

Short communication

## Validation of a capillary electrophoresis method for the analysis of ibandronate related impurities

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### Abstract

A capillary zone electrophoretic (CZE) method has been developed for the determination of impurities (phosphite and phosphate) in technical-grade ibandronate, which is a potent nitrogen-containing bisphosphonate. Successful separation of the drug from the impurities was achieved using 1 mM tetradecyl-trimethyl-ammonium bromide (TTAB) and 5 mM potassium chromate (pH 10.0) as background electrolyte with an indirect detection at 254 nm. The optimised method was validated for specificity, precision, linearity and accuracy. The limit of detection (LOD) was 2 µg/mL and the limit of quantification (LOQ) was 7 µg/mL for both phosphite and phosphate. The developed CZE method used to determine phosphite and phosphate as bisphosphonates impurities can be used to evaluate the quality of regular production samples of ibandronate.

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### 1. Introduction

The impurities in drugs often possess unwanted pharmacological or toxicological effects. Therefore, it is quite obvious that the products intended for human consumption must be characterized as completely as possible. Thus, the analytical activities concerning impurities in drugs are among the most important issues in modern pharmaceutical analysis [1–3]. In this way the use of high performance liquid chromatography has been found to be a very good technique for many years in pharmaceutical industry [4].

The use of capillary electrophoresis (CE) techniques has become increasingly popular in recent years [5]. CE is a powerful technique used for the separation of both charged and neutral compounds [6–8]. The wide application range, similar to HPLC, includes assay of drugs [9–11], determination of drugs-related impurities [12–14], analysis of vitamins [15–18] proteins [19] and pharmaceutical excipients [20]. Several advantages can be mentioned in the pharmaceutical analysis using CE, including

speed and cost of analysis, reduction in solvent consumption and disposal, and the possibility of rapid method development, and throughput.

Ibandronate is a potent, nitrogen-containing bisphosphonate with proven efficacy in the treatment of metastatic bone disease, including hypercalcemia of malignancy and in the management of postmenopausal osteoporosis [21]. It is chemically designated as 3-(*N*-methyl-*N*-pentyl) amino-1-hydroxypropane-1,1-diphosphonic acid, monosodium salt, monohydrate.

Because there is no chromophore for UV or fluorescence detection and no suitable groups for derivatization, few analytical methods had been described for ibandronate. Previously reported methods applying ion-exchange chromatography [22] or ion-pair reverse-phase liquid chromatography [23] require more than 15 min for the analysis. These methods, though able to determine all the impurities, require sample preparation, derivatization, or preconcentration steps for satisfactory results.

This work proposed a validated capillary electrophoresis technique with indirect-UV detection, with low migration times and without sample preconcentration requirements, for the analysis of phosphate and phosphite, impurities related to nitrogen-containing bisphosphonates.

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## 2. Experimental

### 2.1. Chemicals

Tetradecyl-trimethyl-ammonium bromide (TTAB), potassium chromate ( $\text{CrO}_4\text{K}_2$ ) and disodium phosphate were purchased from Sigma (St. Louis, MO, USA). Disodium phosphite was obtained from Riedel-de Haën (AG, Seelze, Germany). Working standard of Ibandronate was provided from a local laboratory (Buenos Aires, Argentina). Water was filtered and deionised with a Milli-Q, Millipore system (Milford, MA, USA).

### 2.2. Instrumentation

Electrophoresis was carried out on a Quanta 4000 capillary electrophoresis system with UV detector (Waters Chromatography Div., Millipore Corp., Milford, MA, USA) controlled by the Millennium 2010 Chromatography Manager (Waters) and a Power Mate 433 computer (NEC Technologies Inc., Boxborough, MA). CE analyses were performed using uncoated fused-silica capillary column of 50  $\mu\text{m}$  internal diameter and 50 cm total length (40 cm to detector).

### 2.3. Preparation of background electrolyte (BGE)

The buffer was prepared daily by mixing 10 mL of 10 mM potassium chromate with 4 mL of 5 mM TTAB. After adjusting to pH 10.0 with 0.1 M sodium hydroxide solution, Milli-Q water was added to obtain 20 mL final volume. BGE solution was filtered through 0.45  $\mu\text{m}$  syringe filter and sonicated (Transsonic 540 sonicator, 35 kHz; Elma, Singen, Germany) before used.

