Application note | 000900

Ion chromatography

Determination of phosphite and phosphate in ibandronate sodium

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Keywords

HPIC, Dionex IonPac AS28-Fast-4µm column, RFIC, bisphosphonates, Dionex ADRS 600, Dionex Integrion HPIC

Goal

To design an ion chromatography method that uses a Thermo Scientific™ Dionex™ IonPac™ AS28-Fast-4µm anion exchange column, electrolytically generated KOH eluent, and suppressed conductivity detection to determine phosphite and phosphate in ibandronate sodium

Introduction

Ibandronate belongs to a class of compounds called bisphosphonates, which have a biological effect on bone tissue and osteoclasts.[1] Bisphosphonates are the most commonly used treatments for postmenopausal osteoporosis, Paget's disease, and bone tumor disease.[2,3] Chemically, bisphosphonates are analogs of pyrophosphate.[3] Synthesis of ibandronate sodium from *N*-methyl-*N*-pentyl--alanine HCl leads to the generation of impurities, such as sodium phosphate and sodium phosphite, which are by-products of the synthesis reaction.[4]

Methods with good specificity and sensitivity are required to determine phosphite and phosphate. The method described in the United States Pharmacopeia's (USP) proposed Ibandronate Sodium monograph[5] uses refractive index (RI) detection. However, RI detection does not provide specificity or sensitivity.[6] There are usually very small differences in absolute refractive indices of many substances. The RI is also

affected by temperature and pressure changes. Moreover, a RI detector cannot be used with gradient separations as it is a bulk property detector,[7] and thus is impacted by the change in eluent composition. An ion chromatography with tandem mass spectrometry detection (IC/MS/MS) method was recently reported for phosphite determination, but this method required isotopically labeled standards.[8] Raman spectroscopy has been used,[9] but this method is difficult to automate and different spots of the same sample can give different results. There is a need for an improved method for the determining of phosphite and phosphate that is sensitive, reproducible, and easy to operate.

The goal of this work is to design an ion chromatography (IC) method that uses a Dionex IonPac AS28-Fast-4µm anion exchange column, electrolytically generated KOH eluent, and suppressed conductivity detection to determine phosphite and phosphate in ibandronate sodium. The Dionex IonPac AS28-Fast-4µm column uses 4 µm resin particles for efficient separations, resulting in more accurate peak integration and more reliable results compared to larger particle size resins often used for IC. Its selectivity makes the Dionex IonPac AS28-Fast-4µm column particularly useful for determining trace levels of common anions. The eluent used in this separation is generated with a Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge and purified online using a Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column. The Thermo Scientific™ Dionex™ ADRS 600 2 mm suppressor produces the regenerant ions necessary for eluent suppression and allows continuous operation with minimal maintenance. Because the RFIC system requires only deionized (DI) water as the carrier, it significantly simplifies system operation and improves analytical reproducibility. The method also requires no time-, resource-, or money-consuming isotope labeling. Moreover, IC provides significant improvement to the existing assays because organic solvents are not needed as they are for HPLC methods. This is environmentally friendly and eliminates organic solvent acquisition and disposal costs.

The method proposed in this application note was validated following the guidelines outlined in USP General Chapter <1225>, Validation of Compendial Procedures[10] to meet the requirements specified for phosphite and phosphate quantification prescribed in the proposed USP Ibandronate Sodium monograph.

Experimental

Equipment

Thermo Scientific™ Dionex™ Integrion™ HPIC system** including:

- Eluent generator
- Pump
- Degasser
- Conductivity detector
- Column oven temperature control
- Detector-suppressor compartment temperature control

Thermo Scientific™ Dionex™ AS-AP Autosampler with sample syringe, 250 µL (P/N 074306) and buffer line, 1.2 mL (P/N 074989)

