Validated High Performance Liquid Chromatographic Quantification of Pamidronate in Bulk and Dosage Form

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Authors’ contributions

All the work is carried by the three authors equally. All authors read and approved the final manuscript.

ABSTRACT

Aims: The studied drug is lacking the presence of chromophore so a reaction with NBD-CI is optimized to facilitate its chromatographic detection, so the main aim of the work is 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 chromatographic quantification of the studied drug after its derivatization via its reaction with 4-Chloro-7-nitro-2,1,3-benzoxazole and the product is separated on ODS C18 column (5 µm, 15 cm x 5 mm, i.d.) as a stationary phase and methanol : water (8:2, v/v) as a mobile phase. The flow rate was 1 ml/min.

Results: The studied drug can be determined in the range of 900 - 3000 ng/mL after optimizing the assay conditions to get optimum stationary – mobile phases match. Method validation is performed

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according to USP-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.

**Keywords:** Pamidronate disodium; validation; HPLC; 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], gas chromatography [5] and LC-MS/MS [6,7]. 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 (C₉H₅NO₇P₂Na₂ 5H₂O); 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) [8] 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-benzoazole (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 Sterillini Ltd., Staffordshire-UK).

**2.3 Instrumentation**

High performance liquid chromatograph consisted of an isocratic pump (model L–7110), UV-visible wavelength detector (Model 7120) and a Rheodyne injector (model 7161) equipped with 20-µL injector loop. All by LA CHROM MERCK HITACHI.

**2.4 Standard Solution**

PAM stock standard solution (300 µg/mL), 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.

PAM working standard solution (30 µg/mL), was prepared by accurate transferring 10 mL of PAM stock standard solution (300 µg/mL) into 100-mL volumetric flask. The volume was then completed to the mark with distilled water to get the required concentration.

**2.5 Procedure**

**2.5.1 Linearity**

A set of stoppered test tubes was prepared to contain aliquots of PAM (0.3 - 1 mL) working standard solution (30 µg/mL). Addition of 2 mL phosphate buffer solution (pH 8.5 ± 0.2) then 1.5 mL NBD-Cl, 0.1% (w/v) was carried out. Heating in a thermostatic water bath was done at 70 °C for 20 minutes then the contents of each test tube were cooled to the room temperature and transferred quantitatively to 10-mL calibrated volumetric flask. The volume was completed to
the mark with methanol followed by homogenous mixing. Samples were then chromatographed using ODS C18 column (5 µm, 15 cm x 5 mm, i.d.) as a stationary phase and methanol : water (8:2, v/v) as a mobile phase. The flow rate was 1 ml/min.

Spectrophotometric detection was carried out at 470 nm. Peak area ratios (against the corresponding peak area of the external standard) were plotted against the corresponding concentration to obtain a calibration graph then the regression equation was computed.

### 2.5.2 Accuracy

The previously mentioned procedure under linearity was repeated for the determination of different concentrations of PAM (1200, 200 and 2500 ng/mL). The concentrations were calculated from the corresponding regression equation.

### 2.5.3 Precision

Intraday precision (Repeatability) was done by analyzing three concentrations of PAM (1500, 2000 and 2800 ng/mL) three times within the same day using the previously mentioned procedure under linearity. The mean percentage recoveries of the drug and relative standard deviation were calculated using the suggested HPLC-method.

Intermediate (interday) precision was done by analyzing three concentrations (1500, 2000 and 2500 ng/mL) of PAM on three successive days using the procedure stated under linearity. Calculations were carried out to get the mean percentage recoveries of the drug and relative standard deviation using the suggested HPLC-method.

### 2.5.4 Robustness

A slight variation in the method parameters as variation of the composition & flow rate of the mobile phase and the pH required for the reaction completion was done to assess their effects on the suggested method.

### 2.5.5 Application to pharmaceutical formulation using the proposed HPLC method

The necessary dilutions were performed for Aredia® injection (30 mg/10 mL) then the proposed technique was conducted to get the concentration of the studied drug. Standard addition technique was also applied.

