

Determination of Aluminum in OTC Pharmaceutical Products

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Key Words

Dionex IonPac CS10 Column, Tiron, Antacids, Antiperspirants, Dionex ICS-5000+ Reagent-Free HPIC System

Goal

To develop an IC method for the determination of aluminum in over-the-counter (OTC) products using postcolumn derivatization followed by UV absorbance detection.

Introduction

Aluminum (Al) is the most abundant metallic element in the earth's crust.¹ It is mainly found as oxides or silicates (oxidation state +3). Aluminum-containing compounds are used extensively in cosmetics, prescription pharmaceuticals, and OTC drug products. A number of aluminum-containing compounds are used as active ingredients in underarm antiperspirant products. Compounds approved for this purpose do not include alumina or aluminum hydroxide. However, aluminum zirconium octachlorohydrate and aluminum chlorohydrate can be used at concentrations up to 20% and 25% by weight, respectively, in the United States and in Europe. Aluminum chloride has been used in antiperspirant products up to 15% in Europe. The US FDA allows OTC sale of antiperspirants containing 15–25% aluminum (with the amount varying based on the specific compound used). In general, most OTC antiperspirants contain some form of aluminum-based compound as the main active ingredient—mostly aluminum chloride or aluminum chlorohydrate. Aluminum salts in antiperspirants dissolve in the moisture on the skin surface and form a gel, creating a small temporary 'plug' on the sweat gland and reducing the amount of sweat that is secreted to the skin surface.²



Aluminum-based compounds are also used in antacid formulations. Aluminum hydroxide and magnesium hydroxide act as antacids by neutralizing stomach acid that results in an increased pH in the stomach.

The United States Pharmacopeia (USP) has adopted several different assays for aluminum in various OTC products. The analytical techniques used include complexometric titration, chelatometric titration, ion-exclusion chromatography, and reversed-phase liquid chromatography. The choice of assay method is often predicated by the dosage form. Aluminum can be determined using ion chromatography (IC) with an easy setup and fast run time. Dionex (now part of Thermo Scientific) Application Note (AN) 69 describes the determination of aluminum in complex matrices using chelation ion chromatography.³ AN69 uses a Thermo Scientific™ Dionex™ IonPac™ CS5 column for the separation of aluminum from other cations and postcolumn derivatization for detection by UV absorbance.

In this application note, we report the validation of an IC method for the determination of aluminum in OTC formulations with a cation-exchange column and postcolumn derivatization prior to UV absorbance detection. The separation is achieved using a Dionex IonPac CS10 analytical column with detection by postcolumn addition of Tiron™, a colorimetric reagent, followed by measurement of UV absorbance at 310 nm. The method was evaluated in terms of linearity, precision, accuracy, ruggedness, and limit of quantitation for aluminum.

Equipment

- Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ system including the following:
 - DP Dual pump
 - DC Detector/Chromatography module
- Thermo Scientific Dionex AS-AP Autosampler with a 5000 µL syringe (P/N 074308) and 8500 µL buffer line (P/N 075520) assembly.
- Vial kit, polystyrene with caps and blue septa, 10 mL (P/N 074228)
- Thermo Scientific Dionex ICS Series VWD UV-Vis Absorbance Detector (P/N 069117, 4 wavelength or P/N 069116, single wavelength) with PEEK standard flow cell, 11 µL, 10 mm (Victrex P/N 6074.0200)
- Reaction coil, 125 µL (P/N 053640)
- Manifold 3-way (P/N 024313)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) Software version 7.2

Consumables

- Thermo Scientific™ Nalgene™ Syringe Filters, PES, 0.2 µm (Fisher Scientific, P/N 09-740-61A)
- AirTite™ All-Plastic Norm-Ject® Syringes, 5 mL, sterile (Fisher Scientific, P/N 14-817-28)

Reagents and Standards

- Deionized (DI) water, 18 MΩ-cm or better
- Concentrated hydrochloric acid (trace-metal grade)
- Acetic acid, glacial (TraceMetal™ Grade), Fisher Chemical (P/N A507-P212)
- Ammonium hydroxide (TraceMetal™ Grade), Fisher Chemical (P/N A512-P500)
- Ultrapure 2.0 M ammonium acetate, pH 5.5 (Thermo Scientific P/N 033440)
- Tiron (4, 5-Dihydroxy-1, 3 benzene disulfonic acid, disodium salt monohydrate), ACROS Organics™ (Thermo Scientific P/N 174140250)
- Aluminum chloride AlCl₃•6H₂O

