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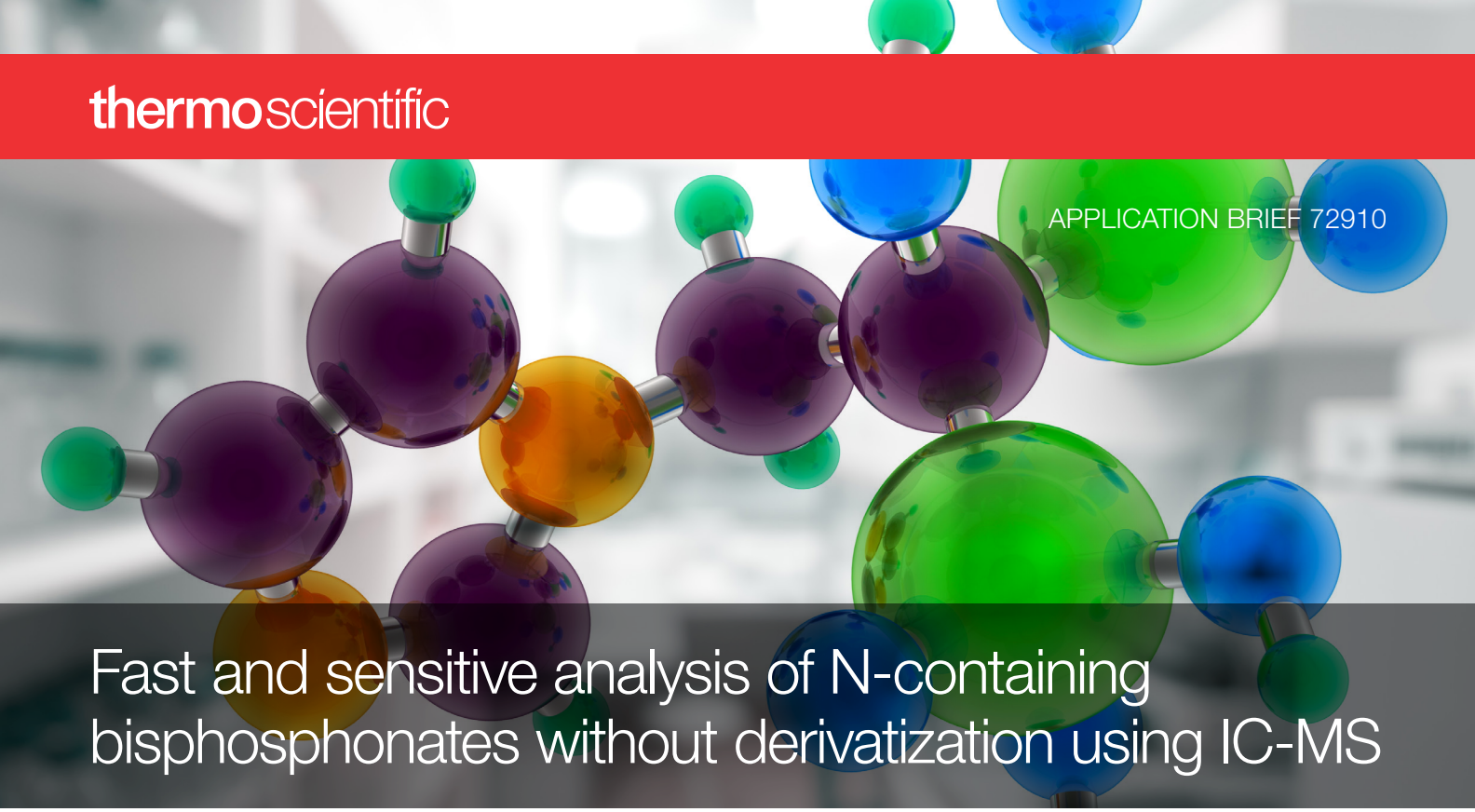

APPLICATION BRIEF 72910

Fast and sensitive analysis of N-containing bisphosphonates without derivatization using IC-MS

