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APPLICATION NOTES

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The determination of aluminium in plasma and dialysate solution using the GBC graphite furnace system

GBC

Introduction

In patients with chronic renal failure, on treatment with haemodialysis, clinical symptoms may develop which are associated with abnormally high levels of aluminium in the blood and tissues. Such patients are exposed to aluminium via the dialysate fluid, from dietary sources, and from the ingestion of aluminium-containing antacids given to reduce phosphate absorption in the gut.

Three syndromes are at present believed to be associated with aluminium toxicity in patients on haemodialysis. The first of these, termed 'dialysis encephalopathy' or 'dialysis dementia' is a progressive, fatal neurological disease first described in 1972,[1] and since reported in many dialysis centres throughout the world. Patients dying with this condition show elevated levels of aluminium in many tissues, including brain grey matter. The brain levels of aluminium are significantly higher than those seen in dialysed patients dying from other causes.[2] The second disorder is a progressive bone disease (dialysis osteodystrophy) distinct from other forms of renal bone disease and which has been shown to occur in regions where there are high concentrations of aluminium in the water supply used to prepare the dialysate.[3] Finally, there is evidence to suggest that aluminium toxicity is responsible for the development of a reversible microcytic anaemia in dialysis patients.[4]

The susceptibility of patients with chronic renal disease to aluminium toxicity has meant that a means to monitor aluminium in the body was required. Serum and plasma are now established as the most suitable body fluids for monitoring aluminium levels in these patients.

The measurement of aluminium in dialysate solution allows the clinician to monitor a patient's level of exposure to this metal. This is particularly important in regions where the quality of water may vary, even from day to day. Although the use of reverse osmosis deionized water treatment to remove aluminium has been able to prevent exposure, contamination of the dialysate concentrate may still occur due to the manufacturing process.[5] Concentrations of aluminium of 1.0 µmol/L (27 µg/L) or less in dialysate solution have been shown to reduce the incidence of fractures,[6] a result that was in agreement with an earlier epidemiological study.[7]

In this study, methods to determine aluminium concentration in plasma and dialysate solution were developed.

Experimental

Instrumentation

A GBC atomic absorption spectrophotometer and GBC automated graphite furnace system were used. The GBC graphite furnace system comprises the graphite furnace power supply (GF) and programmable automatic sample loader (PAL). GBC SavantAA software was used to develop a furnace method, and to collect and store data. Real-time colour graphics of absorbance peaks allowed the operator to optimise furnace conditions. The instrumental conditions for the determination of aluminium in plasma and dialysate solution are given in Figure 1. The purge gas used was argon, as nitrogen produces a loss of sensitivity.[5] The flow rate was 4.2 L/min.

Reagents

A stock solution of 1000 µg Al/mL (aluminium nitrate, standard solution for atomic spectroscopy) was used to prepare standards in a plasma pool supplied by the Red Cross Blood Bank, Melbourne, Australia, and in dialysate solution pool. Dialysate concentrate, commercial name "Renalyte" was diluted (1 + 34) in reverse osmosis deionized water. This dialysate solution pool contained the following constituents: sodium, 136 mmol/L; calcium, 1.55 mmol/L; potassium, 1.54 mmol/L; magnesium, 0.85 mmol/L; chloride, 102.3 mmol/L; acetate, 40 mmol/L; and dextrose, 2 g/L. The manufacturer stated that this solution should contain less than 10 µg Al/L (0.37 µmol/L). AristaR nitric acid was used. Ethylenediaminetetraaceticacid disodium salt (EDTA) and Triton X-100 were also used.

Deionized water for washing and rinsing was obtained from a mixed-bed deionizing unit. Deionized water used for reagents and analysis was from a reverse osmosis, mixed-bed deionizing unit that supplies Type 1 ultrapure water.

Contamination control

All volumetric equipment including pipette tips and sample cups were soaked in 2% m/V EDTA for a minimum of 8 hours and rinsed in copious quantities of deionized water. These items were then dried and used for sample and standard preparation. Sample cups were made of polyethylene. The matrix modifier for the method was 1% m/V Triton X-100 which was stored in a cleaned plastic bottle. This solution contained less than 0.1 µmol Al/L. Deionized water and reverse osmosis deionized water both contained less than 0.1 µmol Al/L and were suitable for use in the method. Vessels that are open to the atmosphere during standard preparation should be covered to avoid contamination from aluminium in room dust.[5]

Graphite furnace system applications report

Pyrolytically coated tube.
Wall atomisation.
1% Triton modifier.

