Screening of UV-absorbing solutes in uremic serum by reversed phase HPLC - change of blood levels in different therapies

Please check the document version of this publication:

Screening of UV-absorbing solutes in uremic serum by reversed phase HPLC – Change of blood levels in different therapies

Ad C. Schoots [a,*], Hittjo R. Homan [a], Marcel M. Gladdines [a], Carel A.M.G. Cramers [a], Rita de Smet [b] and Severin M.G. Ringoir [b].

[a] *Laboratory of Instrumental Analysis, Eindhoven University of Technology, Department of Chemical Engineering, Eindhoven (The Netherlands) and* [b] *Department of Medicine, Nephrological Division, University Hospital of Ghent, Ghent (Belgium)*

Summary

In order to screen UV-absorbing solutes in large numbers of uremic serum samples, an automated liquid chromatographic method was developed. The method proved to be reliable and reproducible in more than 500 analyses. HPLC separation was performed using gradient elution on a 25-cm Ultrasphere Octyl reversed phase column, with 5 μm particles. Characteristic profiles for the uremic state were obtained in the analyses of serum samples of 43 uremic patients before and just after artificial kidney treatment; hemodialysis ($n = 14$), hemodiafiltration ($n = 13$) and hemofiltration ($n = 16$). In these profiles 20–40 peaks were resolved of which nine were 'quantitated' by peak height relative to a standard. Of these solutes creatinine, uracil, uric acid, hypoxanthine, indoxylsulfate, tryptophan and hippuric acid were identified. The heterogeneity of the population of uremic patients, with respect to the UV-absorbing solutes, was estimated. Significant differences of solute blood level changes during hemodialysis, hemodiafiltration and hemofiltration, were observed.

---

* Correspondence to: Ir. A.C. Schoots, Lab. of Instrumental Analysis, SH 2.05, Dept. of Chemical Engineering, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands.

Introduction

Many efforts have been made to separate and identify the solutes accumulating in the blood of patients with chronic renal failure. These solutes with different molecular mass have been analyzed by a range of analytical methods. More recently, with the development of high performance liquid chromatography (HPLC), a number of authors have reported the application of this technique to the analysis of uremic fluids. Senftleber et al [1] and Knudsen et al [2] analyzed hemodialyzate and ultrafiltrated uremic serum by gradient elution reversed phase HPLC, with UV-absorbance detection. Mabuchi and Nakahashi [3] and Swan et al [4] separated fluorescent substances in serum and urine of uremic patients. Mohammed et al [5] determined riboflavin in hemodialyzate by precolumn-enrichment and selective fluorimetric detection. An automatic analyzer for quantitation of biogenic guanidino compounds was described by Hung et al [6]. Some authors separated 'middle molecule' fractions with HPLC [7–9]. Lindblom et al [10] described an efficient procedure for analysis of urinary proteins in renal failure. Only few studies describe the differentiation in uremic patients' populations on the basis of analytical data. In the present study the automated profiling of UV-absorbing solutes in uremic fluids will be described, and the method is applied in a study of patient heterogeneity and in vivo dialysis efficiency.

Materials and methods

Uremic serum samples

Pre- and postdialysis uremic serum samples were ultrafiltrated through membranes with 10 000 M_r cut-off (Diaflo® PM10. Amicon Corp. Danvers, MA, USA), using an 8-cell Multi-micro-ultrafiltration system, MMC (Amicon), at a nitrogen pressure of 2 Bar.

The ultrafiltrated sera were then stored at $-18°C$ until use. Before HPLC analysis 400 μl of the ultrafiltrated sample were diluted by addition of 400 μl of an injection standard solution of naphthalene sulfonic acid (0.037 mg/ml). Fifty microliters of the sample was injected onto the liquid chromatograph.

Dialysis treatment

Fourteen hemodialysis patients were treated with a cuprophane dialyzer and a dialyzate flow of 500 ml/min; 16 hemodiafiltration patients received a 10-l infusion of a substitution fluid together with a dialyzate flow of 500 ml/min and an ultrafiltration of at least 10 l on a more permeable polyacrylonitrile or polymethyl methacrylate membrane. In 16 hemofiltration patients, there was no dialyzate flow, and an infusion of 20 l of substitution fluid was performed, together with an ultrafiltration of at least 20 l. All patients had chronic renal failure (creatinine clearance < 8 ml/min) and were on regular artificial kidney treatment.

HPLC apparatus

The analyses were performed on an automated system, consisting of a model 421

solvent program controller, two model 100 A piston pumps, a model 160 fixed wavelength UV-absorbance detector, operated at 254 nm (0.05 AUFS sensitivity), and a model 500 autosampler (all from Beckman Instr., Berkeley, CA, USA).

An SP4000 laboratory data system was used (Spectra Physics, Santa Clara, CA, USA). UV spectra were recorded on-line with a HP 1040 A photodiode array UV detector (Hewlett Packard, Palo Alto, CA, USA).

