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Derivatization and Spectrophotometric Quantification of Pamidronate in Bulk and Dosage Forms

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Aims: To quantify pamidronate in a sensitive and accurate way either in bulk or dosage forms.
Methodology: The quantification of this group of drugs is a challenging task as they lack the presence of chromophore groups in their structure. The proposed method depends on the derivatization of the studied drug by its reaction with 4-Chloro-7-nitro-2,1,3-benzoxazole and the product is measured spectrophotometrically at 470 nm. The conditions for the reaction are optimized regarding the volume of the reagent, the optimum pH for the reaction completion, the buffer volume, the optimum temperature for the reaction and the optimum heating time.
Results: The studied drug can be determined in the range of 9-30 µg/mL after optimizing the reaction conditions. Method validation is performed according to ICH guidelines and different validation parameters like, linearity, accuracy, precision and robustness are calculated and found to be excellent.
Conclusion: The proposed method is accurate, sensitive and can be applied for the routine analysis of pamidronate in quality control laboratories.

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Keywords: Pamidronate disodium; derivatization; spectrophotometry; analysis; quantification; determination.

1. INTRODUCTION

Pamidronate disodium (PAM) is a bisphosphonate drug that used to treat high levels of calcium in the blood that may be caused by certain types of cancer. PAM is used along with cancer chemotherapy to treat bone damage caused by multiple myeloma or by breast cancer that has spread to the bones. It is also used to treat Paget's disease [1]. Analysis of bisphosphonates is a challenging task. These compounds mostly lack a chromophore. They are extremely polar with several functional groups and very difficult to analyze and not well retained on HPLC columns. Most of the applied methods for the quantification of PAM lie in the category of liquid chromatography [2-4] and gas chromatography [5]. Due to the limited number of the published methods dealing with the determination of PAM, there is an urgent need to develop new methods for its determination either in bulk or dosage forms. So, the main target of this work is to adopt a simple, sensitive and accurate method of analysis of PAM which can be applied in the routine work of quality control laboratories.

2. MATERIALS AND METHODS

2.1 Raw Material and Dosage Form

PAM raw material ($C_3H_9NO_7P_2Na_2 \cdot 5H_2O$); molecular weight 396.1 g/mol was kindly provided by Novartis Pharmaceuticals, San Carlos, CA 94070, United States. Its purity was certified to be 99.81%. Aredia® injection (30 mg/10 mL) for intravenous infusion was manufactured by Novartis Pharmaceuticals, USA.

2.2 Chemicals and Reagents

Methanol (HPLC grade) was purchased from Sigma Aldrich. Phosphate buffer solution (pH 8.5 ± 0.2) [6] 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 then the volume was completed to the mark using the same solvent. Distilled water from "Aquatron" Automotive water Still A 4000 [bibby Sterillin Ltd., Staffordshire-UK).

2.3 Instrumentation

A “Double-beam Spectrophotometer (JASCO, Kyoto, Japan)” containing matched quartz cuvettes with path lengths of 1 cm was used. The spectrophotometer was connected to an “IBM-compatible computer with an HP 680 inkjet printer (Hewlett Packard, USA)”.

2.4 Standard Solution

PAM standard solution (300 µg/mL, 7.57×10^{-4}M), was prepared by accurate weighing and transferring 30 mg of PAM into 100–mL volumetric flask. Dissolution in distilled water was completed by a vortex mixer, then the volume was completed to the mark using the same solvent.

2.5 Procedure

2.5.1 Determination of the λmax of the formed colored product

An aliquot (0.8 ml) of PAM was transferred quantitatively into a 20-ml stoppered test tube, followed by addition of 2 ml phosphate buffer solution (pH 8.5 ± 0.2) then 1.5 ml NBD-Cl, 0.1% (w/v) reagent was added. Heating in a thermostatic water bath was done at 70°C for 20 minutes then the contents of the test tube were cooled to the room temperature and transferred quantitatively to 10-ml calibrated measuring flask. The volume was completed with methanol, then homogenization was carried out. The absorption spectrum was recorded against a reagent blank.