Author

Sylvain Morel, Xavier Deruy,
Thermo Fisher Scientific,
Courtaboeuf, France

Keywords

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Introduction

Bisphosphonates are a class of chemical compounds used in various medical approaches. These active pharmaceuticals ingredients (API) are mainly used against bone disorders including osteoporosis. The older and most used bisphosphonates are etidronate, clodronate, and tiludronate. Now bisphosphonates are used in oncology for treatment of cancer hypercalcemia and metastatic bone diseases. Many new APIs have been developed with the addition of a basic nitrogen in the bisphosphonate side chain. This modification has critically modified the product's potency. For example, pamidronate (N-containing bisphosphonate) is one hundred times more potent than etidronate (non-N-containing bisphosphonate).[1] Regarding the better potency, this new API represents a great potential for doping.

Due to the very polar and strong chelating characteristics of bisphosphonates, their analysis represents a real analytical challenge. The scientific literature reports different approaches to performing bisphosphonates analysis: ion-pairing HPLC with light scattering detection, ion chromatography (IC) with UV indirect detection, HPLC with charged aerosol detection[2] or LC-MS with derivatization[1]. In complex matrices like plasma, only LC-MS with derivatization can provide the required selectivity and sensitivity. However, sample preparation is still required and is a time-consuming step.

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In this application brief, separation of N-containing bisphosphonates and three other bisphosphonates was achieved without derivatization within 15 min (gradient shown in Table 1). The analytes were detected by single quadrupole mass spectrometry in Selected Ion Monitoring (SIM) mode without the aid of a desolvation agent at any step of separation or ionization. Ion chromatography using eluent generation and suppressed conductivity detection provides chromatographic selectivity, low chemical noise, and high compatibility with the MS.[3] Additionally, the analytes leave the IC system as ions further aiding compatibility with MS. The high temperature and aiding voltage coming from the heated electrospray ionization (HESI) probe transforms the aqueous IC stream into a fine spray that can enter the MS detector.

This application brief was developed on the procedure in Thermo Scientific Application Note 1001.[3] It expands the qualified products range to the Thermo Scientific™ Dionex™ ICS-6000 HPIC™ ion chromatography system and the Thermo Scientific™ ISQ EC™ single quadrupole mass spectrometer.

Experimental

Ion chromatography

- Dionex ICS-6000 HPIC system with RFIC capabilities with a dual pump (one pump used for elution and the other to supply water for the suppressor regeneration)
- Conductivity Detector and cell
- Thermo Scientific™ Dionex™ AS-AP thermostatted autosampler

Mass spectrometry

- ISQ EC single quadrupole mass spectrometer
- Thermo Scientific™ syringe pump (P/N 1R76402-5100) for method optimization
- Thermo Scientific™ HESI II probe

Software

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, 7.2.8 version

Method

Column:	Thermo Scientific™ Dionex™ IonPac™ AS18-Fast-4µm column 2 x 150, P/N 076036		
Eluent:	KOH gradient from 40 to 100 mM generated by a Thermo Scientific™ Dionex™ ICS-6000 EG Eluent Generator (Table 1)		
Eluent Source:	Thermo Scientific™ Dionex™ EGC 500 KOH cartridge, P/N 075778, Thermo Scientific™ Dionex™ CR-ATC 600 trap column, P/N 088662, high pressure degasser module		
Flow Rate:	0.25 mL/min		
Injection Volume:	5 µL		
Detection 1:	Suppressed conductivity, AERS-500 2 mm suppressor, P/N 082541, external water mode at 0.5 mL/min		
Typical Conductance Background:	<1 µS/cm		
MS Detection:	-ESI, -3000 V, SIM, HESI		
Temperatures:	Vaporizer: 350 °C; Ion Transfer: 350 °C		
Nitrogen Flow :	Sheath: 60 psi, Aux: 4 psi; Sweep: 0 psi		
Desolvation Agent:	None		
SIM Mode:	N-containing bisphosphonates	Ion *m/z*	CID (V)
Alendronate	✓	248	10
Clodronate		243	10
Etidronate		205	10
Pamidronate	✓	234	10
Risedronate	✓	282	10
Tiludronate		317	10
Zoledronate	✓	271	10

