ISSN: 0974 - 7516 Volume 10 Issue 1

Organic CHEMISTRY

An Indian Journal

Full Paper

OCAIJ, 10(1), 2014 [38-42]

Isolation and characterization of process-related impurity in 5-ethyl-3-methyl-5-phenyl-imidazolidine-2, 4-dione (Mephenytoin-anticonvulsant drug)

Krishnamoorthy Balamurugan[1]*, Tangirala Prakasam[1], K.R.Srinivasan[1], Kathirvel Asokan[2]

[1]Research and Development Centre, Malladi Drugs and Pharmaceuticals Ltd No. 788/1, Irulapalayam, Kuthambakkam 602 107, (INDIA)

[2]National Test House (SR), (INDIA)

E-mail: kbalmurugan@hotmail.com

ABSTRACT

One unknown impurity in Mephenytoin bulk material at a level 0.15% was detected by a reverse phase high performance liquid chromatography (HPLC). This impurity was isolated from a crude sample of Mephenytoin using reverse phase preparative LC. The impurity isolated by preparative LC was characterized by IR, NMR, and MS experimental data. Based on the results obtained from different spectroscopic experiments, these impurity was characterized as 1, 3-Dimethyl-5, 5-phenylethyl hydantoin. Formation of this impurity is also discussed.

KEYWORDS

Mephenytoin;
Impurity;
Preparative LC;
Spectroscopy;
Isolation;
Identification;
Characterization.

INTRODUCTION

Mephenytoin, 5-ethyl-3-methyl-5-phenyl-imidazolidine-2,4-dione is used as an anticonvulsant[1-3]. High performance liquid chromatographic (HPLC) method was developed for testing of purity of Mephenytoin bulk material. The stringent purity requirement from regulatory agencies[4] that all the individual impurity, which are ≥ 0.1%, must be identified and characterized, this paper aims at the identification and characterization of an unknown impurity in Mephenytoin active ingredient by IR, mass spectrometer and NMR. To the best of our knowledge, this impurity is novel and not reported in literature.

Since the impurity level is low, after trying several days, failed to enrich the impurity level by synthetic route.

A thorough study has been undertaken to develop a preparative LC method to isolate, wherever possible and characterize this impurity by chromatographic and spectroscopic techniques[5-8].

EXPERIMENTAL

Samples and chemicals

The samples of bulk Mephenytoin was received from local pharmaceutical industry. HPLC grade acetonitrile was obtained from Merck Co., Mumbai, India. HPLC grade Acetone was obtained form Rankem, Mumbai, India. Ultra pure water was collected from Millipore water purification system. HPLC grade Dichloromethane used for liquid liquid extraction was purchased from Spectrochem, India. Nitrogen and Zero Air used were of ultra pure grade (99.99%).

HPLC

An Agilent HPLC system equipped with 1200 series high pressure binary pump along with pulse dampener, photo diode array detector and auto liquid sampler handling system has been used for the analysis of samples. The data was collected and processed using Agilent EZ Chrome Elite version 3.2.1 software. An Inertsil ODS-3V (250X4.6mm, 5-Micron, GL Sciences, Japan) column was employed for the separation of impurity from Mephenytoin. The column eluent was monitored at detection wavelength 225nm. A isocratic method was optimized for the clear separation of impurity from Mephenytoin active ingredient where the mobile phase ratio was a mixture of 0.04% v/v orthophosphoric acid and acetonitrile in a ratio of 50:50, v/v for 30 min. The Chromatography was performed at room temperature using at a flow rate of 1.0mL min^{-1}.

An Agilent preparative HPLC system equipped with 1100 series pump, photo diode array detector, auto liquid sampler handling system fitted with 900µL loop and 1100 series preparative fraction collector has been used. The data was collected and processed using Agilent EZ Chrome Elite software. A Zorbax C18 column (50 X 50mm, 5-Micron, Agilent Technologies) was employed for loading the sample. An analytical method was developed in isocratic mode separately to resolve this impurity, followed by scaling up the same method for preparative HPLC to collect the required impurity fractions. The mobile phase consisted of water and acetonitrile in the ratio of 20:80 (v/v). The flow rate was set at 25mL min^{-1}. Detection was carried out at 225nm. Approximately 200 mg mL^{-1} of sample was prepared using acetonitrile and water as diluents in the ratio of 50:50, v/v to load on to the column.