2.5.2 Effect of the volume of NBD-Cl solution

The procedure under "*Determination of the λmax of the formed product*" was followed on 0.8 ml aliquots of PAM standard solution (300 µg/mL) but on adding different volumes of the NBD-Cl reagent ranging from 0.5 - 3 ml to each test tube. The absorbance was recorded at 470 nm against its corresponding reagent blank.

2.5.3 Effect of pH on the color development

The procedure under "Determination of the λmax of the formed colored product" was followed on

0.8 ml portions of PAM standard solution (300 µg/mL) but on establishing different pH values ranging from 7 - 9.5. The absorbance of the prepared colored solutions was recorded at the specified wavelength against its appropriate blank.

2.5.4 Effect of buffer volume on the color development

The procedure under "Determination of the λmax of the formed colored product" was followed on 0.8 ml aliquots of PAM standard solution (300µg/ml) but on using different volumes of the buffer solution (pH 8.5 + 0.2) ranging from 0.5 - 3 ml to each tube, then the absorbance of the prepared solutions was recorded at the specified wavelength against its appropriate blank.

2.5.5 Effect of temperature on color development

The procedure under "Determination of the λmax of the formed colored product" was followed on 0.8 ml aliquots of PAM standard solution (300 µg/mL) but on applying variable temperature values from 40-90 ^{o}C in a thermostatic water bath. The absorbance was recorded at the specified wavelength against its corresponding blank.

2.5.6 Effect of heating time on the color development

The procedure under "Determination of the λmax of the formed colored product" was followed on 0.8 ml aliquots of PAM standard solution (300 µg/mL) but recording the absorbance of the prepared colored solutions after variable heating time within 45 minutes, at the specified wavelength against its corresponding blank.

2.5.7 Effect of time on stability of the formed colored product

The previous procedure was applied on 0.8 ml aliquot of PAM standard solution (300 µg/mL) and the absorbance of the colored solution was measured at different time intervals (15, 30 and 90 minutes) against a suitable blank.

2.5.8 Estimation of the reaction stoichiometry

It was done by applying by continuous variation method [7]. Aliquots (0.1, 0.2, 0.3…and 0.9 ml) of $7.57x10^{-4}$ M of PAM standard solution were transferred into a set of stoppered test tubes. Volumes (0.9, 0.8, 0.7…. and 0.1 ml) of 7.57 x 10^{-4} M of NBD-Cl reagent were added, respectively, followed by addition of 2 ml buffer solution (pH 8.5 $\pm$ 0.2). The procedure was followed as under "Determination of the λmax of the Formed Colored Product" starting from:…"Heating in a thermostatic water bath was …." The absorbance of each solution was measured at 470 nm against its appropriate blank.

2.5.9 Linearity

A set of stoppered test tubes was prepared to contain aliquots of PAM (0.3 - 1 ml) standard solution (300 µg/ml), followed by addition of 2 ml phosphate buffer solution (pH 8.5 + 0.2) then 1.5 ml NBD-Cl, 0.1% (w/v) reagent was added. Heating in a thermostatic water bath was done at 70oC for 20 minutes then the contents of each test tube were cooled to room temperature and transferred quantitatively to 10-ml calibrated measuring flask. The volume was completed with methanol and the absorbance was measured at 470 nm against a reagent blank. The calibration graph was constructed by plotting the absorbance against the corresponding concentration.

2.5.10 Accuracy

The previously mentioned procedure under linearity was repeated for the determination of different concentrations of PAM (9 – 30 µg/ml). The concentrations were calculated from the corresponding regression equation.

2.5.11 Precision

Three concentrations of PAM (9, 21 and 30 µg/ml) were analyzed three times at the same day or in three successive days using the previously mentioned procedure under linearity.

