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# Determination of Aluminium with 8-Hydroxyquinoline in the Hemodialysis Waters By Liquid Chromatography of Reversed Phase Polarity

**Soumaya GHARBI[1], Bassem JAMOUSSI[2]**

[1]Unit of Analysis and Synthesis of Macromolecular Advanced Search, IPEST, Tunisia

**Abstract:** ***This study highlights the benefit, as has the use of 8-Hydroxyquinoline (8-HQ), in the liquid chromatography for determination of aluminum in dialysis waters: dissolving in the mobile phase of this chelating agent allows direct injection of the aluminum without prior derivation. The retentions then depend on the one hand of the choice of the chelating agent and, on the other hand, experimental conditions related to the nature and composition of the mobile phase. We have shown that the technique by chromatography by polar reverse-phase on spectrophotometric detection in the ultraviolet (UV) enables the quantitative analysis of aluminum. The detection limit is 0.3ng for injection of 20µL.***

**Keywords:** Aluminum, 8-Hydroxyquinoline, HPLC, Hemodialysis water

## 1. Introduction

Many studies have shown that the aluminum content in the waters of hemodialysis (1-4) could be responsible for a myoclonic encephalopathy in patients with renal hemodialysis. Various toxic effects involving aluminum having also been demonstrated (5-9), with maximum permissible aluminum content for hemodialysis water was fixed.

According to the Directive of the European Communities, Regulation French and O.M.S, this limit is fixed at $30\mu L^{-1}$, although some authors believe that it should not exceed $14\mu L^{-1}$ in the dialysate to prevent aluminum absorption by the patient (10).

Patients with renal insufficiency are faced to multitude of malfunction, including the inability to eliminate the absorbed aluminum. The toxicity associated to exposure of aluminum in the dialysis fluid or which is related to the medical use of long term aluminum compounds for this group of patients is well known. A follow of aluminum levels in the blood is produced in renal insufficiency.

The classical methods of dosing the aluminum trace at various matrices used in colorimetry is based on measuring the absorption spectrophometric aluminum complexes as highly colored chelate with many more chelators like eriochrome cyanine (R) (11) and oxine (12-13). These methods are sensitive because they can achieve a detection limit of 10 micrograms with an accuracy of ± 2% with eriochrome cyanine (R) (11). However, they require a lot of operations that make them uncompetitive.

Numerous articles have been published on the analysis by atomic absorption spectrometry with electro-thermal atomization (EEAS) (14-17) of aluminum trace, especially in biological samples. However, a disadvantage of this technique is the lower test sample that limits the detection threshold of the aluminum.

In addition, this technique has disadvantages and may lead to erroneous results:

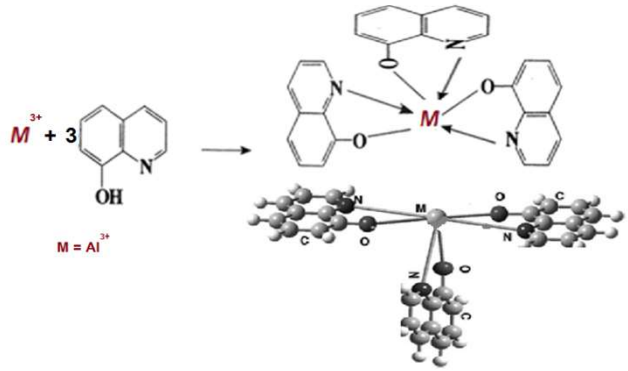
1) Loss of part of the atomic vapor forming molecular species, which reduce the height of the absorbance peak
2) Delay in the formation of the atomic vapor with reducing height and peak broadening

Some authors (19) have suggested the use of the inductively coupled plasma emission spectrometry (ICP) for the determination of aluminum in the blood and urine. However, this technique is often subject to interference from matrix which can cause considerable changes in the signal of the analyte.

Other chromatographic techniques have been tried: besides the usual ion exchange techniques, techniques of gas chromatography have been applied to the analysis of aluminum. K.W.M.Siu et al (20) showed that the aluminum cannot be chromatographed directly but can be derivatized to trifluro-acetylacetonate to impart it a more volatile character (gas chromatography).