Experimental

Conditions

System:	Dionex ICS-5000+ HPIC System
Columns:	Dionex IonPac CG10, Guard, 4 × 50 mm (P/N 043016) Dionex IonPac CS10, Analytical, 4 × 250 mm (P/N 043015)
Eluent:	0.75 M HCl
Flow Rate:	1.36 mL/min
Injection Volume:	25 µL
Inject Mode:	Push full
Loop Overfill Factor:	5
Detection:	Absorbance: 310 nm, after postcolumn derivatization with Tiron reagent
Postcolumn Flow Rate:	0.68 mL/min
System Backpressure:	~1850 psi
Background Absorbance:	~0.1–0.4 mAU
Noise:	0.6–0.9 mAU/min peak-to-peak
Run Time:	5 min

System Setup and Configuration

Install and configure the Dionex AS-AP Autosampler in Push Full mode. Follow the instructions in the *Dionex AS-AP Autosampler Operator's Manual* (Document No. 065361) to calibrate the sample transfer line, thereby ensuring accurate and precise sample injections.

Install the Dionex IonPac CG10 and CS10 columns. Equilibrate the columns with eluent for 30 min and run a system blank. The system will display the background absorbance and noise values similar to those listed under conditions.

The Dionex VWD UV-vis absorbance detector can be equipped with either a standard (PEEK) or a semi-micro (PEEK) flow cell when the Dionex DP or Thermo Scientific Dionex AXP pumps are used to deliver the postcolumn reagent. If the PC10 module is used for postcolumn reagent delivery, the standard (PEEK) flow cell must be used. An end line filter (P/N 045987) can be used for the eluent and postcolumn reagent lines to reduce noise, though none were used for the data shown in this application note.

Postcolumn Reactor Configuration

The system configuration of the IC with postcolumn delivery system is set up as shown in Figure 3 of Thermo Scientific Technical Note (TN) 26.⁴ The Post Column Reagent (PCR) can be delivered in the following three ways.

1. **Dionex DP Pump Module:** The postcolumn reagent can be delivered via the second pump of the Dionex DP module. Operate the Dionex DP pump at a backpressure of 1400–1600 psi. Use green PEEK tubing (P/N 044777) between the pump and backpressure tubing (connect using union P/N 042627) to reduce pump noise. This method of PCR delivery is used in this application note.
2. **Dionex AXP Pump:** Configure the Dionex AXP pump as described in the *Dionex AXP/AXP-MS Manual* and operate at a typical backpressure of 1400–1600 psi. Use green PEEK tubing (P/N 044777) between the AXP pump and backpressure tubing (connect using union P/N 042627) to reduce pump noise. An equilibrated system has peak-to-peak noise of less than 10 μ AU.
3. **PC10:** Configure the IC and the PCR system as shown in Figure 3 of TN26, and as described in the PC10 Postcolumn Delivery System installation instructions. A standard (PEEK) UV cell is recommended with the PC10.

Preparation of Solutions and Reagents

0.75 M HCl

Add 62.0 mL (73.8 g) concentrated HCl to 500 mL deionized (DI) water. Mix and dilute with DI water to 1.0 L. Degas before use.

Tiron Postcolumn Reagent

Prepare the postcolumn reagent in the delivery bottle to minimize contamination. Work in a fume hood. To prepare 1.0 L of Tiron reagent, add 206 mL (186 g) trace-metal grade NH_4OH to 200 mL DI water. Mix and slowly add 172 mL (180 g) trace-metal grade acetic acid. **WARNING:** This solution will become very hot. Add 98.0 mg Tiron to the solution and mix to dissolve. Bring the solution to a final volume of 1.0 L with DI water and degas with ultrasonic agitation. Allow to cool before using.

Standard Solutions

Add 0.494 g of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ to ~50 mL of 50 mM sodium hydroxide in a 100 mL volumetric flask to make a 1000 mg/L stock standard. Dissolve and bring to volume with 50 mM sodium hydroxide. Store the stock standard at 4 °C. Using this stock standard, prepare working standards (1.5–30 mg/L) fresh daily.