Mass spectrometry (LC-MS/MS)

LC-MS/MS analysis has been performed on API 2000, Mass Spectrometer (Applied Biosystems). The analysis was performed in positive ionization mode with turbo ion spray interface. The parameters for Ion source voltage IS = 5500V, declustering potential, DP = 70V, focusing potential, FP = 400V, entrance potential, EP= 10V were set with nebuliser gas as air at a pressure of 40 psi and curtain gas as nitrogen at a pressure of 25 *psi*. An Inertsil ODS-3V (250 X 4.6mm, 5-Micro, GL Sciences, Japan) column was used for the separation. The mobile phase is a mixture of water, acetonitrile in a ratio of 50:50 (v/v). The analysis was performed at a flow rate of 1.0mL min^{-1}

NMR spectroscopy

The ^{1}H and ^{13}C NMR experiments of the impurity carried out at a frequencies of 200 MHz in $CDCl_3$ at 25°C temperature on a Bruker Avance dpx- 200 FT NMR spectrometer. ^{1}H and ^{13}C chemical shifts are reported on the δ scale in ppm, relative to tetra methyl silane (TMS) δ 0.00 and $CDCl_3$ at 77.0ppm in ^{13}C NMR respectively. D_2O exchange experiment was performed to confirm the exchangeable protons.

FT IR spectroscopy

IR spectra of the impurity of Mephenytoin was recorded in the solid state as KBr pellet using IR PRESTIGE-21 IR spectrophotometer.

RESULTS AND DISCUSSION

Detection of impurity by HPLC

Typical analytical LC chromatograms of Mephenytoin and its impurity obtained by using the LC method discussed in the experimental section are shown in figure 1. The targeted impurity under study are marked as Impurity eluted at retention times of about 6.0 min and Mephenytoin eluted at about 9.0 min. Crude sample of Mephenytoin was also injected to check the level of impurity.

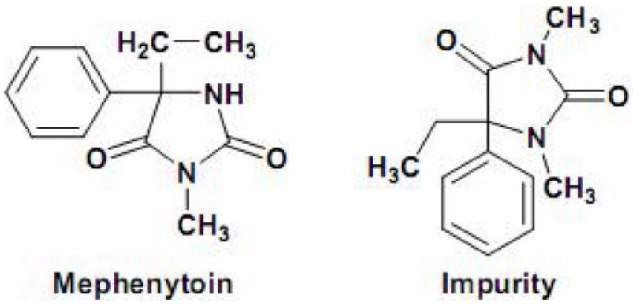

Figure 1 : Chemical structures of Mephenytoin (5-ethyl-3-methyl-5-phenyl-imidazolidine-2,4-dione) and impurity (1.3-Dimethyl-5,5-phenylethyl hydantoin)

Infrared spectrum

The infrared spectrum of mephenytoin - impurity is shown in the following figure 1. The spectrum was obtained as 0.2 percent dispersion of mephenytoin - im-

purity in KBr pellet with a IR PRESTIGE-21 IR spectrophotometer. The following TABLE 1 gives the infrared spectral assignments consistent with the structure of mephenytoin - impurity.

TABLE 1 : Infrared spectral assignments for mephenytoin - impurity

Assignment	IR bands in cm^{-1}
aromatic C – H stretch	3067
aliphatic C – H stretch	2970
amide and imide bands	1769,1715
aromatic bands	1477,1462
C – N stretch	1273
C – H out – of – plane bending of mono – substituted benzene ring	756,729

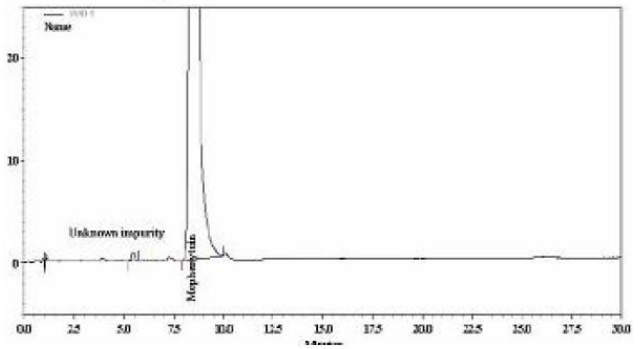

Figure 2 : HPLC chromatogram of Mephenytoin showing an impurity at about 6.0 min

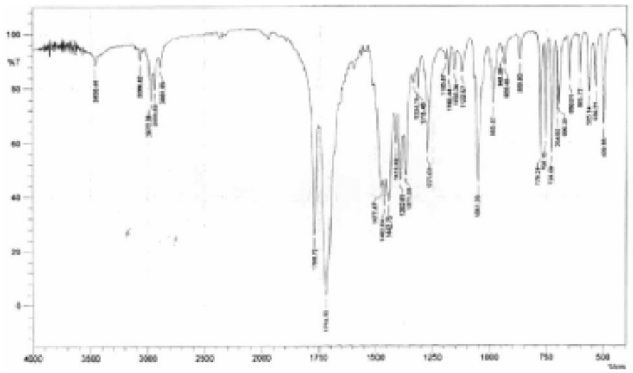

Figure 3 : Infrared spectrum of mephenytoin impurity high performance liquid chromatography (preparative)

LC-MS/MS analysis

LC -M S /MS analysis of crud e sample of Mephenytoin was performed using the solvent system as described in the experimental section. Results of LC-MS/MS analysis revealed that the impurity exhibited molecular ion at m/z (M+1) 233 and mass fragmentation was carried for this molecular ion.