Ion exchange chromatography of aluminum ion using 3-carboxy-2-naphthylamine-N,N-diacetic acid (CNDA) as a fluorescent post-column chelating reagent (21) was also studied. The originality of the chromatographic system we have developed is to achieve dose aluminum as a metal cation, without requiring its prior derivatization chelate form. By analogy with the techniques for the separation of ionized substances after formation of ion pairs, we examined the retention of aluminum after formation of Tris-(8-Hydroxyquinoline) aluminum ($Alq_3$) in the column itself, the chelating agent (8-HQ) was dissolved in the mobile phase.

8-Hydroxyquinoline has a hydrogen atom that is replaceable by a metal, and a heterocyclic nitrogen atom, which forms with this, metals a five-membered ring. It is bidentate ligand and forms stable complexes with several metal ions, a typical reaction is followed:




Suggested reaction of Al(III) with 8-HQ to $Alq_3$ complex

In appropriate chromatographic conditions, the initial head of the column of aluminum in the form of cation ($Al^{3+}$) will be accompanied by various complexing equilibria, giving the metal species and complexed ($Alq_3$) a more hydrophobic character.

By the choice of a chromatographic system of reverse phase polarity (non-polar stationary phase and mobile phase hydroalcoholic), it is possible to expect a metal chelate retention.

We will show how in this work, the suitable choice of chelating agent and chromatographic conditions, provided for the determination of aluminum by absorptiometry (UV) as a metal chelate.

## 2. Materials and Methods

### 2.1 Materials

The equipment used in this study consists of the following elements:

- A pump (LC-9A)
- A Rheodyne injection valve outer loop 20 $mm^3$
- A column of 1/4 "outer diameter (4.6 mm internal diameter) of 15 cm length, filled with Lichrosorb RP8, 5µm (Merck)
- A UV-visible spectrophotometer Schimadzu mark detector (SPD-6AU) allowing the detection of Tris-(8-Hydroxyquinoline) aluminum
- A Schimazu integrator (CR-4A Chromatopac)
- Spectrophotometer (UV-VIS) Schimadzu (2100-S) equipped with quartz cuvettes of 1cm thick for photometric determination of oxine
- pH-meter WTW brand for measuring pH between 0 and 14 to 0.02 units near
- Deionized water is supplied by a device of Millipore (Milli-Q plus 185) equipped with a Kit (Rem 61591) comprising a reverse osmosis membrane and an electrode for measuring the conductivity for the control of the purity of produced water (<0.2 µS)

### 2.2 Reagents

All reagents used are of pure type for toxicological analyzes:

- 8-Hydroxyquinoline (Prolabo, Paris IGT)
- Methanol is HPLC grade (Carlo Erba, Italy) was filtered through a 0.45 mm filter and then degassed by helium
- The solutions were prepared from a standard solution to 1g.$L^{-1}$ Aluminum (Wako Pure Chemical Industries, Tokyo, Japan)

## 3. Experimental

The mobile phases are mixtures of different compositions in water-methanol in which is dissolved oxine. The pH of the aqueous solution is buffered with M/15 phosphate buffer ($Na_2HPO_4$ and $KH_2PO_4$). After dilution of this aqueous solution with methanol to obtain the suitable composition of mobile phase methanol-water, the pH of this new solution is measured using a pH-meter. Vials were then sealed with polytetrafluoroethylene/silicone septa caps. Analyses were immediately done to avoid any risk of VOC losses.

## 4. Results and Discussion

### 4.1 Absorption spectra

To determine the optimum wavelengths, we first studied the spectra absorption of 8-HQ and the system $Alq_3$.

Figure 1 shows the absorbance values obtained based on wavelengths in the range of 190 to 450 nm. It is observed that the 8-HQ and aluminum oxinate ($Alq_3$) complex exhibit an absorption maximum at 240 and 370 nm respectively.