Samples

Antiperspirants (solid):

- 1- Antiperspirant 1 (Aluminum chlorohydrate)
- 2- Antiperspirant 2 (Aluminum zirconium tetrachlorohydrate)

Antacid (suspension):

- 1-Antacid 1 (Aluminum hydroxide)

Sample Preparation

Antiperspirant Sample

Step 1. Accurately weigh 150 mg of deodorant powder sample into a 25 mL volumetric flask.

Step 2. Add 25 mL of 50 mM NaOH, cap, and vigorously shake for 5–10 min.

Step 3. Sonicate the solution from Step 2 for 30 min.

Step 4. Dilute it 20 times using 50 mM NaOH and then pass the liquid through a Nalgene syringe filter before analysis.

Antacid Sample

Step 1. Accurately weigh 250 μ L of antacid suspension into a 25 mL volumetric flask.

Step 2. Add 25 mL of 50 mM NaOH, cap, and vigorously shake for 5–10 min.

Step 3. Sonicate the solution from Step 2 for 30 mins.

Step 4. Dilute it 20 times using 50 mM NaOH and then pass the liquid through a Nalgene syringe filter before analysis.

Note: All the samples and standards used in this study are prepared in 50 mM NaOH. Aluminum chloride ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) is used to prepare the aluminum standard.

Results and Discussion

Determination of aluminum by this method is accomplished by separation on a Dionex IonPac column and postcolumn derivatization followed by UV absorbance detection. The eluent is 0.75 M HCl. Aluminum is detected using selective postcolumn chemistry. Reaction of Tiron with aluminum forms a UV-absorbing complex that can be detected at 310 nm. The reaction of aluminum with Tiron proceeds in the pH range of 4.8–5. This requires the use of a buffer in the postcolumn system to adjust the pH of the eluent. The acid eluent mixes postcolumn with 3 M ammonium acetate in a 2:1 ratio to form 0.5 M acetate buffer. The production of the 0.5 M acetate buffer ensures that the pH of the postcolumn system will remain at approximately 4.8, which is within optimum range for the reaction of aluminum with Tiron.

As there have been numerous improvements in cation-exchange columns for IC since the publication of AN69, a number of cation-exchange Dionex IonPac columns—Dionex IonPac CS5A, Thermo Scientific™ OmniPac™ PCX-100, Dionex IonPac CS11, and Dionex IonPac CS10 columns—were evaluated to replace the Dionex IonPac CS5 column for this analysis. Figure 1 shows chromatograms of a 20 mg/L aluminum standard on Dionex IonPac CS5A, OmniPac PCX-100, Dionex IonPac CS11, and Dionex IonPac CS10 columns, respectively. Among these columns the Dionex IonPac CS10 column gave the best result in terms of peak shape, peak symmetry, and loading capacity. Figure 2 shows chromatograms of 1.5, 5, 10, 15, and 30 mg/L aluminum standards on a Dionex IonPac CS10 column. The retention time (RT) for aluminum was 3.20 min. The ranges in RT in Figure 2 are a result of shifts to lower RT with increasing analyte concentration. These shifts and peak symmetries indicate that the column set is at the initial states of overload at approximately 30–35 mg/L.

