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Determination of trace elements in a large series of spent peritoneal dialysis fluids by atomic absorption spectrometry

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Abstract

An analytical procedure is reported for the determination of six elements in a large series of spent dialysis fluid samples. Determinations of aluminium, chromium, copper, manganese and iron were made by electrothermal atomic absorption spectrometry (ETAAS) with Zeeman background correction, while zinc was analysed by flame atomic absorption spectrometry (FAAS). Because of the complex matrix with high salt content and a high content of proteins, the measurement parameters were optimised for each particular element determined by ETAAS. The samples were collected in polyethylene eppendorf cups and stored in a freezer at -20° C. When the elements were determined by ETAAS the standard addition method was applied in the calibration procedure. The sample (10 µl) was injected into a cuvette and careful drying and long ashing of samples at temperatures between 850 and 1000°C performed. Triton X-100 was added before each determination to reduce the matrix effects of the proteins. Zinc was determined by FAAS in an air–acetylene flame under the usual recommended procedure, calibrating with aqueous standards. The limits of detection (3σ basis) were 1.0 µg 1⁻¹ for aluminium, 0.20 µg 1⁻¹ for chromium, 0.40 µg 1⁻¹ for copper, 0.20 µg 1⁻¹ for manganese, 0.50 µg 1⁻¹ for iron and 5.0 µg 1⁻¹ for zinc. The reproducibility of the measurements for aluminium, copper, iron and zinc was better than $\pm 3.0\%$. It was worse for manganese and chromium (± 6.0 and $\pm 12.0\%$, respectively), since these two elements were present in very low concentrations in all the samples analysed. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

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In order to reduce trace element exposure and to identify imbalances of trace elements in patients on continuous ambulatory peritoneal dialysis (CAPD), it is necessary to analyse spent

0731-7085/99/\$ - see front matter © 1999 Elsevier Science B.V. All rights reserved. *PII* S0731-7085(98)00118-6 dialysis fluid. Spent CAPD fluid is a very complex matrix. It contains high concentrations of various salts, glucose and a high content of proteins. There are several reports in the literature of the determination of various trace elements in fresh peritoneal dialysate [1-7] with the aim of preventing patients becoming intoxicated by CAPD fluid. The most frequently studied elements were aluminium [1,3-6] and chromium [1,2,5,7]. These two elements were determined by ETAAS [1,3-6] or by neutron activation analysis (NAA) [2,7]. To compensate for the matrix effects in ETAAS determinations, the standard addition method was applied in the calibration procedure [1,3], or various modifiers, e.g. nitric acid [4] or orthophosphoric acid [6], were added to the analyte and the elements determined by simple calibration using aqueous standards. Trace element removal through the peritoneum is an important determinant of the status of patients who undergo CAPD treatment alone [5,8-10], or in combined therapy with deferoxamine [11-15]. Aluminium [5,8,11, 12,9,10] and iron [13–15] were most frequently investigated in studies of the transfer of trace elements during CAPD treatment. The measurement parameters for ETAAS determinations were similar to those reported for serum analysis [16,11,9]. Human serum contains a much higher content of proteins (about 60 g 1^{-1}) [17] than spent CAPD fluid (up to 2 g 1^{-1}). To reduce the matrix effects, the serum samples were, in general, diluted before analysis up to four times with various matrix modifiers, e.g. Triton X-100 [18-20], Triton X-100 in combination with $Mg(NO_3)_2$ [21,22], HNO₃ [20] or simply with ultrapure water [18,19,23,20]. Due to the variety of procedures proposed in the literature for the determination of trace elements in complex matrices, e.g. CAPD fluids and biological fluids such a serum and spent CAPD, it is still necessary to optimise the measurement parameters for a particular element, especially for those elements determined by ETAAS. In investigations of trace element exposure in CAPD patients, a large series of spent CAPD fluids needs to be analysed. In such cases special attention should also be paid to efficient cleaning of the cuvette to remove the salt deposit before the next determination.

Therefore, the aim of our study was to optimise the analytical procedure for quantitative and reproducible determination of aluminium, chromium, copper, manganese and iron by ETAAS with Zeeman background correction and zinc by FAAS in a large series of spent CAPD fluids.

