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Speciation of low molecular weight Al complexes in serum of CAPD patients

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Abstract

Speciation of LMW–Al complexes was performed in human serum of six continuous ambulatory peritoneal dialysis (CAPD) patients in order to investigate the individual variability in the percentage and the composition of LMW–Al species. The total concentration of Al in serum ranged from 10 to 120 ng ml^{-1}. The samples with high total concentration of Al were analysed directly, while those of low total Al concentration were spiked with Al^{3+}. Spiked and non-spiked samples (100–120 ng ml^{-1} of total Al) were microultrafiltered through a membrane filter (cut-off 30 000 Da) to separate Al-transferrin from LMW–Al complexes. On an anion-exchange fast protein liquid chromatography (FPLC) column, 0.2 ml of filtrate was injected. An aqueous — 4 mol l^{-1} NH_4NO_3 linear gradient elution was applied for 10 min to separate LMW–Al complexes. Fractions of 0.2 ml collected throughout the chromatographic run were diluted 1:1 with water and Al determined 'off line' by electrothermal atomic absorption spectrometry (ETAAS). The characterisation of LMW–Al species eluted under the chromatographic peaks was performed also by electrospray tandem mass spectrometric (ES-MS-MS) analysis. It was found experimentally that the percentage of LMW–Al species in spiked and non-spiked serum ranged from 25 to 50% (in one non-spiked sample 100%). The following LMW–Al species were separated and identified during the chromatographic run: Al-phosphate and a mixture of Al-citrate and ternary Al-citrate–phosphate complexes. It was found experimentally that the distribution of these species varied among particular patients. Similar distribution of LMW–Al species was found in spiked serum of healthy volunteers.

Keywords: Al-citrate; Al-phosphate; Ternary Al-citrate–phosphate complex; Fast protein liquid chromatography; ETAAS; Electrospray ionisation MS-MS; Serum of CAPD patients

1. Introduction

Al overload in patients with chronic renal failure has been greatly reduced over the past decade, since the use of Al-based phosphate binding drugs has diminished and the quality of the water used

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for dialysis fluids has improved [1]. However, the absorption and accumulation of Al, particularly via the diet (including Al-based medications) [2], still remains a problem in nephrology [3]. Clinical and experimental studies have shown that the gastrointestinal absorption of Al is markedly increased by the presence of citrate [4,5] and that the administration of Al-citrate causes the enhancement of Al concentrations in tissues [6]. It has been proposed that Al is transported as Al-citrate by the monocarboxylic acid transporters (MCT) located at the blood–brain barrier [7]. These observations indicated the important role of low molecular weight (LMW) ligands, particularly citrate, in Al absorption as well as in Al distribution in the body. The body is a dynamic system that is far from chemical equilibrium. The ratio between the complexation reactions of Al with various ligands present in the body plays a critical role in the transportation and distribution of Al. Therefore, it is important to identify different Al complexes in human serum. It has been demonstrated that the dominant Al species in serum is Al-transferrin [8]. The remaining Al is bound to LMW ligands, but reports on the speciation of LMW–Al complexes in serum are rather controversial. The complexation reactions of Al with LMW ligands have been intensively investigated by potentiometry and ^{13}C, ^{1}H and ^{31}P NMR techniques [9,10], while the distribution of LMW–Al species has been calculated using computer simulation programs [11–13]. The LMW–Al species most frequently theoretically predicted to be present in serum are Al-citrate, Al-phosphate, Al-hydroxide and mixed ternary complexes of Al-citrate–phosphate, Al-phosphate–hydroxide and Al-citrate–hydroxide [11,13].

For quantitative determination of LMW–Al species in human serum, microultrafiltration has been used to fractionate high molecular weight (HMW) from LMW–Al complexes in serum. Reported data indicated that 8–20% of total Al in serum corresponded to ultrafiltrable LMW–Al species [14–16]. In order to separate LMW–Al species present in human serum, more powerful separation techniques have been applied. Gel filtration chromatography [17] was used for separation of Al species in serum of chronic-haemodialysis patients and it was demonstrated that beside Al-transferrin, two unidentified LMW fractions of Al were separated. Favarato et al. [18] employed size-exclusion chromatography (SEC) for separation of Al species present in the serum of normal and occupationally exposed subjects and characterised the Al-binding protein complex in one of 3–5 separated fractions which contained Al. A study of the speciation of Al by SEC and ETAAS detection [19] also indicated the presence of two LMW–Al species in the spiked haemofiltrate of uremic patients.

In the above-mentioned studies, the LMW–Al species were not completely characterised or quantified. In order to identify and quantify the LMW–Al complexes present in spiked serum, investigations by anion-exchange FPLC-ETAAS [16] and anion-exchange FPLC-ETAAS-electrospray MS-MS techniques [20] were carried out in our group. It was demonstrated that LMW–Al present in spiked human serum of eight healthy volunteers corresponded to Al-citrate, Al-phosphate and ternary Al-citrate–phosphate complexes [20]. Nevertheless, the LMW–Al species present in the serum of uremic patients has still not been quantified and identified. To better understand the mechanisms of Al transportation and accumulation in renal patients, in this study analyses of spiked and non-spiked serum samples of six CAPD patients were performed by FPLC-ETAAS and ES-MS-MS with the aim of determining the percentage and the composition of LMW–Al species.

2. Materials and methods

2.1. Instrumentation

A strong anion-exchange fast protein liquid chromatography (FPLC) column of Mono Q (Pharmacia, Uppsala, Sweden) was employed for the separation of negatively charged Al species. The column was connected to a Merck-Hitachi (Darmstadt, Germany) 6200 gradient high-pressure pump equipped with a Rheodyne (Cotati, CA, USA) Model 7161 injector (0.2 ml loop). Total Al in human serum as well as the concentra-

tion of Al in separated fractions was determined by electrothermal atomic absorption spectroscopy (ETAAS) at 309.3 nm on a Hitachi (Hitachi, Tokyo, Japan) Z-8270 polarized Zeeman atomic absorption spectrometer equipped with an autosampler. A Micromass (Micromass UK Ltd., Manchester, UK) Quatro LC tandem quadrupole mass spectrometer equipped with a Z spray ion source as the LC–MS interface, employing negative electrospray ionisation, was used for the identification of LMW ligands in separated Al species. A Heraeus (Osterode, Germany) Model 17S Sepatech biofuge was used in the microultrafiltration procedure.

