viernes 02/09/2011 12:05

Dear Prof. Martin Desimone

We are pleased to inform you that your manuscript “Zoledronate and related impurities analysis by capillary zone electrophoresis” is accepted for publication in a special volume of the Current Analytical Chemistry.

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Guest Editors
Current Analytical Chemistry, Special Volume

Zoledronate and related impurities analysis by capillary zone electrophoresis.

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Abstract
A capillary zone electrophoretic (CZE) method has been developed for the determination of zoledronate and its related impurities (phosphyte and phosphate). Successful separation of the drug from the impurities was achieved using 7.5 mM of phthalic acid adjusted to pH 3.50 with TRIS, as background electrolyte with an indirect detection at 205 nm. The optimized method was validated for specificity, precision, linearity and accuracy. The limit of detection was 1.8 μg ml^{-1} and the limit of quantification was 5.9 μg ml^{-1} for phosphyte. The limit of detection was 2.8 μg ml^{-1} and the limit of quantification was 9.3 μg ml^{-1} for phosphate. The developed CZE method used to determine zoledronate, phosphyte and phosphate as bisphosphonates impurities can be used to evaluate the quality of regular production samples of zoledronate.

Keywords: capillary zone electrophoresis, bisphosphonates, zoledronate, impurities.

Introduction

Bisphosphonates are potent inhibitors of osteoclast-mediated bone resorption. Thus, they are used for the treatment of osteoporosis, bone metastasis, Paget's disease and other conditions that feature bone fragility [1, 2]. These inhibitors of bone resorption contains two phosphonate groups attached to a single carbon atom, forming a 'P-C-P' structure [3]. Changes in the chemical structures of the bisphosphonates have resulted in progressive improvements in their antiresorptive potencies [4]. In general, bisphosphonates can be categorized as the non-N-containing (etidronate, clodronate and tiludronate) and the N-containing (pamidronate, neridronate, olpadronate, alendronate, ibandronate, risedronate and zoledronate).

The reported methods for biphosphonates analysis, in most cases, require sample preparation (i.e.: protein precipitation, solid-phase extraction), derivatization, or preconcentration steps for satisfactory results. Indeed, a volatile derivative is required for gas chromatography analysis or derivatization with a chromophore for UV or fluorescence detection in liquid chromatography [5]. A review covering a wide selection of instrumental analytical techniques ranging from liquid and gas chromatography to enzymatic and automated approaches was recently reported by Zacharis and Tzanavaras [6]. However, the development of analysis methods without requirements of sample preparation, derivatization, or preconcentration steps with satisfactory results is highly desirable.

The use of capillary electrophoresis (CE) techniques has become increasingly popular in recent years due to new developments that can provide highly sensitive and high throughput analysis [7, 8]. CE is a powerful technique used for the separation of both charged and neutral compounds [9-11]. The wide application range includes assay of drugs [12, 13], determination of drugs-related impurities [14-17], analysis of vitamins [18-21] proteins [22, 23] and pharmaceutical excipients [24]. In this context, validated CE techniques with indirect-UV detection, low migration times and, capable to analyze bisphosphonates and related impurities possesses several advantages [25, 26]. The employment of CE for the high reliable analysis of biphosphonates leads to an analysis cost reduction, diminution in solvent consumption and disposal, and the possibility of rapid method development, and throughput.

Indirect photometric detection methods are based on the use of an absorbing co-ion as the principal component of the background electrolyte. The zones of non-absorbing ionic species are revealed by changes in light absorption due to charge displacement of the absorbing co-ion. Moreover, by selecting a suitable absorbing co-ion it is possible to achieve a high sensitivity of detection. Accordingly, a BGE composed of sulphanilic acid, 6-aminocaproic acid and TTAB was reported for the analysis of Foscarnet impurities (phosphite and phosphate). By choosing sulphanilic acid as co-ion, good peak shapes of the impurities were obtained in combination with low detection limits due to the strong absorption properties of this ion [27]. Alternatively, a capillary zone electrophoresis (CZE) separation of ortho-, pyro- and tripolyphosphate anions using a phthalate buffer pH 4.0 and indirect UV detection was also described [28]. Other developments involve the analysis of three alkylphosphonate drugs (fosfomycin disodium, clodronate disodium and alendronate sodium) by using an indirect UV detection with a BGE containing benzoic acid, salicylic acid and CTAB [29] or employed a Nitroso-R salt (1-nitroso-2-naphthol-3,6-disulphonic acid disodium salt) as background electrolyte with indirect UV detection of clodronate [30].