HPLC analysis

Separations were carried out on an Ultrasphere Octyl (C8-modified silica) column (4.6 mm i.d. × 25 cm) with 5-μm particles, and a 2 mm i.d. × 3 cm guard column packed with Ultrasphere Octyl 10-μm material. The solvent gradient was 100% aqueous ammonium formate buffer (0.05 mol/l, pH 4) to 60% methanol (Fisons Ltd. Loughborough, UK) within 45 min. After each analysis, the column was regenerated by pumping 100% methanol for 20 min. Flow rate during analysis was 1 ml/min. The solvent and the column (waterjacket Alltech Assoc. Inc., Deerfield, IL, USA) were kept at a constant temperature of 25°C. Water used for the solvent and sample dilution was obtained from a Milli-Q water purification system (Millipore-Waters, Milford, MA, USA).

Between the water pump and the gradient mixer a 15-cm Lichroprep RP18 (Merck, Darmstadt, FRG) column (with 40 μm particles) was mounted to avoid interference of organic impurities in the buffer during gradient elution on the main column.

Calibration lines for creatinine, uracil, indoxylsulfate and hippuric acid, were determined by injection of buffer and methanolic solutions.

Peak heights of the solutes, relative to the height of the injection standard, naphthalene sulfonic acid, were determined. The equations, correlation coefficients and ranges of the regression lines were: creatinine, $y = 0.054 + 0.001x$ ($r = 0.998$) (88–2,655 μmol/l); uracil, $y = 0.1057 + 0.0329x$ ($r = 0.998$) (2–100 μmol/l); indoxylsulfate, $y = 0.0053 + 0.0099x$ ($r = 0.999$) (2–50 μmol/l); hippuric acid, $y = 0.049 + 0.008x$ ($r = 0.999$) (17–503 μmol/l).

Determination of creatinine

Creatinine was determined in uremic serum samples with a routine clinical method (picrate complex) using a Beckman Creatinine Analyser 2 system.

Mass spectrometry

Lyophilized HPLC fractions were analyzed by desorption chemical ionization MS. The samples were applied on a platinum wire on top of a direct insertion probe. In the ion source an electric current through the wire was programed (0–2.8 A in 20 s). The reagent gas was ammonia/methane 10/90 v : v for reasons of its high proton affinity, resulting in ionization with little fragmentation for the nitrogenous compounds expected. At an ionizer pressure of 0.10 Torr, NH_4^+ was the reactant ion. We used a Finnigan 4000 quadrupole GC-MS system combined with a Data General Nova 4S data system.

Data analysis

Statistical evaluation of analytical data was performed with the statistics program CONSTAT, running on the Institute's Burroughs B7700 mainframe computer.

Experimental

Optimization of the HPLC analysis

Type of column packing and elution conditions were optimized with respect to the number of peaks separated in a uremic serum ultrafiltrate test pool, distribution of the peaks throughout the chromatogram, separation efficiency in terms of plate number, and analysis time. Commercially available reversed phase columns from different manufacturers, packed with octyl as well as octadecyl modified silica, were tested. The Ultrasphere Octyl (C8-modified) reversed phase column was chosen for reasons of selectivity and efficiency.

Reproducibility of retention times between different columns of the same type and manufacturer constitutes a serious problem in modern liquid chromatography especially when large numbers of samples have to be analyzed reproducibly from one column to another. Therefore four columns from the same production batch were tested under isocratic conditions (solvent: methanol/water 60 : 40 v/v). The retention times of the test solutes phenol ($k' = 0.77$), *p*-cresol ($k' = 1.33$) and anisole ($k' = 2.37$) showed reproducibilities which were better than 2.5% (CV). All columns had efficiencies of more than 88000 theoretical plates/meter (4.9% CV).

The HPLC elution conditions were comparable to those published earlier by Senftleber et al [1]; however, for reasons of volatility we used an ammonium formate buffer. This was necessary for mass spectrometric identification, and the application of in vitro toxicity tests on lyophilized HPLC fractions.

The separation of a uremic ultrafiltrated serum pool on the Ultrasphere ODS (C18) and Octyl (C8) columns, under the optimized conditions, is shown in Fig. 1. As can be seen, more than 40 peaks are resolved within 30 min. In the separation on the octyl column the peaks are more evenly distributed throughout the chromatogram.

Reproducibility of the analysis

In order to check column and equipment performance a gradient test mixture (Fig. 2) was injected after every ten samples. Retention times and peak heights showed CV values of 0.6–1.0 and 1.0–3.6%, respectively in 10 gradient test injections during a 1-mth period. Thus these data represent long-term analysis reproducibility. Total reproducibility, including sample pretreatment (ultrafiltration), of retention times and peak heights of four solutes in a uremic serum pool, were 0.1–0.3 and 3.2–6.2%, respectively ($n = 6$).