2.2. Reagents

Merck suprapur acids and water doubly distilled in quartz were used for the preparation of samples and standard solutions. All other reagents were of analytical reagent grade.

A stock Al^{3+} solution (100 μg Al ml^{-1}) was prepared by dissolving $Al(NO_3)_3 \cdot 9H_2O$ (Riedel-de Haën) salt in water. A stock Al-citrate solution (100 μg Al ml^{-1}) was made by mixing citric acid (Merck) and $Al(NO_3)_3 \cdot 9H_2O$ in a 100:1 citric acid to Al molar ratio.

Imidazole ($C_3H_4N_2$, 0.2 mol l^{-1}) (Merck) buffer solution with the addition of an appropriate amount of hydrochloric acid (0.1 mol l^{-1}) was used to adjust the pH of synthetic samples to 7.4.

The 4 mol l^{-1} ammonium nitrate eluent was prepared by dissolving 320.16 g of NH_4NO_3 in 1 litre of water. Chelex 100 (Na^+ form, 100–200 mesh) chelating ion-exchange resin (Sigma) and a silica-based LiChrosorb RP-18 HPLC column (150 × 4.6 mm i.d.) were used for purification of the eluent [16]. Centricon 30 concentrators (Amicon, Germany) with a nominal cut-off of 30 000 Da were used in the ultramicrofiltration procedure of human serum.

2.3. Sample preparation

Synthetic working solutions of LMW–Al complexes were prepared daily in 50 ml Teflon volumetric flasks. A synthetic working solution of Al-citrate (100 ng Al ml^{-1}) was prepared by mixing 0.1 ml of stock Al-citrate solution in imidazole–HCl buffer with a pH of 7.4 (100:1 citrate to Al molar ratio). A synthetic working solution of Al-phosphate (100 ng Al ml^{-1}) was made by mixing 0.1 ml of stock Al^{3+} solution and 0.1 ml of 2 mol l^{-1} H_3PO_4 in an imidazole–HCl buffer solution (pH = 7.4, 1000:1 PO_4^{3-} to Al molar ratio). The Al to citrate and/or phosphate molar ratio was the same as in human serum [21].

Blood from CAPD patients was taken during the ambulatory examination after the informed consent was obtained. It was collected into Al-free Becton–Dickinson vacutainers without additives. Samples were centrifuged for 10 min at 855 g. Serum was transferred to a Teflon flask with a polyethylene pipette and analysed within 12 h. The total concentration of Al was first determined by ETAAS using the standard addition calibration method and 32% nitric acid as a matrix modifier [16,22]. In samples where the concentration of total Al exceeded 60 ng ml^{-1}, serum was immediately microultrafiltered. Otherwise, it was spiked with Al^{3+} (water solution of Al-nitrate), so that the final concentration of Al in the serum ranged from 100 to 120 ng ml^{-1}. Spiked serum was left to equilibrate for 4 h [20]. After that it was microultrafiltered (cut-off 30 000 Da) to separate HMW from LMW–Al species. Speciation of Al was performed in the serum filtrate of spiked and non-spiked samples by the recommended analytical procedure.

2.4. Recommended procedures

Sample preparation, chromatographic separations and determination of Al by ETAAS were carried out under clean-room conditions (class 10000). To avoid contamination by external Al, the Teflon ware, the NH_4NO_3 eluent, the FPLC columns and microultracentrifugation membranes of the Centricon 30 concentrators were purified by the cleaning procedure reported previously [16].

Negatively charged LMW–Al complexes were separated on a Mono Q strong anion-exchange FPLC column. Microultrafiltered serum sample

(0.2 ml) was injected onto the column and aqueous (0–100% 4 mol l^{-1} NH_4NO_3) linear gradient elution was applied for 10 min at a flow rate of 1 ml min^{-1}. Eluate was collected in 0.2 ml fractions and diluted to 0.4 ml with water in Eppendorf polyethylene cups. The concentration of Al in the eluate was determined 'off line' by ETAAS under the optimum measurement conditions described previously [16,20]. For the identification of LMW ligands eluted under the chromatographic peak, fractions were diluted 1:5 with water and 1:2 with acetonitrile and analysed by the ES-MS-MS technique employing a Z spray ion source. The MS measurement conditions were the same as described previously [20].

2.5. Accuracy test

In order to test the accuracy of the determination of total Al in the human serum, the Seronom™ Trace Elements serum standard reference material obtained from Nycomed Pharma AS was analysed as well. Good agreement between the reported certified value (67 ± 4 ng ml^{-1}) and the Al concentration determined (69 ± 1 ng ml^{-1}) was obtained.

3. Results

3.1. Speciation of synthetic working solutions of Al-citrate and Al-phosphate by FPLC-ETAAS and ES-MS-MS

The separation of synthetic working solutions of Al-citrate and Al-phosphate (100 ng ml^{-1} of total Al at pH 7.4) on the Mono Q FPLC column with ETAAS detection was examined and is presented in Fig. 1. The Fig. 1 shows that Al-citrate was quantitatively eluted from 2.6 to 3.4 min. (The data on the quantitative separation were reported in our previous work [16]). The soluble fraction of Al-phosphate ($21 \pm 4\%$) was eluted as negatively charged species from 1.8 to 2.8 min. The remaining 79% of Al-phosphate was strongly adsorbed on the resin column and did not disturb further separations.

In order to identify LMW–Al ligands, the ES-MS-MS technique using a Z spray ion source was applied [20]. The MS analysis was performed by scanning negative ions. The mass spectra and the corresponding MS-MS spectra of Al-citrate and Al-phosphate synthetic working solutions are presented in Fig. 2. In the mass spectrum of Al-citrate (Fig. 2A) a peak with m/z 191 which corresponds to deprotonated citric acid was

