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Reassessment of Matrix Interference in the Pharmacopeial Limit Test for Aluminum in Citric Acid: Toward Method Revision

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ABSTRACT

The pharmacopeial limit test for aluminum in citric acid intended for dialysate production commonly relies on solvent extraction with 8-hydroxyquinoline followed by fluorescence measurement. However, fluorescence intensity (F.I.) readings from citric acid extracts have frequently proven unreliable, often registering lower values than even the blank solution. This study aimed to investigate the impact of the citric acid matrix on the accuracy of the test. A comparison between standard aluminum solutions prepared in water and those in citric acid revealed significant differences in both slope and y-intercept of the calibration curves, with F.I. values markedly lower in the citric acid solutions. Further experiments demonstrated that increasing the concentration of citric acid consistently reduced the F.I. of aluminum solutions. These findings indicate that the acidic nature and metal-chelating properties of citric acid interfere with the assay. Consequently, the current pharmacopeial limit test for aluminum in citric acid requires revision, as it may otherwise underestimate aluminum content and produce misleading results.

Keywords: Matrix effects, Aluminum, Citric acid, Limit test, Interference

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Introduction

Aluminum contamination in dialysis solutions has been linked to serious health issues, including osteomalacia, anemia, and cognitive decline in patients with chronic kidney disease undergoing long-term dialysis [1]. Therefore, monitoring aluminum concentrations in materials used for dialysate preparation is crucial to ensure patient safety. For both anhydrous citric acid and citric acid monohydrate, which are commonly used in citric acid-based dialysates such as Citrasate, pharmacopeias specify a maximum aluminum concentration of 0.2 ppm. This limit is consistently stated in major compendia, including the United States Pharmacopeia and National Formulary [2], European Pharmacopoeia [3], and British Pharmacopoeia [4]. These pharmacopeial methods employ a semi-quantitative approach in which aluminum reacts with 8-hydroxyquinoline to form a fluorescent complex, which is then extracted into chloroform. The sample is deemed compliant if its fluorescence intensity (F.I.) does not exceed that of a standard aluminum solution.

While this assay is routinely applied to determine aluminum in substances such as sodium chloride and calcium phosphate, its application to citric acid has produced inconsistent and questionable results. Both our own data and previous reports indicate that citric acid samples often generate F.I. readings lower than expected, and sometimes even below the blank, potentially causing underestimation of aluminum content. Such inaccuracies could compromise the safety of dialysate produced from these materials.

In analytical chemistry, the sample matrix—the constituents of a sample other than the analyte—can significantly influence the accuracy and reliability of a method [5]. In the case of citric acid, the sample matrix differs from that of the standard aluminum solution, which contains no citric acid. Moreover, at the allowable aluminum concentration of 0.2 ppm, citric acid is present at concentrations exceeding the analyte by over six orders of

magnitude (>10^6 times). This disproportion suggests that citric acid itself may interfere with the assay, causing unreliable results. To evaluate this, standard aluminum curves were prepared both in water and in citric acid solutions. If matrix effects were absent, both calibration curves would align closely. Any variations in slope or intercept would indicate interference from citric acid [5]. Additional experiments were designed to understand the mechanism of this interference and its impact on test performance. The findings aim to support the need for revising or improving the compendial limit test for aluminum in citric acid.

Materials and Methods

Reagent-grade chemicals were used without further purification. Citric acid samples were obtained from Merck (Darmstadt, Germany) and BDH Chem (Poole, England). 8-Hydroxyquinoline was sourced from Sigma-Aldrich (St. Louis, MO) and Fluka (Munich, Germany). Chloroform (RCI Labscan, Thailand) and distilled water were used as solvents.