Table 1. KOH gradient generated by the Dionex ICS-6000 EG

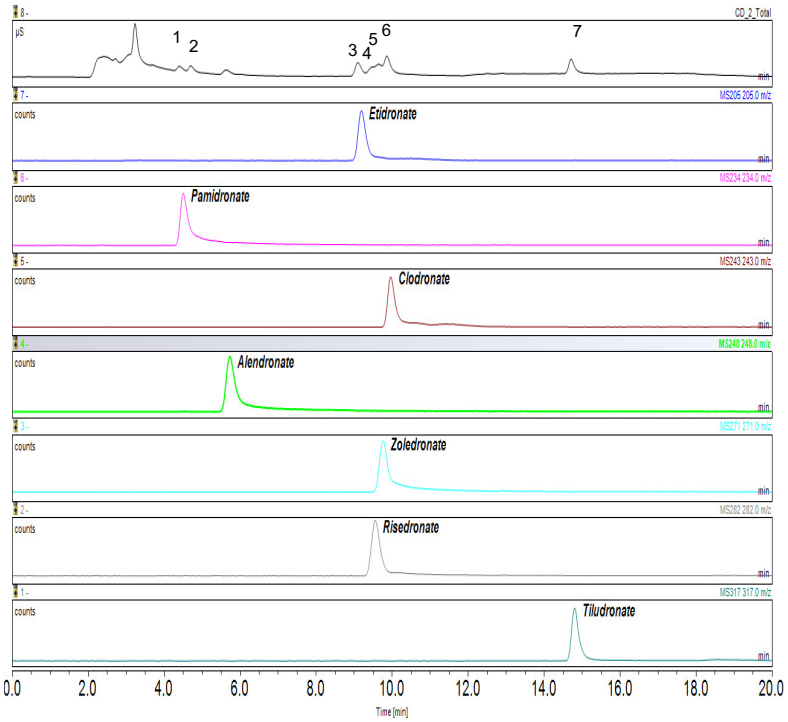
Time	Concentration (mM)	Curve
0.0	Run	
0.0	40	5
4.0	40	5
9.0	60	5
9.0	100	5
15.0	100	5
15.0	40	5
20.0	Stop Run	

Results

Figure 1 shows the detection of each bisphosphonate using the method described in this application brief. The selectivity of the mass spectrometer is useful for this application because the chromatogram with suppressed conductivity detection shows coelution of four compounds. Extractions of each SIM trace allows easier and better quantification for each compound. The IC-MS setup is the best option for any work in complex matrix.

Calibration curves generated for each phosphorylated compound are shown in Figure 2. These show that MS response is linear between 0.01 and 1 ng/µL.

Very low limits of quantification (LOQ) can be reached using this IC-MS method. Under these conditions, a LOQ around 10 µg/L can be expected. This is lower than the previous method's LOQ[3]. At this concentration, signal-to-noise ratios calculated in Figure 3 range between 5 and 22.



Column:	Dionex IonPac AS18-Fast-4µm, 150 × 2 mm
Eluent:	KOH gradient (for details see Table 1)
Eluent Source:	Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 trap column
Flow Rate:	0.25 mL/min
Inj. Vol.:	5 µL
Oven Temp.:	30 °C
Detector 1:	Suppressed conductivity, Dionex AERS 500 suppressor, 124 mA, 15 °C, external water mode, 0.5 mL/min
Detector 2:	ISQ EC, -ESI, -3000 V source, HESI
Scan Mode:	SIM
Make-up Solvent:	None
Source Temp.:	Vaporizer 350 °C, Ion Transfer 350 °C
N_2 Gas Flow (psi):	Sheath 60, Aux 4, Sweep 0
Sample:	1 ng/µL of each compound in MeOH/water solution (φ = 50%)