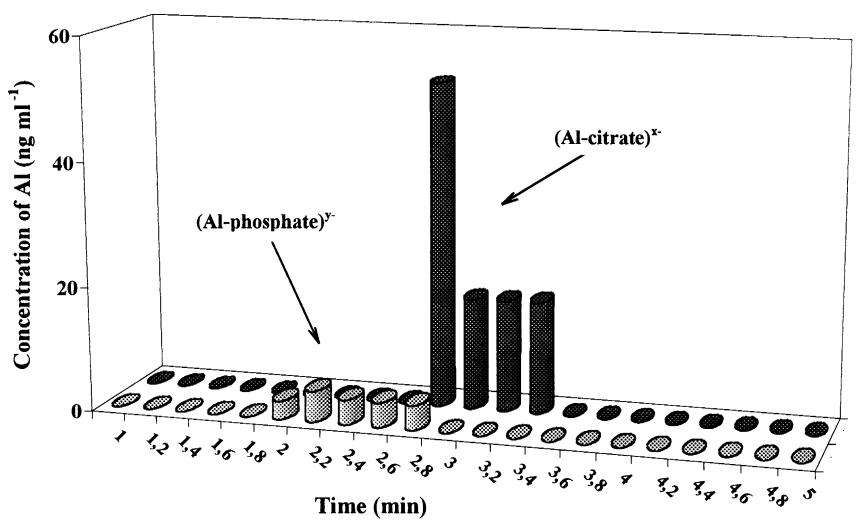

Fig. 1. Typical chromatograms of Al-citrate and Al-phosphate (100 ng ml^{-1} Al) at pH 7.4. Separation was performed on a Mono Q HR 5/5 anion-exchange FPLC column and separated species were detected by ETAAS.

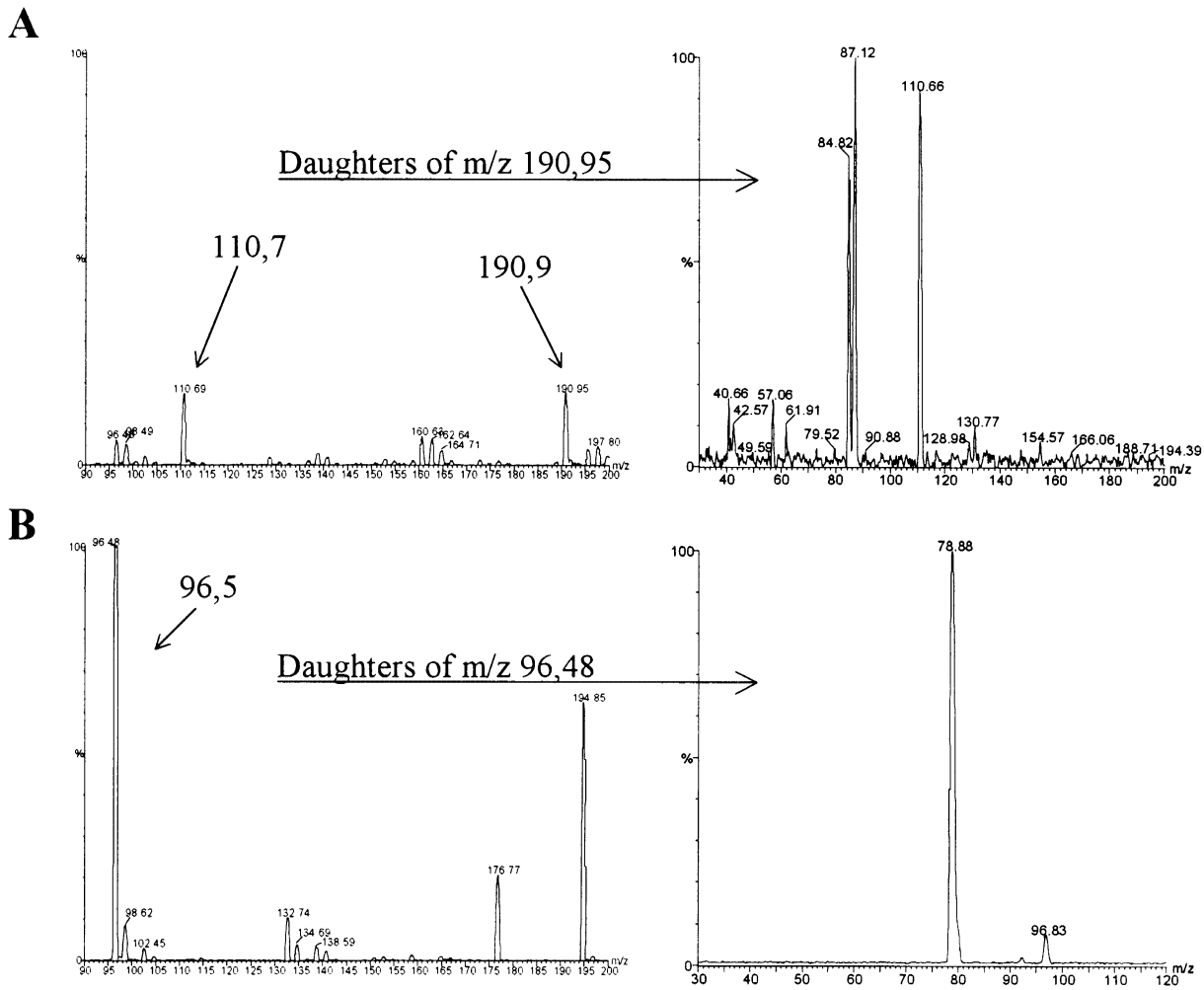

Fig. 2. ES-mass spectra and corresponding daughter ion mass spectra for synthetic solutions of Al-citrate (A) and Al-phosphate (B) (100 ng ml^{-1}) at pH 7.4.

present. This peak was selected as the parent ion for the MS-MS experiment. After fragmentation, masses of m/z 111, 87 and 85 resulted in the daughter ion mass spectrum. In the mass spectrum of Al-phosphate (Fig. 2B) a peak with m/z 97 appeared. In the MS-MS spectrum two peaks with m/z 97 and 79 were present.

The same synthetic working solutions were injected onto the FPLC column and ES-MS-MS spectra recorded for the separated fractions eluted under the chromatographic peaks. It was found experimentally that in the ES-MS scans, additional peaks with m/z 124.6, 127, 142, 147, 188 and 199 corresponded to signals from the eluent of the chromatographic run (NH_4NO_3). ES-MS-MS spectra of fragments with m/z 97 and 191 were the same as in standard solutions before chromatographic separations.

