Analytica Chimica Acta 633 (2009) 232–237

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Positive mode electrospray ionization mass spectrometry of bisphosphonates using dicationic and tricationic ion-pairing agents

Molly M. Warnke, Zachary S. Breitbach, Edra Dodbiba, Jeffrey A. Crank, Tharanga Payagala, Pritesh Sharma, Eranda Wanigasekara, Xiaotong Zhang, Daniel W. Armstrong*

Department of Chemistry and Biochemistry, University of Texas at Arlington, Arlington, TX 76019, USA

ARTICLE INFO

Article history:
Received 15 October 2008
Received in revised form
19 November 2008
Accepted 24 November 2008
Available online 30 November 2008

Keywords:
Electrospray-ionization mass spectrometry
Bisphosphonates
Tricationic pairing reagent
Ion-pairing

ABSTRACT

A general method for detecting bisphosphonate drugs by ESI-MS and LC–ESI-MS as positive ions has been developed. Bisphosphonates can have multiple negative charges in solution. Tricationic ion-pairing reagents were paired with bisphosphonates to form a positively charged complex. It was clear that this facile pairing method worked. However, an appreciable presence of −1 bisphosphonate species were observed in positive mode ESI-MS (i.e. as the +2 complex with tricationic reagents). This led to an extended investigation on the use of dicationic pairing agents. The use of dicationic reagents improved the detection sensitivity for all of the bisphosphonates. Tandem mass spectrometry also improved the limits of detection for most of the bisphosphonates using both the tricationic and dicationic pairing reagents. A tricationic reagent also was used as an ion-pairing reagent in chromatography experiments. Thus the addition of a single reagent produced benefits in that it increased chromatographic retention and enhanced the ESI-MS detection of bisphosphonates.

1. Introduction

Bisphosphonates are a class of drug with a P–C–P backbone structure. These drugs are used to treat bone-related diseases, such as osteoporosis and Paget's disease [1,2]. The detection of bisphosphonates using many traditional separation methods is a challenge because most bisphosphonates either lack or have weak UV chromophores. Bisphosphonates also exist in multiple charge states in solution and are very polar, which can limit chromatographic options. In reverse phase HPLC, bisphosphonates elute at or near the dead volume without the use of ion-pairing agents.

Conductivity and indirect UV detection have been used to detect bisphosphonates in capillary electrophoresis (CE) [3] and ion chromatography (IC) experiments [4,5], respectively. Derivatization methods for fluorescence and spectrophotometric detection have also been reported [6–8]. Evaporative light scattering detection [9] and charged aerosol detection [10] have also been employed for the detection of bisphosphonates. Most separation methods are based on CE, anion-exchange chromatography, or ion-pair chromatography [4,5,7,8].

A few reports on the use of mass spectrometry (MS) for the detection of bisphosphonates have appeared in the literature. Trimethylsilyl-clodronate derivatives were analyzed by GC–MS [11]. Anion-exchange chromatography coupled with ICP-MS detection was used to analyze alendronate and etidronate [12]. Electrospray ionization (ESI)-MS was used in negative-ion mode when coupled with CE and also to investigate bisphosphonate charge states and fragmentation [13,14]. Positive ion-mode IC–ESI-MS–MS was used to detect diazomethane derivatives of the nitrogen-containing bisphosphonates risedronate and alendronate [15].

ESI-MS is a logical choice for bisphosphonate detection because the analyte is negatively charged at a wide range of pH values. Negative-ion mode ESI-MS is the most common method to detect anions, however standard chromatographic solvents, such as methanol and water can lead to poor spray stability, corona discharge, and arcing. These disadvantages ultimately lead to poor detection limits [16,17]. Recently, a method was developed to detect singly charged anions using positive mode ESI-MS by pairing the anion with a dicationic reagent to create a positively charged complex [18–21]. The use of tricationic reagents which pair with divalent anions for the detection of a +1 complex also has been reported recently [22–24]. Using positive mode ESI-MS avoids spray stability problems. Beyond this, pairing the anion with the dicationic or tricationic reagent has other benefits. For example, monitoring the anion/cation pair moves the detected species to a higher mass region where there is lower background noise. Also, anions of low mass may be moved well above the low mass cutoff of quadrupole instruments (e.g., ion traps).

---

* Corresponding author. Tel.: +1 817 272 0632.
E-mail address: sec4dwa@uta.edu (D.W. Armstrong).

0003-2670/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2008.11.056

In this study, tricationic and dicationic pairing agents are examined as a general analytical approach for the ESI-MS and LC–ESI-MS of seven bisphosphonate compounds. Their use in MS–MS and possible dissociation mechanisms also will be discussed. Additionally, these ion-pairing reagents can be used in the mobile phase to enhance the chromatographic retention and separations of bisphosphonates on a C_{18} stationary phase.

2. Experimental

HPLC grade water and methanol was obtained from Burdick and Jackson (Morristown, NJ). Reagent grade sodium hydroxide and sodium fluoride were purchased from Fisher Scientific (Pittsburgh, PA). Etidronic acid sodium, alendronate disodium hydrate, clodronic acid disodium salt, and neridronate were purchased from Sigma–Aldrich (Milwaukee, WI). Risedronate sodium and ibandronate were purchased from AkSci (Mountain View, CA) and zoledronic acid was purchased from Waterstone Technology (Carmel, IN). Stock solutions (1 mg mL^{-1}) were made weekly and diluted serially for analysis.

