Trade Science Inc.

December 2009 Volume 8 Issue 4

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 8(4) 2009 [459-463]

A validated GC method for estimation of process related impurities in 3-(N-methyl-N-pentylamine) propionic acid hydrochloride (ISB), a precursor of ibandronate sodium

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Received: 28th August, 2009 ; Accepted: 7th September, 2009

ABSTRACT

The present paper describes the development of a gas liquid chromatographic method for advanced intermediate of Ibandronate sodium (ISB) in the presence of its impurities. As the molecule is a hydrochloride salt, the sample preparation was critical for the quantification of known and unknown impurities. Successful separation of ISB from the synthetic impurities achieved on a AT-5, 30 m × 0.53 mm, 5µ column. The developed GC method was validated with respect to linearity, accuracy, precision, ruggedness and robustness. To the best of our knowledge, a validated GC method which separates all the impurities disclosed in this investigation was not published elsewhere.

KEYWORDS

Gas liquid chromatography;
Method validation;
Ibandronate sodium.

INTRODUCTION

3-(N-methyl-N-pentylamine) propionic acid hydrochloride, (ISB), is the precursor of Ibandronate sodium, chemically 1-Hydroxy-3-(methylpentylamino) propylidene bisphosphonic acid, monosodium salt, monohydrate, that inhibits osteoclast-mediated bone resorption[1,2].

In order to commercialize an active pharmaceutical ingredient, it is mandatory requirement from regulatory authorities to show the proper qualification of its advanced intermediate through monitoring known and unknown impurities that are present[3]. Three impurities are identified as process related and needs to be quantified[4,5].

Organic impurities can arise during the manufacturing process and storage of the drug substances and the criteria for their acceptance up to certain limits are based on pharmaceutical studies or known safety data[6]. In the present study we describe a simple, economic and time efficient Gas chromatographic method for the separation and quantification of process related impurities of ISB. The accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), ruggedness and robustness of the method was determined in accordance with ICH guidelines[7,8] and found to be suitable for quality assurance of ISB. The present paper provides the validated GC method which separates potential impurities for first time.

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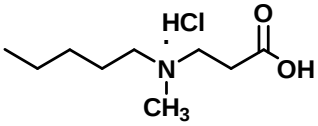
EXPERIMENTAL

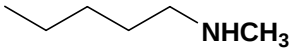
Chemicals

Samples of ISB and three impurities namely imp-A, imp-B and imp-C (Figure 1) were synthesized and characterized by using Mass, IR and NMR in our laboratory. All reagents used were of analytical-reagent grade unless otherwise stated.

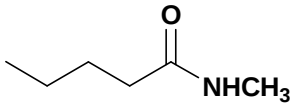


3-(*N*-methyl-*N*-pentylamino)-propionic acid Hydrochloride

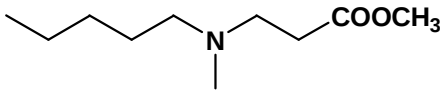
(a) ISB.



(c) Impurity-B
***N*-methyl-*N*-pentyl amine**



(b) Impurity-A
Valeric acid methyl amide



(d) Impurity-C
3-(*N*-Methyl-*N*-pentyl amine) propionic ester

Figure 1 : Chemical structure and name of (a) ISB (b) Impurity-A (c) Impurity-B (d) Impurity-C.

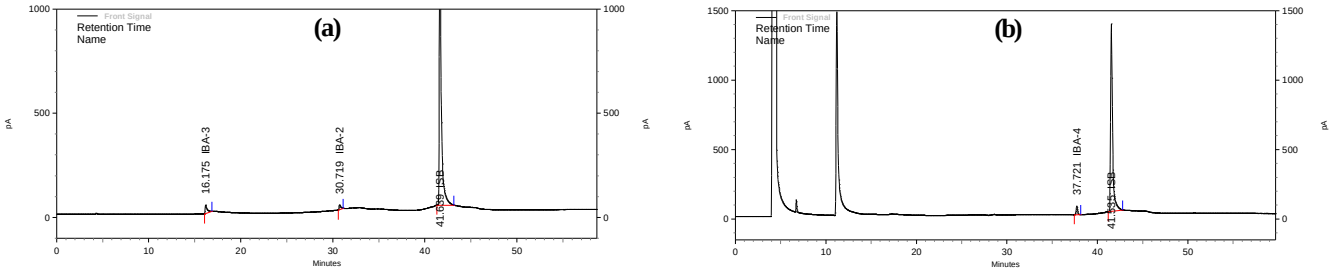


Figure 2 : (a) Impurity blend chromatogram-1 (Impurity-A, Impurity-B and ISB) and (b) Impurity blend chromatogram-2 (Impurity-C and ISB).