2.5.12 Robustness

A deliberate variation in the method parameters was studied as variation of the buffer pH, temperature and volume of the used chromogen and time required for complete reaction.

2.5.13 Application to pharmaceutical formulation

An aliquot of 0.1 mL was quantitatively transferred into a stoppered test tube then the procedure was completed as under linearity.

3. RESULTS AND DISCUSSION

In this study, a simple and sensitive colorimetric method was adopted. The method was based on using NBD-Cl as a chromogen for the determination of the studied drug, in bulk and tablet forms. The reaction of the anti-osteoporotic drug with NBD-Cl gave highly stable colored product which exhibited maximum absorption at 470 nm, Fig. 1.

Optimum conditions affecting the reaction were studied in order to optimize different parameters for the quantitative determination of the studied drug. The variables that were found to affect the intensity of the resulting color namely, volume of the reagent, pH, volume of the buffer, temperature, time of heating and stability of the produced color. Maximum color intensity was attained on using 1.5 ml of NBD-Cl solution (0.1% w/v), as shown in Fig. 2. Different pH values were used for the studied reaction and it was found that, the reaction needed a slight alkaline medium (pH 8.5 $\pm$ 0.2), Fig. 3. The optimum volume of the buffer (pH 8.5 $\pm$ 0.2) was 2 ml, Fig. 4. Maximum color intensity was, also attained at 70oC, Fig. 5, where under this temperature, the reaction was not complete and above it, the colored product started to be destructed and the optimum time of heating was 20 minutes, Fig. 6. The color was stable for up to 90 minutes. The stoichiometry of the reaction was determined using continuous variation method [7], which revealed a 1:1 ratio of drug and NBD-Cl reagent upon using 7.57 x 10^{-4} M solution of both.

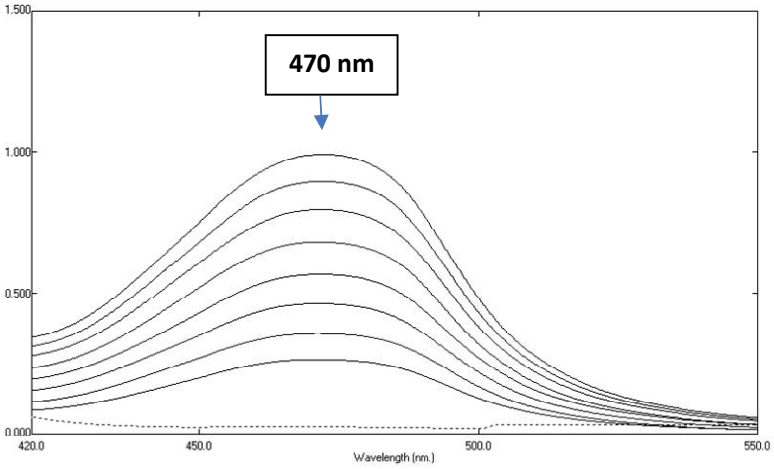

Fig. 1. Absorption spectra of the reaction product in the range of 9-30 µg/mL and the reagents blank