**This method can be executed on any Thermo Scientific™ Dionex™ RFIC system

Conditions

Parameter	Setting
Columns:	Dionex IonPac AG28-Fast-4µm Guard, 2 × 30 mm (P/N 088750) and Dionex IonPac AS28-Fast-4µm Analytical, 2 × 150 mm (P/N 088749)
Eluent source:	Dionex EGC 500 KOH Eluent Generator Cartridge (P/N 075778) with Dionex CR-ATC 600 trap column (P/N 088662)
Eluent:	KOH
Flow rate:	0.3 mL/min
Column temperature:	30 °C
Compartment temperature:	25 °C
Injection volume:	2.5 µL (Full Loop)
Detection:	Suppressed conductivity, Dionex ADRS 600 Suppressor (2 mm) (P/N 088667), recycle mode, constant current mode, suppressor current: 58 mA
Backpressure:	~3,000 psi

Reagents and chemicals

- Sodium phosphate, mono basic (Sigma P/N S8282)
- Sodium phosphite dibasic pentahydrate (Sigma P/N 04283)
- Ibandronate sodium (Sigma P/N PHR2715-500MG)

Preparation of solutions and reagents

Ibandronate stock solution 1,000 mg/L

Ibandronate stock solution is prepared using ibandronate sodium as follows. Accurately weigh 11.2 mg of ibandronate sodium and dissolve in DI water in a 125 mL polypropylene bottle. Adjust the weight to 10 g with DI water. Prepare a 100 mg/L secondary stock solution by 10-fold dilution of the primary stock solution.

Phosphite and phosphate stock solutions 1,000 mg/L

Phosphate and phosphite stock solutions were prepared separately as follows. Accurately weigh 274 mg of sodium phosphite pentahydrate or 126 mg of sodium phosphate and dissolve in DI water in a 125 mL polypropylene bottle. Adjust the weight to 100 g with DI water. Prepare a 100 mg/L secondary stock solution by 10-fold dilution of the primary stock solution.

On the day of the analysis, dilute the secondary stock solutions together to prepare 10 mixed calibration standards with concentrations of 15, 10, 7.5, 5.0, 2.5, 1.5, 0.625, 0.31, and 0.2 mg/L of phosphite and phosphate. For example, for the 10 mg/L standard, perform a 1:10 dilution of the 100 mg/L standard by adding 10 g of 100 mg/L standard to a 125 mL polypropylene bottle. Add DI water to a final weight of 100 g, cap and store the bottle at 4 °C until needed.

Robustness study

Following the guidelines of USP Physical Tests, <621> Chromatography,[11] evaluate the robustness of this method by examining the retention time (RT), peak asymmetry, and resolution after imposing small variations (±10%) in procedural parameters (e.g., flow rate, eluent gradient concentration, column temperature). Inject a standard containing 2.5 mg/L phosphite and phosphate. Apply the same procedure to another column set. Test the following variations:

- Flow rate at 0.27 mL/min, 0.3 mL/min, 0.33 mL/min
- Column temperature at 27 °C*, 30 °C, 33 °C
- KOH eluent initial concentrations at 31.5 mM, 35 mM, 38.5 mM
- KOH eluent final concentration: 70 mM, 77 mM, 84.7 mM

[Note- The underlined parameters belong to the main method and the other two are the tested variations.]

*Because the Dionex Integrion HPIC system does not have column cooling capacity for testing lower temperature conditions, the columns were allowed to equilibrate with the room temperature, which was 28.7 and 28.2 °C for columns 1 and 2, respectively.

Results and discussion

Separation

Separation of phosphite and phosphate was achieved using a Dionex IonPac AS28-Fast-4µm column set under step change elution conditions. Figure 1 shows the separation of a solution containing 1 mg/L each of phosphite and phosphate. To achieve good separation of phosphite from the nearest anion, i.e., carbonate, a lower eluent concentration (35 mM) was required i n the first step. The eluent concentration was increased to 77 mM for the second step to quickly elute phosphate. Table 1 the shows the complete eluent step change program used for this work. Both peaks are well separated from nearby peaks. The resolution between phosphite and carbonate is 2.31. The total method run time is 25 min. This run time is sufficiently long to ensure that any anions that elute after phosphate will elute prior to the next injection. It also enables handling an increase in retention time encountered during some of the conditions of the robustness studies. If an increase in phosphate retention time is not a concern, then the run time can be decreased by reducing the duration of the 77 mM eluent concentration step.