Interference of drugs

The most commonly used drugs in the population of the uremic patients were: Co-trimoxazol, fenoterol, clonidine, isosorbidedinitrate, cyclandelate, dihydrotachysterol, dimetindeen, propanolol, prazosine, nitrazepam, dihydralazine, thiamin, pyridoxin, cyanocobalamin, metoclopramide, flunitrazepam and cimetidine.
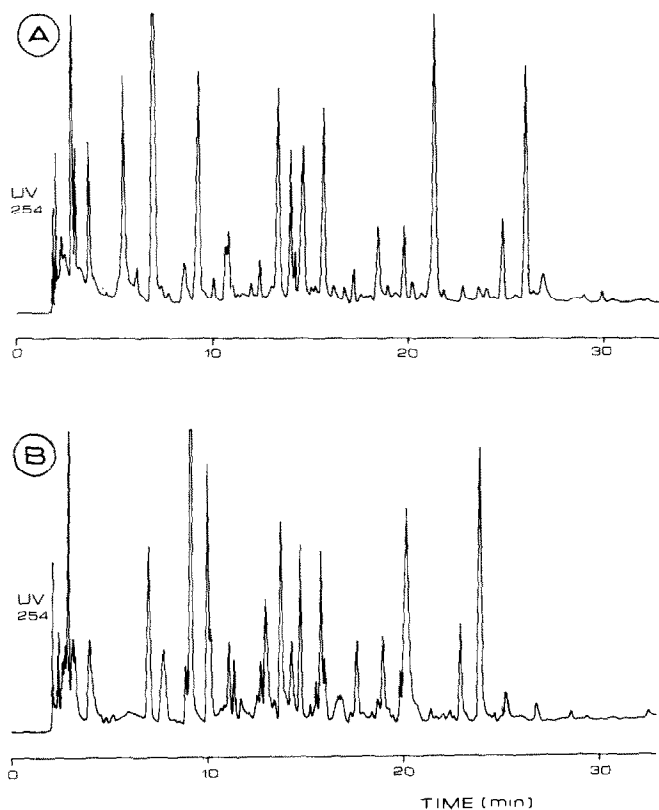
Fig. 1. HPLC gradient separation of UV-absorbing substances, accumulated in uremic serum. An ultrafiltrated uremic serum pool is shown. A, Ultrasphere Octyl; B, Ultrasphere ODS column. Solvent gradient: 0.05 mol/l ammonium formate (pH 4) to 60% methanol within 45 min in both chromatograms A and B. Flow rate 1 ml/min.
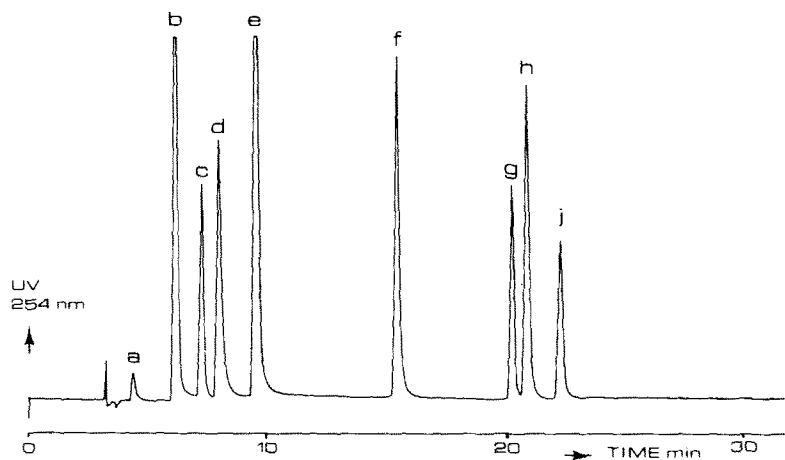
Fig. 2. Separation of the gradient test mixture with the same operational conditions as in Fig. 1. a, creatinine; b, uracil; c, uric acid; d, nicotinic acid; e, hypoxanthine; f, nicotinamide; g, indoxylsulfate; h, tryptophan; j, hippuric acid.

Taking into account expected therapeutic serum concentrations in uremic patients and extinction coefficients at 254 nm of the active substances in the drugs, only Co-trimoxazol (trimethoprim, sulfamethoxazol) and neurobion[R] (thiamin, pyridoxin, cyanocobalamin) were suspected of interference with the HPLC analysis.

Injection of methanolic solutions of these drugs, in the expected concentrations, resulted in peaks at retention times of 1,935 and 1,999 s for Co-trimoxazol and at 848 s for neurobion. However, the possible interference of metabolites of the drugs was not estimated, as only the native drugs were available.

Identification of peaks

Peaks were identified by HPLC retention times of known injected solutes of which the presence was expected, by comparing UV-spectra of the peaks with those of known solutes with the same retention time, and by mass spectrometric analysis. A list of solutes and their retention times is given in Table I. Until now the compounds creatinine, uracil, uric acid, hypoxanthine, indoxylsulfate, tryptophan and hippuric acid were identified by a combination of the described methods (see Fig. 3). UV-spectra of the peak at retention time 350 s and an injected sample of uracil are compared in Fig. 4.

TABLE I

Retention times of solutes expected to accumulate in uremic serum in the HPLC gradient separation

Solute	Molecular mass	Retention time (s)
Ascorbic acid	176	211
Creatinine	113	259
Uracil	112	350
Urocanic acid	138	388
Uric acid	168	415
Nicotinic acid	123	469
Hypoxanthine	136	561
Xanthine	152	590
Uridine	244	653
Hydroxytryptophan	220	810
Phenylalanine	165	911
Nicotinamide	122	917
Indoxylsulfate	251	1,207
Tryptophan	204	1,244
Kynurenic acid	189	1,310
Hippuric acid	179	1,326
p-Hydroxybenzoic acid	138	1,527
p-Hydroxyphenylacetic acid	152	1,555
o-Hydroxyhippuric acid	195	1,620
Caffeine	194	1,764
Ferulic acid	194	2,190
3-Indole-acetic acid	175	2,387
Indole	117	2,708