This work proposed a validated CE technique with indirect-UV detection, with low migration times and without sample preconcentration requirements, for the analysis of zoledronate and its related impurities, phosphite and phosphate. The reported method can be successfully applied in quality control and stability studies.

Experimental

Reagents

Phthalic acid and disodium phosphate were purchased from Sigma (St. Louis, MO, USA). Disodium phosphyte was obtained from Riedel-de Haën (AG, Seelze, Germany). Working standard of Zoledronate was provided from a local laboratory (Buenos Aires, Argentina). Water was filtered and deionised with a Milli-Q, Millipore system (Milford, MA, USA).

Instrumentation

Electrophoresis was carried out on a Agilent capillary electrophoresis system with diode array detector (Agilent Technologies). CE analyses were performed using uncoated fused-silica capillary column (Polimicro Technologies, Phoenix, Arizona, USA) of 50 μm internal diameter and 50 cm total length (40 cm to detector).

Preparation of background electrolyte (BGE)

The buffer was prepared daily at a concentration of 7.5 mM of phthalic acid adjusted to pH 3.50 with TRIS. BGE solution was filtered through 0.45 μm syringe filter and sonicated (Transsonic 540 sonicator, 35 kHz; Elma, Singen, Germany) before used.

Electrophoretic system and capillary conditioning

New capillaries were flushed with water for 5 min, sodium hydroxide 0.5 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 BGE for 2 min. The voltage applied was −20 kV. All samples were introduced into the capillary by pressure at 20 psi for 5 s. Indirect on-line UV detection was achieved at 205 nm.

Preparation of standard solutions

The method described has been applied to technical grade zoledronate samples supplied by a local laboratory (Buenos Aires, Argentina). One hundred milligrams of zoledronate was accurately weighed and transferred to a 5 mL volumetric flask, dissolved in NaOH 0.1 N with the aid of brief sonication, filtered through 0.45 μm syringe filter and then introduced into the capillary electrophoresis system. The recovery was studied by spiking different amounts of phosphate and phosphyte to zoledronate samples. Individual stock solutions of disodium phosphate and disodium phosphite containing 1 mg ml^{-1} of each one of them were prepared in water. Working solutions were obtained by appropriate dilution with milli-Q water.

Results and discussion

Validation

Specificity

In the present method complete resolution of Zoledronate and its related substances has been achieved. Typical electropherograms of technical-grade Zoledronate and Zoledronate working standard with disodium phosphate and disodium phosphite are shown in Figure 1A and B, respectively. These results indicate that there is a good resolution between zoledronate and its related impurities.

Precision

The intraday and interday (5 days) repeatability were calculated by injecting six replicate samples of each compound. Statistical evaluation was expressed as relative standard deviation. The results regarding intraday repeatability were 1.1 % for Zoledronate, 2.3 % for phosphyte and 3.2 % for phosphate. The results regarding interday repeatability were 1.9 % for Zoledronate, 3.0 % for phosphyte and 4.5 % for phosphate. In all cases peak area was measured.

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

Linearity of the detector response to sample concentration was assessed by measuring five calibration points ranging from 100 to 1200 μg ml^{-1} for zoledronate and 25 to 120 μg ml^{-1} for phosphate and phosphyte. 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 100 to 1200 μg ml^{-1} for zoledronate and 25 to 120 μg ml^{-1} for phosphate and phosphyte. The regression equations of these curves (average peak-area, y, versus concentration, x, μg ml^{-1}) and the correlation coefficients were: $y = 2.454x + 18.58$ ($r^2 = 0.998$) for zoledronate, $y = 3.609x + 11.98$ ($r^2 = 0.992$) for phosphate and $y = 6.5066x - 27.94$ ($r^2 = 0.991$) for phosphyte. The limit of detection was 1.8 μg ml^{-1} and the limit of quantification was 5.9 μg ml^{-1} for phosphyte. The limit of detection was 2.8 μg ml^{-1} and the limit of quantification was 9.3 μg ml^{-1} for phosphate. It is worth mention that the acceptable impurities levels were 0.5 % p/p for phosphate and 0.2% for phosphate.