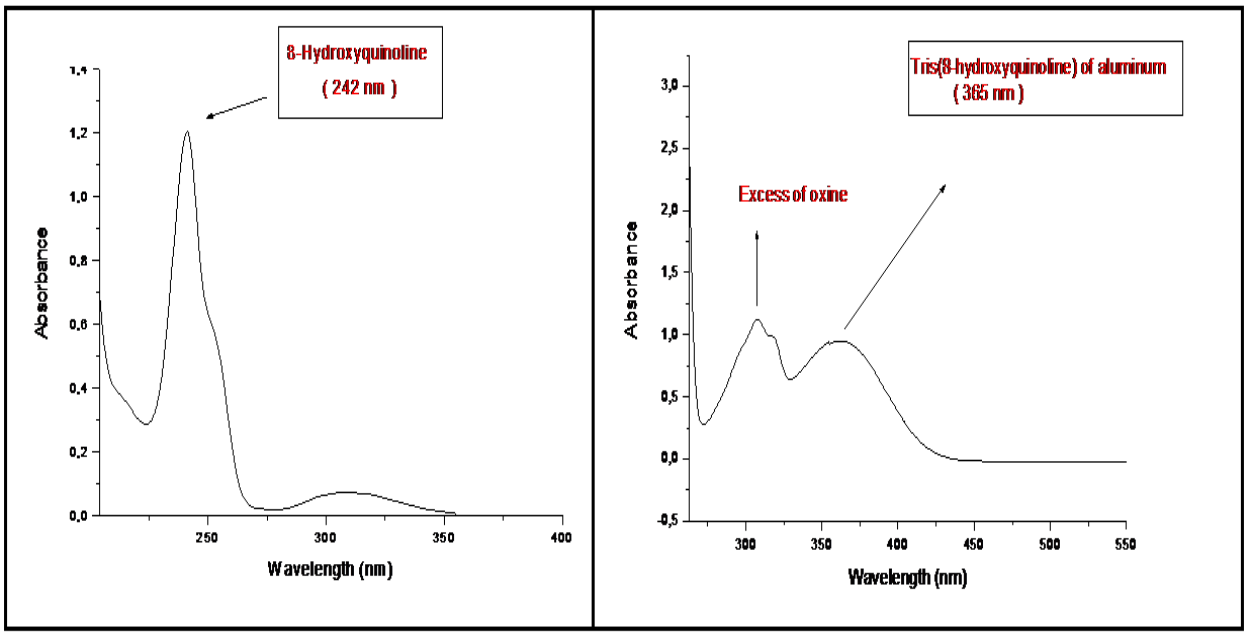



**Figure 1:** Absorption spectrum of oxine and aluminum oxinate in a solvent mixture of CH3OH - H2O (53-47 v / v)

### 4.2 Oxine sharing between the mobile phase and stationary phase

Preliminary tests of various chelating agents allowed retaining the interesting nature of the 8-hydroxyquinoline or oxine - extractant agent well known in the field of liquid-liquid extraction.

The chromatographic system then consists of a water-methanol mobile phase in which is dissolved oxine and an apolar stationary phase (Lichrosob RP8). Should be considered in advance how to distribute oxine in such system.

At pH where we work (5 <pH <8), oxine is mainly as HOx, the forms $H_2Ox^+$ and $Ox^-$ are minority, being given pK values near 4 and 11 in such media-water-methanol [21-23]. Thus, the equilibrium of oxine sharing between each two mobile and stationary phases can be represented by a general expression of the type:

$$[Ox]_s = K_{ox}.[\ [Ox]_m]^p \qquad (1)$$

s and m representing successively the stationary and mobile phases.

After equilibrium by successive passages on column with different mobile phase methanol-water (40-60v / v), pH 8,5-6 and 4,7, and with different concentrations of oxine, the column is eluted after each passage by a previous mobile phase water-methanol (40-60 v / v) pH 8.5, until the complete recovery of oxine deposited on the stationary phase. A UV photometric dosage at 370 nm then allows the determination of $[ox]_s$.

Due to the dead volume of the column, the results for concentrations of oxine on the mobile phase in the range of $10^{-2}$M and $10^{-4}$ M are plotted in Figure 2.