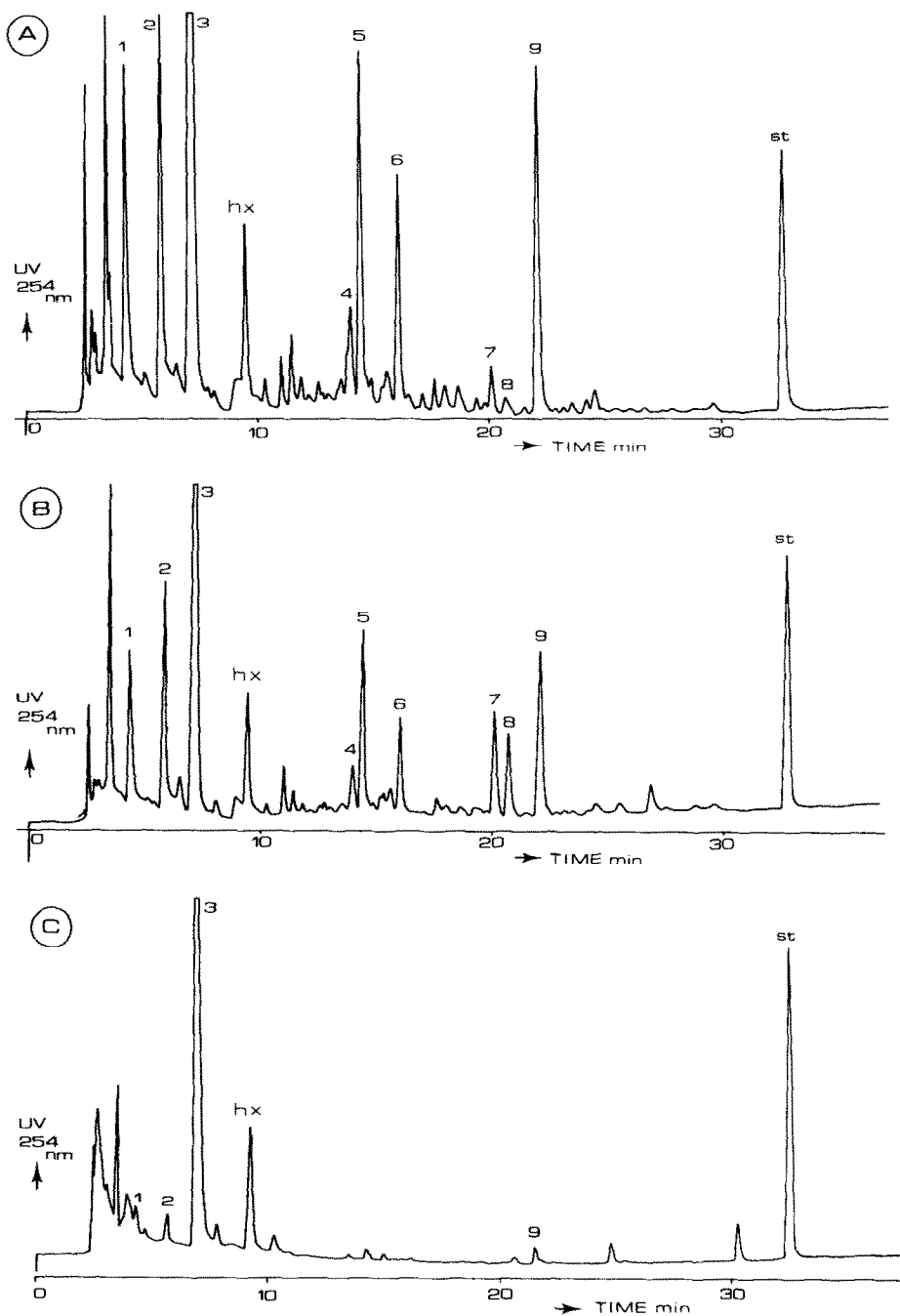Fig. 3. Characteristic HPLC profiles of ultrafiltrated uremic serum before (A) and just after hemodialysis treatment (B) and a normal ultrafiltrated serum pool (C). 1, creatinine; 2, uracil; 3, uric acid; hx, hypoxanthine; 4, unknown; 5, unknown; 6, unknown; 7, indoxylsulfate; 8, tryptophan; 9, hippuric acid; st, injection standard naphthalene sulfonic acid. This patient belongs to group 2, as described in the text.

Although the spectra of Fig. 4 are not enough for a positive identification, we based the identification of the solute uracil on retention time, UV-spectrum and mass spectrometric data. The characteristic and, in most cases, abundant peaks 4, 5 and 6 (with retention times of 825, 860 and 960 s) remain to be identified.

Results

Estimation of heterogeneity

Ultrafiltrated uremic serum samples of 33 patients on regular hemodialysis ($n = 17$) and hemodiafiltration ($n = 16$) were analyzed, in order to estimate the heterogeneity of this population with respect to the accumulation of UV-absorbing solutes. Some of the patients were followed for several months to investigate the variability of the patterns with time. The patients could be divided into three main groups according to the predialysis HPLC profiles.

The first group of 5–10 patients showed profiles (Fig. 5A) which were similar to those of healthy persons (Fig. 3C), although their creatinine levels were significantly higher.