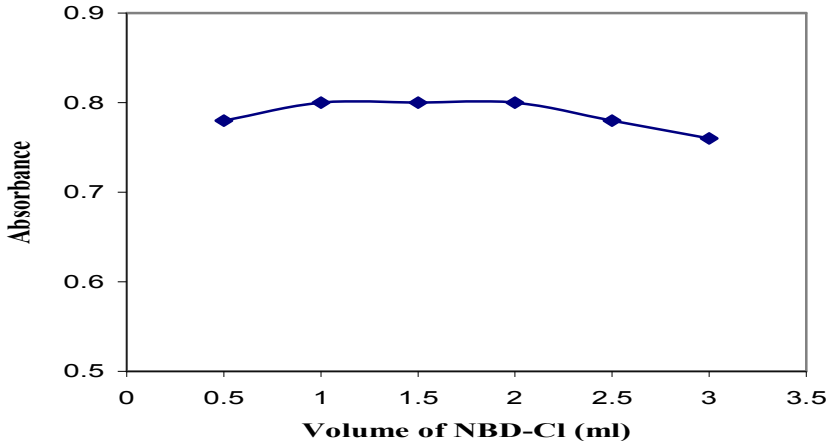

Fig. 2. Effect of the volume of NBD-Cl on the formation of the reaction product

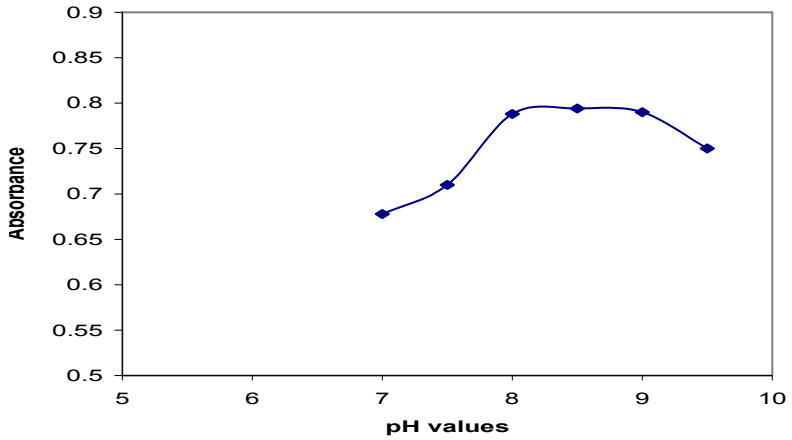

Fig. 3. Effect of different pH of phosphate buffer on the formation of the reaction product

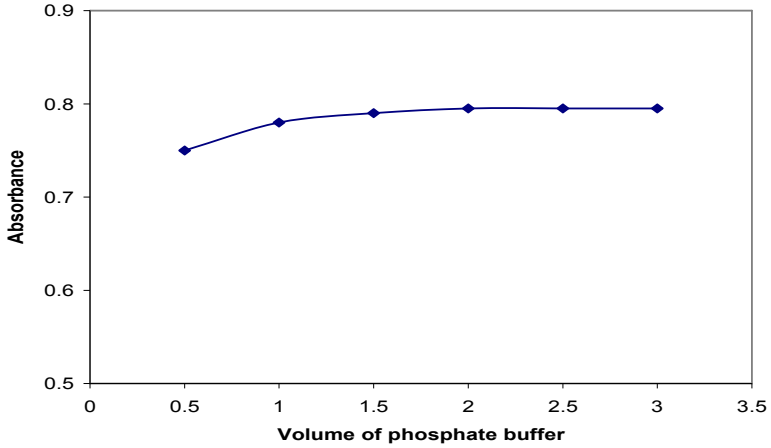

Fig. 4. Effect of different volumes of phosphate buffer (pH 8.5 + 0.2) on the reaction product

