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Determination of Inorganic Anion Impurities in a Water-Insoluble Pharmaceutical by Ion Chromatography with Suppressed Conductivity Detection

INTRODUCTION

The U.S. Food and Drug Administration (FDA) is responsible for protecting consumers by ensuring that pharmaceuticals are safe by requiring the manufacturers to verify their identity, strength, quality, and purity characteristics. Impurities that are present even in small amounts may influence the safety and efficacy of the pharmaceutical product. According to the International Conference on Harmonization, impurities are defined as any component of the active pharmaceutical ingredient (API) that is not the chemical entity defined as the API.¹

Pharmaceutical impurities are categorized as organic, inorganic, or residual solvents. Inorganic impurities that may be derived from the manufacturing process of bulk drugs include reagents, catalysts, ligands, heavy metals, and other materials (e.g., filter aids, charcoal).² For example, inorganic impurities may be present in the raw materials or may be derived from reagents, such as phosphate buffers, used during the production of the pharmaceutical. While the presence of many inorganic impurities at low concentrations have few toxicological consequences, significant variation in the impurity levels from batch-to-batch can indicate that the manufacturing process of the drug product is not adequately controlled.^{3,4} In most cases, these impurities should be removed or at least minimized in the final product. Therefore, the identification, quantification, and control of impurities are important during drug development in the pharmaceutical industry.

Ion chromatography (IC) with suppressed conductivity detection is a well-established technique for the determination of inorganic and organic ions in pharmaceuticals.⁵⁻⁷ For the determination of anions, a hydroxide eluent is commonly used. Hydroxide is suppressed to water, which provides exceptionally low background conductivity and baseline noise and, therefore, very low detection limits. In Application Note 190 (AN190), we demonstrated the determination of sulfate counter ion and anionic impurities in several water-soluble aminoglycoside antibiotics.⁸ Most of the samples described in AN190 could be analyzed by direct injection after dilution with deionized water. In this Application Note (AN), we demonstrate the development of an IC method for the determination of anionic impurities in a proprietary water-insoluble pharmaceutical. A 2-mm IonPac[®] AS15 column with an electrolytically generated potassium hydroxide eluent was used for the determination of sub-mg/L concentrations of inorganic anion impurities in a proprietary pharmaceutical dissolved in 100% MeOH. A 100 μ L sample was concentrated on an IonPac UTAC-ULP1 concentrator followed by elimination of the MeOH matrix and pharmaceutical with 1 mL of deionized water to permit the determination of the target inorganic anions without matrix interferences. The linearity, detection limits, precision, and accuracy of the method are described.

EQUIPMENT

Dionex ICS-3000 Reagent-Free™ Ion Chromatography (RFIC) system consisting of:
DP Dual Pump module (an SP Single Pump module can also be used)
EG Eluent Generator module
DC Detector/Chromatography module (single or dual temperature zone configuration)
AS Autosampler with a 1-mL syringe (P/N 055066)
EluGen EGC II KOH cartridge (P/N 058900)
Continuously-Regenerated Anion Trap Column, CR-ATC (P/N 060477)
Chromeleon® 6.8 Chromatography Data System

REAGENTS AND STANDARDS

Deionized water, Type I reagent grade, 18 MΩ-cm resistivity or better
Combined Seven Anion Standard, 100 mL (Dionex P/N 056933)
Fluoride Standard 1000 mg/L, 100 mL (Dionex P/N 037158 or Ultra Scientific, VWR P/N ULICC-003)
Chloride Standard 1000 mg/L, 100 mL (Dionex P/N 037159 or Ultra Scientific, VWR P/N ULICC-002)
Sulfate Standard 1000 mg/L, 100 mL (Dionex P/N 037160 or Ultra Scientific, VWR P/N ULICC-006)
Nitrate Standard 1000 mg/L, 100 mL (Ultra Scientific, VWR P/N ULICC-004)
Phosphate Standard 1000 mg/L, 100 mL (Ultra Scientific, VWR P/N ULICC-005)
Methanol, ACS grade (99.8% min), BDH (VWR P/N BDH1135-4LG)

CONDITIONS

Columns: IonPac AG15 Guard, 2 × 50 mm (P/N 053943)
IonPac AS15 Analytical, 2 × 250 mm (P/N 053941)
Eluent: 10 mM potassium hydroxide 0–8 min,
10 – 40 mM from 8 – 14 min, 40 – 60 mM from 14 – 20 min, 60 mM from 20 – 30 min*