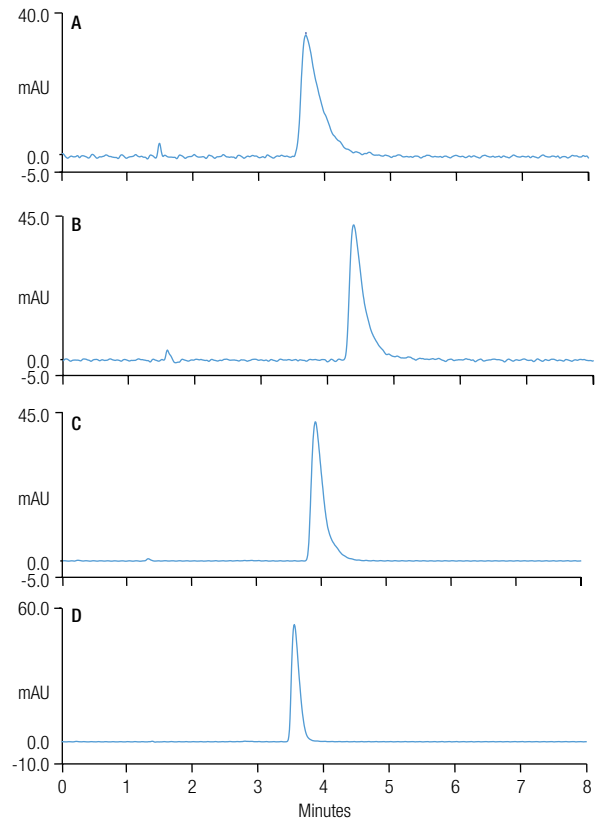


Figure 1. Chromatogram of a 20 mg/L aluminum standard on A) Dionex IonPac CS5A, B) Dionex IonPac CS11, C) OmniPac PCX-100, and D) Dionex IonPac CS10 columns.

Column:	Dionex IonPac CG10 4 mm Dionex IonPac CS10 4 mm
Eluent:	0.75 M HCl
Flow Rate:	1.36 mL/min
Injection Volume:	25 μ L
Column Temperature:	30 $^{\circ}$ C
Post Column Reagent (PCR):	0.3 mM Tiron in 3 M Ammonium acetate
PCR Flow Rate:	0.68 mL/min
Detection:	UV Absorbance @ 310 nm

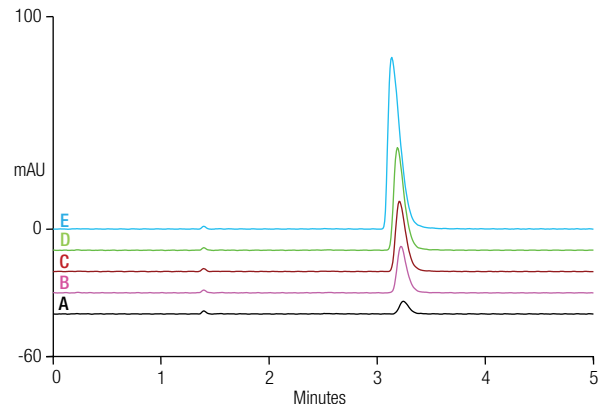


Figure 2. Chromatograms of 1.5 (A), 5 (B), 10 (C), 15 (D), and 30 mg/L (E) aluminum standards on a Dionex IonPac CS10 column. A 10% signal offset has been applied.

Calibration, Limit of Detection (LOD), and Limit of Quantitation (LOQ)

For calibration, three injections of seven concentrations were acquired using aluminum chloride as the standard.

Note: Aluminum sulfate, $Al_2(SO_4)_3 \cdot 18H_2O$, mol wt. 666.42 g/mol (as a standard) was tried first and was found during spiking experiments to be incompatible with the samples used in this study. When mixed with the samples (deodorants and antacid) for recovery, it was not recovered or gave negative recovery (in the case of deodorant samples). Figure 3 shows the chromatogram of unspiked and spiked (at 100%) antacid using aluminum sulfate as the standard. The calibration plots of peak area versus concentration were fit using linear regression. The calibration curve shown in Figure 4 is linear over the calibration range 1.5–30 mg/L for aluminum, with a coefficient of determination (r^2) greater than 0.9999 for the Dionex IonPac CS10 column. To determine the limit of detection (LOD) and limit of quantification (LOQ), the baseline noise was first determined by measuring the peak-to-peak noise in a representative one-minute segment of the baseline where no peaks elute, but close to the peak of interest. The signal was determined from the average peak height of three injections of 2.5 $\mu\text{g/L}$ of aluminum. The LOD and LOQ were then calculated by multiplying the signal-to-noise ratio three times and ten times, respectively. Table 1 summarizes the results of the calibration, LOD, and LOQ.

Minimum Detection Limit (MDL)

MDL is a measure of the precision of replicate injections of a low-level standard and is defined as the minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero. In this application, the MDL for aluminum was determined by analyzing seven replicate injections of 50 mM NaOH fortified with aluminum at 65 $\mu\text{g/L}$ (i.e., approximately 3–5 times the estimated method detection limit). This produced a calculated MDL value of 13.2 $\mu\text{g/L}$.

