

Rapid and Sensitive Analysis of Aluminum Ions using On-column Derivatization (Part 1)

Aluminum ions are known to react with 8-hydroxyquinoline (8-HQ) to create a fluorescent chelate. HPLC analysis of this aluminum/8-HQ chelate permits the highly sensitive analysis of the aluminum ions. Generally, the aluminum/8-HQ chelate is created prior to injection into the column (pre-column

derivatization). However, this Application News introduces a method for the rapid and sensitive analysis of aluminum ions using a unique on-column derivatization method, in which the 8-HQ is pre-added to the mobile phase.

■ Benefits of the On-column Derivatization Method

8-HQ alone does not fluoresce. However, it reacts with aluminum ions in a 1:3 mol ratio to form a strongly fluorescent chelate (Fig. 1).

A pre-column derivatization using this reaction has been reported. However, this method involves first creating the chelate and then injecting it into the column. This is a complex operation, subject to contamination, errors, and human mistakes.

Since the reaction between aluminum ions and 8-HQ proceeds quickly, the on-column derivatization method can be applied, where the 8-HQ pre-added to the mobile phase and the injected aluminum ions react to create chelate before the aluminum ions reach the column.

As the reaction forming the chelate occurs inside the HPLC, the on-column derivatization method avoids

contamination, errors, and human mistakes during pretreatment and allows the easy, rapid, and highly sensitive analysis of aluminum ions.

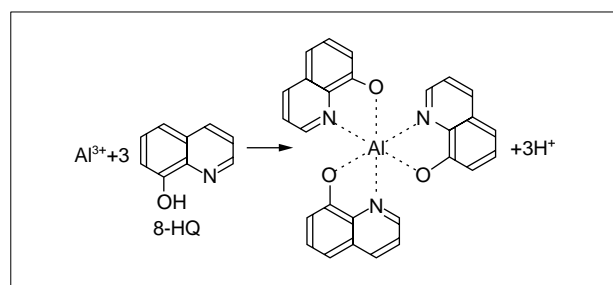


Fig. 1 Reaction of Aluminum with 8-hydroxyquinoline

■ Standard Analytical Conditions

As the chelate created is hydrophobic, analysis is possible with a reversed-phase chromatography column. Table 1 shows the optimal analytical conditions.

Fig. 2 shows the analysis results for a 50 µg/L aluminum standard solution. The standard solution was prepared by adding a small quantity of nitric acid (ultra-high purity analysis reagent) as an adsorption inhibitor to a 1,000 mg/L raw standard solution for atomic absorption analysis, and then diluting with pure water.

Table 1 Analytical Conditions

Column	:Shodex Asahipak ODP-50 (150mmL.×4.6mmI.D.)
Mobile Phase	:A/B = 4/6 :A:12mM 8-hydroxyquinoline acetonitrile solution :B:100mM imidazole buffer (pH=7.1) adjusted by perchloric acid
Flow Rate	:0.6mL/min
Inj. Volume	:20µL
Temperature	:40°C
Detection	:RF-10AXL Super at Ex=380nm, Em=520nm (Cell Temperature 25°C)

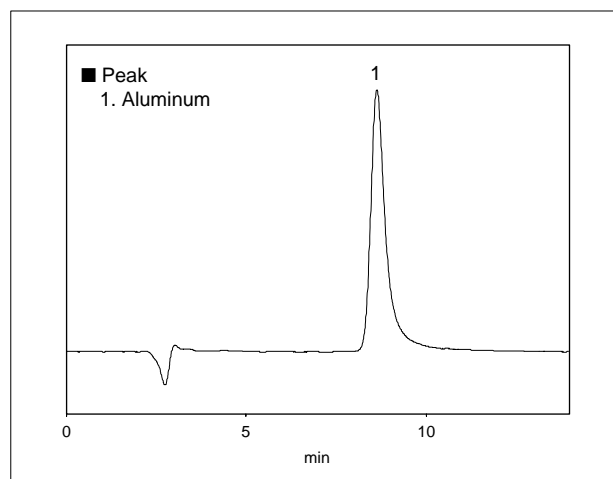


Fig. 2 Chromatogram of Aluminum Standard Solution

■ Effects of Detector Cell Temperature

Fluorescence is generally temperature-dependent: the response drops as the temperature rises. Fig. 3 shows the results of investigating changes in fluorescence intensity (peak area) while changing the detector cell temperature between 25°C and 35°C in three stages during the analysis of aluminum/8-HQ chelate. It shows that controlling the detector cell temperature is an important factor in achieving higher aluminum-ion quantitation accuracy. Consequently, this system incorporates a fluorescence detector with a cell temperature-control function to conduct analysis at a cell temperature of 25°C.

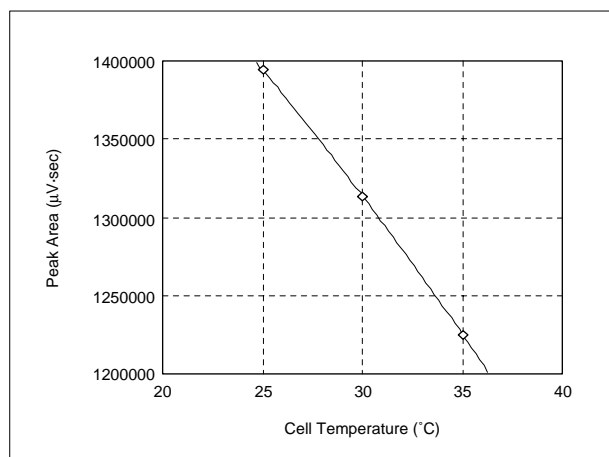


Fig. 3 Relationship between Detector Cell Temperature and Peak Area

■ Linearity

Fig. 4 shows the linearity of the peak-area calibration curve for an aluminum ion concentration up to 50 µg/L. Highly accurate analysis is possible up to several µg/L.

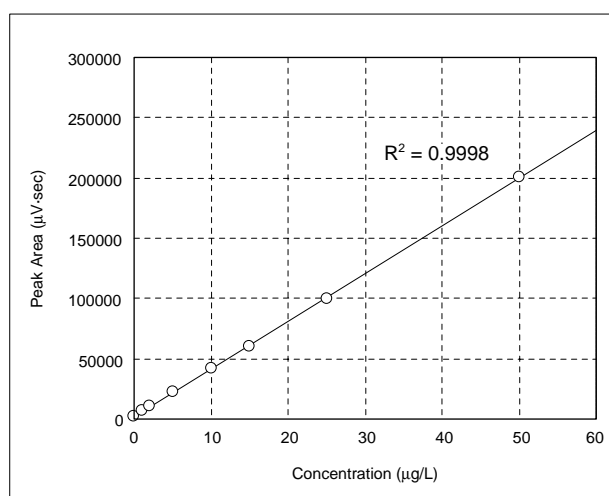


Fig. 4 Calibration Curve for Aluminum Ions

■ Repeatability

Table 2 shows the peak area repeatability for aluminum ions. Sufficient accuracy is achieved at low concentrations.

Table 2 Peak Area Repeatability for Aluminum Ions

	Blank	10µg/L	25µg/L
1st	2294	41764	99605
2nd	2259	42103	99798
3rd	2311	41480	98212
4th	2342	40105	97503
5th	2194	42220	99125
6th	2236	40165	100891
Average	2272.7	41306.2	99189.0
RSD%	2.37	2.29	1.21



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