Peaks:	*m/z*	CID (V):
1. Pamidronate	234	10
2. Alendronate	248	10
3. Etidronate	205	10
4. Risedronate	282	10
5. Zoledronate	271	10
6. Clodronate	243	10
7. Tiludronate	317	10

Figure 1. SIM extraction for each phosphorylated compound and comparison with suppressed conductivity detection (top chromatogram)

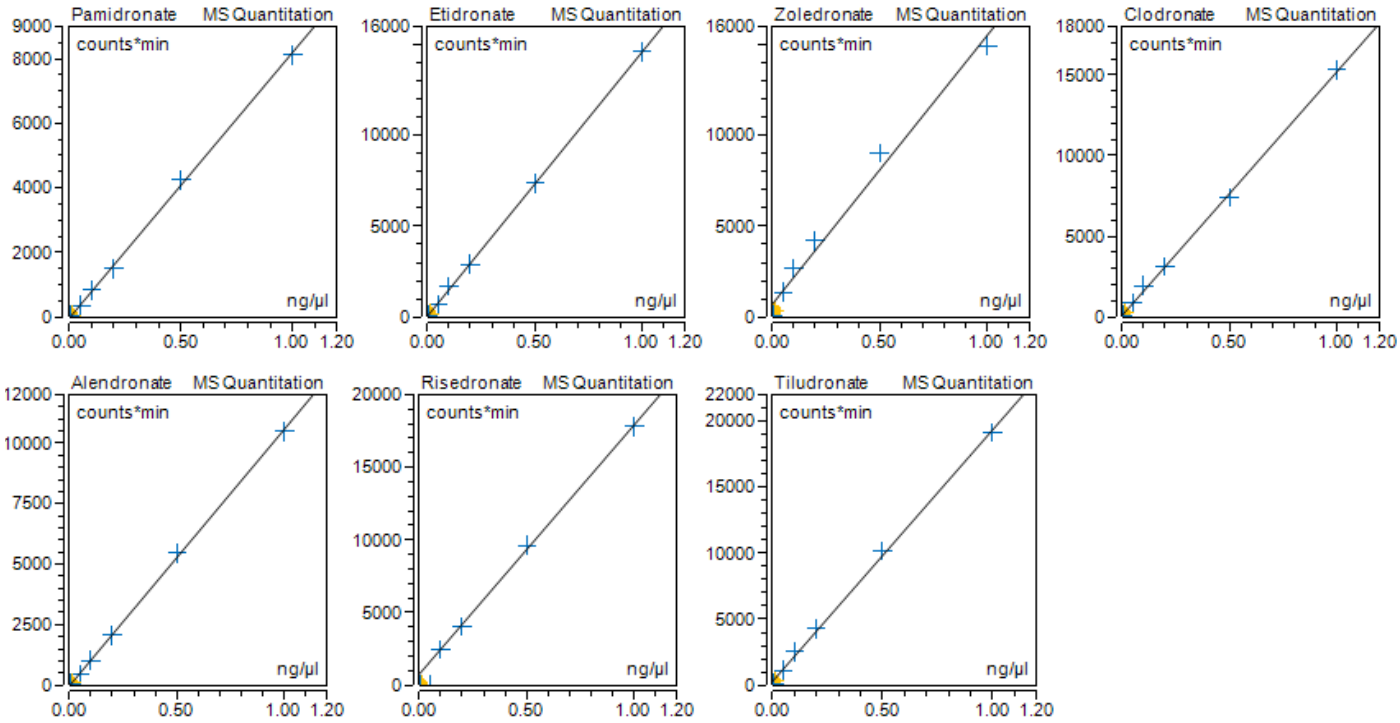


Figure 2. Calibration curve (linear model) for each phosphorylated compound. Concentration ranges from 0.01 to 1 ng/µL.