Assessment of matrix effects

To investigate the impact of citric acid on the assay, aluminum calibration curves were constructed both in water and in 0.2 g/mL citric acid solution. Aluminum solutions ranging from 0 to 0.06 mg/mL were prepared, followed by the addition of acetate buffer (pH 6.0) and extraction with 0.5% w/v 8-hydroxyquinoline in chloroform in three successive steps, according to USP procedures. Fluorescence of the chloroform extracts was measured at 492 nm excitation and 518 nm emission, with chloroform used to zero the instrument. All measurements were performed in triplicate.

Reproducibility was ensured by using citric acid from two suppliers, 8-hydroxyquinoline from two manufacturers, and two Shimadzu spectrofluorometer models (RF-6000 and RF-1501, Japan) with different instrumental settings. For RF-6000, excitation and emission bandwidths were 3 nm with high sensitivity; for RF-1501, the bandwidths were 10 nm with high sensitivity. Aluminum concentrations in Merck and BDH Chem citric acid samples were determined using inductively coupled plasma-mass spectrometry (ICP-MS) to be 0.033 and 0.048 ppm, respectively.

Determination of aluminum by ICP-MS

Aluminum concentrations in citric acid samples and in the aqueous phase before and after extraction were measured using an Agilent ICP-MS spectrometer (Model 7500ce). To minimize spectral interferences, collision/reaction cell technology was employed. The instrument was operated at an RF power of 1500 W, with ultrapure argon (99.9995%) as the carrier gas at a flow rate of 1.5 L/min, and a nebulizer pump speed of 0.1 rps. Signal intensities were calibrated against a standard aluminum curve ranging from 0.5 to 100 mg/L. All measurements were performed in triplicate, and results are reported as the mean values.

Results and Discussion

Appearance and pH of aqueous phases in different matrices

Prior to extraction, the pH of the aluminum solutions was recorded. Despite the addition of a buffer, the citric acid-containing solution had a much lower pH (2.2) compared to the solution prepared in water (5.7). Upon addition of 8-hydroxyquinoline in chloroform and subsequent mixing, the aqueous phase from the citric acid solution displayed a faint yellow tint, in contrast to the colorless aqueous phase from the water-based solution. Since 8-hydroxyquinoline is sparingly soluble in water but soluble in chloroform and acidic aqueous solutions [6], the low pH of the citric acid solution likely enhanced the migration of 8-hydroxyquinoline into the aqueous phase, accounting for the observed yellow coloration.

Matrix effects on the assay

Within the aluminum concentration range of 0–0.06 mg/mL, which encompasses the compendial standard (0.04 mg/mL), the calibration curves of aluminum prepared in 0.2 g/mL citric acid solution differed markedly from those prepared in water in both slope and y-intercept **(Figure 1)**, demonstrating significant interference from citric acid. Furthermore, the F.I. values for the citric acid solutions were consistently lower than those obtained for

water-based solutions. These observations were reproducible across different sources of chemicals and instrumentation.

In an additional experiment, aluminum solutions (0.04 mg/mL) containing increasing concentrations of citric acid showed a progressive decline in F.I. **(Figure 2)**, confirming that higher citric acid levels suppress the analytical signal. Collectively, these results indicate that citric acid acts as a matrix interferent, substantially diminishing the fluorescence response. As a result, reliance on the current pharmacopeial procedure, which evaluates compliance by comparing sample F.I. to that of the standard, may lead to underestimation of aluminum content and produce misleading conclusions.