Element	Al
Matrix	Plasma
Instrument Mode	Absorbance BC on
Beam Mode	Double Beam
Measurement Mode	Peak Area
Wavelength (nm)	309.3
Slit Width (nm)	1.0
Slit Height	Reduced
Lamp 1 Current (mA)	0.0
Lamp 2 Current (mA)	10.0
Integration Time (sec)	0.1
No. of Replicates	2
Calibration Mode	Concentration
Recal. Std No.	2

Calibration Table		
	Standard or Added Concentration	**Mean Standard Absorbance**
Blank/Sample	0.00	0.000
Std 1	1.84	0.148
Std 2	3.67	0.262
Std 3	5.48	0.362

Concentration Units	µmol/L

Graphite Furnace Parameters					
Step No.	**Final Temp ˚C**	**Ramp Time (sec)**	**Hold Time (sec)**	**Gas Type**	**Read On**
1	80	5.0	5.0	Aux.	No
2	140	25.0	10.0	Aux.	No
3	1500	20.0	10.0	Aux.	No
4	1500	1.0	1.0	None	No
5	2700	1.0	4.0	None	Yes

Sampling Mode	Auto Sampling
Standards Preparation	Pre-mix

Pre-mix Sampler Volumes	
Sample Volume (µL)	10
Modifier Volume (µL)	10
No. of Injections	1
Inject before Step No.	1
Recalibration Rate	30

Figure 1: Operating parameters for aluminium in dialysate solution

Preparation of calibration standards

Aluminium stock standard 1000 µg Al/mL (37.06 µmol Al/mL) was diluted to make up the intermediate standard by taking 10 mL and making up to a litre with deionized water. Volumes of 50, 100 and 150 µL of this intermediate standard were added to 10 mL aliquots of the plasma pool and the dialysate solution pool. The plasma standards and the dialysate standards each had concentrations of 1.84, 3.67 and 5.48 µmol Al/L respectively (Figure 1). An aliquot of each pool was used as the blank for the particular calibration curve and was placed in the blank position on the PAL. Either of the pool samples should contain less than 0.2 µmol Al/L. The computer subtracts the absorbance of the particular blank from all standards and then plots the standard curve.

Preparation of samples

Patient samples should be collected using a standard venepuncture technique with particular reference to preventing aluminium contamination of the blood sample from the needle or the plastic syringe. A glass syringe or a glass sampler tube should not be used for blood collection. Collected whole blood should be dispensed into a pre-cleaned polyethylene or polypropylene tube that has a suitable quantity of heparin to prevent coagulation. The added heparin should not elevate the aluminium concentration of the sample to a detectable level. After centrifugation, the plasma sample should be decanted into a second clean tube for storage. Refrigerate at 4°C if samples will be analysed in a few days. However for longer periods of storage samples should be frozen at -20°C. Plasma samples for the analysis consisted of Seronorm Trace Elements human reference serum batch no. 105 (Nycomed AS Diagnostics, Oslo, Norway) and samples provided by the Department of Clinical Chemistry, Northern General Hospital, Sheffield, England. Plasma samples were measured directly using the autosampler to dispense 10 µL into the graphite tube followed by 10 µL of Triton X-100 matrix modifier.

Dialysate solution samples should be collected into washed containers that have concentrated nitric acid added to give a final concentration of 1% V/V to preserve the sample. Refrigerate the samples at 4°C if they will be analysed in a few days. However for longer periods of storage samples should be frozen at -20°C. Four dialysate samples, three of which had been spiked with known concentrations of aluminium, were obtained from the Department of Clinical Chemistry, Northern General Hospital, Sheffield, England. Dialysate solution samples were measured directly. The autosampler dispensed 10 µL into the graphite tube followed by 10 µL of Triton X-100 matrix modifier.

Instrument calibration and operation

The instrument was set to calibration mode and allowed to run. Once the calibration was complete the instrument was stopped to examine the calibration graph (Figure 2). At the instrument wavelength of 309.3 nm the calibration graph is curved since there are actually two lines at 309.27 and 309.28 nm with a predicted relative absorption of 10:1 respectively.[8] The addition of aluminium to a pool blank is a form of standard additions and the operator must be aware that due to this curvature errors will occur in the calibration if the endogenous aluminium concentration of the pool blank is not at the recommended low concentration.