2. Materials and methods

2.1. Instrumentation

Aluminium, chromium, copper, manganese and iron were determined by ETAAS on a Hitachi Z-8270 polarized Zeeman atomic absorption spectrophotometer equipped with an autosampler. Zinc was determined by FAAS on a Varian AA-5 atomic absorption spectrometer in an airacetylene flame.

2.2. Reagents

Merck Suprapur acids and water doubly distilled in quartz were used for the cleaning of the laboratory ware and the preparation of the standard solutions. All other chemicals were of analytical reagent grade.

Stock standard solutions of aluminium, chromium, copper, manganese, iron and zinc $(1000 \pm 0.002 \text{ mg } 1^{-1} \text{ in } 5\% \text{ HNO}_3)$ were obtained from Merck. Fresh working standard solutions were prepared by dilution of particular stock solutions with water before each new series of samples were analysed.

Triton X-100 (98–102%, for molecular biology) was purchased from Merck.

2.3. Sample preparation

To avoid contamination by extraneous aluminium, polyethylene ware was used. Sample preparation and determination of trace elements by ETAAS was carried out under clean-room conditions (class 10000). Before use the plastics were treated with 10% HNO₃ for 24 h, rinsed with an adequate amount of water doubly distilled in quartz and dried at room temperature. The samples of the spent CAPD fluids were collected after CAPD exchange in polyethylene beakers, divided to 1 cm³ aliquots in polyethylene eppendorf cups, and immediately stored in a freezer at -20° C. Before analysis the sample aliquots were left in ambient air to equilibrate to room temperature. After being refrozen, the samples were analysed within 24 h to avoid hydrolysation of the proteins in the spent CAPD fluids. For that reason 1 cm³ sample aliquots were used for determination of only one particular element.

3. Results and discussion

3.1. Optimisation of measurement parameters for determination of aluminium, chromium, copper, manganese and iron by ETAAS

Spent CAPD fluid is a complex matrix with a high salt content and a high content of proteins. Before being used, CAPD fluid contains 132 mmol 1^{-1} sodium, from 1.25 to 1.75 mmol 1^{-1} calcium, from 0.25 to 0.75 mmol 1^{-1} magnesium, from 95 to 102 mmol 1^{-1} chloride, from 35 to 40 mmol 1^{-1} lactate and from 1.36 to 3.86% glucose. After application, the spent CAPD fluid also contains up to 2.0 g 1^{-1} proteins. In order to reduce the matrix effects in such a complex analyte, the measurement parameters were optimised for each particular element. The standard addition method was applied in the calibration procedure. Before each determination 5 µl Triton X-100 was added to the cuvette to reduce the matrix effects of proteins and to improve the drying characteristics of the sample [24]. After that 10 µl sample was injected and careful dried by a slow temperature ramp to 140°C and long ashing of samples was performed. In order to reduce nonspecific absorption originating mainly from molecules of NaCl, the ashing was carried out for 30-40 s at temperatures between 850 and 1000°C. Special attention was paid to efficient cleaning of the cuvette of the salt deposit before the next determination. For that purpose prolonged cleaning times were applied for 10 s at a temperature of 2800°C. In addition, after analysis of five successive samples, pure water was introduced into the cuvette and

the calibration curve resloped. When a large series of spent CAPD fluid samples are analysed it is of particular importance to apply the cleaning step exactly. Only by following the recommended analytical procedure can reproducible results and no loss in sensitivity after successive determinations be obtained. Integrated absorbances $(A \cdot s)$ were used in the calculation of trace element concentrations. Pyrolytically coated graphite tubes were used throughout the measurements. Zeeman background correction was employed to compensate for nonspecific absorption. The measurement parameters for the determination of aluminium, chromium, copper, manganese and iron in the samples of spent CAPD fluid by ETAAS are shown in Table 1. It was found experimentally that Triton X-100 (0.1% solution) did not contribute to the sample blank for any of the trace elements determined. The same parameters as shown in Table 1 with the exception that no Triton X-100 was added to the sample analysed were used for the determination of trace elements in fresh CAPD fluids.

3.2. Determination of zinc by FAAS

Zinc in samples of spent CAPD fluid was determined by FAAS at 213.9 nm in an air-acetylene flame. Nonspecific background absorption was corrected by a deuterium lamp. It was found experimentally that the sample matrix does not influence the sensitivity of the measurements in the determination of zinc by FAAS. The same results as with the samples analysed were obtained when aqueous standard solutions and a standard addition calibration were applied. For that reason determination of zinc in the samples of spent CAPD fluid by FAAS was performed against aqueous standards.

