1590(aromatic C=C stretching) 1500,1458(aliphatic C-H bending), 748,700 (aromatic C-H bending)	$m/z$ 380.2, 362.2,288.1,243.1.
2	Donepezil Open ring	3330(carboxylic acid O-H stretching) 1690 (carboxylic acid C=O stretching),2930(aromatic C-H stretching),1594(aromatic C=C stretching),	$m/z$ 412.2, 394.2,368.2,

Donepezil HCl and open ring impurity of donepezil,  $^1H$  NMR, structure prediction and detailed assignment shown in table-2 and table-3 respectively

Table 2:  $^1H$  NMR Assignment of Donepezil.

Chemical shift (ppm)	Number of protons	Multiplicity	Assignment
7.43 – 7.64	5H	Multiplet	19,20,21,22,23
7.09	1H	Singlet	7
6.82	1H	Singlet	6
3.95	1H	Singlet	25
3.82	1H	Singlet	24
3.66	2H	Multiplet	2
2.65 – 3.00	2H	Multiplet	3
1.84 – 2.06	9H	Multiplet	11,12,13,15,16
1.54 – 1.85	2H	Multiplet	10

Table 3:  $^1H$  NMR Assignment of Donepezil open ring impurity.

Chemical shift (ppm)	Number of protons	Multiplicity	Assignment
7.615	1H	Singlet	2
7.261	-	-	$CHCl_3$
7.468- 7.375	5H	Multiplet	19,20,21,22,23
6.6	1H	Singlet	5
4.052 – 3.857	10H	Multiplet	1Me,6Me,7,17
3.384 – 3.362	2H	Doublet	10
2.576 – 2.461	4H	Multiplet	13, 15
1.813 – 1.713	5H	Multiplet	11, 12, 16

**CONCLUSION**

This research paper describes the synthesis, isolation and structure elucidation of process related open ring impurity of donepezil. The impurity was separated by reverse phase chromatographic technique, by using Flash Chromatography and High performance liquid chromatography (prep-HPLC). The isolated impurity was characterized by using IR, <sup>1</sup>H NMR, and LC-MS/MS spectroscopic technique. The synthesis of impurity was also discussed in brief.

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