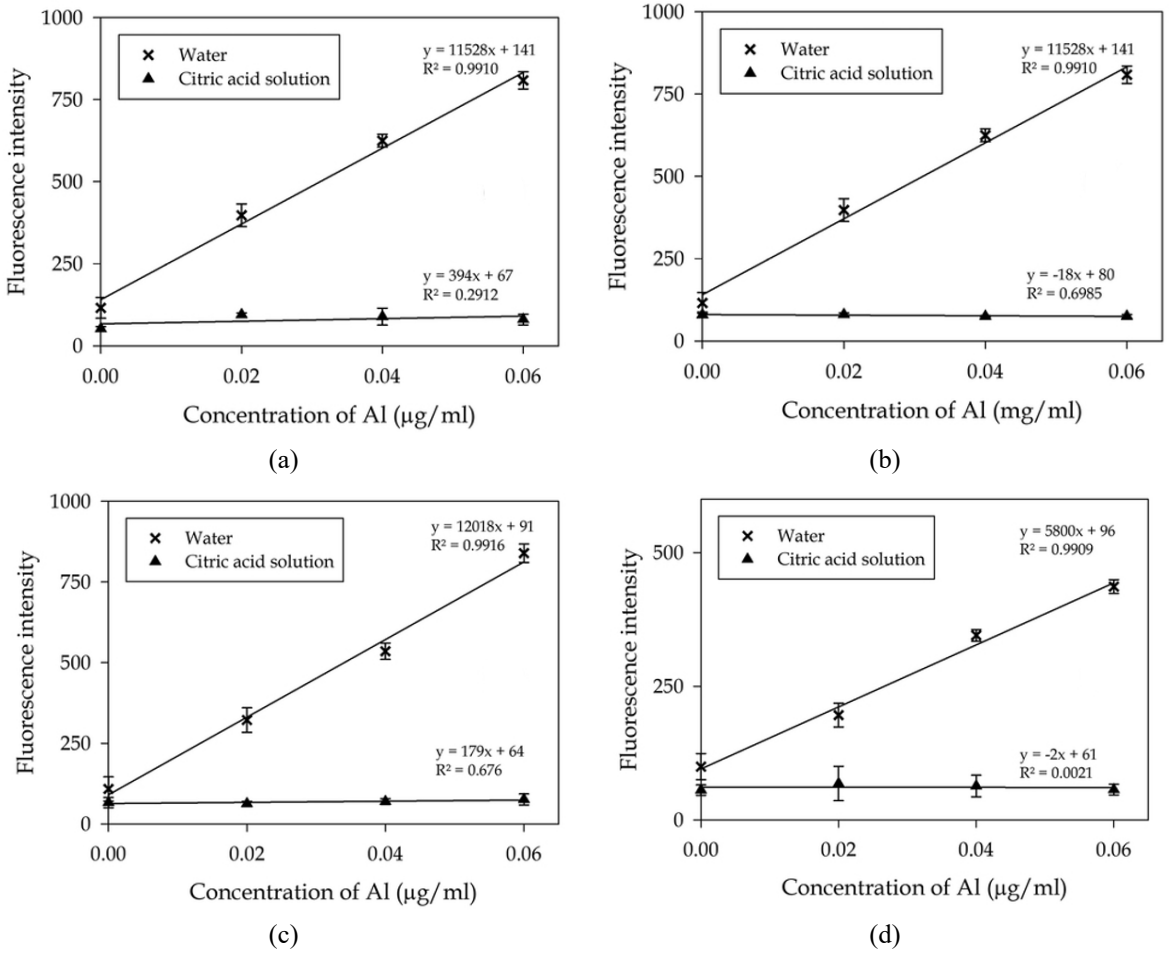

Figure 1. Calibration curves for aluminum in both water and citric acid solutions, prepared using chemicals and instruments from various manufacturers: (a) Merck citric acid, Sigma-Aldrich 8-hydroxyquinoline, spectrofluorometer RF-1501; (b) BDH Chem citric acid, Sigma-Aldrich 8-hydroxyquinoline, spectrofluorometer RF-1501; (c) Merck citric acid, Fluka 8-hydroxyquinoline, spectrofluorometer RF-1501; (d) Merck citric acid, Sigma-Aldrich 8-hydroxyquinoline, spectrofluorometer RF-6000.