Accuracy

Three solutions of 20 mg ml^{-1} zoledronate with concentrations at three different levels from 80 to 120 μg ml^{-1} of phosphate and phosphyte 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 95 to 104 % for phosphate and from 99 to 104 % for phosphyte. In the case of zoledronate as a pure drug, the accuracy was evaluated by application of the analytical procedure to a reference standard. The differences in the quantitative analysis were lower than 1 %.

Robutness

Different parameters have been modified to study the system robustness for the quantification of zoledronate including the temperature (20 to 30 ºC), BGE pH (3.0 to 4.0), voltage (-15 to -25 kV). No significant changes have been found in the quantitation of the drug.

Conclusion

Herein, were present a validated capillary zone electrophoresis method suitable for the determination of zoledronate and related compounds such as phosphites and phosphates. Advantages of the CZE method include short analysis times, efficiency and unique selectivity. Samples are usually solved in less than 12 minutes allowing fast quantification in bulk and finished product for quality control purposes. The CZE method described in this work demonstrates obvious advantages such as low detection limits and shorter analysis times, and requires no preconcentration procedures. The results show that all compounds under study were detected below the required limits. The validation parameters support this method, so that it can be included in analytical control.

Acknowledgements
G.S.A is grateful for her postdoctoral fellowship granted by the National Research Council (CONICET). M.L.F is grateful for her doctoral fellowship granted by the University of Buenos Aires. The authors would like to acknowledge the support of grants from the University of Buenos Aires UBACYT B049 (to L.E.D.) and 20020090200051 (to M.F.D.) and from Agencia Nacional de Investigaciones Científicas y Técnicas BID 1728/OC-AR PICT 1783.

References

[1] Devogelaer, JP.; Modern therapy for Paget's disease of bone: Focus on bisphosphonates. *Treat. Endocrinol.,* **2002**, 1, 241-257.
[2] Body, JJ.; Bisphosphonates in the treatment of metastatic breast cancer. *J. Mammary Gland Biol. Neoplasia*, **2001**, 6, 477-485.
[3] Russell, RGG.; Rogers, MJ. Bisphosphonates: From the laboratory to the clinic and back again. *Bone*, **1999**, 25, 97-106.
[4] Russell, RGG. Ibandronate: Pharmacology and preclinical studies. *Bone* **2006**, 38, S7-S12.
[5] Sparidans, RW.; Den Hartigh, J.; Chromatographic analysis of bisphosphonates. *Int. J. Clin. Pharm.,* **1999**, 21, 1-10.
[6] Zacharis, CK.; Tzanavaras, PD. Determination of bisphosphonate active pharmaceutical ingredients in pharmaceuticals and biological material: A review of analytical methods. *J. Pharm. Biomed. Anal.* **2008**, 48, 483-496.
[7] Marsh, A.; Broderick, M.; Altria, K.; Power, J.; Donegan, S.; Clark, B. Capillary electrophoresis for pharmaceutical analysis. *Meth. Mol. Biol.* (Clifton, NJ) **2008**, 384, 205-245.
[8] Suntornsuk, L. Capillary electrophoresis in pharmaceutical analysis: A survey on recent applications. *J. Chrom. Sci.* **2007**, 45, 559-577.
[9] Priestap, HA.; Iglesias, SL.; Desimone, MF.; Diaz, LE. Determination of aristolochic acids by capillary electrophoresis. *J. Cap. Electroph. Microchip Technol.,* **2003**, 8, 39-43.
[10] Pañak, KC.; Ruiz, OA. ; Giorgieri, SA. ; Diaz, LE. Direct determination of glutathione in human blood by micellar electrokinetic chromatography: Simultaneous determination of lipoamide and lipoic acid. *Electrophoresis,* **1996**, 17, 1613-1616.
[11] Pañak, KC.; Giorgieri, SA. ; Diaz, LE. ; Ruiz OA. Simultaneous determination of S-adenosylmethionine and S-adenosylhomocysteine by capillary zone electrophoresis. *Electrophoresis,* **1997**, 18, 2047-2049.
[12] Hsieh, YC.; Whang, CW. Analysis of ethambutol and methoxyphenamine by capillary electrophoresis with electrochemiluminescence detection. *J. Chromatogr. A* **2006**, 1122, 279-282.
[13] Wise, DD.; Barkhimer, TV.; Brault, PA.; Kirchhoff, JR.; Messer, Jr WS.; Hudson, RA. Internal standard method for the measurement of choline and acetylcholine by capillary electrophoresis with electrochemical detection. *J. Chromatogr B,* **2002**, 775, 49-56.
[14] Jouyban,A.; Kenndler, E. Impurity analysis of pharmaceuticals using capillary electromigration methods. *Electrophoresis* **2008**, 29, 3531-3551.