Eluent Source: EGC II KOH with CR-ATC
Flow Rate: 0.40 mL/min
Temperature: 30 °C (lower compartment)
30 °C (upper compartment)
Inj. Volume: 100 µL
Matrix Elim. Vol.: 1000 µL (DI water)
Concentrator: IonPac UTAC-ULP1, 5 × 23 mm (P/N 063475)
CRD: CRD 200, 2-mm (P/N 062986)
Detection: Suppressed conductivity, ASRS® 300 (2 mm), Recycle mode, 60 mA current
System
Backpressure: ~2400 psi
Background
Conductance: ~0.5-0.7 µS
Noise: ~1-2 nS/min peak-to-peak
Run Time: 30 min

*The column equilibrates at 10 mM KOH for 5 min prior to the next injection

PREPARATION OF SOLUTIONS AND REAGENTS

Mixed Inorganic Anion Stock Solution

To estimate the concentration of the target anions in the sample, prepare a 1000-fold dilution of the Combined Seven Anion Standard. Inject 100 µL of this standard followed by 1000 µL of deionized water. The separation should be similar to that shown in Figure 1. For this application, nitrite and bromide were excluded from the calibration standards because these anions were not detected in the sample or matrix blank.

Stock Standard Solutions for Target Anions (1000 mg/L)

For several of the analytes of interest, 1000 mg/L standard solutions are available from Dionex or other commercial sources. When commercial standards are not available, 1000 mg/L standards can be prepared by dissolving the appropriate amounts of the required analytes from ACS reagent grade salts (or better) in 100 mL of deionized water. Standards are stable for at least one month when stored at 4 °C.

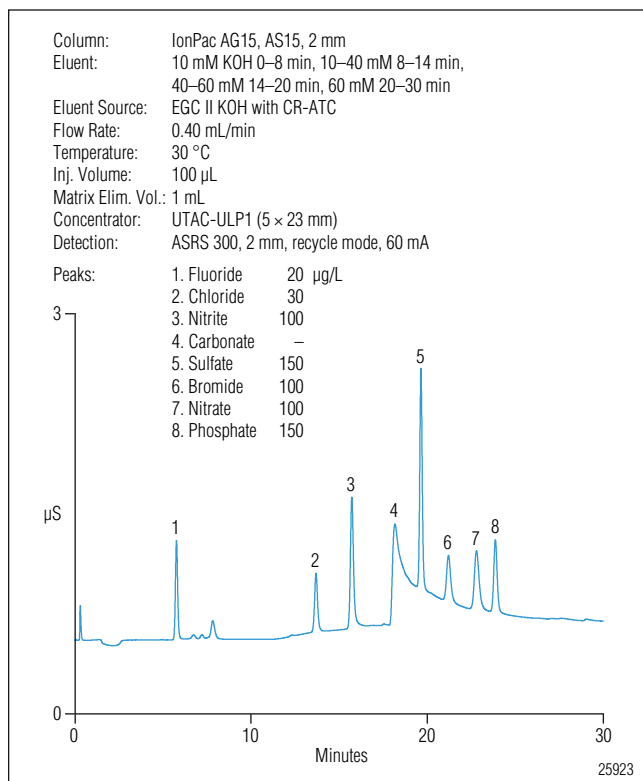


Figure 1. Separation of common inorganic anions in a 1000-fold dilution of a mixed common anion standard on the IonPac AS15 column.

Primary Dilution Standards

Prepare 100 mg/L each of fluoride and phosphate standards in separate 20 mL scintillation vials by combining 2 mL of the respective 1000 mg/L stock solutions with 18 mL of deionized water. Prepare 1 mg/L each of chloride, sulfate, and nitrate standards in separate 125 mL HDPE bottles by combining 100 µL of the respective 1000 mg/L stock solutions with 99.9 mL of deionized water.

Calibration Standards

Prepare calibration standards in the low-µg/L to mg/L range by adding the appropriate volumes from the target anions primary dilution standards to separate 125 mL HDPE bottles and dilute to 100 mL with deionized water. Four levels of calibration standards were used in this study to cover the expected concentrations found in the pharmaceutical sample.

SAMPLE PREPARATION

Weigh approximately 30 ± 2 mg of sample on an analytical balance and then transfer to a previously weighed 100 mL polypropylene volumetric flask. Dissolve the solid in 100 mL of ACS grade MeOH ($d = 0.7918$ g/mL) to prepare a final sample concentration of 0.30 mg/mL (w/v). *Caution: MeOH is flammable. Work under a hood.* Record the weight of this solution in the volumetric flask and subtract from the weight of the empty volumetric flask and solid to obtain the weight of MeOH used to prepare the sample. To completely dissolve the solid material, sonicate the solution for approximately 15 min.