The second group, comprising some 20 patients, had patterns that seem to be characteristic of uremia (Fig. 3A), although different gradations of accumulation are observed.

In the profiles of the third group (5–10 patients) a large number of additional high peaks are found (Fig. 5B).

The profiles of a number of patients from these three different groups were determined during a 5-mth period, and were found to be fairly constant although slight variation in peak heights did appear (Fig. 6).

In Fig. 6 HPLC profiles of two patients recorded at two months intervals, are shown. Preliminary observations indicate that differences in the profiles are at least partly related to residual renal function. No relation to the type of renal failure seems to be present in this population of patients, who all have residual renal function of less than 8 ml/min creatinine clearance.

A correlation of the patterns to patient specific clinical parameters, using multivariate statistical analysis, is presently under investigation.
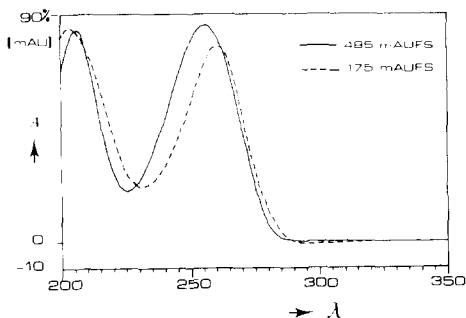
Fig. 4. Photodiode array UV-spectra of HPLC peak 2 (- - - - - -) and test solute uracil (————).

Correlation between peaks within the patterns

Nine prominent peaks, present in almost all chromatograms (as numbered in Fig. 3) were quantitated by measuring predialysis peak heights and dividing by the height of the injection standard. The peak eluting after 560 s, hypoxanthine, was not evaluated, because it showed dialysis ratios greater than, as well as smaller than unity, and also high short term variance in the patients sera. Frequency distributions of the levels of the solutes were determined. Table II presents the pretreatment free serum levels of four solutes. These data were obtained by means of the calibration lines, as described under 'Materials and Methods'. Table III shows the correlation

Fig. 5. HPLC profiles representing groups of uremic patients with low (A) and high (B) degree of accumulation of UV-absorbing solutes. Compare creatinine levels (CR) and levels of other solutes. i.s., Injection standard naphthalene sulfonic acid.
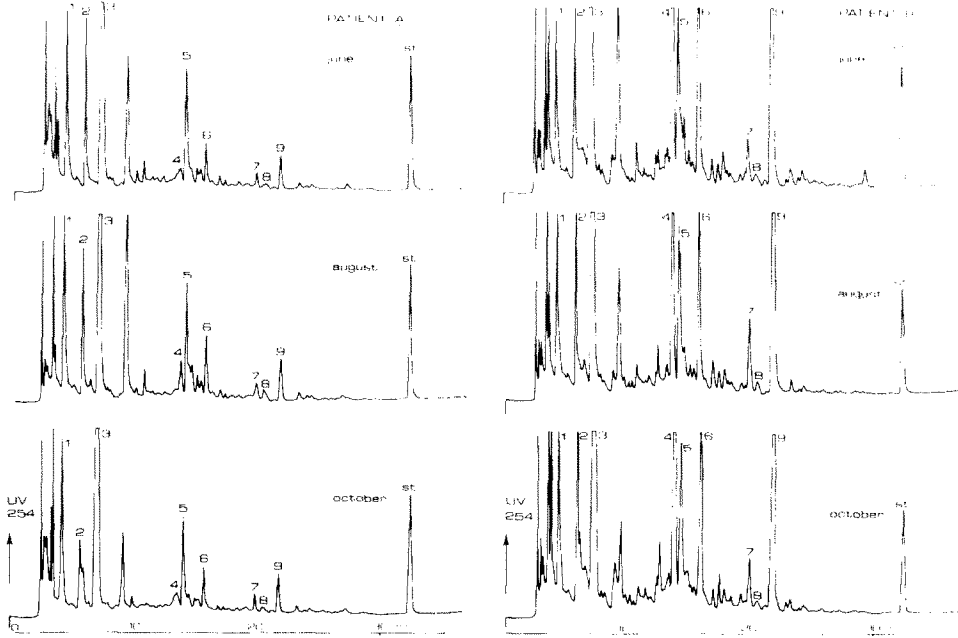
Fig. 6. Longitudinal change of uremic serum profiles during a 5-mth period.