[15] Castro-Puyana, M.; Garcia-Ruiz, C.; Cifuentes, A.; Crego, AL.; Marina, ML. Identification and quantitation of cis-ketoconazole impurity by capillary zone electrophoresis-mass spectrometry. *J. Chromatogr. A,* **2006**, 1114, 170-177.
[16] Carvalho, AZ.; Pauwels, J.; De Greef, B.; Vynckier, AK.; Yuqi, W. Hoogmartens J, et al. Capillary electrophoresis method development for determination of impurities in sodium cysteamine phosphate samples. *J. Pharm. Biomed. Anal.,* **2006**, 42, 120-125.
[17] Toro, I.; Dulsat, JF.; Fabregas, JL.; Claramunt, J. Development and validation of a capillary electrophoresis method with ultraviolet detection for the determination of the related substances in a pharmaceutical compound. *J. Chromatogr A,* **2004**, 1043, 303-315.
[18] Aurora-Prado, MS.; Silva, CA.; Tavares, MFM.; Altria, KD. Determination of folic acid in tablets by microemulsion electrokinetic chromatography. *J Chromatogr. A,* **2004**, 1051, 291-296.
[19] Law, WS.; Kuba, P.; Zhao, JH.; Li, SFY.; Hauser, PC. Determination of vitamin C and preservatives in beverages by conventional capillary electrophoresis and microchip electrophoresis with capacitively coupled contactless conductivity detection. *Electrophoresis,* **2005,** 26, 4648-4655.
[20] Pañak, K.; Giorgieri, S.; Ruiz, O. ; Diaz, LE. Postharvest analysis of Vitamin C and inorganic cations in lettuce by CZE. *J. Cap. Electroph. Microchip Technol.,* **1998**, 5, 59-63.
[21] Piehl, LL.; Facorro, GB.; Huarte, MG.; Desimone, MF.; Copello, GJ.; Diaz, LE.; et al. Plasmatic antioxidant capacity due to ascorbate using TEMPO scavenging and electron spin resonance. *Clin. Chim. Acta,* **2005**, 359, 78-88.
[22] Giorgieri, S.; Pañak, K. ; Diaz, LE. ; Etchepare, G. ; Ruiz, OA. Comparative analysis of storage proteins of Lotus spp. seeds by CGE and SDS-PAGE. *J. Cap. Electroph. Microchip Technol.,* **1998**, 5, 177-179.
[23] Salmanowicz, BXP. Capillary electrophoresis of seed 2S albumins from Lupinus species. *J. Chromatogr. A,* **2000**, 894, 297-310.
[24] Altria, KD. Application of microemulsion electrokinetic chromatography to the analysis of a wide range of pharmaceuticals and excipients. *J. Chromatogr. A*, **1999**, 844, 371-386.
[25] Foret, F.; Fanali. S.; Ossicini. L.; Bocek, P. Indirect photometric detection in capillary zone electrophoresis. *J. Chromatogr.,* **1989**, 470, 299-308.
[26] Bertinatto Rodriguez JA, Desimone MF, Iglesias SL, Giorgieri SA, Diaz LE. Validation of a capillary electrophoresis method for the analysis of ibandronate related impurities. *J. Pharm. Biomed. Analysis*, **2007**, 44, 305-308.
[27] Andersson, EKM. Impurities at a level of 0.01% in foscarnet sodium determined by capillary zone electrophoresis with indirect UV detection and sample self-stacking. *J. Chromatogr. A,* **1999**, 846, 245-253.
[28] Stover, FS.; Keffer, SS. Separation of condensed phosphates using capillary zone electrophoresis with indirect UV detection. *J. Chromatogr. A,* **1993**, 657, 450-454.
[29] Prutthiwanasan B, Suntornsuk L. Rapid analysis of alkylphosphonate drugs by capillary zone electrophoresis using indirect ultraviolet detection. *J. Sep. Sci.,* **2010**, 33, 228-234.
[30] Perjési, P. ; Kim, T. ; Zharikova, AD. ; Li, X. ; Ramesh, T. ; Ramasubbu, J. ; et al. Determination of clodronate content in liposomal formulation by capillary zone electrophoresis. *J. Pharm. Biomed. Analysis*, **2003**, 31, 929-935.

