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Analysis of aluminium in pharmaceutical products by post-column derivatization ion chromatography

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Abstract

Aluminium can be determined in pharmaceutical products using a chromatographic method with a cation-exchange separation and fluorescence detection after post-column derivatization with 8-hydroxyquinoline-5-sulphonic acid. The method had a detection limit of less than 0.5 ng/ml and the aluminium peak response was linear up to at least 1200 ng/ml. Chromatographic reproducibility was approximately 1–3% over the concentration range of 50–1200 ng/ml. Sample preparation involved boiling in 0.05 *M* sulphuric acid for 5 min. The average recovery for Al spikes in the range 10–1200 ng/ml, from a variety of pharmaceutical samples, was 99% with an R.S.D. of 5%. No interferences from either sample matrix effects or transition metals were found and the results of the chromatographic method agreed well with those obtained by inductively coupled plasma atomic emission spectroscopy.

1. Introduction

Chronic aluminium toxicity in animals and humans is well documented [1,2]. The clinical biochemistry of aluminium is mostly associated with its neurotoxicity (epilepsy, encephalopathy and Alzheimers disease) and renal failure in patients undergoing regular dialysis due to aluminium accumulation in kidneys [1]. Its toxicity in aquatic and soil systems has been shown to be dependent on its chemical speciation, while its mobility from soils into lakes and rivers is enhanced under conditions of increased acidity [3–5]. Hence, there is a continuing interest in the determination of trace levels of aluminium, and its various chemical species, in pharmaceutical products, soils and environmental samples.

Current analytical methods for the trace determination of aluminium in such samples mostly involves the use of spectroscopic techniques, such as graphite furnace atomic absorption spectroscopy (GFAAS) [6]. Such techniques suffer from a number of drawbacks, including high equipment costs, matrix interference effects and the ability to determine only total aluminium. Consequently, increasing attention has been focussed on the use of ion chromatography (IC) for the analysis of aluminium in a variety of sample matrices [7–12]. A number of reports have recently described the use of 8-hydroxyquinoline-5-sulphonic acid (8-HQS) as a post-column reagent for the fluorimetric detection of aluminium following chromatographic separation [7–10]. The use of micelle-forming surfactants in the post-column reagent, such as cetyltrimethylammonium bromide (CTAB), has been reported [9], whilst optimisation of the post-column reaction conditions has also been described [7]. The aim of this work was to investigate the applicability of IC for the quantitative determi-

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nation of aluminium in injectable pharmaceutical preparations. As a concentration of 10 ng/ml was a regulatory requirement for the maximum allowable Al limit in water to be used for the preparation of such products, a principal objective of the chromatographic method was to be able to quantitate below this value and to achieve a detection limit in the order of 1 ng/ml Al.

2. Experimental

2.1. Instrumentation

The liquid chromatograph consisted of a Waters Chromatography Division of Millipore (Milford, MA, USA) Model 625 polyether ether ketone (PEEK) solvent-delivery system, 625 loop injector, 470 programmable fluorescence detector (excitation 395 nm, emission 500 nm) and a Millennium chromatography data manager. The post-column reagent was delivered with a Waters pneumatic reagent-delivery module (RDM) and mixed with the column effluent in a 1.0-ml knitted PTFE ninhydrin reaction coil. The column used was a Waters Protein-Pak SP-5PW (75 × 7.5 mm I.D.) cation-exchanger. Eluent and RDM flow-rates were both 1.0 ml/min and a volume of 50 μl was used for all injections.

2.2. Reagents

Water purified (18 MΩ) using a Millipore Milli-Q water-purification system (Bedford, MA, USA) was used for all solutions. The mobile phase consisted of 0.1 *M* potassium sulphate (Ajax, Sydney, Australia) adjusted to pH 3.0 with 5 *M* sulphuric acid. The post-column reagent (PCR) solution contained 0.004 *M* 8-HQS and 0.002 *M* CTAB in a 1.0 *M* sodium acetate–acetic acid buffer at pH 4.4. The PCR solution was prepared by dissolving 41.7 *g* sodium acetate trihydrate in Milli-Q water, adding 39.7 ml glacial acetic, 100 ml 0.04 *M* 8-HQS (Sigma, St. Louis, MO, USA) stock solution, 100 ml 0.02 *M* CTAB (Fluka, Buchs, Switzerland) stock solution and diluting the mixture to 1.0 l. A 1000-μg/ml stock aluminium solution in 0.1 *M* HNO_3 was prepared from $Al(NO_3)_3 \cdot 3H_2O$ (Ajax). A 10-μg/ml solution was prepared by dilution in 0.05 *M* sulphuric acid of the stock standard. All working standards, covering the concentration range of 2 to 1200 ng/ml, were freshly prepared daily from the 10-μg/ml solution by dilution in 0.05 *M* sulphuric acid. All eluents and post-column reagents were prepared daily, filtered and degassed with a Waters solvent-clarification kit.

