Comparison of Solid-Phase Extraction and Dialysis on Pretreatment Efficiency of Blood Urea Analysis

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Abstract

Although the analysis of urea in blood using high-performance liquid chromatography has been reported, its automated solid-phase extraction (SPE) has not been reported. In addition to these two methods, dialysis may also be used to pretreat blood samples. The separation efficiency of automated SPE is discussed and compared with automated dialysis using a condensing column. The results indicate that automated SPE and dialysis have an almost identical separation efficiency for urea analysis. However, the dialysis technique is not as efficient for the removal of admixtures eluted after urea.

Introduction

Urea is a major toxin that accumulates in the blood of uremia patients. It causes disorders of the urea cycle due to feedback inhibition, and it denatures several proteins. There are two types of urea: free and protein bound. Free urea analysis is essential in the evaluation of patients. However, in the currently used clinical test, only the total urea content in blood is measured using an ammonium-selective electrode and an immobilized urease membrane (1,2). This method is insufficient for the differential analysis of urea from endogenous ammonium. Additionally, this procedure is insufficient for the differential analysis of free from bound urea. Therefore, for differential analysis, an appropriate pretreatment method is required, such as dialysis and ultrafiltration. Native blood is used in the analysis of free urea, and denatured blood with added acid is used in total (free plus bound) urea analysis with the dialysis or the ultrafiltration method. The bound urea amount is found by subtracting the free urea amount from the total amount.

Recently, several papers have reported the analysis of urea in blood using an immobilized urease column set preceding or following the analytical column. However, in order to attain the differential analysis of endogenous ammonium and urea, a complicated procedure that is not appropriate for routine analysis is required (3–5). The analysis of urea in food has been reported using urease, but differential analysis of endogenous ammonium and urea was not attainable following this method (6).

Several pretreatment methods for separating admixtures in the matrix have been reported (1). Techniques including ultrafiltration, dialysis, supercritical fluid extraction, and solid-phase extraction (SPE) are currently available and are being researched as pretreatment methods (7–9). Supercritical fluid extraction is the only technique that has a restriction in that hydrophobic compounds cannot be extracted.

In this work, urea was separated from blood admixtures using pretreatment rather than column separation. The separation efficiency of automated on-line SPE using a conventional strong cation-exchange resin is discussed and compared with the efficiency of an automated on-line dialysis method using a condensing column.

Experimental

Materials

Urea and blood were obtained from Wakoh and Kantoh Kagaku (Tokyo, Japan), respectively. The other reagents used were HPLC grade.

Methods

Automated SPE

The BenchMate was from Zymark (Hopkinton, MA). A strong cation-exchange resin column (H type, Bond Elut SCX) was obtained from Varian (Harbor City, CA). It had a weight of 500 mg and a void volume of 2.8 mL. The column was conditioned with 3 mL methanol followed by 3 mL water at a flow rate of 3 mL/min. It was rinsed with 1 mL water at a flow rate of 3 mL/min. The column was eluted with 4 mL 5% phosphoric acid solution at a flow rate of 1 mL/min. Conditioning, rinsing, and elution were carried out under vacuum using a vacuum pump from Iwaki (AP-115 AN; Tokyo, Japan).

High-performance liquid chromatograpy (HPLC) was carried

out after SPE on an MCI GEL CK 08S column, which is a polymer-based strong cation-exchange resin column (Na type). Its dimensions were 4.6 × 150 mm, 11–14 µm particle diameter. The pore diameter was unspecified by the supplier (Mitsubishi Kasei; Tokyo, Japan). Other conditions were as follows: eluent, 1mM HCl solution; flow rate, 1 mL/min; detection, 200 nm; application volume, 20 µL; and column temperature, 35°C. The Model PU-980 HPLC and Model PU-970 UV detector were from Nihonbunkoh (Tokyo, Japan).

Automated dialysis

The ASTED and trace enrichment column were from Gilson (Villiers-le-Bel, France). A polymer-based strong cation-exchange resin column (Na type) was used for condensing the sample. The resin weight was 20 mg. The column was conditioned with 1.5 mL 1M sulfuric acid followed by 0.9 mL water at a flow rate of 2 mL/min. The other conditions were as follows: dilutor 1, 0.01% TritonX 100; dilutor 2, 5mM phosphate buffer at pH 7.4. The cut-off molecular weight for dialysis membrane (cellulose) was 15,000 Da.

The HPLC procedure used after the ASTED treatment was identical to the one used for SPE except that the flow rate was 1.5 mL/min.