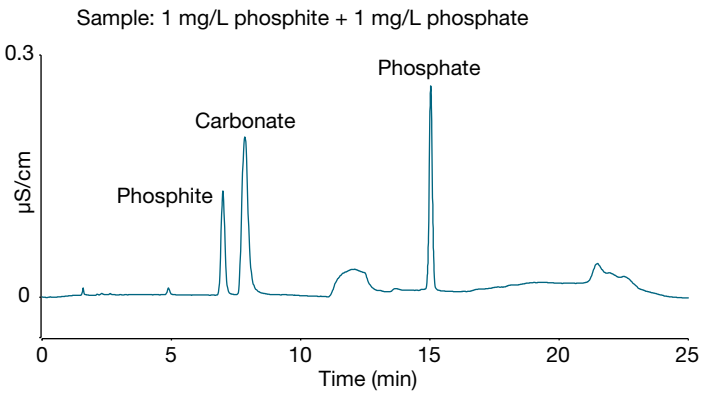

Figure 1. Phosphite and phosphate separation using a Dionex IonPac AS28-Fast-4µm column

Table 1. Elution program

Time (min)	Eluent concentration (mM)
0	35
8.5	35
8.5	77
18.5	77
18.5	35
25.0	Stop Run

Next, a commercially available sample of ibandronate reference standard was analyzed. Figure 2 shows a chromatogram obtained by analyzing a sample containing 100 mg/L ibandronate and that sample spiked with a 0.25 mg/L mixed standard of phosphite and phosphate and a 1 mg/L mixed standard of phosphite and phosphate. Phosphate is well resolved from a large peak eluting just after it.

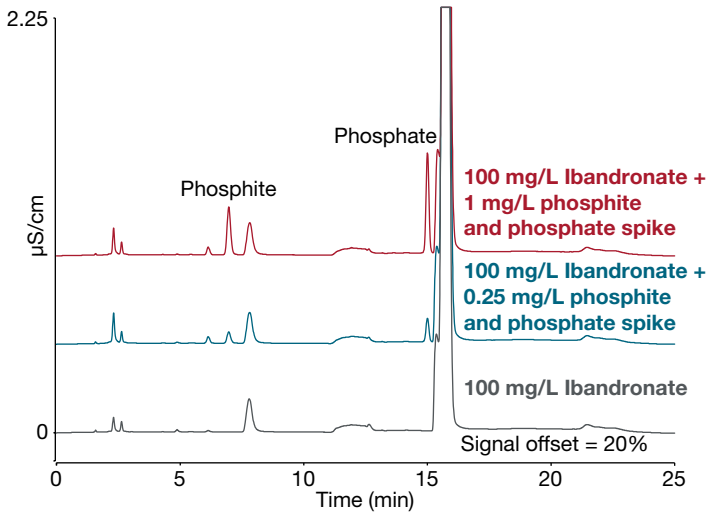

Figure 2. Analysis of a 100 mg/L ibandronate sample on a Dionex IonPac AS28-Fast-4µm column

Method linearity and precision

The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the USP General Chapter <1225> guidelines recommend a minimum of five concentrations to establish linearity in an assay.[10] For a drug substance or finished product, the minimum specified range is 80 to 120% of the test concentration. Method linearity was studied using phosphate and phosphite standards at ten concentration levels ranging from 0.2 to 15 mg/L (ppm). For a linear fit, the coefficient of determination value determined was 0.9995 for phosphite and 0.9997 for phosphite.

Assay precision was evaluated by injecting three replicates at three phosphite and phosphate concentration levels, 0.2, 1, and 5 mg/L, and expressed as the RSDs of retention time and peak area from the series of measurements. The RT RSDs were ≤0.12% and the peak area RSDs were ≤1.99% (Table 2).