Isolation of the impurity (m/z; 233) by preparative HPLC

A simple reverse phase solvent system discussed in the experimental section was used for isolating the impurity with m/z 233(M+1). In this solvent system, Mephenytoin eluted at about 9.0 min whereas the impurity eluted at about 6.0 min. The impurity fractions isolated, were concentrated by removing acetonitrile layer at room temperature under high vacuum on a Buchii Rotavapour Model R124. The aqueous layer containing this impurity was extracted into methylene chloride using the separating funnel. The methylene chloride layer which is having impurity is dried through anhydrous sodium sulphate. These fractions were concentrated at room temperature under high vacuum on a Rotavapour. Purity of this impurity was tested in analytical mode and was found to be 99.5% before carrying out spectroscopic experiments.

Structural elucidation of impurity

LC-MS/MS spectral data of impurity was compared with the spectral data of Mephenytoin. This impurity exhibited molecular ion at m/z 233(M+1) in LC-MS/MS analysis. This molecular ion is exactly 14 more than that of Mephenytoin. The mass fragmentation pattern of impurity is shown in the figure 4.

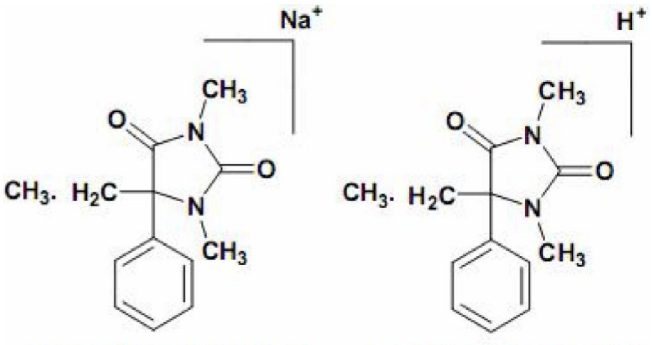

Figure 4 : Mass fragmentation of impurity

The mass fragmentation of impurity supports to the chemical structure predicted in figure 1.

Proton nuclear magnetic resonance (NMR) spectrum

The proton NMR spectrum of mephenytoin impurity is shown in the following Figure. The spectrum was obtained with a Bruker Avance-300 MHz FT3

NMR spectrometer. $CDCl_3$ was used as solvent with TMS as the internal standard. The following TABLE 2 gives the proton NMR spectral correlations which are consistent with the structure of mephenytoin ñ impurity.

TABLE 2 : Proton NMR spectral correlations of mephenytoin – impurity

Protons	No.of protons	Chemical shift (δ_H)	Multiplicity
A	3	0.87	t
B	1	2.03	m
C	1	2.59	m
D	3	2.84	s
E	3	3.06	s
F	5	7.31	m