3.3. Calibration graph, limit of detection and reproducibility of measurements

The calibration graph for aqueous standard solutions were linear up to $100 \ \mu g \ l^{-1}$ aluminium, $40 \ \mu g \ l^{-1}$ chromium, $80 \ \mu g \ l^{-1}$ copper, $40 \ \mu g \ l^{-1}$ manganese, $100 \ \mu g \ l^{-1}$ iron and up to $500 \ \mu g \ l^{-1}$ zinc. Therefore, in the linear concentration range,

Table 1

Measurement parameters for determination of aluminium,	, chromium, copper,	, manganese and iron	in spent CAPD f	fluid samples by
ETAAS with Zeeman background correction				

	Ala	Cr ^b	Cu ^c	Mn^d	Fe ^e	
Wavelength (nm)	309.3	359.3	324.8	279.6	248.3	
Spectral bandwidth (nm)	1.03	1.30	1.30	0.40	0.20	
Lamp current (mA)	10.0	5.0	5.0	10.0	15.0	
Sample volume (µl)	10	10	10	10	10	

Electrothermal atomization programme

Stage No.	Stage	Start temperature (°C)	End temperature (°C)	Ramp time (s)	Hold time (s)	Gas flow (ml min ⁻¹)
1	Dry	60	90	15	5	200
2	Dry	90	100	10	5	200
3	Dry	100	110	15	5	200
4	Dry	110	40	0	5	200
5	Ash	140	1000 ^{a,b}	10	20 ^{a,b}	100
			850 ^{c,d,e}		30 ^{c,d,e}	
6	Atomization	2700 ^{a,b}	2700 ^{a,b}	0	4	0
		2400 ^c	2400 ^c			
		2300 ^d	2300 ^d			
		2500 ^e	2500 ^e			
7	Clean	2800	2800	0	10	200
8	Cool	_	—	0	5	200

Before each determination 5 µl Triton X-100 (0.1% solution) was added.

it was possible to use the standard addition method of calibration. The limits of detection (LOD), calculated on a 3σ basis (a value of three times the S.D. of the blank) were found to be 1.0 µg l⁻¹ for aluminium, 0.20 µg l⁻¹ for chromium, 0.40 µg l⁻¹ for copper, 0.20 µg l⁻¹ for manganese, 0.50 µg l⁻¹ for iron and 5.0 µg l⁻¹ for zinc. The reproducibility of measurements (R.S.D.) was tested on one representative sample of spent CAPD fluid by six parallel determinations for each particular element. The results are shown in Table 2. It is evident from these data that the R.S.D. for aluminium, copper, iron and zinc is better than $\pm 3.0\%$. It is worse for manganese ($\pm 6.0\%$) and chromium ($\pm 12.0\%$), since the concentrations of these two elements in spent CAPD fluid are very low.

Table 2

Reproducibility of measurements of trace elements in a test sample of spent CAPD fluid determined by ETAAS and FAAS*

Test sample replicate	Al ($\mu g l^{-1}$)	Cr (µg 1 ⁻¹)	Cu (µg 1 ⁻¹)	Mn ($\mu g l^{-1}$)	Fe (µg 1 ⁻¹)	$Zn^* \ (\mu g \ l^{-1})$
1	3.6	0.85	14.8	1.71	18.7	66.6
2	3.0	0.80	13.8	1.59	18.1	69.7
3	2.7	0.78	14.3	1.84	18.6	66.6
4	3.1	1.07	13.8	1.63	17.6	66.6
5	3.5	0.90	14.6	1.63	17.8	69.7
6	3.5	0.93	13.9	1.59	18.3	66.6
Mean	3.2 ± 0.4	0.89 ± 0.11	14.2 ± 0.4	1.67 ± 0.1	18.2 ± 0.4	66.7 ± 1.6
R.S.D. (%)	1.0	12.4	3.1	6.0	2.4	2.4

Table 3			
Concentrations of trace elements in a series of	f 48 spent CAPD	fluid samples determined	by ETAAS and FAAS*