Column: Dionex IonPac CS10 4 mm
 Eluent: 0.75 M HCl
 Flow Rate: 1.36 mL/min
 Injection Volume: 25 μL
 Column Temperature: 30 $^{\circ}\text{C}$
 Post Column Reagent (PCR): 0.3 mM Tiron in 3 M Ammonium acetate
 PCR Flow Rate: 0.68 mL/min
 Detection: UV Absorbance @ 310 nm

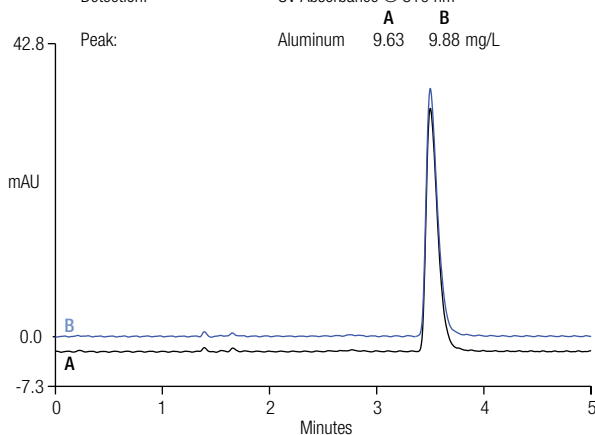


Figure 3. Chromatogram of A) antacid 1 sample and B) antacid 1 sample spiked using aluminum sulfate standard, demonstrating that it is not suitable for this method.

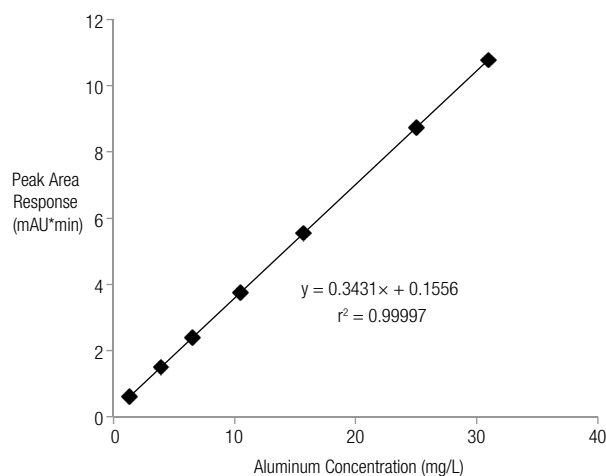


Figure 4. Calibration curve for aluminum on a Dionex IonPac CS10 column.

Table 1. Results of aluminum calibration, LOD, and LOQ.

Range (mg/L)	Cal. Type	Number	RSD	(r^2) ^a	LOD ^b ($\mu\text{g/L}$)	LOQ ^c ($\mu\text{g/L}$)	MDL ^d ($\mu\text{g/L}$)
1.5–30	Linear, with Offset	21	0.3448	0.99997	31.84	107.9	13.20

^aCoefficient of determination

^bLOD = 3 \times signal-to-noise ratio (S/N)

^cLOQ = 10 \times S

^dMDL = $\sigma \times 3.14$

Sample Analysis

An antacid suspension and two antiperspirant sticks were purchased from a pharmacy and analyzed for their aluminum content. Samples used in the study were found to be only slightly soluble in water. After the solution was kept for 2–3 days, the sample tended to settle at the bottom, giving incorrect aluminum concentrations. Various concentrations of different acids and bases were tested to improve the dissolution of the aluminum sample and 50 mM NaOH was chosen for this work as it gave the best solubility. Thus all samples and standards were prepared in 50 mM NaOH instead of DI water. The concentration range for aluminum as calculated in antiperspirant /antacid samples was approximately 200–250 mg/L for 150 mg/250 μ L sample dissolved in 25 mL of 50 mM NaOH. To avoid overloading the column, antiperspirant and antacid samples were diluted 20 times with 50 mM NaOH before analysis. Figure 5 shows chromatograms of samples (Antiperspirant 1, Antiperspirant 2, and Antacid 1) containing aluminum on a Dionex IonPac CS10 column.

Most of the aluminum containing compounds such as aluminum chloride, aluminum chlorohydrate, aluminum sulfate, and aluminum zirconium tetrachlorohydrate can be analyzed using this method with the exception of aluminum oxide which is insoluble in water.