### 3. RESULTS AND DISCUSSION

In this work, a specific and sensitive HPLC-method was established by using NBD-Cl as a derivatizing agent for the studied drug to get over the problem of difficulty of direct UV detection (non chromophoric bisphosphonate drug).
Table 1. Established system suitability parameters of the HPLC-method

| Parameter                                      | PAM-NBD-Cl reaction product | Reference value(15) |
|------------------------------------------------|-----------------------------|---------------------|
| \( t_R \) (min.)†                               | 3.22 ± 0.15                 | -                   |
| Number of theoretical plates (N)                | 8320                        | Increase with the increase in efficiency of separation |
| Height equivalent to theoretical plates (HETP)  | 0.018                       | Decrease with the increase in efficiency of separation |
| Tailing factor (T)                              | 1.41                        | \( T=1 \) for a typical symmetric peak. |

†Triplicate runs per a sample

Fig. 2. Calibration curve for the HPLC determination of PAM via derivatization using NBD-Cl reagent

Table 2. Validation results of the proposed HPLC method

| Parameters                                         | The proposed HPLC method |
|----------------------------------------------------|--------------------------|
| **Accuracy (Mean Recovery % ± SD)**                | 100.18 ± 1.393           |
| **Precision (RSD):**                               |                           |
| Repeatability*                                     | 99.88 ± 2.071            |
| Intermediate precision*                            | 98.59 ± 2.454            |
| **Robustness:**                                    |                           |
| Variation of the mobile phase composition          | 100.29 ± 2.216            |
| Variation of the pH                                | 99.89 ± 2.390             |
| Variation of the flow rate                         | 100.59 ± 1.828            |
| **Linearity:**                                     |                           |
| Slope                                              | 0.0006                    |
| Intercept                                          | - 0.1421                  |
| Correlation coefficient (r)                        | 0.9995                    |
| Standard error of the intercept                    | 0.03525                   |
| Confidence limit of the intercept                  | -0.2260 - -0.0535         |
| Standard error of the slope                        | 0.000017                  |
| Confidence limit of the slope                       | 0.000559 - 0.000643       |
| Range(ng/mL)                                       | 900-3000                  |
| LOD (ng/mL)                                        | 600                       |
| LOQ( ng/mL)                                        | 900                       |
Table 3. Application of the proposed method for the determination of PAM in its dosage form

| Aredia® injection for intravenous infusion | Content uniformity | Standard addition |
|------------------------------------------|--------------------|-------------------|
| labelled to contain 30 mg/10 mL          | 101.54 ± 0.654     | 102.41 ± 0.638    |
| Mean ± SD                               | 0.644              | 0.623             |
| %RSD                                    |                    |                   |

*Standard deviation, average of three determinations

Table 4. Statistical comparison between the results obtained by applying the proposed HPLC method and that obtained by the reference method for the determination of pure PAM

| Items                         | The proposed method | The reference method [4] |
|-------------------------------|---------------------|--------------------------|
| Mean ± SD                     | 100.18 ± 1.393      | 99.81 ± 0.587            |
| RSD                           | 1.39                | 0.588                    |
| n                             | 8                   | 5                        |
| Variance                      | 1.940               | 0.345                    |
| F-value (6.09)                | 5.62                | -                        |
| Student’s t-test (2.201)      | 0.556               | -                        |

Values in parenthesis are the theoretical values of t and F at P = 0.05

Several trials have been carried out to obtain good peak characters for alendronate – NBD-Cl reaction product. Fig. 1. These trials involved the use of different mobile phases with different flow rates. The mobile phase of choice was methanol: water (8: 2, v/v). On the other hand, the stationary phase was ODS C18 column (5µm, 15 cm x 5 mm, i.d.) and the flow rate was 1 mL/ min. Optimum separation was attained at retention time of 3.22 minutes.

Acceptable chromatographic parameters for system suitability were obtained, Table 1. The results confirmed good column efficiency and peak symmetry.

A linear correlation was obtained between peak area ratios and concentration of the drug in concentration range 900 – 3000 ng/mL, Fig. 2. The following regression equation was computed to be:

\[ A = 0.0006C - 0.1421 \quad r=0.9995 \]

Where,

A: peak area ratio.
C: Concentration (ng/mL).
r: Correlation coefficient.

Validation parameters according to USP [9] guidelines like accuracy, repeatability and intermediate precision for the proposed method are presented in Table 2. The given data ensures good sensitivity and reproducibility of the proposed method. The method was successfully applied for the determination of the studied drug in the pharmaceutical preparation. Standard addition technique was also applied and gave acceptable results, Table 3. The results obtained by applying the proposed method were statistically compared with those obtained by reference method [4]. Table 4 shows that, the calculated t-test and F-value [10] are less than the corresponding tabulated ones. This confirms the accuracy and precision of the proposed method.

*Intra-day and interday relative standard deviation of the average of three concentrations of alendronate sodium.

LOD and LOQ are obtained experimentally.

4. CONCLUSION

The conditions for the PAM derivatization reaction are well optimized and the product is well separated and detected chromatographically. The method is validated according to USP-guidelines. The proposed method is applied for the quantification of PAM in its dosage form. 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

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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