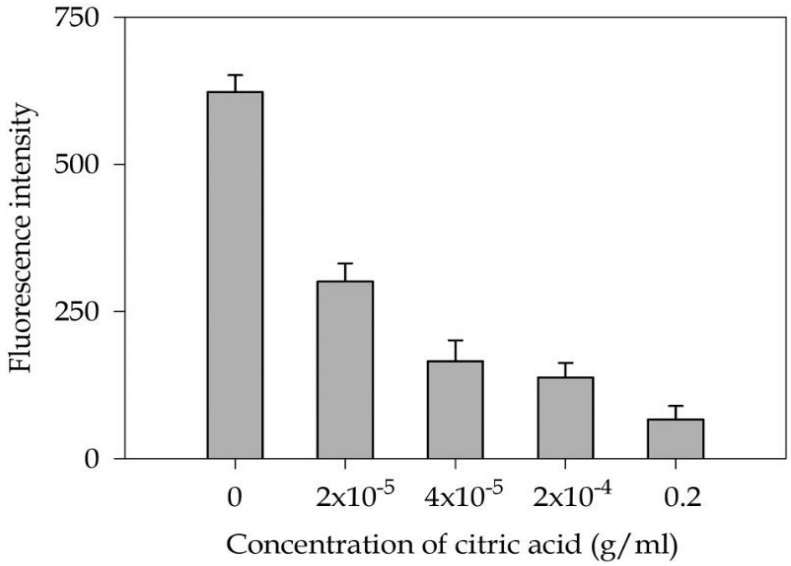

Figure 2. Impact of different citric acid concentrations on the fluorescence intensity of a 0.04 mg/ml aluminum solution, measured using a Shimadzu RF-6000 spectrofluorometer.

Since the aluminum solution with citric acid had a noticeably lower pH than the solution in pure water, we examined whether this acidity contributed to the interference observed. When the pH of the citric acid solution was raised from 2.2 to 4.0, the fluorescence intensity increased **(Figure 3)**. Nevertheless, further adjustment to pH 5.7 still did not restore the fluorescence intensity to the level seen in water-based aluminum solutions. These observations indicate that while citric acid's low pH can partially alter the behavior of amphoteric 8-hydroxyquinoline and the extraction efficiency of the aluminum complex into chloroform—effects that are more pronounced at neutral pH [6]—acidity alone cannot fully explain the interference.

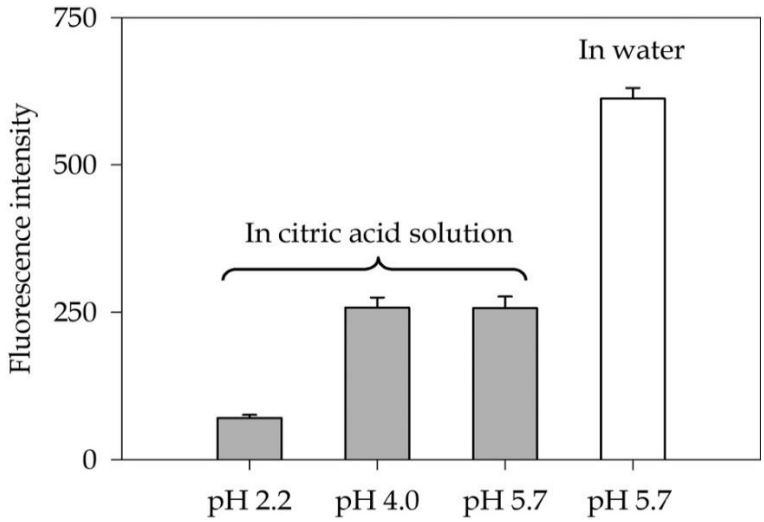

Figure 3. Influence of pH on the fluorescence intensity of a 0.04 mg/ml aluminum solution prepared in 0.2 g/ml citric acid, compared to aluminum in water. Measurements were performed using a Shimadzu RF-6000 spectrofluorometer.