### 2.4. Capillary preconditioning

New capillaries were flushed with water for 5 min, sodium hydroxide 0.1 M for 10 min, water for 5 min and finally BGE for 10 min. Prior to daily usage, the capillary was conditioned by flushing water for 2 min, sodium hydroxide 0.1 M for 2 min, water for 2 min, and finally the BGE for 10 min. Between each run, the capillary was flushed with NaOH 0.1 M for 1 min and then BGE for 2 min.

### 2.5. Electrophoretic separation conditions

The voltage applied was  $-10\text{ kV}$ . For sample introduction the hydrostatic mode was used, with the inlet position at 10 cm high during 10 s. Indirect on-line UV detection was achieved at 254 nm.

### 2.6. Determination of phosphate and phosphite in ibandronate

The method described has been applied to technical grade ibandronate samples supplied by a local laboratory (Buenos Aires, Argentina). Hundred milligrams of ibandronate was accurately weighed and transferred to a 5 mL volumetric flask,

dissolved in Milli-Q water with the aid of brief sonication, filtered through 0.45  $\mu\text{m}$  syringe filter and then introduced into the capillary electrophoresis system. The recovery was studied by spiking different amounts of phosphate and phosphite to ibandronate samples.

## 3. Results and discussion

### 3.1. Effect of pH

The effect of pH on the migration time of each analyte was investigated over the pH range 8.0–11.0, in these conditions each compound were present as anion. This showed that migration of ibandronate and related impurities increased with increasing BGE pH, this behaviour was assigned to an increase in the electroosmotic flow, which is dependent on pH.

BGE with pH under 9.5, results in lower resolution between ibandronate and its impurities. In the pH range 9.5–11.0 the method performs well. The choice of pH 10.0 for the BGE not only enabled excellent separation and reasonable retention time but also resulted in a long column life.

### 3.2. Specificity

In the present method complete resolution of Ibandronate and its related substances has been achieved. A typical electropherogram of working standard and technical-grade ibandronate is shown in Fig. 1.

Commercial formulation of ibandronate injection reported by Boehringer Mannheim (Germany) also contains sodium chloride and sodium acetate in injection water. Complete resolution of phosphite and phosphate from these formulation anions was obtained, a typical electropherogram is shown in Fig. 2.

### 3.3. Precision

The intraday and interday repeatability were calculated by injecting five replicate samples of each compound. Statistical

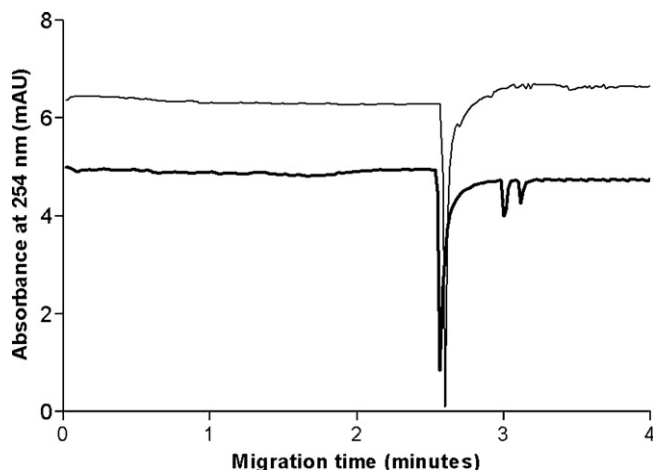


Fig. 1. Electropherogram of: (—) working standard and (---) technical-grade Ibandronate. Peaks: (1) Ibandronate; (2) phosphite; (3) phosphate.

Table 1  
Accuracy data

HNa <sub>2</sub> PO <sub>4</sub>			HNa <sub>2</sub> PO <sub>3</sub>		
Amount added (μg/mL)	Amount found (μg/mL)	Recovery (%)	Amount added (μg/mL)	Amount found (μg/mL)	Recovery (%)
120	118	99	120	123	103
120	121	101	120	122	101
120	124	103	120	123	103
100	94	94	100	96	96
100	97	97	100	95	95
100	98	97	100	97	97
80	81	102	80	84	104
80	81	101	80	83	104
80	80	100	80	82	102

evaluation was expressed as relative standard deviation. The results regarding intraday repeatability were 3.9% for phosphite and 2.6% for phosphate. The results regarding interday repeatability were 4.2% for phosphite and 3.2% for phosphate. In all cases peak area was measured.