Equipment

The GC system was equipped with auto sampler connected with a Flame ionization detector.

Preparation of standard and sample solutions

1 gram of ISB sample was dissolved in 6mL of water, the resulted solution pH adjusted to 9.5 with 5.0N sodium hydroxide solution and made up to 10mL for estimation of imp-A and imp-B.

200mg of ISB sample dissolved in 8mL of acetonitrile, the resulted solution pH adjusted to 7.0 with triethylamine and diluted to 10mL with acetonitrile for estimation of imp-C

A stock solution of a mixture containing the two impurities imp-A and imp-B, were prepared at 1000 µg mL-1 in water.

A stock solution of imp-C was prepared at 200 µg mL-1 in acetonitrile.

Chromatographic conditions

High pure helium gas was used as mobile phase. The system was equilibrated for 30 minutes and analysis was carried out under temperature gradient using a flow rate of 2.5psi. Injector temperature was maintained at 90°C for estimation of imp-A, imp-B and unknown impurities and 110 °C for estimation of imp-C. Detector temperature was maintained at 260°C. AT-5, 30m X 0.53mm, 5µ column was used for successful separation. The injection volume was 1.0 µL with a split ratio of 1:5. The initial column temperature was held at 50°C for 5 minutes, then raised to 105°C @ 6°C per minute, held at 105°C for 8 minutes. Again the temperature was increased to 180°C @10°C per minute, held at 180°C for 8 minutes. Finally the column temperature was increased to 260°C @ 20°C and held at 260°C for 18 minutes.

Method development & optimization of chromatographic conditions

The 3-(N-methyl-N-pentylamine) propionic acid hydrochloride and the related impurities are non chro-

mophoric, and cannot be detected by conventional HPLC detector like UV, fluorescence, etc. Hence Gas chromatography was chosen for the estimation of impurities. Since the molecule is a hydrochloride salt, the regular diluents like water, acetonitrile, did not yielded ISB peak by using the above chromatographic conditions due to the presence of hydrochloric acid in the resultant solution. Known impurities can be quantified using external quantification method by injecting known quantities of impurities. But quantification of unknown impurities may not be easier, without ISB peak. Hence acidic nature of resultant sample solution makes the peak elution and determination difficult. Selection of diluents and preparation of sample solution was found to be critical. A good peak of ISB was obtained by injecting the sample solution, prepared by dissolution of sample in 6ml of water, adjusting the pH of the resulting solution to basic, i.e., 9.5 and made to 10ml with water. However, imp-C, 3-(N-methyl-N-pentylamine)-propionate, being an ester, found to be hydrolyzed in presence of water giving back ISB. Hence Acetonitrile was used as diluent for the estimation of imp-C and pH adjusted to 7.0 with triethylamine and external quantification method was used.

In optimized chromatographic conditions, the relative retention time for imp-A and imp-B was found to be 0.4 and 0.74 respectively and relative retention time for imp-C was found to be 0.91.

Method validation

Precision

The precision of the method was determined by injecting six individual preparations of ISB spiked with 0.1% of imp-A, 0.5% of imp-B and 1.0% of imp-C with respect to ISB analyte concentration. % RSD was calculated for the area of each imp-A, imp-B and imp-C respectively. The intermediate precision of the method was evaluated by a different analyst and different instrument in the same laboratory.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were determined by signal to noise ratio method by injecting a series of dilute solutions with known concentrations.