SYSTEM PREPARATION AND SETUP

1. Install an EGC II KOH cartridge in the EG-3000 module.
2. Install backpressure tubing in place of the columns to produce a total backpressure of ~2000-2500 psi at a flow rate of 1 mL/min.
3. Condition the cartridge by setting the KOH concentration to 50 mM at 1 mL/min for 30 min.
4. Disconnect the backpressure tubing installed in place of the column set.
5. Install a CR-ATC between the EGC II KOH cartridge and the EGC degas.
6. Hydrate the CR-ATC prior to use by following the instructions outlined in the EluGen Cartridge Quickstart Guide.
7. Install 2 × 50 mM AG15 and 2 × 250 mm AS15 columns in the lower compartment of the DC using red PEEK™ tubing (0.005" i.d.) between connections.
8. Install a 5 × 23 mm UTAC-ULP1 concentrator in place of the sample loop on valve #1 using black PEEK (0.010" i.d.) tubing. Direction of sample loading should be opposite of analytical flow.
9. Make sure the pressure is ~2200-2500 psi using the operating conditions described earlier to allow the degas assembly to effectively remove electrolysis gases. If necessary, install additional backpressure tubing or trim tubing between degas assembly and the injection valve to achieve the recommended pressure.
10. Hydrate and install ASRS 300 suppressor and Carbonate Removal Device (CRD 200) according to the instructions in the product manuals.
 - a. Install both in recycle mode using red PEEK tubing for all connections.

The AS autosampler was used in this AN to concentrate the sample and eliminate the matrix from the UTAC-ULP1 concentrator column. To install and configure the AS autosampler:

1. Install a 1-mL sample syringe (P/N 055066).
2. From the front panel (and under System Parameters), configure the AS autosampler sample mode to Concentrate.
3. Connect the AS injection port tubing directly to the injection valve. Be sure the tubing is properly calibrated before operating the autosampler.
4. The AS autosampler Concentrate option allows the AS to deliver sample to a low pressure concentrator at a maximum pressure of 100 psi. Therefore, the sample syringe dispense speed should be no greater than 2 in the Chromeleon program.

This application requires a matrix elimination step using deionized water to remove MeOH from the concentrator column. There are two possible procedures to accomplish this task:

1. Rinse a 10 mL AS sample vial several times with deionized water and then fill the vial with deionized water. When performing the matrix elimination step in the program, direct the AS autosampler to aspirate 1 mL from the vial. Separate vials are strongly recommended for different calibration standards and samples to minimize cross contamination. The deionized water in the vial should be changed frequently. For ease-of-use, this option for performing the matrix elimination step was used.
2. Alternatively, the matrix elimination step can be performed by using the sample prep syringe of the AS autosampler with a 5 mL syringe installed. However, this setup requires an 8.2-mL sampling needle assembly (P/N 061267) to accommodate the larger volume. The use of the sample prep syringe for eliminating the matrix from the concentrator will require more time per injection.
3. To setup the concentrate and matrix elimination steps in Chromeleon, use the program wizard and go to the Sampler Options section. By default, the first line of the Sampler Options steps should appear. The first line should read:

1 Concentrate Loadposition Aspirate = 3 Dispense = 1
Change the dispense speed from 1 to 2 and click

Enter. Click the mouse pointer on the next line and then select Reagent Flush from the drop down menu. To use a vial as the source of the matrix elimination solution, as described in #1 above, enter the appropriate vial # in the box. To use the second option, described in #2 above, choose the appropriate Reagent Reservoir that contains the solution used to eliminate the matrix. For the volume, enter 1000 μ L and Valve Position should equal No Change. Click insert to insert the line in the sampler options steps. This completes the steps required to concentrate the sample and eliminate the matrix from the concentrator column.

RESULTS AND DISCUSSION

A primary consideration in the development of a suitable IC method for pharmaceuticals is the solubility of the API in water. Many drugs and intermediates are insoluble in water and other aqueous solutions that are typically used in IC systems. This poses a potential analytical challenge as it could lead to precipitation of the API in the chromatography system and therefore cause excess backpressure and column contamination.⁹ To overcome this challenge, a sufficient amount of organic solvent can be added to the eluent to maintain the solubility of the API or the API can be precipitated and the resulting solution filtered prior to analysis.⁹ The former approach requires a manually prepared eluent and therefore precludes the use of a Reagent-Free ion chromatography (RFIC) system, while the latter increases analysis complexity that can lead to potential contamination and measurement errors.