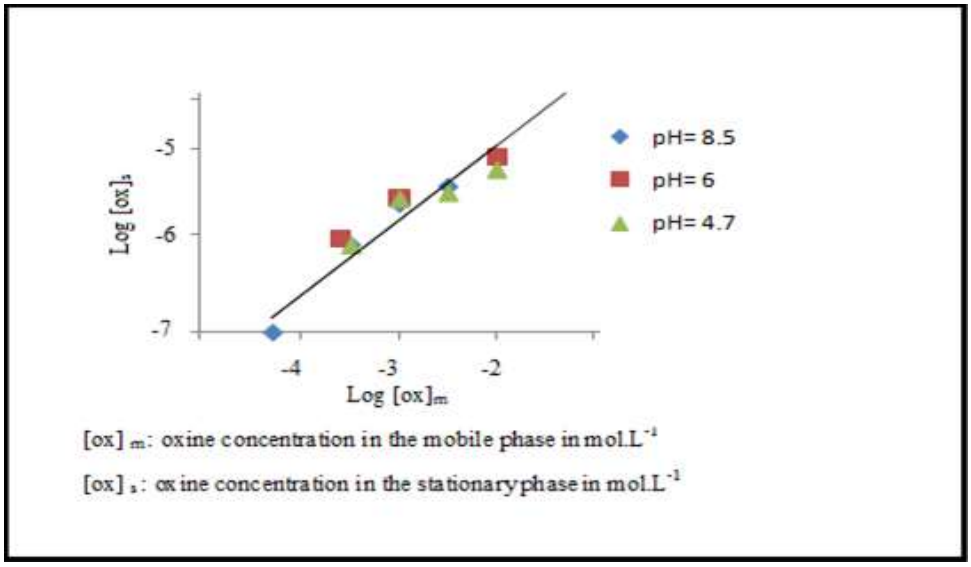



**Figure 2:** Distribution of oxine between mobile phase CH3OH - H2O (60-40 v / v) and stationary phase

For different values of pH between 8,5 and 4,7 of a straight slope close to 1 is obtained: thus; whatever the pH between 8,5 and 4,7 the distribution of oxine is based on a simple equilibrium of the type:

$$HOx_m \rightleftarrows HOx_s \quad (2)$$

Whose $K_{HOx}$ equilibrium constant is given by:

$$[HOx]_s = K_{HOx}.[Ox]_m \quad (3)$$

### 4.3 Retention of metal aluminum

We can represent different equilibrium involved by:

$HOx_m \rightleftarrows HOx_s$ ; equilibrium constant $K_{HOx}$ **(2)**

$Al^{3+}_m + 3\ HOx_m \rightleftarrows Al(Ox)_{3m} + 3\ H^+$ ; equilibrium constant $K_m$ **(4)**

$Al^{3+}_m + 3\ HOx_s \rightleftarrows Al(Ox)_{3s} + 3\ H^+$ ; equilibrium constant $K_s$ **(5)**

The capacity factor K 'of a solute is given by:

$$K' = cte.\ [Al]_s / [Al]_m = cte.\ [Al(Ox)_3]_s / [Al^{3+}]_m + [Al(Ox)_3]_m$$

Or by developing:

$$K' = cte.\ K_s/K_m\ .\ (K_{HOx})^3.1 / 1 + [H^+]^3 / Km.\ [HOx]^3_m \quad (6)$$

### 4.4 Influence of chromatographic parameters on retention

Figure 3 illustrates the variation of the capacity factor **K '**of aluminum, for a concentration of oxine in the mobile phase equal to $5.10^{-3}$M and a pH of 8.5 as a function of the composition of water-methanol phase mobile. It is noted that the retention of aluminum decreases with the increase of the composition of methanol in the mobile phase.

