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Original Research Article

Sensitive and accurate method for chromatographic quantification of ibandronate in bulk and dosage forms

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Abstract

Purpose: *To develop a simple, accurate and reproducible chromatographic method for determination of ibandronate.*
Methods: *Lack of chromophore groups in their structure is a problem with the determination of bisphosphonates. The developed method relies on chromatographic determination following derivatization of ibandronate with NBD-Cl. Separation and quantification of reaction products were done using HPLC system (LA CHROM MERCK HITACHI) composed of an isocratic pump (model L–7110) supplied with UV-visible wavelength detector (Model 7120) with Rheodyne injector (model 7161) and equipped with a 20-µL injector loop.*
Results: *Ibandronate was successfully derivatized with NBD-Cl to enhance its HPLC separation and quantification. The reaction conditions were optimized to achieve maximum sensitivity of the assay. The studied drug was satisfactorily quantified in the concentration range of 500 - 5000 ng/mL. Method validation was successfully performed in line with ICH directives in terms of robustness, accuracy, linearity and precision of the test method. The proposed method was satisfactory for the determination of ibandronate in either bulk and dosage forms.*
Conclusion: *An accurate and reproducible method for regular quantification of ibandronate has been developed, and it is suitable for use in analytical laboratories, including those of regulatory agencies.*

Keywords: *Ibandronate; HPLC; Analysis; Chromophore; Determination*

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INTRODUCTION

Ibandronate sodium monohydrate (IBN) or sodium hydroxy-[1-hydroxy-3-[methyl (pentyl) amino]-1-phosphonopropyl] phosphonate hydrate inhibits bone resorption associated with osteoclast activity [1]. It elevates bone mineral density, and it can be used to prevent and treat osteoporosis [2]. The analytical process for bisphosphonates in dosage form is challenging since they do not have light-absorbing groups. Bisphosphonates are very polar compounds which are not well retained on HPLC columns [1]. Alendronate, a member of bisphosphonates, was quantified only after it was extracted and derivatized using 9-fluorenylmethyl [3]. Different amino-bisphosphonates have been quantified with GC-MS after their conversion to readily

volatile compounds [4]. In addition, different bisphosphonates and their impurities have been quantified using capillary electrophoresis [5]. Moreover, IBN has been determined *via* enzyme-linked immunosorbent assay **(**ELISA) procedure [6]. Amino-bisphosphonates in biological samples were recently quantified using liquid chromatography coupled with tandem mass spectrometry after their liquid-liquid extraction and derivatization [7,8].

Molecular spectrophotometry is a key method for the quantification of pharmaceuticals, where indirect spectrophotometry was applied for the determination of IBN after the substitution of salicylate from its complex with Fe^{3+} [9], and direct spectrophotometry was used to determine IBN [10]. The direct spectrophotometric method has the drawback of lower sensitivity due to lower absorptivity at the selected wavelength, arising from absence of a chromophore in the chemical structure of the studied drug.

Therefore, the aim of this study was to develop an accurate, rugged and simple chromatographic method that can be easily applied in quality control laboratories to facilitate the process of quality assurance.

EXPERIMENTAL

Raw material and dosage form

The IBN reference material ($C_9H_{24}NNaO_8P_2$; molecular weight 359.23 g/mol) was purchased from Bio Vision (USA), with declared purity of 99.97 % (CAS # 138844-81-2). Boniva® injection (3 mg/3 mL), NDC 0004-0191-09, was obtained from Genentech Inc. (San Francisco, United States of America).

Chemicals and reagents

Acetonitrile and methanol (HPLC grade) were purchased from Sigma Aldrich. Phosphate buffer solution (pH 8.5 ± 0.2) [11] was prepared by dissolving specific amounts of potassium dihydrogen orthophosphate and sodium hydroxide (Sigma Aldrich) in distilled water. 4-Chloro-7-nitro-2,1,3-benzoxazole [NBD-Cl, E. Merck Darmstadt-Germany, 0.1% (w/v)] was prepared by dissolving 100 mg of NBD-Cl in methanol in 100-mL measuring flask, and making up to volume with methanol. Distilled H_2O was purchased from "Aquatron" (Staffordshire).