This AN describes the development of an IC method for the determination of monovalent to polyvalent inorganic anions commonly found in pharmaceuticals. The method combines preconcentration with matrix elimination to detect trace concentrations of inorganic impurities in a proprietary water-insoluble drug. A 100 μ L of the pharmaceutical dissolved in 100% MeOH is concentrated on a UTAC-ULP1 concentrator column to trap the inorganic anion impurities, while the MeOH matrix is eliminated with 1 mL of deionized water before analysis. This approach eliminates the need for organic solvent in the eluent or the offline precipitation of the API and therefore improves the methods ease-of-use.

The IonPac AS15 column was chosen as the separation column because it is a high-capacity, hydroxide-selective column specifically developed for the rapid and efficient separation of trace concentrations of inorganic anions in matrices with varying ionic strength. The use of an electrolytically generated hydroxide eluent for this application produces an exceptionally low background and baseline noise and therefore lower detection limits, which enables the detection of inorganic impurities that are less than 0.001% (w/w) in the 0.30 mg/mL pharmaceutical sample analyzed in this study.

It is important to establish a matrix blank and ensure its stability before proceeding to analyze the sample. In this AN, MeOH was required to dissolve the pharmaceutical sample. In general, organic solvents are known to contain trace concentrations of inorganic anions and low molecular weight organic acids as discussed in AU163.¹⁰ However, trace anions in solvents can also be derived from sample handling procedures and contaminated materials used to transport the solution for analysis. Therefore, it is critical to use the same set of containers and other components used to prepare the samples to obtain a representative blank. As shown in Figure 2, trace concentrations of fluoride, chloride, sulfate, and nitrate were detected in 100% MeOH.

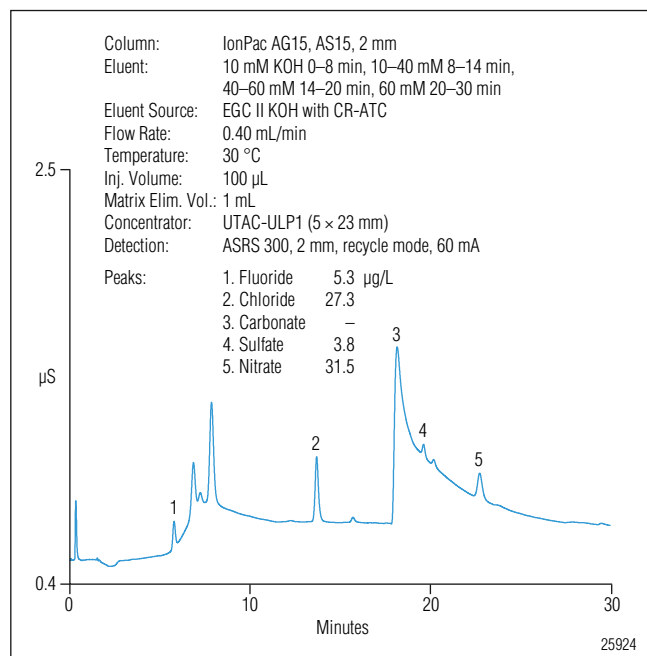


Figure 2. Target anions detected in a representative MeOH blank.

To establish a suitable concentration range for the target anions, the anions detected in the pharmaceutical sample (0.30 mg/mL) and MeOH matrix blank were compared against a 1000-fold dilution of a mixed common anion standard (Figure 1). Table 1 summarizes the range of the calibration curves and the linearity for each target anion. The results demonstrate that the calibration curves for the target anions were linear with correlation coefficients (r^2) greater than 0.997. Table 1 also summarizes the estimated limits of detections (LODs) for the target analytes, calculated based on three times the signal-to-noise ratio (S/N).

Analyte	Range (µg/L)	Linearity (r^2)	Estimated Limits of Detection ^a (µg/L)
Fluoride	500–2000	0.9996	0.16
Chloride	10–100	0.9989	0.39
Sulfate	5.0–50	0.9974	0.46
Nitrate	10–100	0.9997	1.3
Phosphate	250–1000	0.9997	1.7

^aLODs estimated from $3 \times S/N$

The method performance was evaluated by analyzing three different preparations of the pharmaceutical sample over three days. Figure 3 demonstrates the applicability of the method for determining trace anions in a 0.30 mg/mL proprietary pharmaceutical product. As shown, the pharmaceutical sample primarily consists of fluoride and phosphate with only trace concentrations of chloride, sulfate, and nitrate. When the sample is corrected for the MeOH blank, the concentrations of the trace anions (chloride, sulfate, nitrate) were determined to be significantly less than the background concentrations. Therefore, this AN focuses on the primary anion constituents, fluoride and phosphate, in the pharmaceutical sample. The average concentrations for fluoride and phosphate detected in the sample over three days were 967 ± 12 µg/L and 339 ± 10 µg/L, respectively. The presence of fluoride in some inorganic raw materials used for the preparation of pharmaceuticals is well-known. Calcium salts are the most contaminated with fluoride due to their manufacturing process. The determination