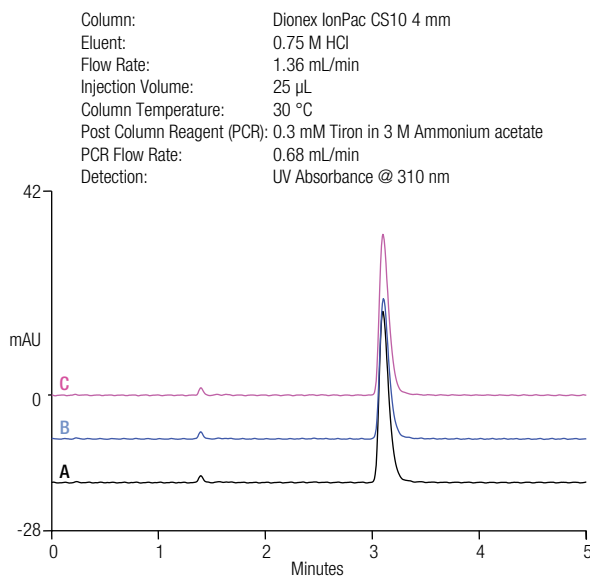


Figure 5. Chromatogram of A) Antiperspirant 1, B) Antiperspirant 2 sample, and C) Antacid 1 on a Dionex IonPac CS10 column. A 25% signal offset has been applied.

Sample Recovery

Method accuracy was evaluated by calculating the recovery of aluminum after spiking directly into the antacid and antiperspirant samples at 50% and 100% of the expected amount. Samples were prepared and injected in triplicate. Figures 6, 7, and 8 show an overlay of the spiked and unspiked antacid and antiperspirant samples, respectively. Recoveries for aluminum spiked at 50% and 100% of the expected amount into the antacid sample were 99.9% and 100%, respectively (Table 2). For antiperspirant samples recoveries were 78.1–80.7% and 74.7–104%. A reason for the low recoveries of aluminum in antiperspirant samples could be the sticky nature of the sample, which tends to make it cling to the bottom or sides of the flask giving inconsistent results. Another reason could be non-uniformity of aluminum content in the antiperspirant stick.

Note: Due to the insolubility of aluminum oxide in aqueous solution, this method is not applicable to the OTC products containing aluminum oxide as an active ingredient.

Table 2. Results of spike recovery for aluminum in antiperspirant and antacid samples.

		Found (ppm)	Added (ppm)	Recovered (ppm)	Recovery (%)
Antacid 1	+50%	9.66	5.21	14.9	99.9
	+100%	9.82	9.53	19.4	100
Antiperspirant 1	+50%	10.2	4.77	13.0	80.7
	+100%	10.7	13.3	24.4	104
Antiperspirant 2	+50%	8.40	4.75	11.3	78.1
	+100%	8.68	7.23	13.5	74.7

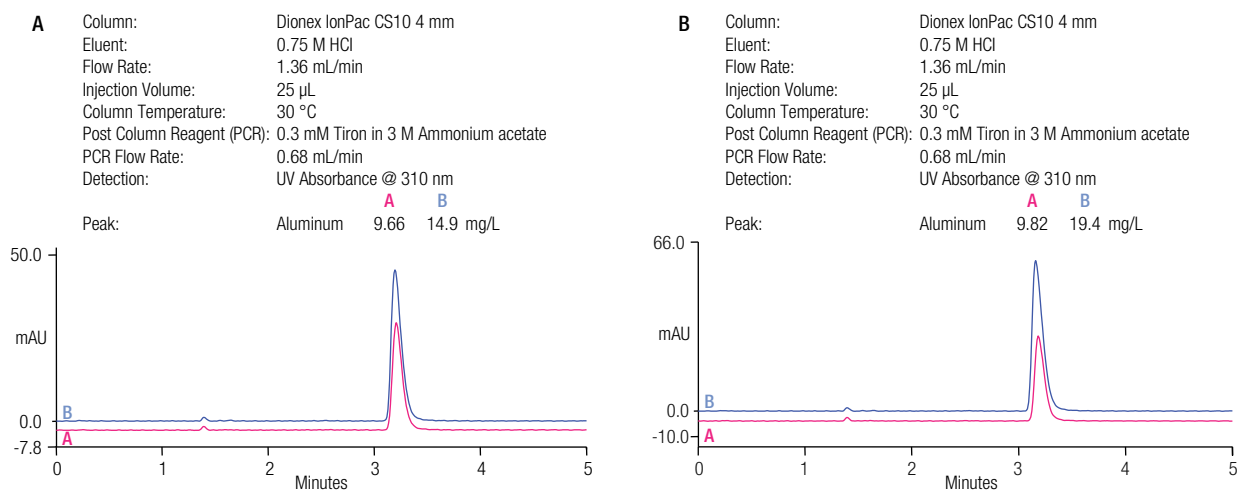


Figure 6A. Chromatogram of A) Antacid 1 and B) Antacid 1 sample spiked (50% spike). Figure 6B. Chromatogram of A) Antacid 1 sample and B) Antacid 1 sample spiked (100% spike). A 5% signal offset has been applied.

