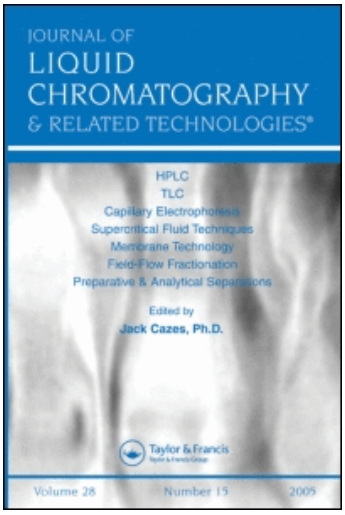

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Selection of Columns for Analysis of Blood Urea

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SELECTION OF COLUMNS FOR ANALYSIS OF BLOOD UREA

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ABSTRACT

For the analysis of blood urea, the author used several kinds of columns such as conventional ion exchange chromatographic column, ion chromatographic column and reverse phase columns. The result of comparison for separation efficiency will be discussed and conventional ion exchange chromatographic column indicated a best separation efficiency. Prior to application to the columns, blood urea was treated with solid phase extraction (SPE) with a strong cation exchange column with SO_3H functional group.

INTRODUCTION

In order to avoid overlapping of blood admixtures to determine the compound of interest in blood, pretreatment using solid phase extraction (SPE), liquid-liquid extraction and chromatographic separation will be required. In advance before the determination of the compound, the reader must clarify whether the eluted peak was not overlapped and confirm totally pure using several kind of detection method such as photo diode array or mass spectrometry and so on. These detectors are expensive. The simple way to avoid overlapping in HPLC is to purify the compound of interest prior to HPLC application. Using solid phase extraction (SPE) prior to HPLC application, sufficient separation of compound of interest from blood admixtures will be attainable. Concerning SPE of blood

urea, the paper is now under evaluation[1]. In this paper the author will present the comparison of separation efficiency of conventional ion exchange chromatographic column, ion chromatographic column and reverse phase columns for the separation and determination of blood urea.

MATERIALS AND METHOD

Blood was supplied from Wako and other reagents were special grade commercially available.

For the analysis of blood urea, ion exchange chromatographic column of Mitsubushi Kasei MCI^R CK 08S strong cation exchange column (SO_3H type, 4.6X 150 mm), ion chromatographic column of Wescan strong cation exchange column 269-004 (SO_3H type, 4.6X 250 mm), reverse phase column of Capcell Pak C-18 SG120 (4.6X 250 mm, 5 μm) from Shiseido and reverse phase column column of Capcell Pak C-18 AG120 (4.6X 250 mm, 5 μm) were compared with separation efficiency. The column capacity and particle size of MCI^R CK 08S and Wescan strong cation exchange column 269-004 was as follows: more than 1.9 meq/ml and 11-14 μm and 0.03 meq/ml and 10 μm, respectively. The material of the former is SDB polymer base (degree of crosslinking of DVB is 8%) and that of the latter is silica base, therefore residual silanol effect must be considered for the latter. The eluent of ion exchange columns is 1.5 mM HCl aqueous solution and urea is detected by ultraviolet (UV) at the wavelength of 210 nm. Flow rate is 2 ml/min.

The material of the reverse phase columns was silica with a trace amount of heavy metals and silicone was coated to prevent the residual heavy metal effect as well as residual silanol effect which may cause tailing phenomena. The eluent of reverse phase columns is a mixture of an aqueous solution of water and acetonitrile at a ratio of 90/10 adjusted to pH 9 with phosphate buffer and detection is by UV at the wavelength of 210 nm. Flow rate is 1 ml/min. HPLC apparatus of ion exchange chromatography and reverse phase chromatography was SP-8750 from Spectra-Physics.

SPE of blood urea using strong cation exchange column is as follows: blood is acidified at pH 3 with HCl or used as is. These were applied for ultrafiltration by centrifugation at 4,000 rpm using a Centricon^R (cut-off molecular weight 10,000 daltons) supplied by Amicon and the centrifugated solution was acidified to pH3 with HCl. These were applied to the strong cation exchange column (SO_3H type) of Bond Elut^R SCX (500 mg of resin weight and 0.6 ml of void volume) supplied by Analytichem (Harbor

City, CA, USA). The column was conditioned with 2 ml of methanol followed by 2ml of 0.1M HCl aqueous solution[1]. One ml of blood was applied to the conditioned SCX column at an application flow rate of 0.3 ml/min and washed with 2 ml of water. The retained urea on SCX column was eluted with 2 ml of 1M HCl aqueous solution at the flow rate of 0.3 ml/min. Conditioning and elution in SPE were carried out using a Model AP-115 AN vacuum pump supplied by Iwaki (Tokyo, Japan)[1]. Thus the treated blood urea was applied to HPLC with a column of MCI^{R} CK 08S.

When using ion chromatographic column and reverse phase columns for blood urea analysis, the recommended SPE procedure is as follows: the series of C-18 column and the strong cation exchange column were connected in this order. C-18 column is for removing hydrophobic compounds in blood. The treatment procedure of the strong cation exchange column used for SPE is the same as mentioned above. The conditioning method of C-18 column used for SPE is as follows: C-18 column has 500 mg resin weight and 0.6 ml void volume, thus the conditioning procedure is 2 ml acetonitrile followed by 2 ml water and the conditioned column was set prior to the strong cation exchange column. The conditioning procedure was identical to that presented in the paper[2]. The above treated blood was applied to ion chromatographic column and reverse phase columns.

