Asian Journal of Chemistry Vol. 21, No. 6 (2009), 4425-4428

Isolation, Identification and Characterization of Process Related Impurity in 2-Isopropyl-4-hydroxy Anisole

M. THAPA*, R. VANSADIYA, R. NAUTIYAL, C.C. SHAH† and KESHAV DEO†
Analytical Development Laboratory, Alembic Research Centre
Alembic Limited, Vadodara-390 003, India
Fax: (91)(265)2281173; Tel: (91)(265)2307946
E-mail: manish.thapa@alembic.co.in

One impurity in 2-isopropyl-4-hydroxy anisole at level *ca.* 0.2 % at relative retention time *ca.* 2.30 is detected by reversed phase HPLC. This impurity is isolated from the enriched impurity sample using reversed phase preparative HPLC. Based on the spectral data (IR, NMR, ^{13}C NMR and MS) impurity is characterized as dimer of 2-isopropyl-4-hydroxy anisole.

Key Words: 2-Isopropyl-4-hydroxy anisole, Impurity, HPLC.

INTRODUCTION

In-house HPLC method was developed to check the relative purity of the compound. During the analysis of different laboratory batches of 2-isopropyl-4-hydroxy anisole, one impurity is detected consistently in almost all batches, whose area percentage ranged from *ca.* 0.13-0.20 % by reversed phase HPLC method. A comprehensive study has been done to isolate and characterize this impurity by spectroscopic techniques. The impurity profile study has to be carried out from any final drug substances to identify and characterize the unknown impurity that is present at a level above 0.17 %. The requirement of identification and characterization of the impurity in the final drug substances is extremely necessary to meet the stringent regulatory or customer requirements[1].

EXPERIMENTAL

The investigated samples of 2-isopropyl-4-hydroxy anisole drug substances and enriched impurity samples were obtained from Custom Research laboratory of Alembic Research Centre, Alembic limited, Vadodara, Gujarat (India).

High performance liquid chromatography (analytical): A Shimadzu LC-2010 HT separation module equipped with UV detector was used. Inertsil C-18 column having dimensions 250 mm × 4.6 mm i.d. and 5 µm particle size was used

†Chemical Research Development Laboratory, Alembic Research Centre, Alembic Limited, Vadodara-390 003, India.

for analysis. The column was maintained at 25 °C and the eluent was monitored at 220 nm and the data was recorded using LC Solution software[2]. Mobile phase A (Water HPLC grade) and mobile phase B (acetonitrile) is used for the separation. The diluent was mobile phase A:B::45:55. The gradient programme is depicted in Table-1.

TABLE-1
GRADIENT PROGRAMME

Time (min)	Mobile phase A	Mobile phase B
0.01	45	55
20	20	80
35	20	80
40	45	55
45	45	55

Mobile Phase A = Water HPLC grade; Mobile phase B = Acetonitrile HPLC grade.

High performance liquid chromatography (preparative): A Waters LC-2000 separation module equipped with 2487 UV detector and system controller were used. Luna C-18 column having dimensions 250 mm × 50 mm i.d and 15 µm particle size was used for the impurity isolation work. A 10 mL injection loop was used and the eluent was monitored at 220 nm and the data was recorded using Millenium software[3,4]. About 200 mg of the sample was dissolved in a mixture water and acetonitrile in the ratio (30:70) and loaded on preparative column. Mixture of water and acetonitrile in the ratio (30:70) is at a flow rate of 35 mL/min and the eluent was monitored at 220 nm.

NMR spectroscopy: NMR measurement (^{1}H and ^{13}C) were performed on a Bruker Avance 300 MHz instrument at 25 °C in $CDCl_3$. The chemical shift values were reported on the d scale relative to TMS.

Mass spectrometry: Mass spectra were obtained using AB Sciex API-2000 LC/M-MS Mass Spectrometer in Negative Ion Ionization Mode.

FT-IR spectroscopy: IR spectrum of impurity is recorded in the solid state as KBr dispersion using Perkin-Elmer, spectrum-one FT-IR spectrophotometer.

RESULTS AND DISCUSSION

Detection of impurity: A typical analytical LC chromatogram (Fig. 1) of a laboratory 2-isopropyl-4-hydroxy anisole recorded using liquid chromatography method[5]. The target impurity under study is marked as dimeric impurity. Retention time (RT), relative retention time (RRT) and structures of this impurity and 2-isopropyl-4-hydroxy anisole are shown in Table-2. Impurity is isolated from enriched impurity sample of 2-isopropyl-4-hydroxy anisole on preparative LC. Attempts were also made to synthesize the impurity.