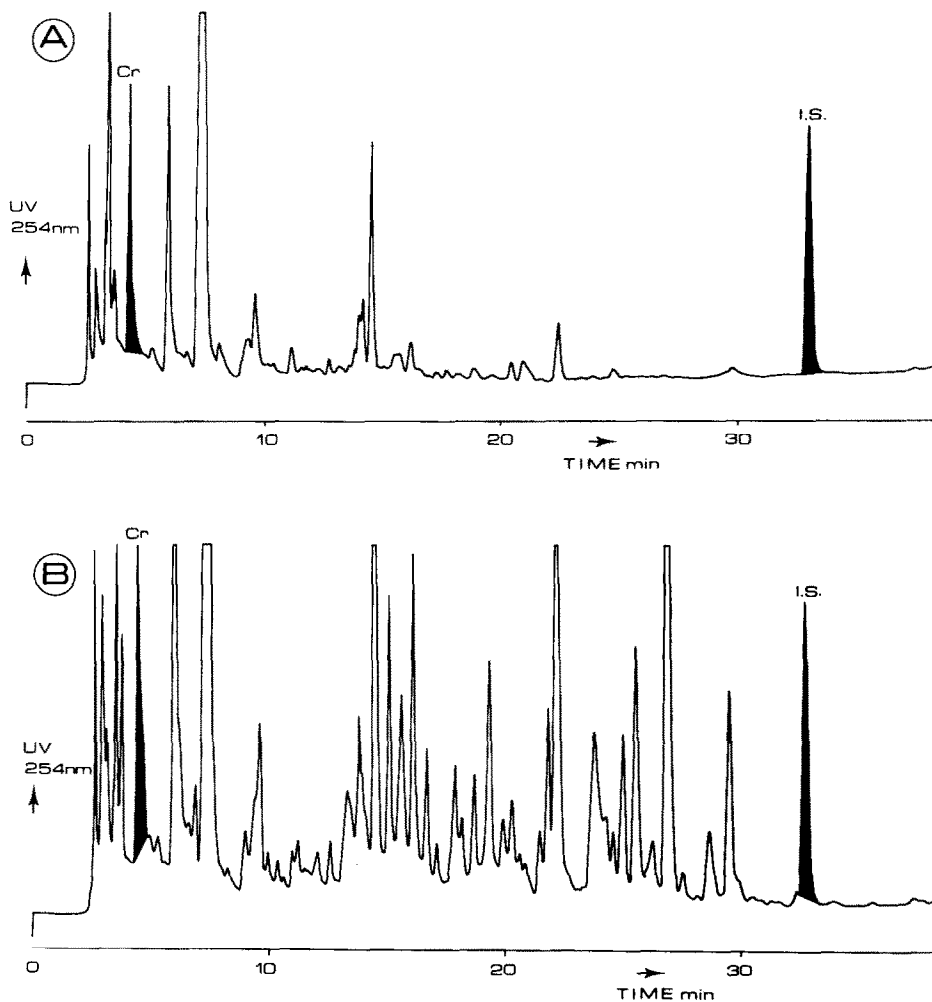matrix of the 9 peaks, and total UV-absorbance (total peak area at 254 nm) for 33 patients on hemodialysis or hemodiafiltration treatment. (This is the population from columns 4 and 5 in Table IV, with 6 additional patients on HD (3) and HDF (3) of which only predialysis serum samples were available.)

The values of the correlation coefficients and their significance (between parentheses) are given. Some interesting phenomena are observed in this matrix. There is no significant correlation between creatinine levels and those of the other solutes, nor with total peak area, at $p = 0.05$ ($r < 0.3$). This is also illustrated by creatinine peak heights in Fig. 5. On the other hand, the peak at 960 s (peak no. 6,

TABLE II

Pretreatment uremic serum levels of the non protein bound fraction of four solutes in patients on regular dialysis treatment ($n = 30$)

Solute	Concentration $\bar{x}$ (SD) (μmol/l)	Range (μmol/l)
Creatinine	1071 (285)	525–1,681
Uracil	34.2 (12.6)	8.3–77.6
Indoxylsulfate	17.9 (9.9)	1.47–40.6
Hippuric acid	240 (184)	27–635

TABLE III

Correlation matrix of predialysis solute concentrations, expressed in peak heights for nine substances present in the characteristic uremic HPLC profiles, and total UV expressed in total peak area ($n = 33$)

Solute	Creatinine	Uracil	Uric	Peak 4	Peak 5	Peak 6	Indox	Trypt	Hippuric	Total UV
Creatinine	1									
Uracil	n.s.	1								
Uric	n.s.	0.57 (0.001)	1							
Peak 4	n.s.	n.s.	n.s.	1						
Peak 5	n.s.	n.s.	n.s.	n.s.	1					
Peak 6	n.s.	0.49 (0.005)	n.s.	0.35 (0.05)	n.s.	1				
Indox	n.s.	0.37 (0.05)	0.33 (0.05)	n.s.	n.s.	0.57 (0.001)	1			
Trypt	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	1		
Hippuric	n.s.	n.s.	n.s.	0.81 (0.0005)	n.s.	0.52 (0.0025)	n.s.	n.s.	1	
Total UV	n.s.	0.66	0.49 (0.005)	0.46 (0.01)	n.s.	0.62 (0.0005)	0.53 (0.0025)	n.s.	0.66 (0.0005)	1

Correlation coefficients are presented (Pearson correlation). Significance is given between parentheses., n.s.. not significant at $p = 0.05$ ($r < 0.3$). uric = uric acid; indox = indoxylsulfate; trypt = tryptophan. Peaks 4–6 unknown.