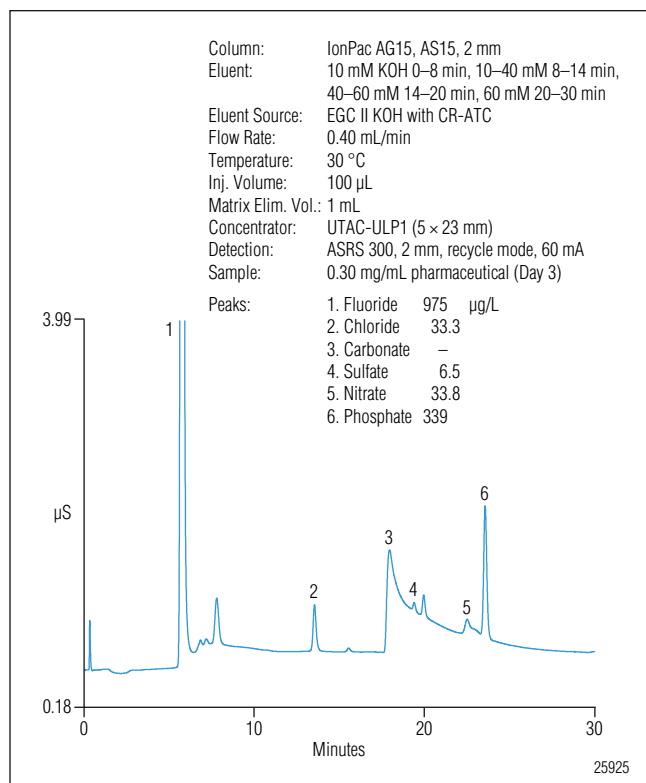


Figure 3. Determination of inorganic anion impurities in a proprietary water-insoluble pharmaceutical compound

of fluoride in pharmaceuticals is critical because excess fluoride is toxic and can cause bone diseases, such as fluorosis, osteoporosis, and skeletal fragility.¹¹ The presence of phosphate is also not uncommon in pharmaceuticals as phosphate buffers are commonly used during the preparation of the final formulation. Table 2 summarizes the results for the determination of fluoride and phosphate in the pharmaceutical sample. For the three day study, the retention time and peak area RSDs were <0.1% and <1.2%, respectively, for the target anions. The method accuracy was also evaluated by determining the recoveries of fluoride and phosphate spiked into the sample at concentrations that were nearly equivalent to the unspiked sample. The calculated recoveries for fluoride and phosphate were 102.6% and 107.7%, respectively. The good recoveries obtained in this study indicate that the method performed well for the determination of the target anions in a proprietary water-insoluble pharmaceutical compound.

Table 2. Summary of Data Obtained for Target Anions in a Water-Insoluble Pharmaceutical Product

Day	Analyte	Amount Found (µg/L)	% (w/w) in a 0.30 mg/mL Pharmaceutical	Retention Time RSD ^a	Peak Area RSD ^a
1	Fluoride	973.5	0.25	0.06	0.12
	Phosphate	328.9	0.08	0.02	1.1
2	Fluoride	953.9	0.24	0.12	0.41
	Phosphate	349.0	0.09	0.01	0.76
3	Fluoride	974.6	0.25	0.04	0.38
	Phosphate	339.0	0.09	0.01	0.40

^an = 6

CONCLUSION

In this AN, we demonstrated the ability to determine trace anions in a proprietary water-insoluble pharmaceutical using preconcentration with matrix elimination. This method was designed to provide a simpler approach that avoids the potential complications of column contamination and excess column backpressure that can occur when analyzing water-insoluble samples. The use of a hydroxide-selective AS15 column provided an efficient separation of common anions from low to high µg/L concentrations that are typically found in pharmaceuticals. In addition, the combination of a hydroxide-selective column with an electrolytically generated potassium hydroxide eluent eliminates the problems associated with the manual preparation of hydroxide eluents and therefore further increases the ease-of-use and method automation. This method demonstrated good linearity, sensitivity, precision, and accuracy for determining inorganic anion impurities in a water-insoluble pharmaceutical compound.

LIST OF SUPPLIERS

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