Table 2. Retention time and peak area precision (n=5)

	Phosphite (mg/L)			Phosphate (mg/L)		
	0.2	1	5	0.2	1	5
RT RSD	0.11	0.10	0.12	0.05	0.04	0.04
Peak area RSD	1.77	1.59	0.46	0.65	1.60	1.99

Method sensitivity

Method sensitivity is important when assaying phosphite and phosphate as impurities. Method sensitivity was determined by analyzing phosphate standards and adjusting concentrations until S/N ratios of ~3 (LOD) and ~10 (LOQ) were obtained. To determine the LODs and LOQs, the baseline noise was first determined by measuring the peak-to-peak noise in a representative 1 min segment of the baseline where no peaks elute but close to the peaks of interest. The signal was determined from the average peak height of three injections of phosphite and phosphate. The results are included in Table 3. The LOD and LOQ for phosphite were 0.003 mg/L and 0.01 mg/L, respectively. Figures 3 and 4 show chromatograms corresponding to LOD and LOQ concentrations of phosphite and phosphate, respectively.

Table 3. Method sensitivity (n=3)

	Phosphite	Phosphate
LOD (mg/L)	0.003	0.002
LOQ (mg/L)	0.010	0.006

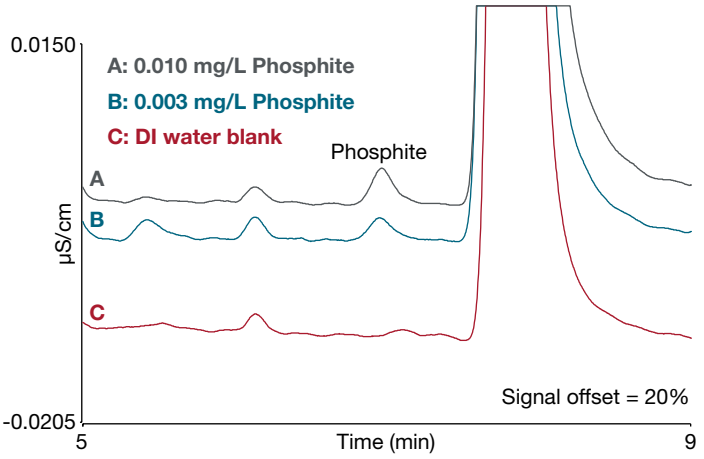

Figure 3. Phosphite sensitivity

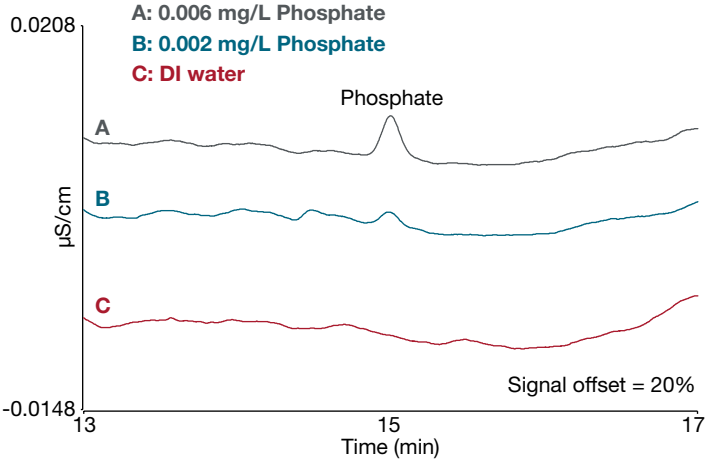

Figure 4. Phosphate sensitivity

Method accuracy

The acceptance criteria for phosphite and phosphate impurities in the proposed USP Ibandronate Sodium monograph are set at NMT 0.5%. This level corresponds to 0.5 mg/L phosphite or phosphate in a 100 mg/L ibandronate solution. A suitable analytical method needs to be able to accurately quantify phosphate and phosphite at this level.