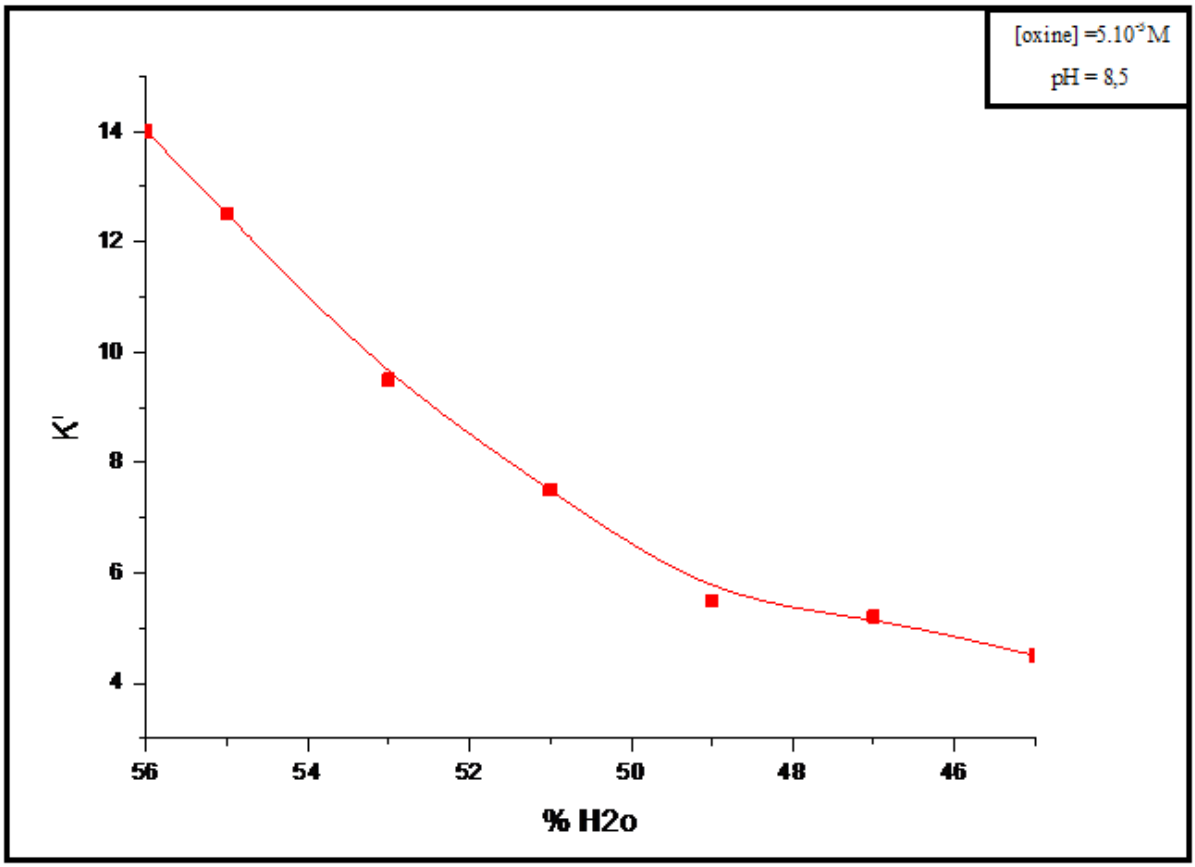



**Figure 3:** Values K 'in function of the composition of the mobile phase

This variation is logic in such technical chromatography with reverse phase. The factors that will govern the retention of solutes, further composition of the water-methanol of the mobile phase, are pH and the concentration of oxine in the mobile phase. Figure 4 illustrates the variation of K 'obtained for $Al^{3+}$ as function of pH for an oxine concentration equal to $5.10^{-3}$M.