3.2. Determination of LMW–Al species in serum of CAPD patients

Sera of six CAPD patients were analysed by the FPLC-ETAAS and ES-MS-MS methods. The to-

Table 1
Concentrations of total Al (ng ml^{-1}) in serum from six CAPD patients before and after spiking with Al^{3+} solution, determined by ETAAS ($n = 3$)

Serum sample (number)	Total concentration of Al (ng ml^{-1})	Total concentration of Al after spiking (ng ml^{-1})
I	10.0 ± 0.5	115 ± 3
II	120 ± 3	120 ± 3[a]
III	130 ± 4	130 ± 4[a]
IV	11.5 ± 0.5	108 ± 3
V	9.5 ± 0.5	113 ± 3
VI	80 ± 2	80 ± 2[a]

[a] Samples were not spiked with Al.

tal Al was first determined by ETAAS. It is evident from Table 1 that the total concentrations of Al in serum of patients I, IV and V ranged from 9 to 12 ng ml^{-1}, while in samples II, III and VI from 80 to 130 ng ml^{-1}. The source of elevated total Al concentrations in serum was most likely the Al-based drugs which patients No. II, III and VI were taking. Total concentrations of Al in samples I, IV and V were too low for performance of reliable speciation analysis, therefore the samples were spiked with Al^{3+} solution. The final concentrations of Al in the spiked serum ranged between 110 and 120 ng ml^{-1} (Table 1) and were comparable to the concentrations of Al in the non-spiked serum of patients II, III and VI.

Before speciation analysis, spiked and non-spiked serum samples were microultrafiltered. The filtrate was injected on to the anion-exchange FPLC column and the concentration of Al was determined in fractions collected throughout the chromatographic run. The distribution of LMW–Al species in serum filtrate is presented in Table 2. It can be seen that the concentration of microultrafiltrable Al in serum ranged from 24 up to 53% (one sample 100%) of the total Al. Data from Table 2 further indicate that two LMW–Al species were separated on the column and that the distribution of these species varied among particular patients. LMW–Al species were eluted from 2.0 to 2.6 min (a similar retention time of Al-phosphate), and from 2.6 to 3.4 min at the reten-

Table 2
Separation of microultrafiltrable Al (LMW–Al) in spiked (I, IV, V) and non-spiked (II, III and VI) human serum from six CAPD patients on an anion-exchange FPLC column and determination of Al (ng ml^{-1}) in separated fractions by ETAAS ($n = 3$)

Time (min)	Concentration of Al in separated fractions of six CAPD patients (ng ml^{-1})					
	I (Spiked)	II (Non-spiked)	III (Non-spiked)	IV (Spiked)	V (Spiked)	VI (Non-spiked)
0–0.5	*	*	*	*	*	*
0.5–1.0	*	*	*	*	1.0 ± 0.2	4.0 ± 0.2
1.0–1.5	1.0 ± 0.2	*	*	*	*	*
1.5–2.0	*	*	*	*	*	*
2.0–2.2	*	2.0 ± 0.2	*	*	4.1 ± 0.2	*
2.2–2.4	6.1 ± 0.4	2.1 ± 0.2	11.0 ± 0.2	2.0 ± 0.2	17.0 ± 0.2	40 ± 0.5
2.4–2.6	1.0 ± 0.2	1.9 ± 0.2	3.9 ± 0.2	2.1 ± 0.2	*	20 ± 0.3
2.6–2.8	*	15.0 ± 0.3	*	11.5 ± 0.2	6.0 ± 0.2	10.1 ± 0.2
2.8–3.0	15.0 ± 0.4	7.2 ± 0.2	4.1 ± 0.2	12.5 ± 0.2	*	9.9 ± 0.2
3.0–3.2	16.9 ± 0.3	25.5 ± 0.2	8.3 ± 0.2	*	*	5.0 ± 0.2
3.2–3.4	2.0 ± 0.2	10.3 ± 0.2	5.7 ± 0.2	*	*	*
3.4–3.6	*	*	*	*	*	*
3.6–3.8	*	*	*	*	*	*
3.8–4.0	*	*	*	*	*	*
4.0–4.5	*	*	*	*	*	*
4.5–5.0	*	*	*	*	*	*
5.0 → 10.0	*	*	*	*	*	*
% of separated LMW–Al	37	53	25	26	24	100