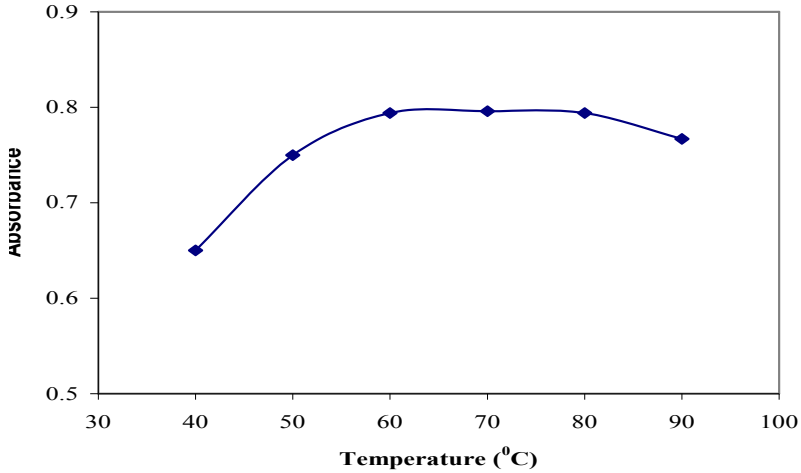

Fig. 5. Effect of temperature on the formation of the reaction product

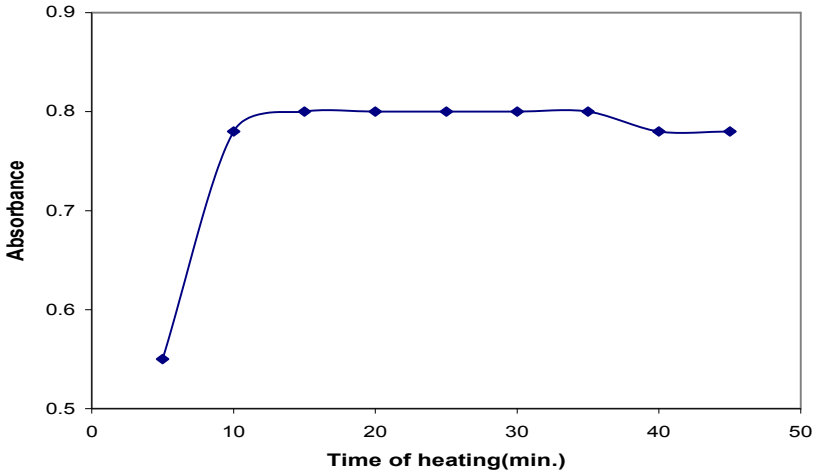

Fig. 6. Effect of the heating time on the formation of the reaction product

Table 1. Application of the proposed method for the determination of PAM in its dosage form

Aredia® injection for intravenous infusion labelled to contain 30 mg/10 mL	**Content uniformity**	**Standard addition**
Mean ± SD*	99.54 ± 0.874	100.11 ± 0.541
%RSD	0.878	0.540

**Standard deviation, average of three determinations*

By applying the specified optimum conditions, the calibration graph was plotted. Beer's law was obeyed with well-fitted linear relationship, between the absorbance of the reaction product and the corresponding concentrations, in the range of 9-30 µg/mL. The regression equation was computed to be:

$$A = 0.0353\ C - 0.0621 \quad r = 0.9996$$

Where,

A: Absorbance of the reaction product at 470 nm
C: Concentration (µg/ml)
r: Correlation coefficient.

To check accuracy of the method, different concentrations of the pure drug were analyzed by the proposed method. The suggested method was successfully applied for the determination of the studied drug in its dosage form. Also, the standard addition technique was also carried out to show the accuracy of the proposed method showing satisfactory results, Table 1.

Validation parameters according to USP [8] guidelines like accuracy, repeatability, intermediate precision and linearity parameters for the proposed method are presented in Table 2.

Table 2. Validation results of the proposed method

Parameters	**The proposed spectrophotometric method using NBD-Cl regent**
Accuracy (Mean+SD)	100.33 ± 0.634
Precision	
Repeatability*	100.98 + 0.758
Intermediate precision*	99.58 + 0.987
Robustness	101.34 + 0.896
Linearity:	
Slope	0.0353
Intercept	-0.0621
Correlation coefficient (r)	0.9996
Range(µg/mL)	9-30
LOD (µg/ml)	4.5
LOQ(µg/ml)	9
A(1%, 1cm)	333.333
Apparent molar absorptivity (ε)	10837

** Intra-day and interday relative standard deviation of the average of three concentrations of alendronate sodium*
LOD and LOQ are obtained experimentally

One can conclude that the proposed method is simple, accurate, precise and reproducible therefore it could be used in quality control laboratories for the analysis of the studied drug in bulk and dosage forms.

4. CONCLUSION

One can conclude that the proposed method is simple, accurate, precise and reproducible. Therefore, it could be used in quality control laboratories for the analysis of the studied drug in pure and tablet forms.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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