47

TABLE IV

Dialysis efficiencies (in vivo), expressed in terms of mean dialysis ratio ($\bar{D}$) for nine solutes and total UV, in three types of artificial kidney treatment

Peak	RT (s)	$\bar{D}_{HF}$(SD) (n = 16)	$\bar{D}_{HD}$(SD) (n = 14)	$\bar{D}_{HDF}$(SD) (n = 13)	Significance of difference (Wilcoxon)		
					HF-HD	HD-HDF	HF-HDF
Creatinine	259	1.70 (0.28)	2.09 (0.33)	2.40 (0.41)	< 0.01	n.s.	< 0.005
Uracil	350	1.57 (0.23)	1.71 (0.37)	2.32 (0.88)	n.s.[a]	< 0.025	< 0.005
Uric acid	415	2.04 (0.54)	2.61 (0.72)	2.93 (0.51)	< 0.01	n.s.	< 0.005
Peak no. 4	825	2.18 (0.44)	2.25 (0.75)	2.82 (0.80)	n.s.	< 0.05	< 0.01
Peak no. 5	860	1.50 (0.30)	1.77 (0.38)	1.99 (0.32)	< 0.02	< 0.05	< 0.002
Peak no. 6	960	1.99 (0.43)	2.28 (0.50)	2.86 (0.53)	< 0.01	< 0.025	< 0.001
Indoxyl-sulfate	1,270	0.50 (0.25)	0.47 (0.23)	0.61 (0.16)	n.e.	n.e.	n.e.
Tryptophan	1,244	n.e.[b]	0.42 (0.28)	0.31 (0.13)	n.e.	n.e.	n.e.
Hippuric acid	1,326	1.64 (0.43)	1.66 (0.36)	1.97 (0.37)	n.s.	< 0.025	< 0.01
Total UV	–	1.45 (0.32)	1.70 (0.26)	2.05 (0.43)	< 0.02	< 0.025	< 0.001

[a] n.s. = not significant (at p = 0.05).

[b] n.e. = not evaluated.

Fig. 3) correlated well with four other solutes and the total peak area, at different levels of significance. The correlation of peak heights of uracil, peak no. 6, and hippuric acid, with total UV (total peak area) were very significant ($p = 0.0005$). Creatinine levels as determined by the picrate method in serum relate quite well to creatinine levels in ultrafiltrated serum determined by HPLC ($r = 0.92$, $p < 0.001$).

These results suggest that the serum creatinine level, which is commonly determined, is not an optimal measure of the degree of accumulation of UV-absorbing substances in serum of patients on chronic dialysis treatment.

Hippuric acid, uracil and peak no. 6 seem to be more optimal as a measure of accumulation of UV-absorbing substances. Moreover, from Table III it can be seen that there is a highly significant correlation between peak heights of hippuric acid and the unknown peak 4, possibly indicating a metabolic relationship between the two.

Estimation of artificial kidney treatment efficiency

In this study, the efficiency of the in vivo removal of different UV-absorbing substances during hemodialysis (HD), hemodiafiltration (HDF) and hemofiltration (HF) was estimated. Treatment duration was 4 h, 3 times/wk, in all cases. For each of the nine solutes in the HPLC profiles the concentration ratio of the substance in predialysis and postdialysis serum was determined, as a measure of the efficiency of the treatment (dialysis ratio) [11,12]. These ratios were determined for each patient treated with one of the three methods. Then a mean dialysis ratio, $\bar{D}$, was calculated per group (or per type of treatment). In Table IV these data are presented. In the last three columns the differences of the dialysis ratio in the three treatment groups are evaluated with the Wilcoxon distribution free test. It is seen that most solutes show the following sequence of treatment efficiency: $\bar{D}_{HDF} > \bar{D}_{HD} > \bar{D}_{HF}$. This phenomenon is the most prominent for the solutes uracil, hippuric acid and the unidentified peak no. 6. This also holds for the dialysis ratio calculated from predialysis and postdialysis total UV (254 nm).

It must be stressed that the dialysis ratio is only indicative of the efficiency of treatment. Blood concentrations during and after dialysis are influenced by mass transfer across the dialysis membrane, but also by generation rate and transfer across membranes between compartments.

It is seen that, while the difference of $\bar{D}_{Creat,HD}$ and $\bar{D}_{Creat,HDF}$ is not significant at $p = 0.05$, the corresponding values for uracil, hippuric acid peak no. 6, and total UV peak area are ($p < 0.025$).

The difference of $\bar{D}$ values for indoxylsulfate and tryptophan in the three groups are not evaluated for reasons of high variance. In all patients the solutes indoxylsulfate and tryptophan had dialysis ratios smaller than unity. This means that the concentration of the free solutes is higher in the patient's serum just after treatment compared to predialysis levels. Mean values (and SD) of pre- and post dialysis, non-bound indoxylsulfate levels in the sera of 30 uremic patients were 17.9 (9.9 SD) and 35.8 (20.8 SD) μmol/l, respectively.

This possibly is a result of heparinization during artificial kidney therapy. It is suggested [13] that heparin releases the enzyme lipoprotein lipase from tissue, which

promotes the hydrolyzation of triglycerides to form free fatty acids (FFA), which could act as competitors to tryptophan and indoxylsulfate protein binding, resulting in high postdialysis levels of the free solutes.

The data on the relative efficiencies of solute removal in the three methods are in accordance with observations reported in literature. It is known that hemofiltration, which was designed to remove so-called 'middle molecules' is less efficient with respect to low molecular mass substances than hemodialysis [14].

Hemodiafiltration, which is applied with the purpose of combining the benefits of both diffusion (hemodialysis) and convection (hemofiltration) [15] is shown to remove the low molecular mass solutes better than each of the methods separately. This suggests that the average therapy duration in the whole group of patients might be shorter in hemodiafiltration compared to the 4-h hemodialysis.