Instrumentation

The HPLC system (branded LA CHROM MERCK HITACHI) was composed of an isocratic pump (model L–7110) supplied with UV-visible wavelength detector (Model 7120), injector and an injector loop.

Stock and working standard solutions

A stock solution of IBN (500 µg/mL) was made by dissolving 50 mg of IBN powder in a 100-mL volumetric flask with distilled water. The solution was made up to volume. To prepare IBN working solution (50 µg/mL), the stock (500 µg/mL) was accurately diluted with distilled H_2O.

Validation studies

Linearity

Different volumes of IBN working standard (0.1 – 1 mL) were transferred to a set of stoppered test tubes. Two (2) mL phosphate buffer solution was added, followed by 1.5 mL of 0.1% (w/v) NBD-Cl. After warming to 70 ºC for 20 minutes, the contents of each test tube were cooled to room temperature, transferred quantitatively to 10-mL calibrated volumetric flask and made up to volume with methanol. Samples were then chromatographed using a stationary column of length 15 cm and internal diameter of 5 mm, which was packed with 5-µm ODS C18 packing material. The mobile phase composition was methanol: acetonitrile: water (6:2:1, by volume) programmed at 1 mL/min, and absorbance was read at 470 nm. A calibration curve was produced by plotting peak areas against the corresponding IBN levels. Then, a regression equation was generated.

Accuracy

It can be considered as the percentage of the recovered analyte from a definite quantity. It was checked by analyzing the studied drug at three concentration levels (1000, 2000, and 3000 ng/mL) using the procedure under linearity. The concentration of each sample was calculated from the regression equation.

Precision

Intra-day precision (repeatability) was assessed by analyzing three concentrations of IBN (1000, 2000 and 3000 ng/mL) three times within the same day using the above-mentioned procedure under linearity. On the other hand, inter-day precision was checked by analyzing three concentrations (1000, 2000 and 3000 ng/mL) of IBN on 3 continuous days with the same steps as indicated in linearity.

Robustness

This was done by introducing small changes in method parameters such as flow rate, mobile phase composition and the pH required to complete the reaction, followed by evaluation of their effects on the developed method.

Application to a pharmaceutical formulation

One (1) mL of Boniva® injection (3 mg/3 mL) was diluted to 1000-mL using distilled water. Then, the procedure under linearity was followed to get the concentrations of IBN in the dosage forms. Furthermore, addition test was performed by spiking the dosage form with bulk IBN quantities at three concentration levels (1000, 2000 and 3000 ng/mL). Then, the procedure under linearity was followed.

RESULTS

In this work, overcoming the difficulty of direct spectrophotometric detection of the studied drug was achieved using NBD-Cl as a derivatizing agent. Several trials were carried out with various types of mobile phase at varying speeds to determine optimum separation, and excellent peak for IBN – NBD-Cl reaction product was obtained (Figure 1). The best mobile phase and flow rate were methanol: acetonitrile: water (6:2:1, by volume) and 1 mL per minute.

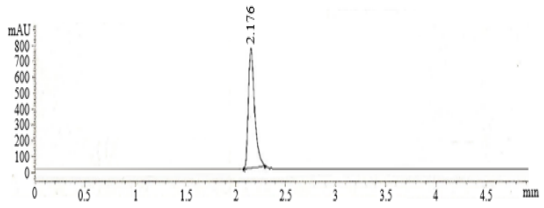

Figure 1: HPL chromatogram of the separated IBN – NBD-Cl reaction product

On the other hand, ODS C-18 column was the best stationary phase. Optimum separation was attained at t_R of 2.17 min.

System suitability parameters were calculated for the developed method. The results are presented in Table 1. The results indicate excellent efficiency of the column and acceptable peak symmetry.

Linearity was determined by plotting the relationship between peak area ratio and concentration of IBN across a range of 500 – 5000 ng/mL (Figure 2). The regression equation computed was as:

$PA = 0.9855C + 22.154$ ..... (1) $r = 0.9995$

where *PA* is peak area, *C* is concentration (ng/mL), and *r* is correlation coefficient.