Legend

Figure 1: Electropherogram of technical-grade Zoledronate (A) and Zoledronate working standard (B, peak 3) with disodium phosphite (peak 2) and disodium phosphate (peak 1).

Table 1. Accuracy data

HNa_2PO_4			HNa_2PO_3		
Amount added (µg/mL)	Amount found (µg/mL)	Recovery (%)	Amount added (µg/mL)	Amount found (µg/mL)	Recovery (%)
120	122	102	120	123	103
120	121	101	120	125	104
120	125	104	120	124	103
100	96	96	100	103	103
100	99	99	100	99	99
100	98	98	100	97	97
80	81	102	80	80	100
80	79	99	80	82	103
80	83	104	80	82	102

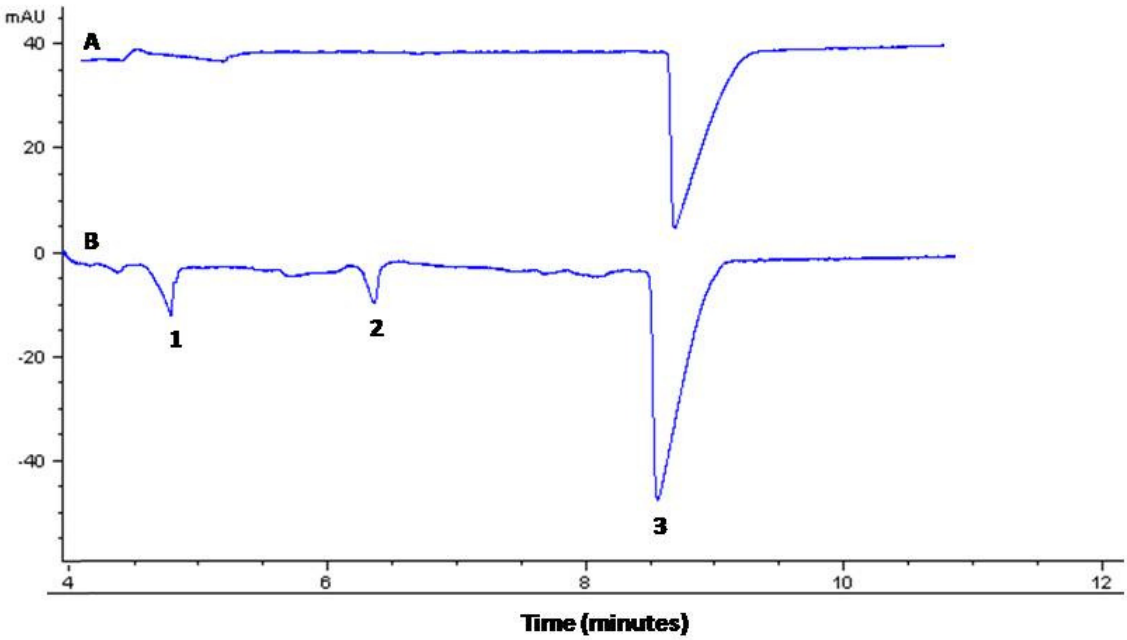

Figure 1: Electropherogram of technical-grade Zoledronate (A) and Zoledronate working standard (B, peak 3) with disodium phosphite (peak 2) and disodium phosphate (peak 1).