Results and Discussion

When a small-capacity strong cation-exchange resin was used for ion chromatography, the separation of urea from blood admixtures was not satisfactory (10). Therefore, the use of a conventional large-capacity strong cation-exchange resin was studied. There are two types of strong cation-exchange resin columns: a silica-based column (4.6 × 250 mm, 10-µm particle diameter) (TSK SP2SW, Toso; Tokyo, Japan) and a polymer-based column (MCI GEL CK 08S). The polymer-based column was selected because the primary amine in urea may cause tailing when it comes in contact with residual silanol in the silica-based column. It was expected that the polymer-based column might have a smaller exchange capacity compared with the silica-based column; this would result in a smaller theoretical plate number and possibly in an unsatisfactory separation of urea from blood admixtures if pretreatment of SPE or dialysis was not successfully attained (especially in the case of the dialysis method). However, this kind of inferiority was not significantly observed in this experiment.

HPLC chromatograms obtained after automated SPE or

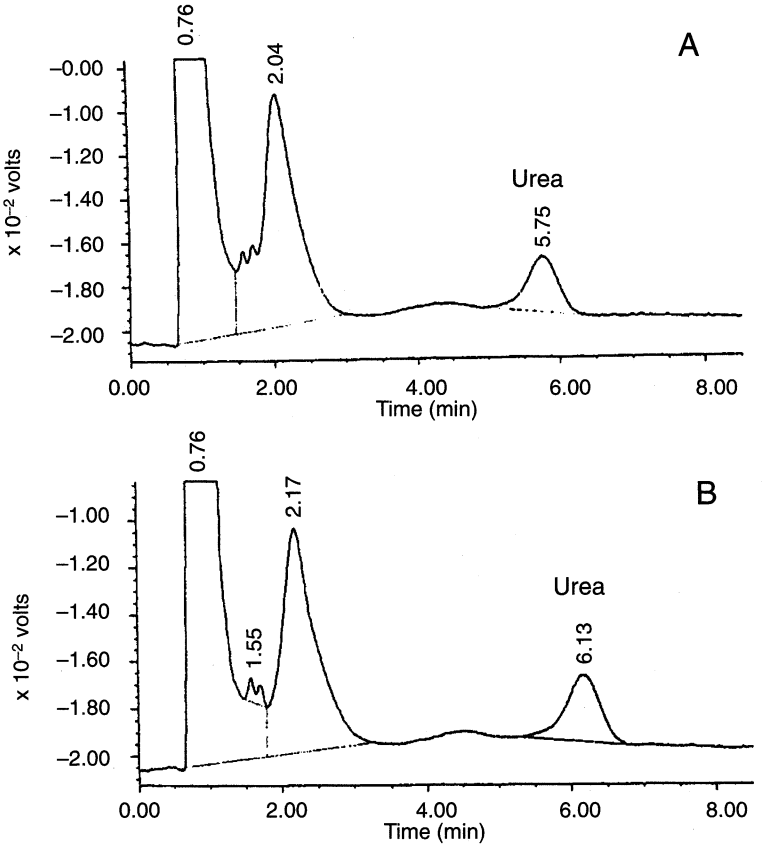

Figure 1. HPLC chromatogram after SPE treatment: A, blank blood; B, blood spiked with urea (0.1 mg/mL).

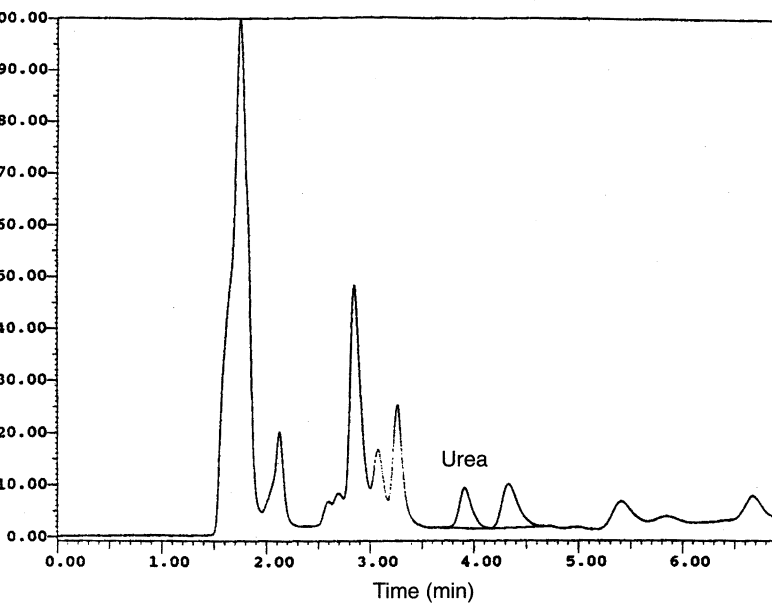

Figure 2. HPLC chromatogram of blood after pretreatment with dialysis.