### 3.4. Linearity evaluation

Linearity of the detector response to sample concentration was assessed by measuring five calibration points ranging from 20 to 120 μg/mL for phosphate and phosphite. Each calibration point was prepared for triplicate and each sample was injected for duplicate. Regression coefficients were obtained by plotting the average peak area versus concentration, using the least squares method. The method presents linearity in the range of 20–120 μg/mL for HNa<sub>2</sub>PO<sub>4</sub> and HNa<sub>2</sub>PO<sub>3</sub>. The regression equations of these curves (average peak-area, *y*, versus concentration, *x*, μg/mL) and the correlation coefficients were:  $y = 31.333x + 80.089$  ( $r^2 = 0.9975$ ) for phosphate and  $y = 18.506x - 98.06$  ( $r^2 = 0.9989$ ) for phosphite.

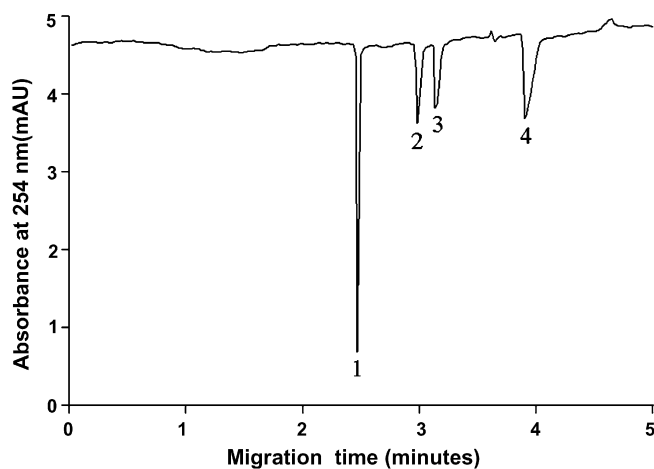


Fig. 2. Electropherogram of standard solution of phosphite and phosphate in the presence of other formulation anions. Peaks: (1) chloride; (2) phosphite; (3) phosphate; (4) acetate.

### 3.5. Accuracy

Three solutions of 20 mg/mL Ibandronate, with concentrations ranging from 80 to 120 μg/mL of HNa<sub>2</sub>PO<sub>4</sub> and HNa<sub>2</sub>PO<sub>3</sub> were prepared for triplicate. Each solution was determined twice. The results of standard addition recoveries are listed in Table 1. The percentage recovery ranged from 94 to 103% for phosphate and from 95 to 104% for phosphite.

### 3.6. Limit of detection (LOD) and limit of quantification (LOQ)

The results obtained in linearity test were used to calculate LOD and LOQ for phosphite and phosphate. The standard deviation of each group of determinations was calculated for HNa<sub>2</sub>PO<sub>4</sub> and HNa<sub>2</sub>PO<sub>3</sub>. Then the regression equations of curves (standard deviation, *y*, versus concentration, *x*, μg/mL) were calculated as follows:  $y = 0.0324x + 22.565$  for phosphate and  $y = 0.1098x + 13.555$  for phosphite.

The limit of detection was 2 μg/mL and the limit of quantification was 7 μg/mL for both, phosphite and phosphate.

### 3.7. Determination of phosphate and phosphite in ibandronate

Phosphite and phosphate are synthesis by-products and degradation products, respectively. FDA allowed limit for these impurities in ibandronate are 5 g/kg. It was found that the levels of impurities in commercial ibandronate samples were lower than the legal limit. We found values between 0.41 and 0.50 g/kg for phosphate and between 0.39 and 0.45 g/kg for phosphite in technical-grade ibandronate samples. Potential formulation components such as sodium acetate and sodium chloride, did not interfere with the assay procedure.

## 4. Conclusion

This work presents the possibility of use capillary electrophoresis for the routine determination of impurities such as phosphite and phosphate in bisphosphonates during production and storage.

CE seems to be an advantageous alternative to traditional methods because fasting, simple and accurate simultaneous determination of phosphite and phosphate in ibandronate. This technique is very useful, especially when analysis of large numbers of samples is required. The developed method could be applied by the quality control department to determine these impurities in regular Ibandronate production samples. The optimised method was validated and was found to be sensitive, accurate and precise.

The result has led us to investigate a wide range of applications of this methodology, especially for quality control of other bisphosphonates and related substances.

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