The predialysis levels of the nine solutes in hemodialysis and hemodiafiltration were compared. Only peak no. 4 (825 s), indoxylsulfate and hippuric acid were present in significantly lower concentrations in predialysis serum of patients on hemodiafiltration ($p = 0.005, 0.05, 0.03$ respectively). This phenomenon is presently under investigation.

Conclusion

The automated HPLC profiling technique for UV-absorbing solutes was found to be reliable and reproducible in the analysis of more than 500 samples. It yields information on accumulating substances with rather low molecular masses, related to protein, amino acid and nucleic acid metabolism.

About 75 samples can be analyzed weekly, enabling studies in which a large number of samples are to be investigated, such as patient heterogeneity, longitudinal, time-concentration, and in vivo and in vitro dialysis studies. Here, only nine solutes were 'quantitated' by peak height. However, it seems possible to quantitate 15–20 substances, in a single run HPLC gradient elution analysis, after appropriate identification of peaks. A number of peaks were identified, but the nature of three, very characteristic and prominent peaks remains to be established.

The population of uremic patients in the present study, could roughly be divided into three groups, as described in the text. Only slight longitudinal changes of the HPLC patterns of individual patients were found. These findings suggest the possibility of multivariate statistical analysis and correlation to patient specific parameters. This is presently under investigation and will be reported elsewhere. A comparison of in vivo dialysis efficiency in hemofiltration, hemodialysis and hemodiafiltration showed that the latter is the superior method for the removal of most solutes in the HPLC chromatograms.

Again, this in vivo dialysis ratio is only indicative for the efficiency of treatment. In vitro dialysis, and time concentration studies are necessary to obtain more exact data on the behavior of these solutes in the uremic patient/artificial kidney system. Individual data on dialysis ratio are presently related to and possibly corrected for, body weight, residual renal function, membrane type, protein catabolic rate etc. Selectivity data on the removal of different solutes with different dialysis strategies,

variations of membrane surface area and filtration rate, as suggested earlier by Sprenger [14], may lead to the design of, more patient-aimed, therapies.

Acknowledgement

References

1 Senftleber FC, Halline AG, Veening H, Dayton DA. Reversed phase liquid chromatographic analysis of hemodialysate from uremic patients. Clin Chem 1976; 22: 1522–1527.

2 Knudsen EJ, Lau YC, Dayton DA. Time-concentration studies by high-performance liquid chromatography of metabolites removed during hemodialysis. Clin Chem 1978; 24: 686–691.

3 Mabuchi H, Nakahashi H. Liquid-chromatographic profiling of endogenous fluorescent substances in sera and urine of uremic and normal subjects. Clin Chem 1983; 29: 675–677.

4 Swan JS, Kragten EY, Veening H. Liquid chromatographic study of fluorescent materials in uremic fluids. Clin Chem 1983; 29: 1082–1084.

5 Mohammed HY, Veening H, Dayton DA. Liquid chromatographic determination and time-concentration studies of riboflavin in hemodialysate from uremic patients. J Chromatogr 1981; 226: 471–476.

6 Hung Y, Kai M, Ohkura Y. High performance liquid chromatographic analyzer for guanidino using benzoin as a fluorogenic reagent. J Chromatogr 1984; 305: 281–294.

7 Schoots AC, Mikkers FEP, Claessens HA, De Smet R, Van Landschoot N, Ringoir SMG. Characterization of uremic middle molecular fractions by gas chromatography, mass spectrometry, isotachophoresis and liquid chromatography. Clin Chem 1982; 28: 45–49.

8 Gallice P, Fournier N, Crevat A, Briot M, Frayssinet R, Murisasco A. Separation of one uremic middle molecules fraction by high performance liquid chromatography. Kidney Int 1983; 23: 764–767.

9 Mabuchi H, Nakahashi H. Medium-sized peptides in the blood of patients with uremia. Nephron 1983; 33: 232–237.

10 Lindblom H, Axiö-Frederiksson UB, Cooper EH, Turner R. Separation of urine proteins on the anion-exchange resin mono Q$^{(TM)}$. J Chromatogr 1983; 273: 107–116.

11 Schoots AC, Mikkers FEP, Cramers CAMG, Ringoir SMG. Profiling of uremic serum by resolution gas chromatography, electron impact, chemical ionization mass spectrometry. J Chromatogr 1979; 164: 1–8.

12 Schoots A, Mikkers F, Cramers C, De Smet R, Ringoir S. Uremic toxins and the elusive middle molecules. Nephron 1984; 38: 1: 1–8.

13 Farrell PC, Grib NL, Fry DL, Popovich RP, Broviac JW, Babb AL. A comparison of in vitro and in vivo solute protein binding interactions in normal and uremic objects. Trans Am Soc Art Intern Organs 1972; 18: 268–276.

14 Sprenger KBG. Review haemodiafiltration. Life Support Systems 1983; 1: 127–136.

15 Leber HW, Wizemann V, Goubeaud G, Rawer P, Schütterle G. Simultaneous hemofiltration/hemodialysis: an effective alternative to hemofiltration and conventional hemodialysis in the treatment of uremic patients. Clin Nephrol 1978; 9: 115–121.