The tricationic and dicationic reagents used in this study were synthesized according to previous reports [19,22,23,25–27]. Before analysis, each cationic reagent was anion exchanged to the fluoride form as previously reported [18,22].

For direct injection analysis, a 40 μM dication-fluoride or trication-fluoride solution was pumped into a Y-type mixing tee at 0.1 mL min^{-1} using a Shimadzu LC-6A pump (Shimadzu, Columbia, MD). A 67:33 methanol:water mixture was also directed into the mixing tee at a flow rate of 0.3 mL min^{-1} using the Surveyor MS pump (Thermo Fisher Scientific, San Jose, CA). The resulting flow into the mass spectrometer is 50:50 methanol:water with a cationic pairing reagent concentration of 10 μM and a total flow rate of 0.4 mL min^{-1}. The six-port injection valve on the mass spectrometer (5 μL loop) was used for sample introduction.

A Finnigan LXQ (Thermo Fisher Scientific, San Jose, CA) ESI-MS was used for the analysis of anions in this study. The ESI-MS conditions used were: spray voltage 3 kV; sheath gas flow, 37 arbitrary units (AU); auxiliary gas flow rate, 6 AU; capillary voltage, 11 V; capillary temperature, 350 °C; tube lens voltage, 105 V. The trication–anion complex was monitored in SIM mode with a width of 5 *m*/*z* units. For SRM experiments, the isolation width was 1–5 units with a normalized collision energy of 30 and an activation time of 30 ms. These conditions were used for all compounds for comparison purposes, but the optimization of parameters for a given complex often leads to an improvement in sensitivity. Data was analyzed using the Xcalibur and Tune Plus software. The limits of detection were determined when multiple injections of a given concentration resulted in a signal-to-noise ratio of three.

For the chromatography experiments, the mobile phase was pumped isocratically using a Shimadzu LC-6A pump. Sample introduction was made by a six-port injection valve (Rheodyne, Cotati, CA). The stationary phase used was a Supelco Ascentis C_{18} (25 cm × 2.1 mm, 5 μm particle). The mobile phase was 80:20 50 μM tricationic reagent:methanol with a flow rate of 0.3 mL min^{-1}. Methanol (0.1 mL min^{-1}) was teed into the flow post-column. The MS was operated in SIM mode, monitoring the mass of each bisphosphonate/trication complex throughout the chromatographic run.

3. Results and discussion

The first facet of this study was an investigation on the use of tricationic pairing reagents with bisphosphonate analytes. In aqueous solution at pH 6, the predominant bisphosphonate species has a −2 charge, so paired with the tricationic reagent, a +1 overall complex will be observed by MS. The tricationic reagents used in this study were chosen to represent the best performing trigonal and linear trications identified in previous studies [22–24]. These four tricationic reagents (Fig. 1) offer a variety of functional groups as well as differences in rigidity. The linear trications (TriA and TriB) contain an imidazolium core with different chain lengths and terminal charged groups. TriA (Fig. 2) has C_{10} linkages between the central imidazolium and tripropylphosphonium (TPP) terminal charged groups. TriB has benzylimidazolium terminal charge groups and C_6 linkage chains. TriC and TriD (Fig. 2) are trigonal trications, which have a more rigid structure. TriC consists of a mesitylene core with three *n*-butylimidazolium groups in the 2,4,6 positions. TriD has a benzene core with three TPP-charged groups.

The seven bisphosphonates used in this study are illustrated in Fig. 2. Table 1 shows the LOD results for each of the bisphosphonates using the four tricationic reagents. In the SIM mode, ibandronate had the lowest limit of detection (1.3 ng, TriA) of all of the bisphosphonates. In general, ibandronate and the other nitrogen-containing bisphosphonates (alendronate, risedronate, neridronate, zoledronate) had lower LODs than etidronate and clodronate. The LODs for some of the analytes varied significantly with

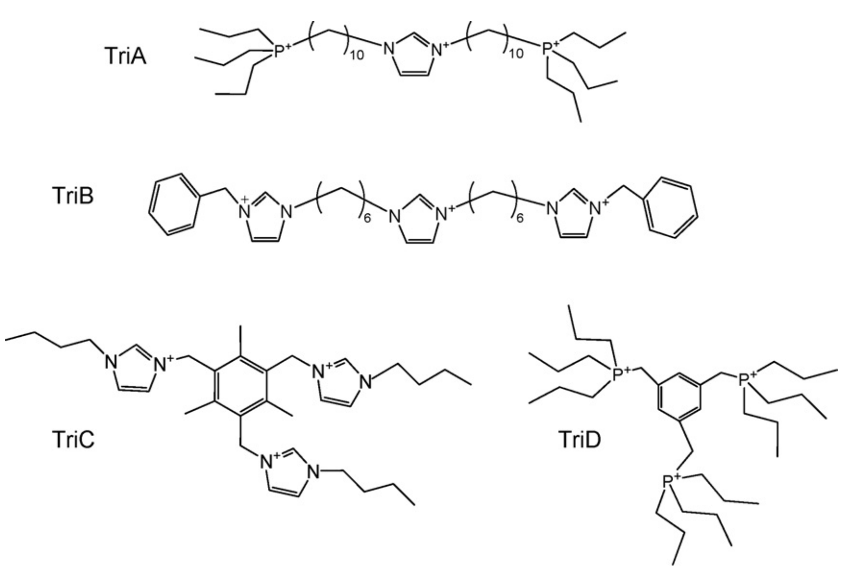

Fig. 1. Structures of the tricationic ion-pairing reagents used in this study. Each reagent is labeled with an abbreviation used throughout the text.