Citric acid is known to form complexes with metals, including aluminum [7-9]. Consequently, citric acid present in the aqueous phase may compete with 8-hydroxyquinoline for aluminum binding, causing a portion of the aluminum to remain in the aqueous layer. Supporting this idea, ICP-MS analysis of the aqueous phase after extraction indicated that most of the aluminum persisted in solution when citric acid was present **(Figure 4)**, likely as a water-soluble aluminum–citric acid complex. By contrast, in the absence of citric acid, aluminum was nearly fully transferred into the organic phase, reflecting the chloroform solubility of the aluminum–8-hydroxyquinoline

complex. These findings demonstrate that citric acid interferes with the assay through a combination of its acidic nature and its metal-chelating properties.

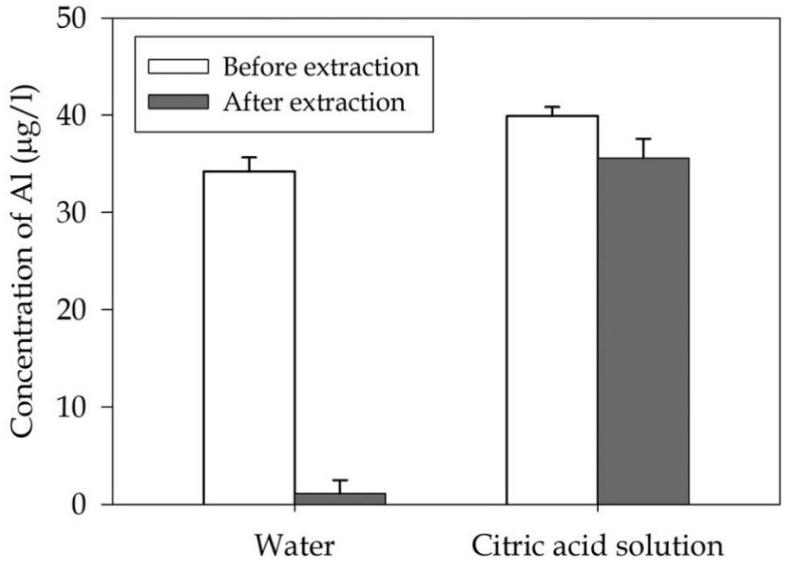

Figure 4. Aluminum levels remaining in the aqueous phase after extraction from a 0.04 mg/ml aluminum solution prepared in water versus a 0.2 g/ml citric acid solution.

In pharmaceutical quality control, interference from sample matrices can compromise the accuracy of active ingredient tests, potentially leading to misleading results and posing safety risks for consumers. To address such issues, it is advisable to revise the current assay or explore alternative, more robust methods that are less sensitive to matrix effects. Options include atomic absorption-based limit tests described in certain pharmacopeias for materials such as potassium chloride, sodium acetate, and sodium carbonate [2], or techniques reported in earlier studies [10, 11]. Other promising approaches include sensitive atomic emission-based methods like ICP-MS [12], as well as spectrophotometric [13, 14] and fluorimetric assays [15, 16] previously applied to aluminum quantification in pharmaceutical or biological matrices. Any alternative method must undergo thorough validation, including assessment of potential interference from citric acid, to ensure reliable results.

Conclusion

The standard compendial limit test for aluminum, which depends on forming the aluminum–8-hydroxyquinoline complex and extracting it into an organic solvent, is strongly influenced by the presence of citric acid. Experimental findings revealed that the acidic environment of citric acid promotes partial dissociation of 8-hydroxyquinoline, leading to its migration into the aqueous phase. At the same time, citric acid competes with 8-hydroxyquinoline for aluminum binding, preventing complete extraction of the complex into chloroform. As a result, samples containing citric acid exhibit lower fluorescence intensity compared to standard solutions, which could lead to erroneous conclusions. These observations highlight the necessity to either modify the current compendial test or develop an alternative method to ensure accurate aluminum measurement and maintain patient safety.

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Ethics Statement: None

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