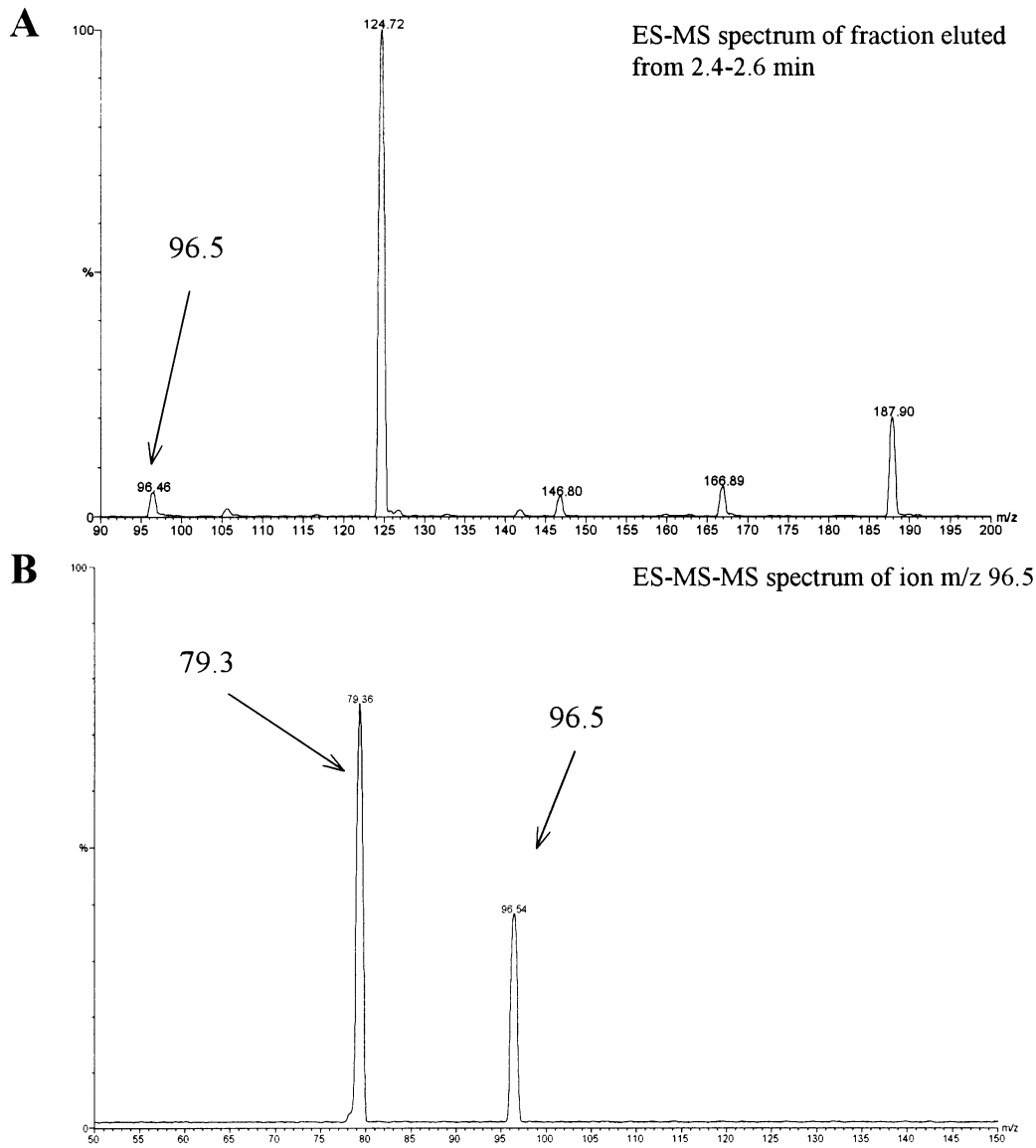

Fig. 3. ES-MS spectrum (A) and corresponding daughter ion mass spectrum of m/z 97 (B) for fraction eluted from 2.4–2.6 min, on an anion-exchange FPLC column for serum sample No. I.

tion time typical of Al-citrate. In order to identify the Al-binding ligands, ES-MS-MS analysis was performed on each separated fraction containing Al. Typical MS and the corresponding MS-MS spectra for samples No. I and II are presented in Fig. 3 Fig. 4 Fig. 5 Fig. 6.

Data of Table 2 shows that 6.1% of total Al in spiked serum sample No. I was eluted from 2.2 to 2.6 min on the anion-exchange column. In the mass spectrum (Fig. 3) of this fraction, a characteristic peak with m/z 97 was present. In the daughter ion mass spectra, two peaks with m/z 97 and 79 were observed, which confirmed the presence of phosphate as a binding ligand. Another LMW–Al species present in spiked serum No. I was eluted from 2.8 to 3.4 min and represented

29.5% of total Al. The mass analysis (Fig. 4) of this fraction indicated the presence of two characteristic peaks with m/z 97 and 191. In the corresponding daughter ion mass spectrum of m/z 97, ions with characteristic masses of m/z 97 and 79 were present, while in the daughter ion mass spectrum of m/z 191 fragments with m/z 111, 87 and 85 appeared. These data confirmed the presence of citrate and phosphate-binding ligands. On the basis of these results, it can be presumed that Al, which was eluted from 2.8 to 3.4 min, was present as Al-citrate and/or ternary Al-citrate–phosphate complexes.

Due to its high total Al concentration (120 ng ml^{-1}) serum sample No. II was not spiked. It can be seen from the data in Table 2 that the Al

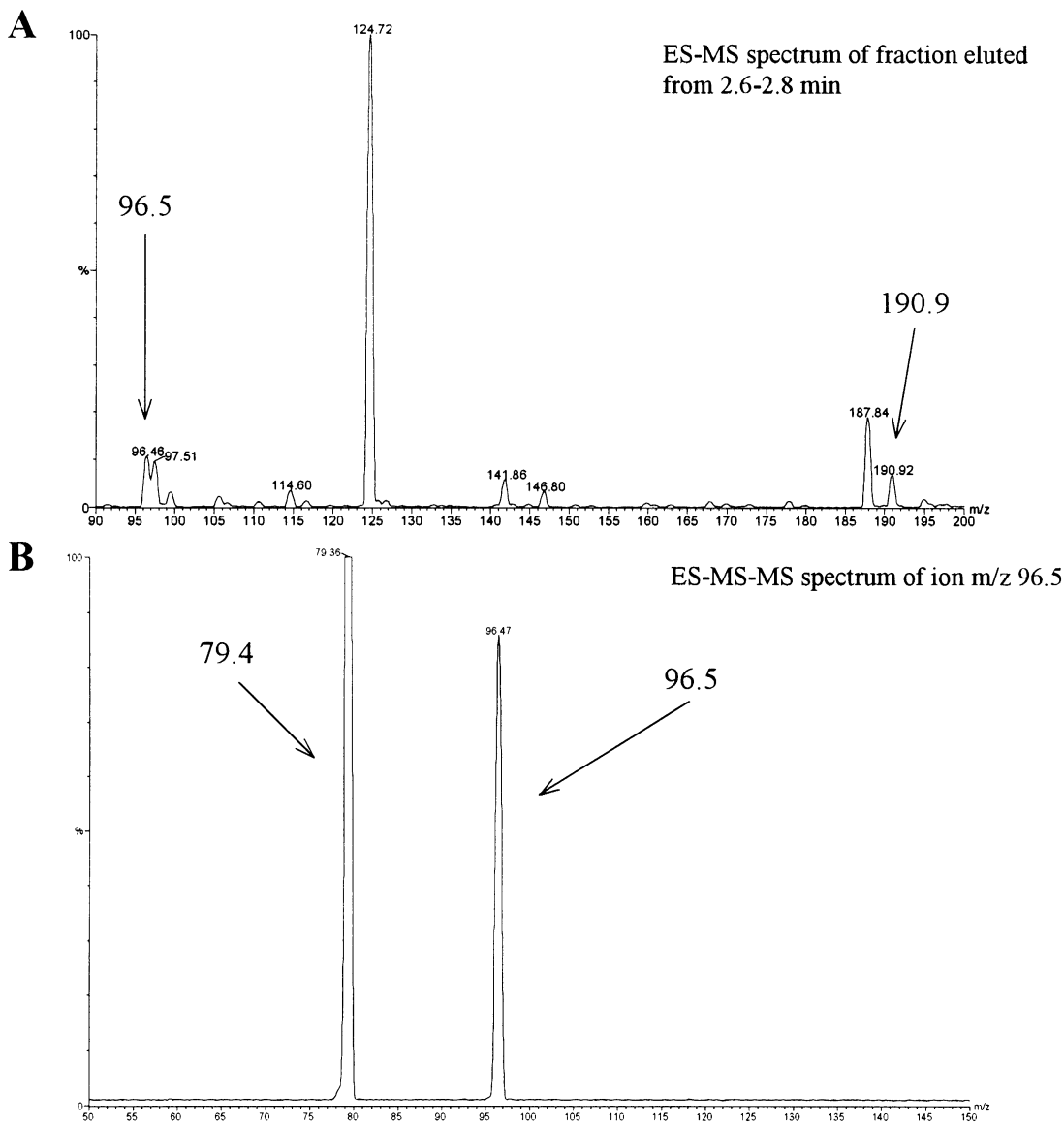

Fig. 4. ES-MS spectrum (A) and corresponding daughter ion mass spectrum of m/z 97 (B) for fraction eluted from 2.6–2.8 min, on an anion-exchange FPLC column for serum sample No. I.