2.3. Sample preparation procedures

All sample quantitation was performed using standards run at the same time as the unknowns. The general sample preparation procedure involved adding a sample aliquot (up to 5 ml) to an acid-washed 25-ml beaker, after which 100 μl of 5 *M* sulphuric acid were added and the solution boiled gently for 5 min. The solution was allowed to cool, quantitatively transferred to a 10-ml volumetric flask and the volume adjusted to the mark with Milli-Q water. Duplicate injections (50 μl) of these sample solutions were made. For samples such as 5% injectable glucose and injectable water, where dilution was undesirable, 100 μl 5 *M* sulphuric acid was added to 10 ml of sample in a 25-ml beaker and the solution boiled gently until the volume was reduced to approximately 7 ml. After cooling, this was quantitatively transferred to a 10-ml volumetric flask and the volume adjusted to the mark with Milli-Q water.

3. Results and discussion

3.1. Chromatographic method

This work describes the development of a procedure, based on that of Gibson and Willett [7], for the analysis of aluminium in pharmaceutical products, such as injectable glucose, saline solution and water. A higher-capacity cation-exchange column (Protein-Pak SP-5PW) was chosen for this work to allow for the direct injection of high ionic strength samples, such as

injectable saline. Mobile phase parameters, such as ionic strength and flow-rate, were initially optimised for the separation of aluminium on the Protein-Pak SP-5PW column. A 1.0-ml knitted PTFE reaction coil was added to the chromatographic system to allow sufficient time for the reaction of aluminium with the post-column reagent, which was delivered at an optimal flow-rate of 1.0 ml/min. A typical chromatogram of a 50-μl injection of a 2.0 ng/ml aluminium standard obtained using the Protein-Pak SP-5PW column and an eluent of 0.1 *M* potassium sulphate at pH 3.0 is shown in Fig. 1. The retention time of the Al peak was 6.1 min. The response was found to be linear over the range examined (2 to 1200 ng/ml) with a correlation coefficient >0.999. The chromatographic detection limit was calculated (at 3× baseline noise) to be less than 0.5 ng/ml for a 50-μl injection. Typical chromatographic reproducibilities for 50 and 100 ng/ml standards were 2–3% and <1%, respectively.

3.2. Sample preparation and interferences

A number of potential problems could arise during the quantitation of aluminium by IC. These include the presence of other aluminium complexes in solution which could interfere with the separation of the free Al^{3+} peak. The presence of other metal ions in the samples could interference either through the formation of fluorescent complexes that co-elute with the Al^{3+} peak or by quenching the aluminium peak fluorescence. Also, fluorescence quenching could occur as a result of co-elution with other components, such as glucose, active compounds and other matrix materials which are often present at high levels in pharmaceutical samples.

The problem of multiple Al complexes and their dissociation to form free Al has generally been addressed by preparing the sample in acidic conditions [7–9]. Figs. 2(1), and 3(1) show chromatograms obtained from the direct injection of saline and glucose solutions. Apart from the Al peak, two additional peaks (retention times 3.6 and 4.3 min) were detected in the saline and glucose samples. These additional peaks could be due to the elution of various Al complexes, to other metals, or both. Figs. 2(2) and 3(2) show the corresponding chromatograms obtained after the samples had been boiled in 0.05 *M* sulphuric acid, as detailed in the Experimental section. In both cases, there was a large increase in the size of the Al peak (and an increase in the total peak area) after sample treatment. In the case of the glucose sample, the two minor peaks almost disappeared, whilst in the case of the saline solution, the peak at retention time 4.3 min was still significant but markedly diminished.