B and C protons are diastereotopic

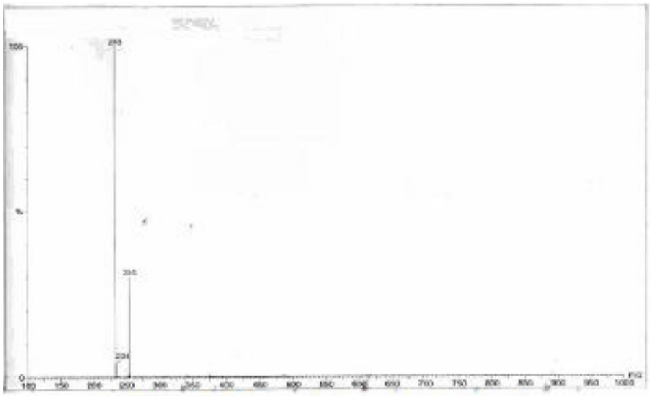

Figure 5 : Mass spectrum of impurity

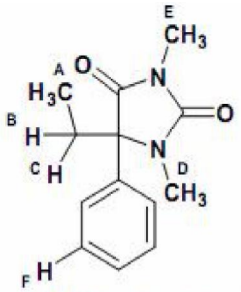

Figure 6 : The proton NMR spectrum of mephenytoin-impurity

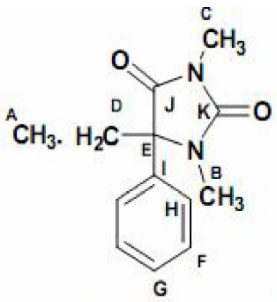

Figure 7 : The carbon-13 NMR spectrum of mephenytoin-impurity

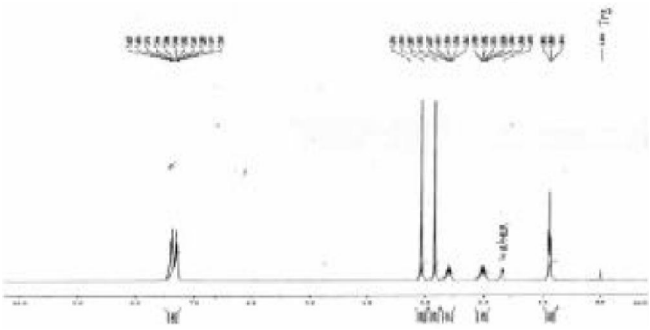

Figure 8 : ^{1}H NMR spectrum of impurity

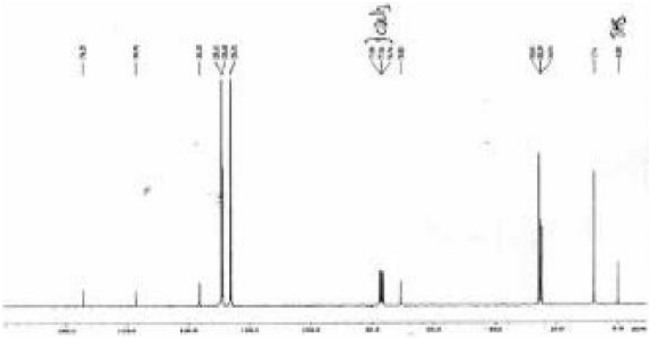

Figure 9 : ^{13}C NMR spectrum of impurity

TABLE 3 : Carbon-13 NMR spectral assignments of mephenytoin - impurity Chemical shift (δ_C) values

Carbon	Chemical shift (δ_C)
A	7.74
B	24.96
C	25.39
D	25.66
E	70.83
F	126.01
G	128.68
H	129.11
I	136.35
J	156.96
K	174.23

Carbon-13 (^{13}C) nuclear magnetic resonance (NMR) spectrum

The carbon-13 NMR spectrum of mephenytoin-impurity is shown in the following figure 3. The spectrum was obtained with a Bruker Avance-300 MHz FT NMR spectrometer. $CDCl_3$ was used as solvent with TMS as the internal standard. The following TABLE 3 gives the ^{13}C NMR spectral correlations consistent with the structure of mephenytoin impurity. Based on these spectral results, it was confirmed that the impurity

having the molecular formula $C_{13}H_{16}N_2O_2$ and the same was characterized as 1,3-Dimethyl-5, 5-phenylethyl hydantoin.

Formation of impurity (m/z 233)

Alkaline methanol was used in the final synthesis of Mephenytoin, which leads to the formation of this impurity 1,3-Dimethyl-5,5-phenylethyl hydantoin.

CONCLUSION

This research paper describes the identification, isolation and structural elucidation of a process related impurity present in the Mephenytoin. The impurity was separated by reverse phase chromatographic technique, further isolated this impurity by semi preparative liquid chromatography. The isolated Mephenytoin impurity was characterized using spectroscopic techniques. This isolated impurity was used for calculating the mass balance of Mephenytoin technical material. The formation of the impurity was also discussed in brief.

REFERENCES

[1] The Treatment of Epilepsyí, (Ed); by S.D.Shorvon, R.David Fish, Emilio Perucca, W.Edwin Dodson; Blackwell Publishing, ISBN 0-632-06046-8 **(2004)**.

[2] The Medical Treatment of Epilepsyí, by R.Stanley Resor; Published by Marcel Dekker, ISBN 0-8247-8549-5 **(1991)**.

[3] R.Ian Phillips, A.Elizabeth Shephard; Methods in Molecular Biology Cytochrome $P_{450,}$ 2nd Edition, 150

[4] World Health Organisation Recommended Classification of Pesticides by Hazard (ref: WHO/PCS/98.2), WHO, Geneva, **(1998-99)**.

[5] S.C.Hou, Z.Q.Zhou, Z.Qiao, H.C.Guo, X.Y.Shi, M.Wang; Chromatographia, **57(3,4)**, 177-180 **(2003)**.

[6] J.M.Clough, C.R.A.Godfrey; Azoxystrobin, A Novel Broad-Spectrum Systemic Fungicidei, Pesticide Outlook, **7**, 16-20 **(1996)**.

[7] C.D.S.Tomlin, (Ed); The Pesticide Manual, British Crop Protection Council (BCPC), UK, **(2000)**.

[8] S.Gorog; Identification and Determination of Impurity in Drugsi, Elsevier, Amsterdam, **(2000)**.