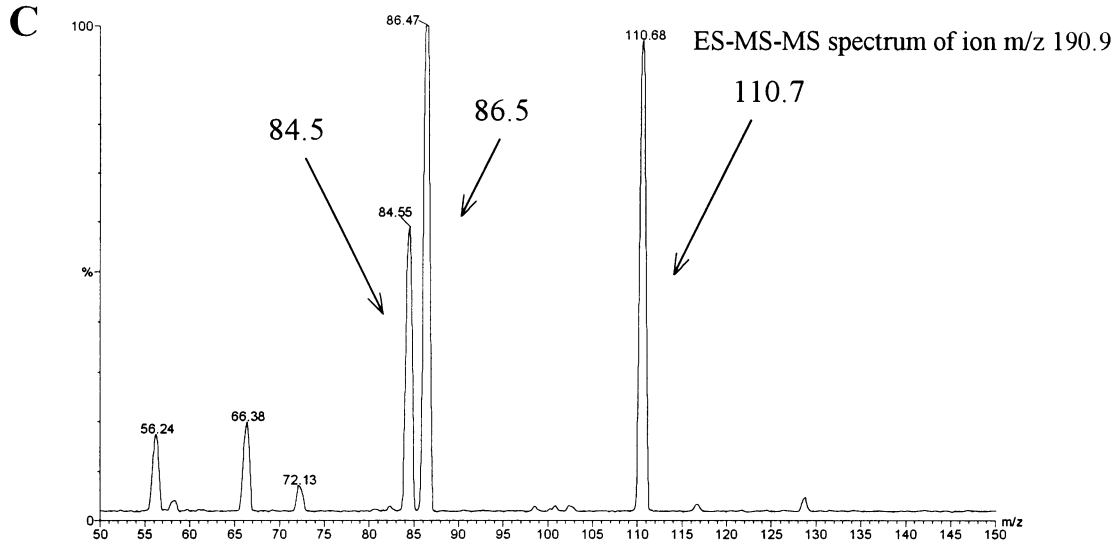

Fig. 4. (*Continued*)

species originally present in this non-spiked serum eluted as a broad peak from 2.0 to 3.4 min. On the basis of the retention time, it was presumed that 5% of the total Al in serum No. II separated from 2.0 to 2.6 min corresponded to Al-phosphate. In the mass spectrum (Fig. 5) a characteristic peak with m/z 97 was present. The daughter ion mass spectrum of m/z 97 (masses m/z 97 and 79) confirmed the presence of phosphate as the binding ligand. Forty-eight percent of total Al was eluted from 2.6 to 3.4 min. The mass spectrum of this fraction (Fig. 6) indicated the presence of characteristic peaks with m/z 191 and 97. The corresponding daughter ion mass spectrum of m/z 191 displayed characteristic masses of 111, 87 and 85, while in the MS-MS spectrum of m/z 97 peaks with m/z 97 and 79 were present. These data confirmed the presence of Al-citrate and ternary Al-citrate–phosphate species.

It is further evident from the data in Table 2 that in serum samples No. III (non-spiked), No. IV and V (spiked) approximately 25% of total Al corresponded to LMW–Al species. On the basis of the retention time, it can be presumed that the LMW–Al species eluted from 2.0 to 2.6 min corresponded to Al-phosphate. This fraction represented 11% of total Al in sample No. III, 4% in sample No. IV and 18.7% in sample No. V. The mass spectra of these separated fractions confirmed the presence of phosphate as the binding ligand. Another LMW–Al species was eluted from 2.6 to 3.4 min and represented 14% of the total Al in sample No. III, 22% of total Al in sample No. IV and 5.3% of total Al in sample No. V. Mass spectra of these fractions indicated the presence of Al-citrate and ternary Al-citrate–phosphate species.

Data from Table 2 further indicate that the Al present in the non-spiked serum sample No. VI was quantitatively microultrafiltered. On the basis of the retention times and mass analyses it was found that 78% of total Al in serum No. VI separating from 2.2 to 2.6 min corresponded to Al-phosphate and 29% of total Al eluted from 2.6 to 3.2 min to Al-citrate and ternary Al-citrate–phosphate species. One small peak, which represented 3% of total Al, was eluted from 0.5 to 1.0 min. We were not able to identify it by the ES-MS-MS technique.

4. Discussion

A method for quantitative determination of Al-citrate in a wide pH range has been developed and validated in our group previously [16]. Combined with the ES-MS-MS technique it was used in an investigation of the distribution of LMW–Al species in human serum of healthy volunteers [20]. The same method [20] was applied in a study of the distribution of LMW–Al species in the serum of six CAPD patients. Data for total Al indicated that concentrations in serum of patients No. II, III and VI, who consumed antacid Al-based drugs, were significantly higher (80–130 ng