These results indicated that Al was present in the samples as a number of complexed species, which could be broken down using acid treatment. The effect of increasing total peak area after sample acidification has also been reported

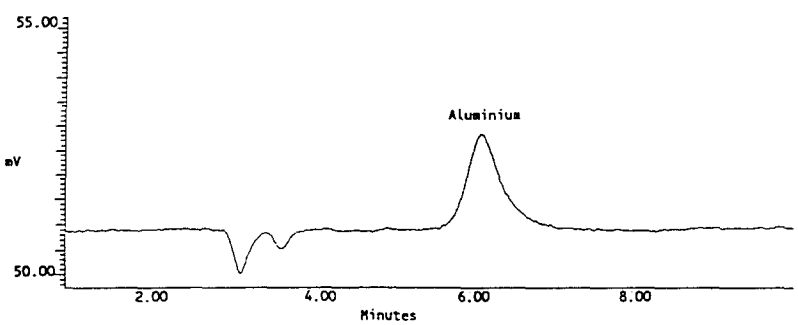

Fig. 1. Chromatogram of aluminium standard. Conditions: column: Waters Protein-Pak SP-5PW cation-exchanger; eluent: 0.1 *M* potassium sulphate at pH 3.0; flow-rate: 1.0 ml/min; injection volume: 50 μl; post-column reagent: 0.004 *M* 8-hydroxyquinoline-5-sulphonic acid, 0.002 *M* cetyltrimethylammonium bromide in 1.0 *M* sodium acetate–acetic acid buffer at pH 4.4, delivered at 1.0 ml/min; detection: fluorescence, excitation 395 nm, emission 500 nm. Solute: aluminium (2.0 ng/ml).

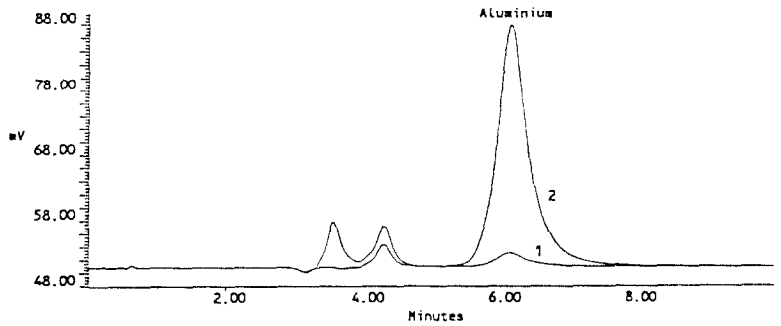

Fig. 2. Chromatogram of 0.9% NaCl saline solution obtained using (1) no acid treatment and (2) after boiling in 0.05 *M* sulphuric acid. Conditions as for Fig. 1 except sample dilution 10×. Solutes (concentration): 1 = aluminium (2.6 ng/ml); 2 = aluminium (30.6 ng/ml).

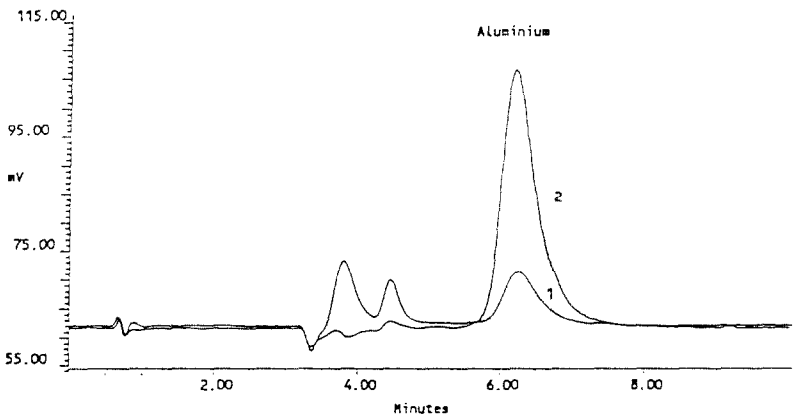

Fig. 3. Chromatogram of 50% glucose solution obtained using (1) no acid treatment and (2) after boiling in 0.05 *M* sulphuric acid. Conditions as for Fig. 1 except sample dilution 10×. Solutes (concentration); 1 = aluminium (14.5 ng/ml); 2 = aluminium (103.5 ng/ml).

previously and was shown to depend upon the specific nature of the Al complex [7]. Species, such as oxalate and fluoride, were shown to have no effect on the fluorescence response, indicating that Al complexes of these ions were fully decoupled by the 8-HQS. Alternatively, citrate reduced the fluorescence response, suggesting that these complexes were sufficiently stable to resist decomplexation and substitution by the 8-HQS [7]. In the case of both the glucose and saline samples, the significant increase in total peak area after acidification suggests that the complexing ions present in the samples must form relatively stable complexes with aluminium.