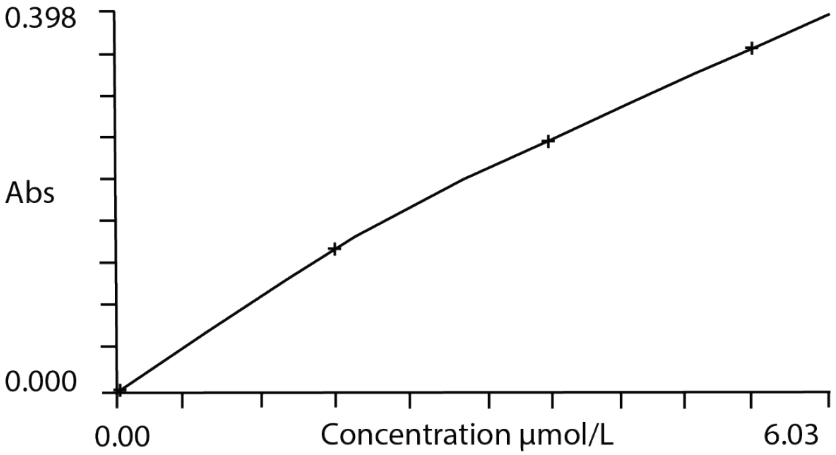

Figure 2: Calibration curve for aluminium in plasma

If the calibration is suitable the analysis should be recommenced and the samples analysed without a calibration routine being performed. The software allows the analyst to quickly select "run application" and "sample selection" so that the run will start with the samples only. This procedure will prevent the absorbance of the blank being subtracted from the samples to give falsely lower results. The rinse bottle for the PAL was also soaked in 2% m/V EDTA. The rinse solution contained 0.1% V/V nitric acid in 0.1% V/V Triton X-100. This solution provided adequate cleaning of the tip and prevented sample carryover.

Results and discussion

The furnace program listed in Figure 1 allowed complete drying of the plasma sample and modifier, and dialysate solution and modifier. The ashing temperature was chosen as 1500°C because high sensitivity was achieved at this temperature. The organic components of plasma were ashed at approximately 700°C and the inorganic salts in plasma and dialysate solution were distilled off at approximately 1000°C. The atomise temperature was chosen as 2700°C, which is in agreement with most other methods.

Sample	Al Added	Result	% Recovery
Pool	0	0.17	–
1	1.11	1.16	90.5
2	2.22	2.35	98.1
3	3.33	3.37	96.1

Table 1: Concentrations (µmol/L) and percent recovery of aluminium added to dialysate solution

Background correction mode was used in the plasma analysis, because biological samples have two possible sources of non-specific absorption, namely organic and inorganic matrix components. Although results for background-only mode showed negligible absorbance for the plasma standards or Seronorm, it is important to understand that considerable variation may occur in the composition of plasma samples from these patients. Also, for measurements at low concentration, reference range determination, or concentrations close to the detection limit, a falsely increased result may occur if background absorbance contributes significantly to the total absorbance.[5] Seronorm has a recommended value of 2.6 µmol Al/L. In this study the result obtained using peak height mode was 2.24 µmol Al/L compared to a value of 2.70 µmol Al/L for peak area mode (Figure 3). For both modes of data acquisition the precision obtained was very good. Consequently peak area was chosen as the measurement mode. The results for Seronorm indicate that the method is accurate in determining plasma aluminium. The method gave a characteristic mass of 14.8 pg Al for a 10 µL plasma sample.

Blank	0.016	0.013
0.00 µmol/L	RSD = 13.47%	Mean Abs. = 0.015
Standard 1	0.149	0.148
1.84 µmol/L	RSD = 0.86%	Mean Abs. = 0.148
Standard 2	0.262	0.262
3.67 µmol/L	RSD = 0.11%	Mean Abs. = 0.262
Standard 3	0.362	0.363
5.48 µmol/L	RSD = 0.16%	Mean Abs. = 0.362
SN 105	0.210	0.199
2.70 µmol/L	RSD = 3.90%	Mean Abs. = 0.205
510 <48>	0.167	0.160
2.07 µmol/L	RSD = 2.81%	Mean Abs. = 0.164
511 <90>	0.264	0.265
3.70 µmol/L	RSD = 0.08%	Mean Abs. = 0.264
512 <140>	0.378	0.366
5.66 µmol/L	RSD = 2.39%	Mean Abs. = 0.372

Figure 3: Standards and results for aluminium in plasma

In clinics where reverse osmosis water purification systems are connected to haemodialysis machines the aluminium concentration in dialysate should be less than 10 µg/L (0.37 µmol/L).[9] Therefore, at low aluminium concentration a falsely increased result may occur if the background absorbance of a dialysate solution contributes significantly to the total absorbance.[5] Hence background correction was

used for the analysis of dialysate solution. In the dialysate method the precision obtained was also very good. Peak area was chosen as the measurement mode. Percent recoveries for dialysate samples (Table 1) indicate that the method is accurate in determining dialysate solution aluminium concentration. The method gave a characteristic mass of 15.2 pg Al for a 10 µL dialysate solution sample.

Conclusion

These methods have been developed to allow simple and rapid analysis of aluminium in plasma and dialysate solution. If the analytical chemist takes due care to prevent contamination of reagents and samples both methods will be accurate and sensitive. The GBC atomic absorption spectrophotometer with GBC graphite furnace system allowed an automated analysis to be developed for methods that have been difficult to perform.

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