Isolation of impurity by preparative HPLC: A reversed phase solvent system was used for the isolation of impurities. The enriched impurity sample was loaded on the preparative column and the fraction collected were pooled together and analyzed

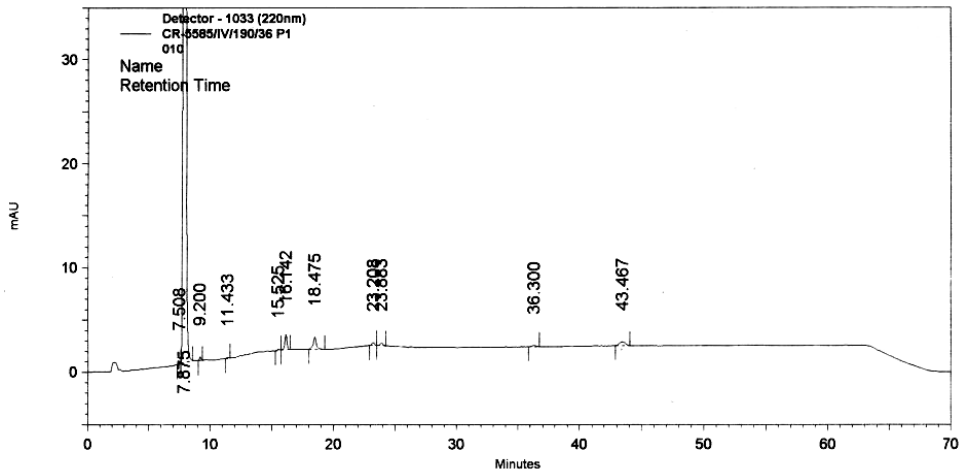
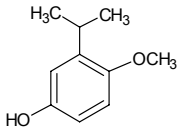
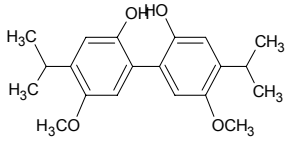


Fig. 1. HPLC chromatogram of 2-isopropyl-4-hydroxy anisole

TABLE-2
IMPURITY ASSIGNMENT

Compd.	Retention time (min)	Relative retention time (min)	Structure	Mass	Root cause
2-Isopropyl-4-hydroxy anisole	7.87	1.00		165.6	
Dimeric impurity of 2-isopropyl-4-hydroxy anisole	18.48	2.34		329.6	Process Related

using analytical HPLC to confirm the RRT and purity of the isolated impurity. The pooled fraction was concentrated under high vacuum Buchi Rotavapour R-124 to distill out the acetonitrile solvent[6,7]. The remaining aqueous layer is subjected to lyophilization in vertis 6L lyophilizer to get a pure compound. The chromatographic purity of the impurity is tested by analytical liquid chromatography. Separately before and after concentration and found to be 99.77 %. The isolated fluffy solid mass used for spectral studies.

Structure elucidation of dimeric impurity: The EI mass spectrum of unknown impurity exhibited a molecular ion peak at m/z 329.5 atomic mass unit (amu) which was 164 amu more than 2-isopropyl-4-hydroxy anisole which itself shows the unknown impurity is dimer. In the FT-IR spectrum a characteristic absorption band is appeared at 1682 cm^{-1} for -CH_3 symmetric and asymmetric deformations, stretching and at

2870 and 2927 cm^{-1} shows-CH_3 asymmetric and symmetric deformation[8], respectively. IR spectra also shows -OH stretching at 3421 cm^{-1}. In the ^1H spectrum of the unknown impurity in $CDCl_3$, 12 protons appeared in the up field region (1.14-1.16 ppm), 6 in the up field region (3.72) and two protons appeared at 6.67 and 6.82 ppm. ^{13}C shows presence of 4-CH_3 groups at 23.03 ppm.

Based on the above spectral data the molecular formula of dimeric impurity was confirmed and the corresponding structure was characterized as 5,5'-dimethoxy-4,4'-*bis*(1-methylethyl)biphenyl-2,2'-diol.

ACKNOWLEDGEMENTS

The authors wish to thank Management of Alembic Limited for supporting this work. Cooperation from the colleagues of synthetic and analytical research is appreciated.

REFERENCES

1. ICH Guideline Q3A (R), Impurities in New Drug Substances, 7 February (2002).
2. K.D. Altria, *Isolation Purification*, **2**, 113 (1996).
3. S. Ahuja, Impurities Evaluation of Pharmaceuticals, Marcel Dekker Inc. (1988).
4. S. Ahuja and S. Scypinski, Handbook of Modern Pharmaceutical Analysis, Academic Press, NY (2001).
5. J.E. Swagzdis and B.A. Mico, *J. Pharm. Sci.*, **75**, 90 (1986).
6. I.G. Beatie and T.J.A. Blake, *J. Chormatogr.*, **474**, 123 (1989).
7. P. Coufal, K. Stulik, H.A. Claessens, M.J. Hardy and M. Webb, *J. Chormatogr. B*, **732**, 437 (1999).
8. K.D. Zissis, R.G. Brereton and R. Escott, *Analyst*, **122**, 1007 (1997).

(*Received*: 9 June 2008; *Accepted*: 16 March 2009) AJC-7347