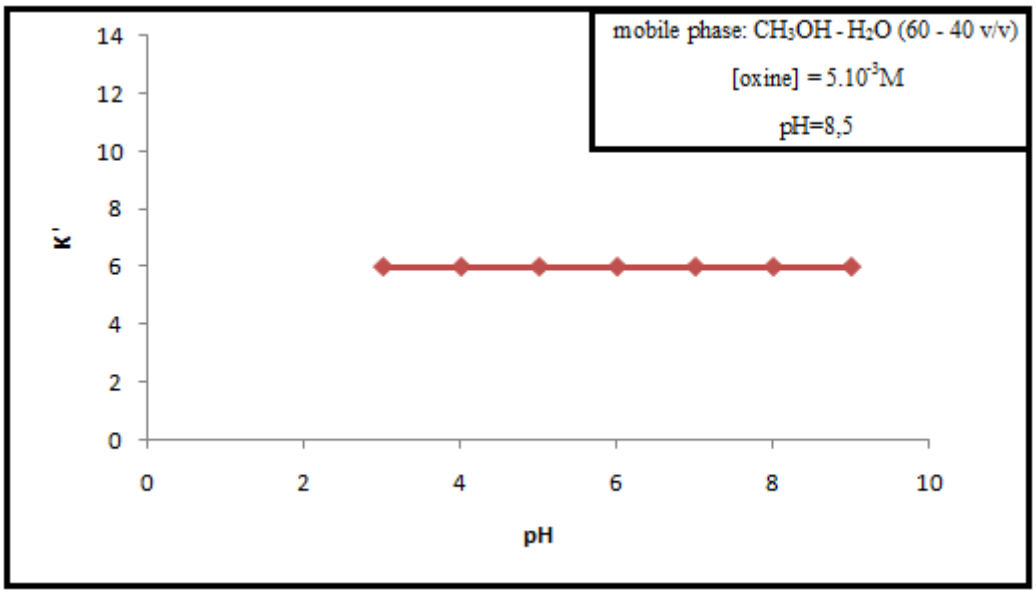



**Figure 4:** Variation of K 'as a function of pH

Similarly, Figure 5 illustrates the variation of K 'obtained for $Al^{3+}$ as function of pH for various concentrations of oxine between $2.10^{-3}$ and $10^{-4}$M.

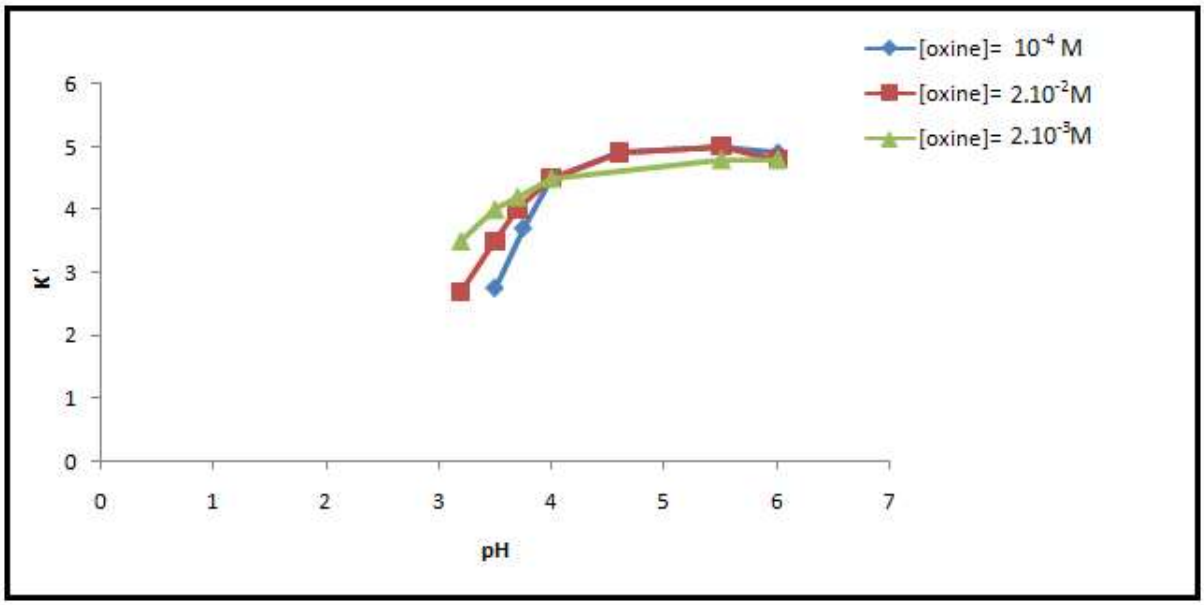



**Figure 5:** Variation of K 'as a function of pH for various concentrations of oxine

The examination of these two curves shows that under the experimental conditions we have set us (HOx is largely dominant in the chromatographic system), retentions are little affected by changes in pH and the concentration of oxine in mobile phase. Under these conditions, equation (6) can be simplified as follows:

$$K' = \text{cte.}\ K_s/K_m\ .\ (K_{HOx})^3$$

That the term $[H+]^3 / Km.\ [HOx]^3_m$ remains well below 1.