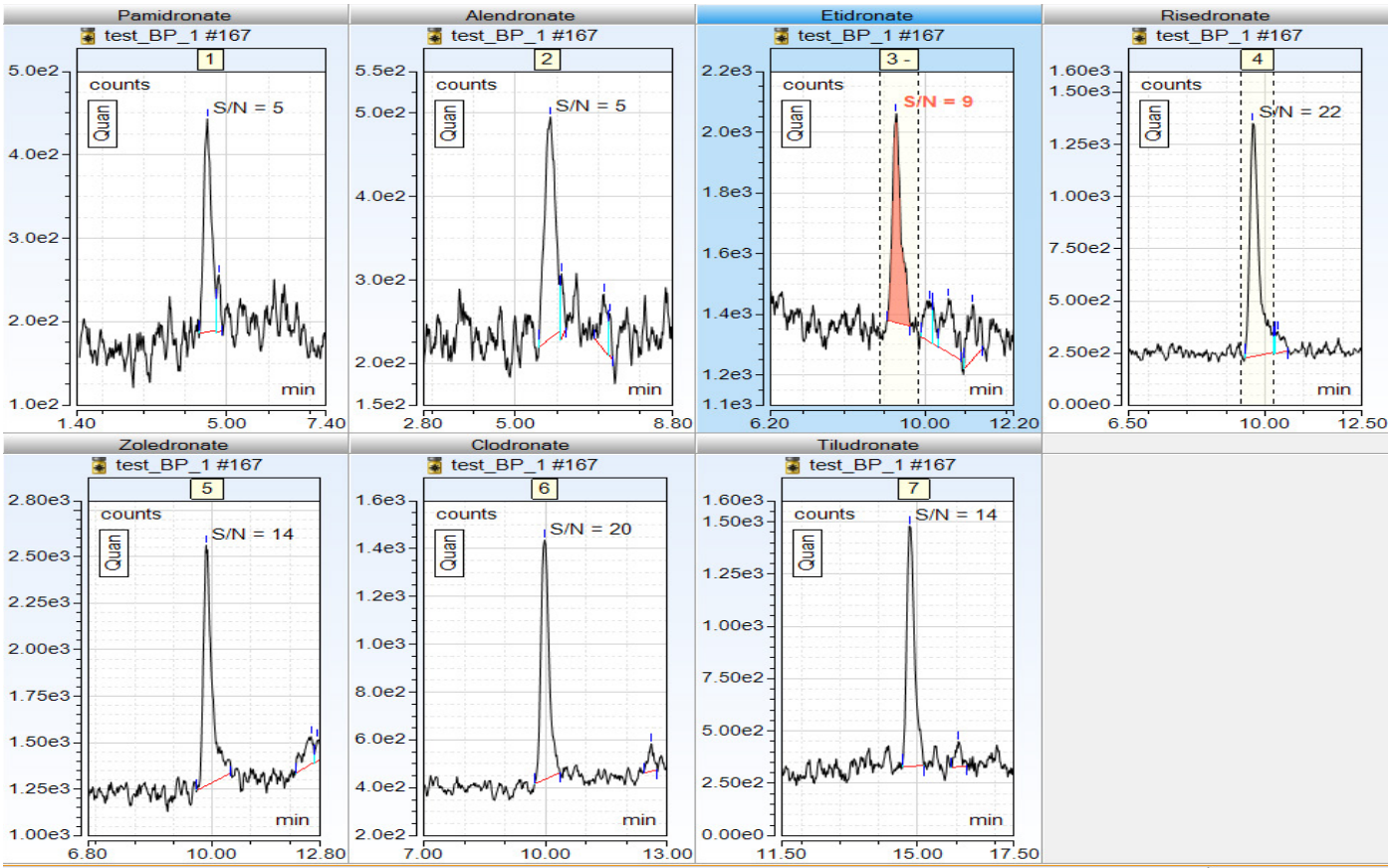


Figure 3. Signal-to-noise ratios according to the European Pharmacopoeia equation for a 5 µL injection of standard solution (10 µg/L for each compound)

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References

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