Sample	Al ($\mu g \ l^{-1}$)	Cr (µg 1 ⁻¹)	Cu ($\mu g l^{-1}$)	$Mn~(\mu g~l^{-1})$	Fe ($\mu g \ l^{-1}$)	$Zn^* \ (\mu g \ l^{-1})$
1	<1.0	0.30 ± 0.05	14.4 ± 0.3	< 0.20	10.0 ± 0.2	25 ± 2
2	<1.0	0.40 + 0.10	14.3 + 0.8	0.50 + 0.05	102 + 0.7	25 + 2
3	<1.0	0.50 ± 0.10	35.2 ± 0.3	< 0.20	135 ± 0.9	36 ± 2
4	<1.0	0.50 ± 0.10	13.9 ± 0.5	0.33 ± 0.06	12.8 ± 0.8	38 ± 2
5	<1.0	0.40 ± 0.10	22.3 ± 0.3	< 0.20	8.2 ± 0.3	29 ± 2
6	<1.0	0.50 ± 0.10	15.1 ± 0.3	0.25 ± 0.05	21.9 ± 0.5	38 ± 2
7	5.1 ± 0.4	0.20 ± 0.03	16.5 ± 0.2	0.22 ± 0.02	14.2 ± 0.9	26 ± 2
8	1.4 ± 0.1	0.30 ± 0.10	29.4 ± 0.8	0.30 ± 0.03	17.1 ± 0.3	48 ± 2
9	<1.0	0.30 ± 0.10	14.7 ± 0.3	0.22 ± 0.02	13.6 ± 0.07	145 ± 2
10	13.3 ± 0.5	0.65 ± 0.03	6.3 ± 0.4	0.37 ± 0.06	5.4 ± 0.4	124 ± 2
11	<1.0	0.40 ± 0.10	18.9 ± 0.8	0.36 ± 0.06	17.7 ± 0.2	71 ± 2
12	7.0 ± 0.2	0.33 ± 0.06	17.7 ± 0.8	0.34 ± 0.06	21.3 ± 0.4	36 ± 2
13	3.2 ± 0.2	0.29 ± 0.09	20.9 ± 0.7	< 0.20	10.3 ± 0.06	43 ± 2
14	<1.0	0.33 ± 0.05	13.7 ± 0.6	0.26 ± 0.02	9.8 ± 0.2	29 ± 2
15	< 1.0	0.50 ± 0.20	32.0 ± 0.8	0.25 ± 0.10	18.4 ± 0.3	33 ± 2
16	3.6 ± 0.3	1.00 ± 0.05	94.9 ± 0.6	0.42 ± 0.04	74.2 ± 0.6	43 ± 2
17	<1.0	0.25 ± 0.05	35.2 ± 0.4	0.77 ± 0.05	14.7 ± 0.04	43 ± 2
18	5.8 ± 0.2	0.45 ± 0.05	33.1 ± 0.5	0.25 ± 0.03	32.5 ± 0.8	57 ± 2
19	3.2 ± 0.4	0.41 ± 0.01	11.1 ± 0.4	0.30 ± 0.03	6.8 ± 0.3	67 ± 2
20	4.2 ± 0.6	0.20 ± 0.02	17.9 ± 0.4	< 0.20	7.0 ± 0.1	52 ± 2
21	<1.0	0.30 ± 0.05	21.7 ± 0.1	< 0.20	11.3 ± 0.6	38 ± 2
22	3.4 ± 0.3	0.27 ± 0.05	35.2 ± 0.2	0.31 ± 0.01	23.0 ± 1.0	60 ± 2
23	2.2 ± 0.1	1.60 ± 0.10	23.1 ± 0.3	0.33 ± 0.03	9.7 ± 0.05	48 ± 2