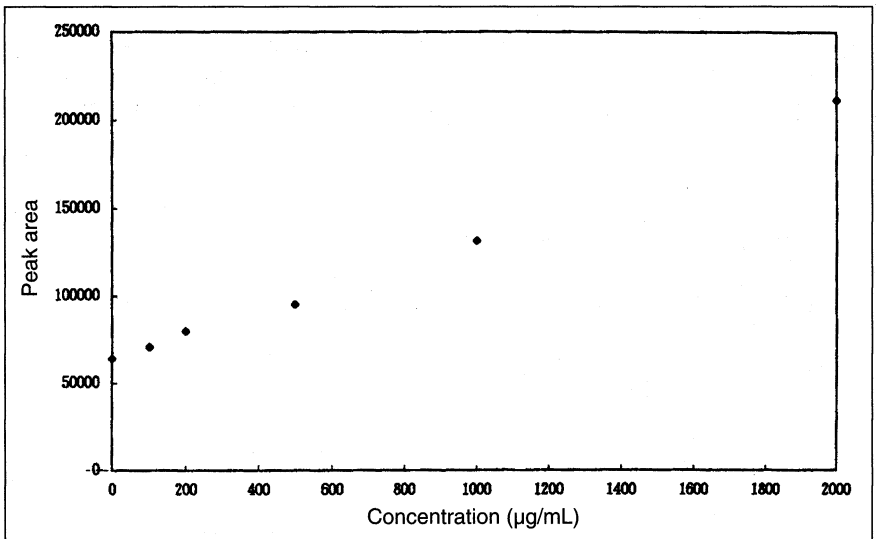

Figure 3. Calibration curve of spiked urea after dialysis treatment with ASTED.

ASTED treatment (automated dialysis) are shown in Figures 1 and 2, respectively. Figure 1 shows the chromatograms of a blank blood sample (A) and a spiked sample (0.1 mg/mL) (B). As shown in Figure 1, chromatograms obtained after SPE treatment indicated that the urea was successfully separated from blood admixtures. The recovery rate of spiked urea was 101.9%, with a relative standard deviation of 2.2% ($n = 4$), indicating a satisfactory separation. The standard curve used for HPLC indicated linearity over the endogenous urea amount.

Figure 2 shows the chromatogram obtained after ASTED treatment; several peaks appear in addition to the urea peak because compounds less than 15,000 Da were eluted into the dialysate. The capacity of the trace enrichment column was insufficient, so urea and the blood admixtures were not well separated compared with the results obtained for SPE. The separation was satisfactory, however, when dialysis was used. In addition, when trace-enrichment columns are used, cleanup is occasionally necessary in routine analysis. Recovery results of urea from blood by dialysis were reproducible even though the recovery rate was lower (around 10%). Reproducibility of the recovery rate was confirmed by the linearity of the spiked urea peak area, as shown in Figure 3 (the coefficient of correlation of the regression line in Figure 3 was 0.98). The amount of endogenous urea was calculated from Figure 3 by the standard addition method and found to be 0.82 mg/mL.

In Figure 2, baseline separation of urea from blood admixtures is obtained both before and after urea elution. The benefit of using dialysis is that the differential analysis of bound and free urea can be attained. It is not possible to attain differentiation using SPE alone. However, if this technique is combined with ultrafiltration, the method is successful.

In terms of separation, both SPE and automated dialysis using a condensing column had identical separation efficiency. Dialysis treatment was inferior to SPE due to its insufficient capacity to remove admixtures, but it was superior to SPE because differential analysis could be obtained simultaneously.

The combination of ASTED and automated SPE in series would be desirable to attain a differential analysis, remove admixtures, and attain a correct diagnosis. Dialysate can be injected into an automated SPE system and analyzed by HPLC. This method is appropriate for differential analysis and removal of admixtures.

Conclusion

The separation efficiency of automated SPE and automated dialysis using ASTED were almost identical for the separation of urea in blood even though the recovery rate between the techniques differed. Dialysis treatment was inferior to SPE for the removal of blood admixtures, but it was superior for simultaneous differential analysis. The use of ASTED and automated SPE in series would be desirable to obtain differential analysis, remove admixtures, and attain superior diagnosis.

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