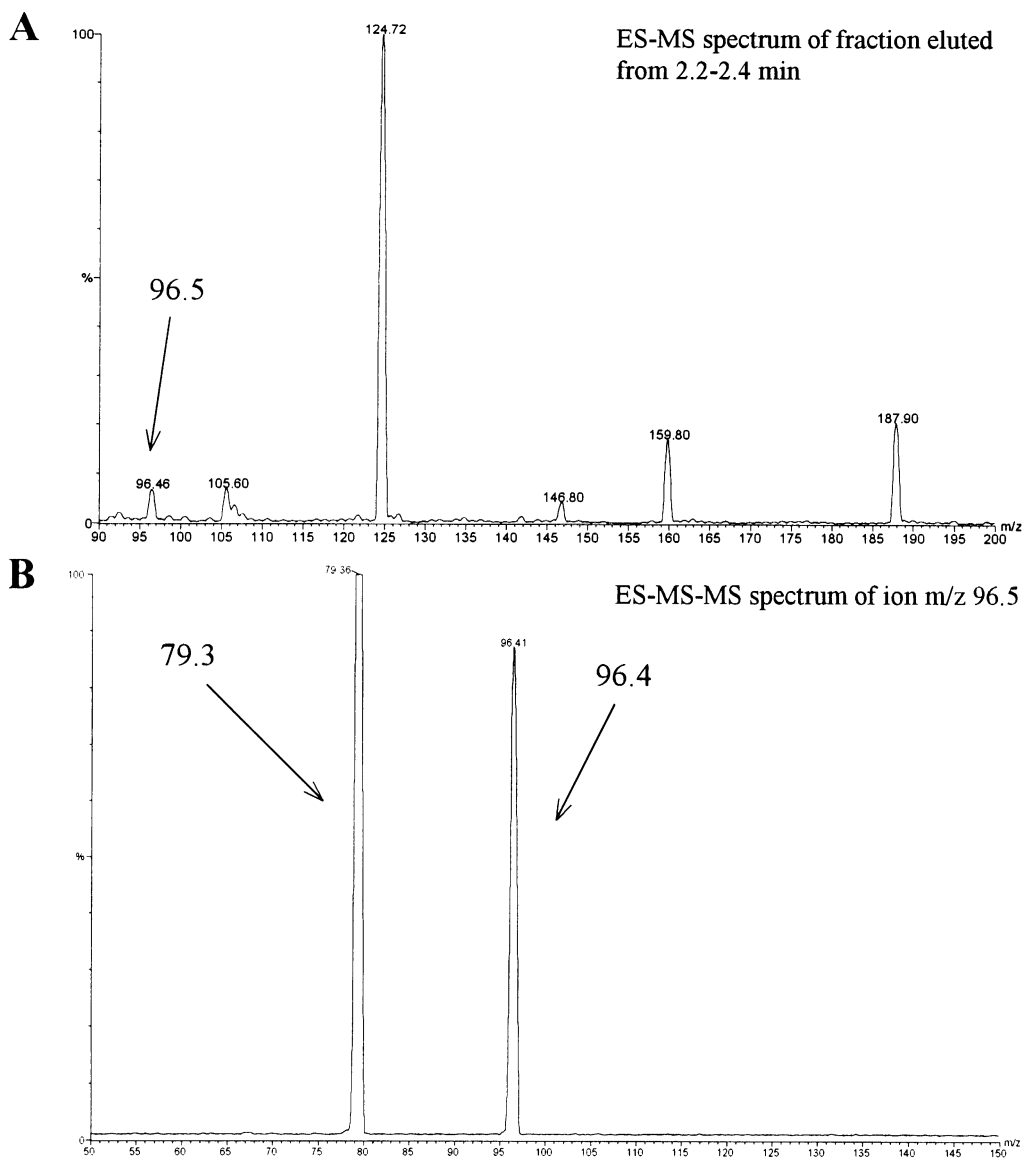

Fig. 5. ES-MS spectra (A) and corresponding daughter ion mass spectrum of m/z 97 (B) for fraction eluted from 2.2–2.4 min, on an anion-exchange FPLC column for serum sample No. II.

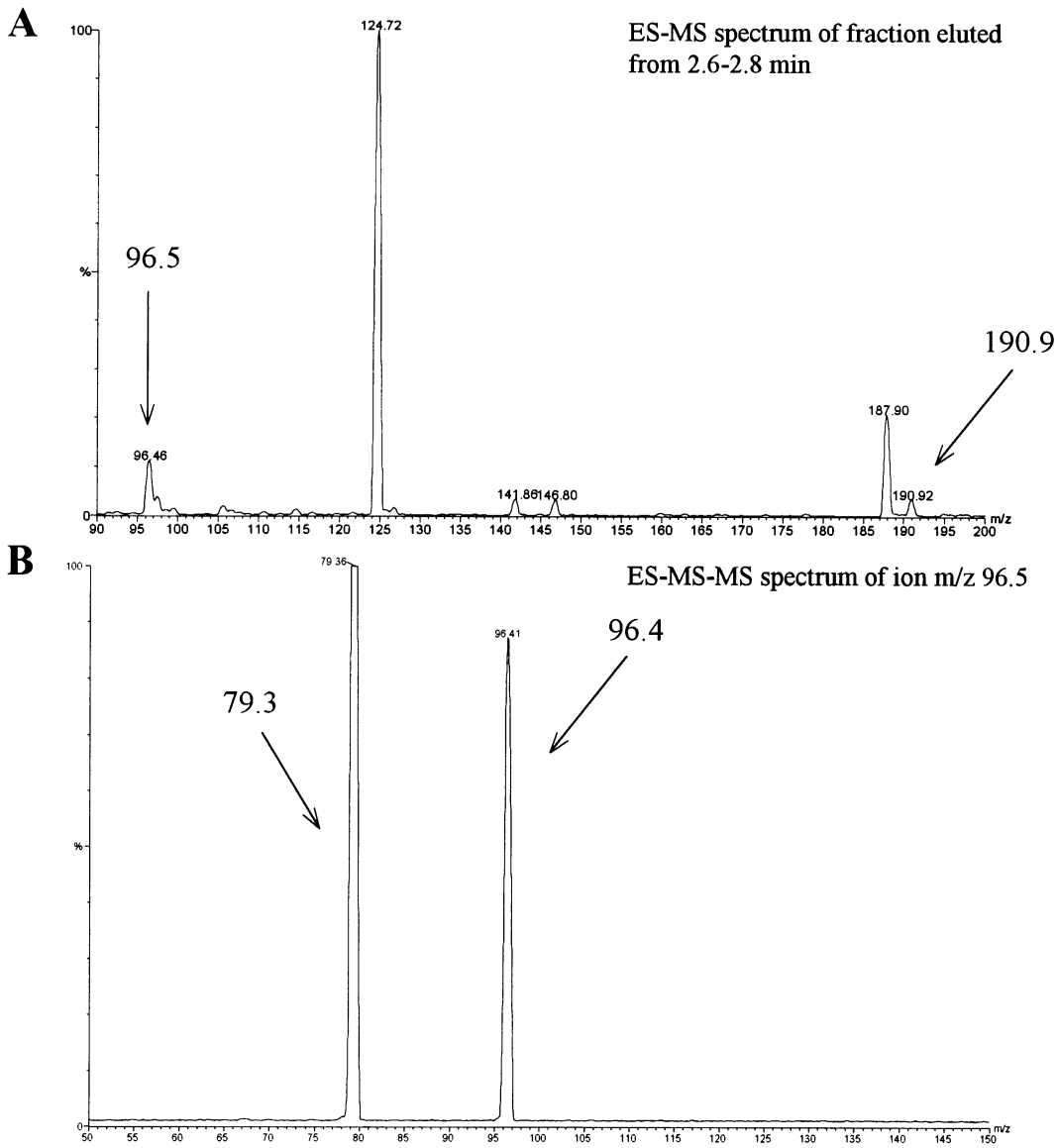

Fig. 6. ES-MS spectra (A) and corresponding daughter ion mass spectrum of m/z 97 (B) and 191 (C) for fraction eluted from 2.6–2.8 min, on an anion-exchange FPLC column for serum sample No. II.