### 4.5 Detection of aluminum

Aluminum as a metal chelate can be detected by UV absorptiometry a $\lambda_{max}$ = 370 nm. However, the responses obtained by such detection will be based on the concentration of metal chelate in the mobile phase (equilibrium (4)), therefore chromatographic parameters (pH and concentration of the mobile phase in oxine). Figure 6 illustrates the detection (by UV absorptiometry at 370 nm) of $Al^{3+}$, to a methanol-water mobile phase (60:40 v / v) for a concentration of $5.10^{-3}$M of oxine.

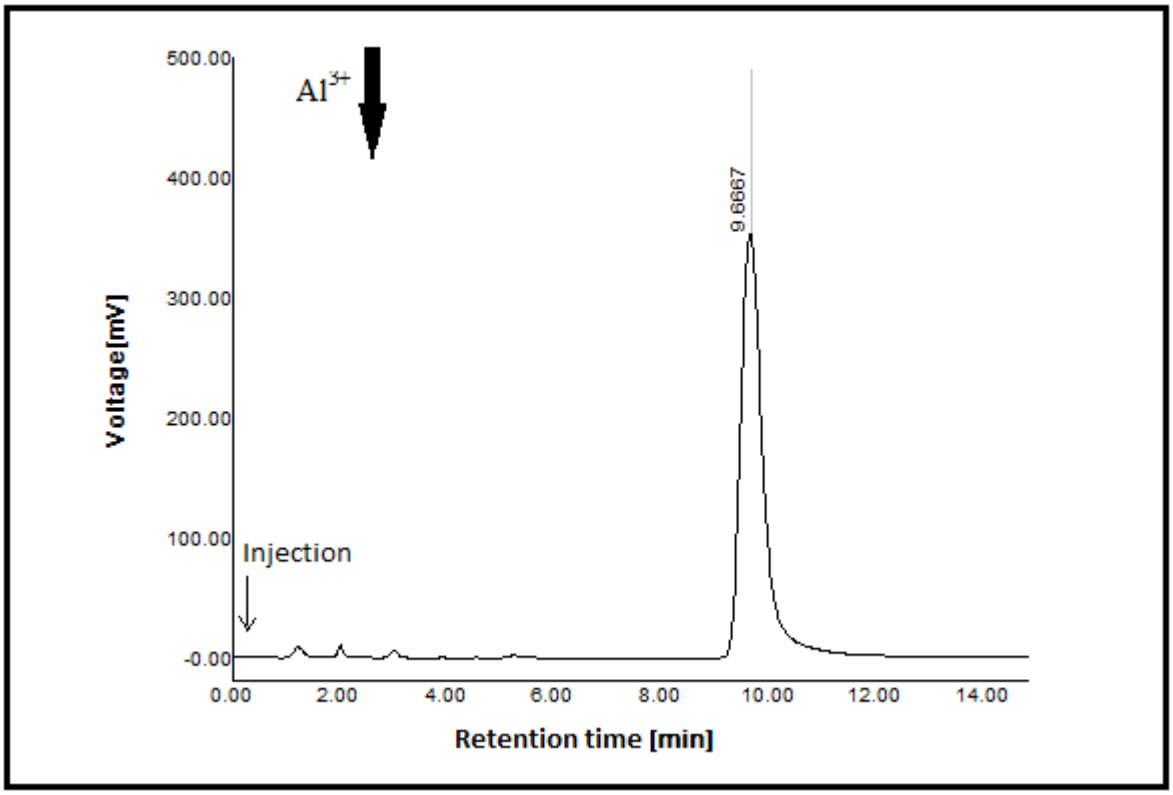



**Figure 6:** Chromatogram of a hemodialysis water enriched by 100 µg.L-1 of aluminum corresponding to 2ng solute for a 20 µL injection
(Column: Lichrorb RP8, Length: 15cm, Mobile phase: CH3OH - H2O (60-40 v / v), [oxine] = 5.10-3M, pH=8.5, Debit: 1cm.min-1, Detection: UV 365)