The effects of acid strength on the Al peak response were investigated for a representative matrix, a 50% glucose sample. The results of varying the sulphuric acid concentration from 0–0.05 *M* are summarised in Table 1. Increasing

Table 1
Effect of acid strength on Al quantitation for a 50% glucose sample

H_2SO_4 concentration (*M*)	Al found (ng/ml)
No acid	145
0.005	660
0.02	840
0.05	1035

Table 2
Al results obtained for duplicate sample injections

Sample	Al (ng/ml)	
	Inject 1	Inject 2
50% Glucose	1181	1201
5% Glucose	54.2	55.6
0.9% NaCl	305.8	303.6
Tap water	7.2	6.4
Production process water	1.6	1.3

the concentration beyond 0.05 *M* did not result in significantly higher Al peak responses and this acid concentration was used for all subsequent analyses. Other acids, such as hydrochloric and nitric acids, gave similar results to those obtained using sulphuric acid, however, the latter was chosen as it was more compatible with the mobile phase.

As previously discussed, the presence of other metals in the samples could potentially lead to chromatographic interferences or the possibility of Al fluorescence quenching. The injection of standard solutions (100 μg/ml) of cations which could potentially occur in pharmaceutical preparations, such as Ca^{2+}, Mg^{2+}, Zn^{2+}, Cd^{2+}, Fe^{3+} and Ni^{2+}, showed that only Zn^{2+} and Cd^{2+} gave a significant detection response. These cations, with retention times of 5.0 min for Zn^{2+} and 4.9 min for Cd^{2+}, however, were well resolved from the Al peak. Potential interferences due to matrix effects were investigated by analysing a 50% glucose sample at dilutions of 10, 20 and 40×. The Al results obtained of 1190, 1170 and 1140 ng/ml, respectively, indicated that there were no significant matrix effects.

3.3. Quantitation

The A1 results and typical reproducibility obtained for duplicate injections of various pharmaceutical samples are shown in Table 2. The duplicate results showed good agreement at levels higher than 50 ng/ml, however, at levels of <10 ng/ml, the repeatability was not as good due to the fact that the Al peak was being quantitated closer to the detection limit. The accuracy of the chromatographic method was assessed by determining the recoveries of Al from standard addition spikes, with the results being shown in Table 3. The recoveries of spikes in the concentration range of 10 to 1200 ng/ml varied from 94 to 106%. The overall average recovery was 99%, which was excellent considering the potential problems of working these at trace levels. The results obtained by IC were also compared to those from inductively coupled plasma atomic emission spectroscopy (ICP-AES), as shown in Table 4. The chromatographic results showed reasonable agreement to those

Table 3
Recovery of Al from spiked samples

Sample	Al present (ng/ml)	Spiked Al (ng/ml)	Al found (ng/ml)	Recovery (%)
50% Glucose	1134	1000	2074	94
5% Glucose (S1)[a]	66.6	50	114.1	96
5% Glucose (S2)[b]	77.7	50	124.5	94
0.9% NaCl (S1)[a]	334	100	437	104
0.9% NaCl (S2)[b]	305	200	516	106
Milli-Q water	ND[c]	10	9.74	97

[a] Sample 1.
[b] Sample 2.
[c] Not detected.

Table 4
Comparison of Al results obtained using IC and ICP-AES

Sample	IC (ng/ml)	ICP-AES (ng/ml)
50% Glucose	1035, 1060, 1090	1030
5% Glucose	67, 78, 54, 56	85
0.9% NaCl	334, 305	306
BP water[a]	1.4	<0.3

The IC results represent single injections from different sample ampoules.
[a] BP water = Sterile water produced according to British Pharmacopeia.

obtained for the same sample types when using ICP-AES.

4. Conclusions

The feasibility of using IC for the trace analysis of aluminium in pharmaceutical products, based on a cation-exchange separation and fluorescence detection after post-column derivatization with 8-HQS, was successfully demonstrated. Sample preparation simply involved boiling in 0.05 *M* sulphuric acid for 5 mins. The chromatographic method had a detection limit of less than 0.5 ng/ml for a 50-μl injection and the aluminium peak response was linear up to at least 1200 ng/ml. The chromatographic reproducibility was approximately 1–3% over the concentration range of 50–1200 ng/ml. The average recovery of Al spikes in the range 10–1200 ng/ml, from a variety of pharmaceutical samples, was 99% with an R.S.D. of 5%, indicating satisfactory method accuracy. No interferences from either sample matrix effects or transition metals were found and the results of the chromatographic method agree well with those obtained by ICP-AES.

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