24	<1.0	0.52 ± 0.03	10.0 ± 0.6	< 0.20	6.8 ± 0.3	60 ± 2
25	<1.0	0.30 ± 0.04	13.4 ± 0.3	< 0.20	12.2 ± 0.3	60 ± 2
26	<1.0	0.40 ± 0.02	14.5 ± 0.5	< 0.20	12.3 ± 0.2	79 ± 2
27	<1.0	0.37 ± 0.10	35.8 ± 0.7	< 0.20	18.5 ± 0.5	50 ± 2
28	<1.0	0.69 ± 0.06	33.2 ± 0.4	< 0.20	17.0 ± 0.2	45 ± 2
29	3.0 ± 0.2	0.31 ± 0.02	39.2 ± 0.9	0.21 ± 0.02	10.3 ± 0.1	93 ± 2
30	<1.0	0.41 ± 0.02	16.7 ± 0.5	< 0.20	8.8 ± 0.2	57 ± 2
31	<1.0	0.35 ± 0.02	23.1 ± 0.9	< 0.20	10.4 ± 0.5	79 ± 2
32	<1.0	0.55 ± 0.05	18.0 ± 0.5	< 0.20	17.3 ± 0.3	48 ± 2
33	1.9 ± 0.2	0.87 ± 0.10	17.0 ± 0.5	< 0.20	21.1 ± 0.1	45 ± 2
34	1.3 ± 0.1	1.90 ± 0.10	15.1 ± 0.5	< 0.20	11.8 ± 0.3	12 ± 2
35	3.6 ± 0.1	0.43 ± 0.09	19.0 ± 0.6	0.25 ± 0.02	11.2 ± 0.2	50 ± 2
36	1.3 ± 0.1	0.50 ± 0.05	10.5 ± 1.0	< 0.20	8.8 ± 0.5	71 ± 2
37	2.8 ± 0.1	0.30 ± 0.05	42.7 ± 0.6	< 0.20	21.5 ± 0.4	67 ± 2
38	1.1 ± 0.2	0.50 ± 0.10	26.1 ± 0.3	< 0.20	35.2 ± 0.5	57 ± 2
39	<1.0	0.75 ± 0.10	16.2 ± 0.8	< 0.20	15.0 ± 0.5	48 ± 2
40	<1.0	0.55 ± 0.13	20.5 ± 0.2	0.29 ± 0.01	14.1 ± 0.5	57 ± 2
41	<1.0	0.70 ± 0.05	43.0 ± 0.5	< 0.20	10.4 ± 0.4	48 ± 2
42	3.1 ± 0.1	0.75 ± 0.01	23.3 ± 0.5	< 0.20	20.5 ± 0.6	60 ± 2
43	<1.0	1.05 ± 0.02	13.2 ± 1.5	0.25 ± 0.04	17.0 ± 1.0	83 ± 2
44	<1.0	0.52 ± 0.06	13.5 ± 0.8	< 0.20	15.1 ± 0.3	48 ± 2
45	1.1 ± 0.1	0.58 ± 0.05	13.0 ± 1.0	0.29 ± 0.05	12.5 ± 0.3	60 ± 2
46	1.2 ± 0.2	0.45 ± 0.05	42.8 ± 1.1	0.25 ± 0.05	29.7 ± 0.2	48 ± 2
47	<1.0	0.95 ± 0.05	11.2 ± 1.2	0.40 ± 0.05	8.4 ± 0.4	33 ± 2
48	< 1.0	0.31 ± 0.04	14.3 ± 0.5	0.75 ± 0.07	18.2 ± 0.4	36 ± 2