### 4.6 Method Validation

- **Calibration curve, linearity of the detector response**
  For a water-methanol mobile phase (40-60 v / v) buffered at pH = 8,5 and a concentration [oxine] = $5.10^{-3}$M, at a flow rate of $1cm^3.min^{-1}$ and column length equal to 15cm, we built a dose-response curve with increasing concentrations of aluminum ranging from 0 to 1 and $100\mu g.L^{-1}$. We deduced that the linear range of the calibration curve was widely extended (from 0 to $100\mu g.L^{-1}$ of $Al^{3+}$) with a linear regression coefficient equal to 0.9998. It shows that, in the chosen concentration range, the response is proportional to the amount of aluminum present.
- **Minimum Quantity Detectable and Limit of Detection**
  The minimum detectable level (limit) is the concentration of $Al^{3+}$ corresponding to an injection of the peak signal (peak area) is three times the background noise (S / B = 3). The minimum detectable quantity is 0.3 ng for a 20µL injection volume, which, reduced to the original sample, provides for minimum detectable concentration (limit): $15\mu g.L^{-1}$. These values depend on the mass spectrometer, the multiplier's voltage, and the chromatographic conditions, as well as the column used, etc.
- **Precision and Accuracy**
  It was calculated on samples of hemodialysis water artificially enriched with two concentrations, 30 $\mu g.L^{-1}$ and 75 $\mu g.L^{-1}$ (which corresponds to two and five times the detection's limit). The results (Table I) illustrate the accuracy of the technique for enriched solutions.

**Table 1:** Relative standard deviation as a function of concentration

| *Concentration ($\mu g.L^{-1}$)* | *Relative standard deviation (%)* |
|---|---|
| 30 | 4,3 |
| 75 | 3,2 |

## 5. Analytical Application

### 5.1 Determination of aluminum in hemodialysis water

Samples of hemodialysis water of standard concentration of aluminum are not commercially available; the study of the limits of validity of the method was carried out using hemodialysis water from an installation operated by ion exchange. The accuracy of the method was evaluated to different concentrations of aluminum in the enriched hemodialysis water using standard solutions of aluminum. The corresponding results are shown in Table II.

**Table 2:** Evaluation of the recovery rate of aluminum additions in a hemodialysis water

| *[Al] added ($\mu g.L^{-1}$)* | *[Al] found($\mu g.L^{-1}$)* | *Recovery rate (%)* |
|---|---|---|
| 30 | 28,4 | 94,6 |
| 60 | 57,6 | 96 |
| 90 | 88,7 | 98,5 |

Through hemodialysis water enriched with 30µg of aluminum per liter (maximum permissible level according to the O.M.S) we studied the reproducibility of the content of the technique used. The results of assays performed on this sample for ten tests are shown in Table III.

**Table 3:** Repeatable tests of the determination of aluminum in a hemodialysis water enriched to 30 µg of aluminum per litre

| *Number of measurements* | *Average [Al] (µg.L$^{-1}$)* | *S= Standard deviation (%)* |
|---|---|---|
| 10 | 29,06 | 4,8 |

In our case the number of degrees of freedom is N = n-1 = 10-1 = 9. Can set confidence limits using the formula: $\mathbf{x - t_s < xi < x + t_s}$. Where x is the average value of [Al]; t being pulled tables Fischer [24] according to the number of measurements n = 10, which were used to determine x and s.

For n = 10 was 95% for the probability, t = 2, 23.
Therefore there's a 95% chance that any new measure comes to lie in the interval (29.06 ± 2.23 x 0.38) = (29.06 ± 0.86) µg.L$^{-1}$.

## 6. Conclusion

It is therefore possible using such a system to ensure quality control "aluminum" for hemodialysis water for the proper functioning of a hospital service. Continuous monitoring of the content of this element in these waters will highlight the accidental administration of this toxic to hemodialysis always possible due to incidents at an installation of water treatment. By choosing a chromatographic system to reverse phase, the direct injection of aluminum without derivation as a chelate allows us to put ourselves in the best possible conditions for its detection. It seems possible that this technique may be used to expand the analysis of metals difficult to detect or requiring the development of post-amplification reactions suitable HPLC column thus opening the possibility of non-limiting detection.

## 7. Acknowledgement

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