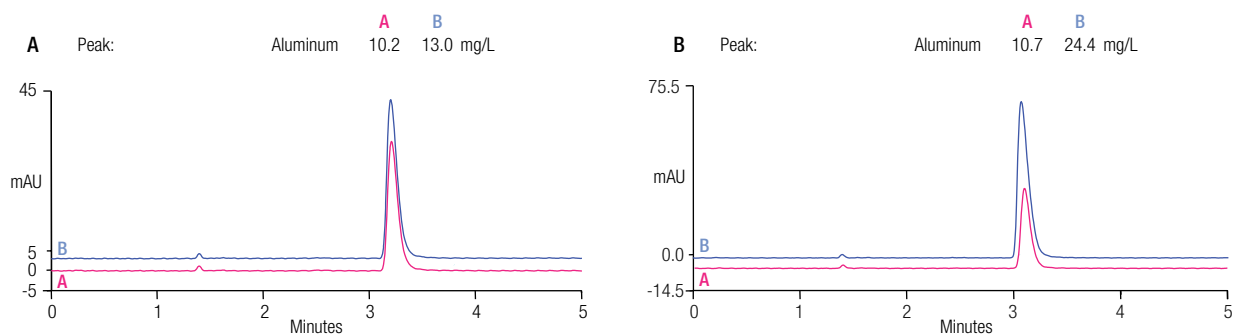


Figure 7A. Chromatogram of A) Antiperspirant 1 sample and B) Antiperspirant 1 sample spiked (50% spike). Figure 7B. Chromatogram of A) Antiperspirant 1 sample and B) Antiperspirant 1 sample spiked (100% spike). A 5% signal offset has been applied.

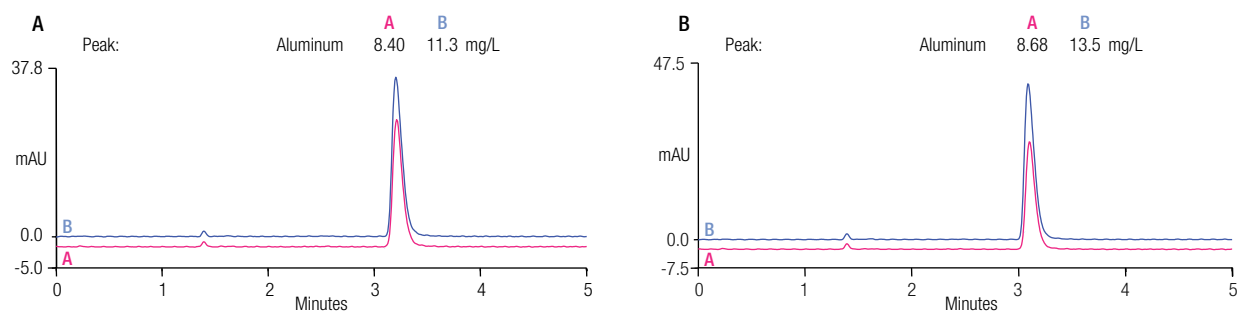


Figure 8A. Chromatogram of A) Antiperspirant 2 sample and B) Antiperspirant 2 sample spiked (50% spike). Figure 8B. Chromatogram of A) Antiperspirant 2 sample and B) Antiperspirant 2 sample spiked (100% spike). A 5% signal offset has been applied.

Conclusion

This application note presents the successful determination of aluminum cation in OTC products. Here we showed the results for two types of OTC products: antacid and antiperspirant. For antacid, a suspension formulation was used, and for antiperspirant, solid sticks were used. This method uses a Dionex IonPac CS10 column and postcolumn derivitization followed by UV detection. Spike recoveries for the antacid sample are 99–101% and 75–110% for the antiperspirants.

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