Linearity of response

The linearity of the method was determined by using test solutions prepared by diluting stock solutions to the required concentrations at 0.025, 0.05, 0.075, 0.10, 0.125 and 0.15% with respect to the ISB test concentration (100mg mL-1) for imp-A, 0.125, 0.25, 0.375, 0.50, 0.625 and 0.75% with respect to the ISB test concentration (100 mg mL-1) for imp-B and 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50% with respect to the ISB concentration (20mg mL-1) for imp-C. The peak response versus concentration data was treated by least-squares linear regression analysis.

Accuracy

The accuracy study of impurities was carried out in triplicate at 0.05% (50%), 0.10% (100%) and 0.15% (150%) with respect to the ISB test concentration (100mg mL-1) for imp-A, 0.25% (50%), 0.50% (100%)and 0.75% (150%) with respect to the ISB test concentration (100 mg mL-1) for imp-B and 0.50% (50%), 1.00% (100%) and 1.50% (150%) with respect to the ISB concentration (20mg mL-1) for imp-C. The percentages of recoveries for impurities were calculated from the respective known concentrations.

Robustness

To determine the robustness of the analytical method, experimental conditions were deliberately altered and the resolution between ISB, imp-A, imp-B and imp-C was recorded. To study the effect of flow rate on the resolution, flow was changed by 10% from 2.25psi to 2.75psi.

Solution stability

The solution stability of ISB was carried out by leaving both the test solutions of sample and reference standard in tightly capped volumetric flasks at room temperature for 24 hours.

RESULTS AND DISCUSSION

Precision

The precision and intermediated precision were successfully demonstrated by achieving % RSD of <4.0% for peak areas of six replicate determinations of all impurities namely imp-A, imp-B and imp-C, respectively (TABLE 1).

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TABLE 1 : Validation data

Validation data			
Parameter	**Impurity-A**	**Impurity-B**	**Impurity-C**
Linearity			
r	0.9995	0.9995	0.9996
Slope	2611.0	2720.3	3283.5
Y-Intercept	0.57	-34.12	-21.47
Accuracy (% recovery)			
LOQ (n = 3)	94.2	95.0	98.9
50% (n = 3)	98.8	100.1	107.3
100% (n = 3)	98.8	100.2	99.2
150% (n = 3)	97.2	100.2	102.5
Precision (n = 6)			
Intraday			
% RSD	2.9	1.6	1.1
Different day and system			
% RSD	0.0	4.0	1.2
Robustness (RRT)			
Actual flow 2.5PSi	0.74	0.39	0.91
Different flow 2.25PSi	0.74	0.40	0.92
Different flow 2.75Psi	0.73	0.37	0.89
LOD	0.0006%	0.003%	0.0045%
LOQ	0.002%	0.01%	0.015%

Limit of detection and limit of quantification

The LOD values were found to be 0.0006mg mL^{-1}, 0.003mg/mL and 0.0009mg/mL and the LOQ values were found to be 0.002, 0.01 and 0.003 mg/mL, respectively, for all the impurities namely imp-A, imp-B and imp-C with respect to the ISB test concentration.

Linearity of response

Linear calibration plot of for the analytical method was obtained over the calibration ranges tested, i.e. LOQ-150% for with respect to the limit of imp-A, imp-B and imp-C. The correlation coefficient obtained from 0.9995 to 0.9996. The above results show an excellent correlation existed between the peak area and the concentration (TABLE 1).

Accuracy

The percentage recovery of impurities in ISB samples varied from 96.1 to 107.3% (TABLE 1).

Robustness

In all the deliberate varied chromatographic conditions (2.25Psi, 2.75Psi flow rate), the relative retention times for all the impurities were found to be comparable. The validation data has been incorporated in TABLE 1.

Solution stability

No significant changes were observed in the content of impurities namely imp-A, imp-B, and imp-C during solution stability study conducted after 24 hours.

CONCLUSION

The GC method developed for determination of impurities of the ISB is precise, accurate and specific. The method has been validated and satisfactory results were observed for all the tested validation parameters. The developed method can be conveniently used for determining the quality control of ISB samples.

ACKNOWLEDGEMENT

The authors wish to thank the management of Dr.Reddy's Laboratories Ltd. for supporting this work. Cooperation from colleagues of Research & Development and Analytical Research & Development of Dr.Reddy's Laboratories Ltd. is appreciated.

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