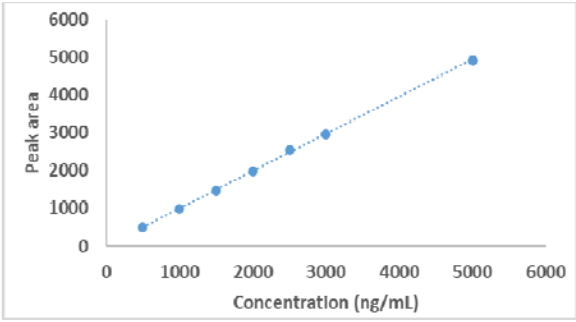

Figure 2: Calibration curve for the HPLC determination of IBN

The new method was validated in line with the recommendations of USP [12]. Table 2 shows the precision, accuracy, robustness and linearity of the method. The data obtained were within acceptable sensitivity, linearity, and reproducibility of the proposed method. The developed procedure was successfully used for quantification of the drug (IBN) in the dosage form. The proposed method was also validated using standard addition test which gave acceptable outcomes (Table 3). When results got using the developed method were compared to a reference method [9], the calculated t-test and F-value [13] were less than the corresponding tabulated ones, indicating accuracy and precision.

Table 1: System suitability parameters for the HPLC method

Parameter	PAM-NBD-Cl reaction product	Reference value [12]
$t_{R\ (min)}$†	2.17 ± 0.12	-
Number of theoretical plates (N)	7520	Increased with increase in efficiency of separation
Height equivalent to theoretical plates (HETP)	0.002	Decreased with increase in efficiency of separation
Tailing factor (T)	1.11	T=1 for a typical symmetric peak.

†Triplicate runs per sample

Table 2: Validation of the HPLC procedure

Parameter	Proposed HPLC method
Accuracy (mean ± SD)	100.18 ± 1.40
Precision (RSD)	
Repeatability*	101.98 ± 1.27
Intermediate precision*	102.81 ± 1.41
Robustness (mean ± SD)	
Variation of the mobile phase composition	99.49 ± 1.11
Variation of the pH	101.81 ± 1.41
Variation of the flow rate	100.87 ± 1.54
Linearity	
Range(ng/mL)	500-5000
Slope	0.9855
Intercept	22.154
Correlation coefficient (r)	0.9995
LOD (ng/mL)	400
LOQ (ng/mL)	500

*Intra-day and inter-day RSD of the mean of 3 alendronate Na concentrations. LOD and LOQ were obtained experimentally

Table 3: IBN levels in dosage form based on the proposed HPLC procedure

Parameter	Content uniformity	Standard addition
Dosage form		
Mean ± SD*	102.87 ± 0.79	103.33 ± 0.57
RSD (%)	0.77	0.55
Pure IBN		
Mean ± SD	100.18 ± 1.40	99.81 ± 0.59
RSD	1.40	0.59
N	8	5
Variance	1.96	0.35
F-value (6.09)	5.62	-
t-test (2.201)	0.556	-

*Standard deviation, average of three determinations
6.09 and 2.201 = hypothetical values of F and t at p = 0.05 in the pure IBN

DISCUSSION

There is no doubt that HPLC technique solves a lot of problems in the pharmaceutical analysis era, especially in the case of the studied drug (IBN), which lacks the presence of a chromophore. This makes its spectrophotometric detection a tough process. Thus, in this work, derivatization of IBN using NBD-Cl reagent facilitated its spectrophotometric detection after chromatographic separation. The method was optimized with respect to the chromatographic conditions to get the best peak shape and system suitability parameters, and it was fully validated in line with the USP stipulations. It demonstrated good sensitivity as indicated by the values of LOD and LOQ, and it was effectively applied for quantification of IBN. In view of all the acceptable results with respect to method optimization, method validation and method application, the developed method is a simple and sensitive alternative that can be applied in quality control laboratories.

CONCLUSION

The developed method is simple, accurate, precise, and reproducible. Therefore, it can be used in quality control and quality assurance laboratories to quantify IBN in bulk and dosage forms.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this study.

Contribution of authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

Data availability

All the data associated with this research have been included in the manuscript.

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