Accuracy studies were conducted by spiking phosphite and phosphate in to a 100 mg/L ibandronate sample at four concentration levels. The unspiked ibandronate sodium sample did not show presence of phosphite or phosphate. The phosphite and phosphate spike levels were 0.25, 0.5, 1, and 2.5 mg/L each. All four levels yielded good phosphite and phosphate recoveries indicating good method accuracy as shown in Table 4.

Method robustness

Method robustness was studied by introducing ±10% changes to method conditions and monitoring changes to key chromatographical parameters—retention time, peak asymmetry, and resolution (phosphite to carbonate). Robustness studies were performed on two different columns. The peak asymmetry was measured using the USP formula.[10] A standard containing 2.5 mg/L of phosphite and phosphate was injected three times (n=3) at each chromatographic condition. Tables 5 and 6 show that all other factors except step 1 eluent concentration do not significantly affect phosphite and phosphate chromatography. The resolution between phosphite and carbonate drops with increased step 1 eluent concentration, but the drop is less than 10% on both columns.

Table 4. Spike recovery experiment (n=3)

Analyte	Recovery (%)			
	0.25 mg/mL spike	0.5 mg/mL spike	1 mg/mL spike	2 mg/mL spike
Phosphite	92.7	95.2	95.4	93.8
Phosphate	87.5	91.3	93.2	94.0

Table 5. Robustness studies on column 1 (n=3)

Condition	Difference (%)				
	Retention time		Peak asymmetry		Resolution
	Phosphite	Phosphate	Phosphite	Phosphate	Phosphite
- 10% Step 1 Eluent	19.3	1.66	-2.10	-0.32	9.52
+ 10% Step 1 Eluent	-13.7	-1.74	0.00	0.32	-9.81
- 10% Step 2 Eluent	0.82	8.76	-0.90	0.63	1.59
+ 10% Step 2 Eluent	0.56	-5.77	-0.90	-0.63	-1.01
- 10% Flow Rate	10.3	4.82	1.50	1.27	0.00
+ 10% Flow Rate	-8.37	-3.92	-1.80	0.00	-0.58
- 4.3% Column Temp.*	-1.92	-0.70	0.30	-0.32	0.72
+ 10% Column Temp.	7.60	3.14	0.30	0.63	-0.72

Table 6. Robustness studies on column 2 (n=3)

Condition	Difference (%)				
	Retention time		Peak asymmetry		Resolution
	Phosphite	Phosphate	Phosphite	Phosphate	Phosphite
- 10% Step 1 Eluent	20.2	1.41	-1.23	-0.95	21.9
+ 10% Step 1 Eluent	-14.6	-1.58	-1.23	0.00	-7.85
- 10% Step 2 Eluent	0.33	1.64	-2.15	0.00	10.4
+ 10% Step 2 Eluent	0.44	-8.25	-0.31	-0.95	-1.00
- 10% Flow Rate	10.2	5.32	0.00	0.32	0.88
+ 10% Flow Rate	-8.61	-4.43	-1.54	-0.32	-0.66
- 6% Column Temp.*	-5.06	-2.62	-0.31	0.95	-1.88
+ 10% Column Temp.	7.93	4.16	0.00	-0.63	0.00

*Since the Dionex Integrion HPIC system does not have column cooling capacity for testing temperatures lower than 30 °C, the columns were allowed to equilibrate with the room temperature, which was 28.7 and 28.2 °C for columns 1 and 2, respectively.

Conclusions

This study describes an IC-based assay to determine phosphite and phosphate in ibandronate sodium. Phosphite and phosphate were separated on an anion-exchange column and detected by suppressed conductivity in 25 min. This method allows the concentration of phosphite and phosphate to be determined in an automated and sensitive manner compared to RI detection. This assay was validated to meet the analytical performance characteristics outlined in USP General Chapter <1225>, Validation of Compendial Procedures, and was shown to measure the phosphite and phosphate content in ibandronate sodium accurately. It offers a simple, accurate, and robust measurement approach to determine phosphite and phosphate in ibandronate sodium.

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