ml^{-1}) than those in samples No. I, IV and V (10–12 ng ml^{-1}). The sampling of blood was performed in the morning, 1–4 h after drug administration. The high concentration of total Al in samples I, IV and VI enabled direct speciation analysis without any pretreatment. Since total Al concentrations in samples No. I, IV and V were too low for performing reliable speciation analysis, these samples were spiked with an appropriate amount of Al^{3+} solution and left to equilibrate prior to speciation.

It was found experimentally that the percentage of LMW–Al in the serum of CAPD patients ranged from 24 to 53% and in one sample was

even 100%. The portion of LMW–Al was very high in two non-spiked samples, while in the others it was similar to healthy persons. In our previous study [20], it was established that the percentage of LMW–Al complexes in eight healthy volunteers ranged from 14 to 30% and in one healthy person exceeded 50% of the total Al. On the basis of these observations it can be concluded that the portion of LMW–Al species varies among particular individuals. In order to assess critically if the proportion of LMW–Al is higher in patients receiving Al-based drugs, a much bigger population should be studied.

The serum samples of CAPD patients did not differ only in the percentage of total LMW–Al species but also in the distribution of particular LMW–Al complexes. On the basis of FPLC-ETAAS and ES-MS-MS techniques, it was found that LMW–Al species separated on the anion-exchange column corresponded to Al-phosphate, Al-citrate and ternary Al-citrate–phosphate complexes. It was found experimentally that the distribution of LMW–Al species varied among particular patients. Our results are in agreement with the theoretical calculations of Lakatos [11] and Hariss [12] who on the basis of computer-aided speciation studies predicted that beside Al-citrate, also Al-phosphate and ternary Al-citrate–phosphate complexes are present in serum. However, with the speciation procedure described it was not possible to distinguish quantitatively between Al-citrate and ternary Al-citrate–phosphate complex.

The data of the present study also demonstrated that spiking of serum with Al^{3+} did not influence the distribution of the LMW–Al species. A similar distribution of the LMW–Al species was found in spiked and non-spiked serum of CAPD patients.

5. Conclusions

FPLC-ETAAS in combination with ES-MS-MS technique was used in an investigation of the speciation of LMW–Al complexes in the serum of six CAPD patients. Total Al concentration in patients who consumed Al-based drugs was significantly higher (80–130 ng ml^{-1}) than those who did not take such medicaments (10–12 ng ml^{-1}). In healthy volunteers, total Al concentration ranged from 5.0 to 11.0 ng ml^{-1} [20]. High total Al concentration in serum of patients who consumed Al-based drugs, enabled determination

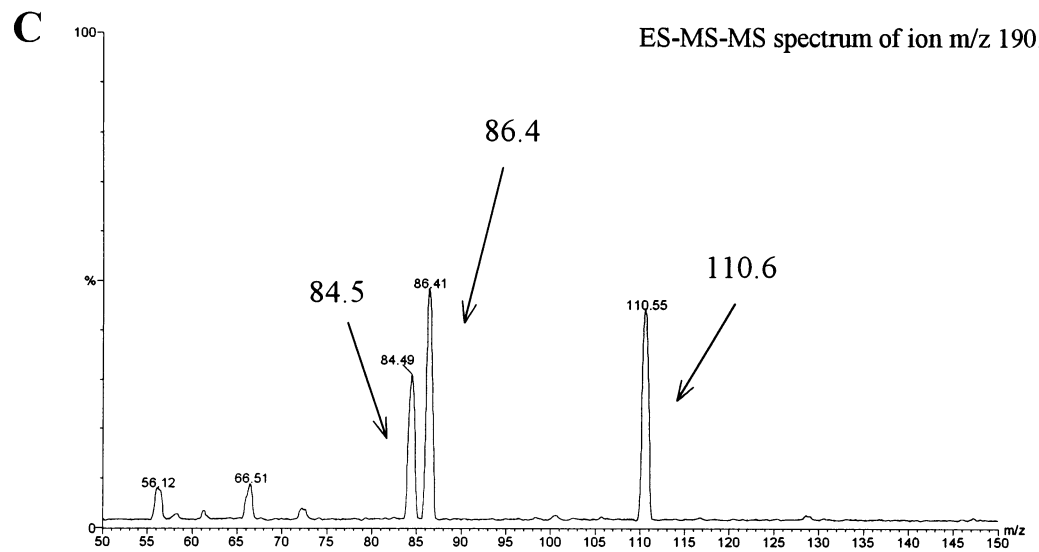

Fig. 6. (*Continued*)

of the percentage and for the first time also the distribution of LMW–Al complexes in non-spiked samples. Samples with low total Al content were spiked before speciation analysis. Data of the present study indicate that the percentage of LMW–Al species in the serum of CAPD patients in spiked and non-spiked samples ranged from 24 to 53% and was in one non-spiked sample even 100%. We did not find the reason for such high content of LMW–Al species in this particular sample. LMW–Al species in spiked and non-spiked samples corresponded to Al-phosphate, Al-citrate and ternary Al-citrate–phosphate complexes. Data of the present study demonstrated that spiking of serum with Al^{3+} did not influence the distribution of LMW–Al species. Spiking is therefore, appropriate procedure to be applied in the investigations of the distribution of LMW–Al complexes in serum, when the total concentration of Al is too low for performing reliable speciation analysis. The distribution of LMW–Al species varied among the particular patients. A similar distribution of the LMW–Al species was found in our previous study in spiked serum of healthy volunteers. However, the percentage of LMW–Al species in healthy volunteers was in general lower (14–30%). Since LMW–Al species play an important role in the transport of Al in the human body, it could be recommended that dialysis patients should reduce to minimum the consumption of Al-based drugs.

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