RESULTS AND DISCUSSION

I Comparison of separation efficiency between ion exchange chromatographic column and ion chromatographic column

The major difference between them is a column capacity. Chromatograms obtained by ion exchange chromatography and ion chromatography, respectively, was presented in Figures I and II. As shown in Figure I, after SPE treatment blood urea was completely separated from blood admixtures. On the contrary, in Figure II by ion chromatography after SPE treatment the chromatogram was found to be successfully separated from blood admixtures, however many admixtures was still present. If the column deteriorates in the process of time and theoretical plate number is decreased, the separation will become worse, thus the former is more superior to the latter.

However there are several ways to overcome these weak points. One is the use of lower flow rate. The other is the use of C-18 column in SPE prior to the strong cation exchange column to remove hydrophobic components by C-18. By the latter procedure, the peaks after elution of urea was diminished. In Figure II, the chromatogram treated

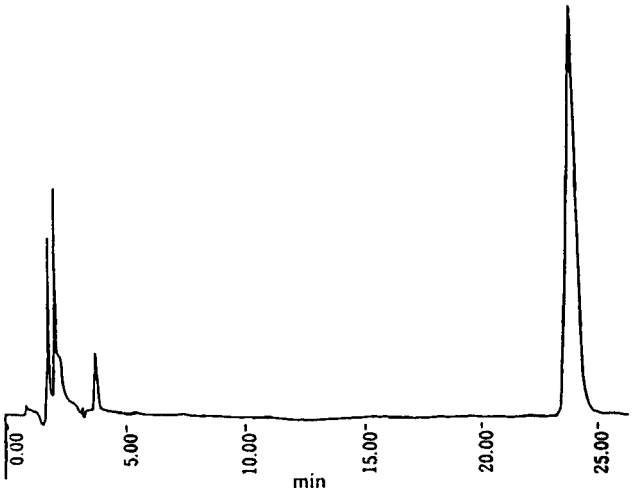

FIGURE I HPLC chromatogram of blood urea after SPE treatment by conventional ion exchange chromatographic column of MCI^{R} CK 08S

The peak eluted at around 24 min is blood urea.

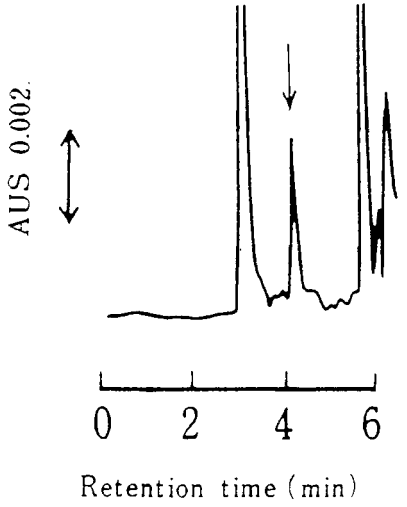

FIGURE II HPLC chromatogram of blood urea after SPE treatment by ion chromatographic column of Wescan 269-004

The peak eluted at around 4 min is blood urea.

without the use of C-18 column for SPE was presented. When using C-18 in addition to the strong cation exchange column for SPE, the peaks eluted after urea in Figure II was diminished.Therefore, both ion exchange columns are found to be appropriate to the analysis of blood urea combined with SPE if the series of C-18 and the strong cation exchange columns were used when using ion chromatographic column of Wescan 269-004. The conventional strong cation exchange column of MCI^{R} CK 08S is superior to ion chromatographic column due to needlessness of C-18 column for SPE.

These results indicated that several factors must be simultaneously considered for the selection of columns.

II Blood urea analysis using SPE combined with reverse phase columns[3-6)]

Urea is a weak alkalinized compound, thus the author suspect when the alkalinized eluent was used, ionization of urea was suppressed and neutralized urea will retain in the reverse phase columns. Therefore, eluent was alkalinized at pH 9 in order to suppress ionization of urea.

Blood urea was treated with SPE procedure as mentioned in the experimental section and applied to HPLC with reverse phase columns. The blood urea was not successfully retained in C-18 column even if ionization of urea was successfully suppressed. This is due to degree of hydrophobicity of suppressed urea. The hydrophobicity of suppressed urea is insufficient as to retain in C-18 column. The k' from void volume to urea elution is around one and around there many blood admixtures eluted, which were mostly hydrophilic compounds unsuccessfully removed by C-18 column for SPE treatment, thus lead to unsuccessful separation. This phenomena is same in AG120 as well as in SG120.

After SPE treatment, urea was acidified and existed as ureonium, however this acidity will be neglected compared with the amount of alkalinized eluent, therefore urea in eluent was sufficiently alkalinized and suppressed ionization.

The alternative method is the use of C-8, phenyl or cyclhexyl in place of C-18 column. However, the author speculates significant improvement of separation will not be attained by those columns. Therefore, ion exchange chromatography will be more appropriate to the analysis of blood urea combined with SPE.

CONCLUSION

Urea was analyzed by conventional ion exchange chromatographic column, ion chromatographic column, reverse phase column after SPE treatment of blood.

Conventional ion exchange chromatographic column was found to be superior to the ion chromatographic column and reverse phase columns. This was found to be due to significant retention in the column with a greater capacity.

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