The results represent the average of three parallel determinations.

3.4. Determination of trace elements in a large series of spent CAPD fluid samples and analysis of CAPD fluids

In order to evaluate the capability of the analytical procedure for determination of trace elements in a large series of spent CAPD fluid samples, 48 samples were analysed in one run under the recommended procedure. Three parallel measurements were performed for each trace element determination. The results are shown in Table 3. In addition, six representative types of fresh CAPD fluid samples were analysed under the same analytical parameters, with the exception that no Triton X-100 was used. The results are shown in Table 4. It is evident from the data in Table 3 that concentrations of chromium and manganese in the samples of spent CAPD fluid are in general lower than 1.0 μ g l⁻¹. Similarly, these concentrations are also low in fresh CAPD fluid itself (see the data in Table 4). In more than 50% of the samples analysed, the aluminium concentrations in the spent CAPD fluid (Table 3) did not exceed 1.0 μ g \overline{l}^{-1} and were higher than 10.0 $\mu g l^{-1}$ only in one sample. The aluminium concentrations were also found to be below 1.0 µg 1^{-1} in all types of fresh CAPD fluid analysed (Table 4). With the exception of one sample of spent CAPD fluid which contained 90 µg copper 1^{-1} (Table 3), the concentrations of copper in general ranged from 6.0 to 40.0 μ g 1⁻¹, whereas in fresh CAPD fluids (Table 4) the copper concentrations did not exceed 14.0 μ g l⁻¹. It is also evident from the data in Table 3 that the concentrations of iron were found to be high in three samples of spent CAPD fluid (between 75 and 135 $\mu g l^{-1}$), while in all the other samples its concentrations ranged between 5.0 and 35.0 μ g l⁻¹. The concentrations of iron in fresh CAPD fluid (Table 4) did not exceed 11.0 μ g 1⁻¹. The concentrations of zinc in the samples of spent CAPD fluid (Table 3) were in general between 25 and 80 μ g 1⁻¹, whereas in fresh CAPD fluids the zinc concentrations (Table 4) ranged between 120 and 200 µg 1^{-1} . On the basis of the present results it could be concluded that fresh CAPD fluid is clean with respect to its concentrations of aluminium, chromium and copper and does not contribute to the transfer of these trace elements through the peritoneum into the body of the dialysis patient. Higher concentrations of aluminium in some samples of spent CAPD fluid indicate the possibility of aluminium clearance from the body into the spent peritoneal CAPD fluid. Although fresh CAPD fluid is slightly contaminated with iron and manganese, the transfer of these two trace elements occurs through the body into the spent CAPD fluid. The opposite is observed in the case of zinc where fresh CAPD fluid is moderately contaminated with this metal but the concentrations in the spent CAPD fluid are lower.

4. Conclusion

An analytical procedure is presented for analysis of aluminium, chromium, copper, manganese and iron by ETAAS with Zeeman background

Table 4

Concentrations of trace elements in six representative fresh CAPD fluid samples determined by ETAAS and FAAS*

Sample	Al (µg 1 ⁻¹)	Cr (µg 1 ⁻¹)	Cu (µg 1 ⁻¹)	Mn ($\mu g l^{-1}$)	Fe (µg 1 ⁻¹)	Zn^* (µg l ⁻¹)
PD1 glucose 2.27%	<1.0	0.30 ± 0.05	< 0.40	0.39 ± 0.03	6.1 ± 0.2	140 ± 2
PD4 glucose 1.36%	<1.0	< 0.20	14.1 ± 0.5	0.32 ± 0.08	7.4 ± 0.4	180 ± 2
PD4 glucose 2.27%	< 1.0	0.70 ± 0.05	< 0.40	0.35 ± 0.04	6.0 ± 0.6	120 ± 5
PD4 glucose 3.86%	<1.0	0.35 ± 0.05	14.4 ± 0.6	0.55 ± 0.12	11.1 ± 0.1	160 ± 5
PD1 glucose 1.36%	<1.0	< 0.20	< 0.40	0.26 ± 0.06	3.2 ± 0.1	150 ± 5
PD1 glucose 2.27%	<1.0	< 0.20	0.5 ± 0.1	0.31 ± 0.09	8.6 ± 0.4	195 ± 5

The results represent the average of three parallel determinations.

PD1 composition: Na, 132 mmol l^{-1} , Ca, 1.75 mmol l^{-1} , Mg, 0.75 mmol l^{-1} , Cl⁻, 102 mmol l^{-1} , lactate, 35 mmol l^{-1} . PD4 composition: Na, 132 mmol l^{-1} , Ca, 1.25 mmol l^{-1} , Mg, 0.25 mmol l^{-1} , Cl⁻, 95 mmol l^{-1} , lactate, 40 mmol l^{-1} .

correction and zinc by FAAS in spent CAPD fluid samples. The protocol for the collection of the samples in polyethylene eppendorf cups in aliquots for determination of each particular element and the storage of the samples at -20° C is recommended. The measurement parameters were optimised for the determination of trace elements by ETAAS, using the standard addition method in the calibration procedure and the addition of Triton X-100 before each determination to reduce the matrix effects. Special attention was directed to efficient cleaning of the cuvette of salt deposits after successive determinations. When zinc is determined by FAAS, a simple calibration with aqueous standards is recommended. The proposed analytical procedure enables trace elements in a large series of spent CAPD fluid samples to be reliably and reproducibly determined which is of great importance when trace element exposure and imbalances of trace elements are studied in patients on CAPD.

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