Reversed-Phase High-Performance Liquid Chromatography with a New Column for Analysis of Urinary Proteins


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Dear Sir,

Analysis of urinary protein is useful for detecting disorders in various renal diseases. The urinary low-molecular-weight proteins such as β2-microglobulin (β2-MG), retinol-binding protein (RBP), and lysozyme (LZM) are very sensitive indices of impaired renal tubular function [1, 2], while the urinary excretion of human serum albumin (HSA), and IgG increases in glomerular damage. Various methods have been used for detecting these urinary proteins [3,4]. Recently, high-performance liquid chromatography (HPLC) has been recommended as a suitable screening method because of its simplicity and fastness [5]. We present here a reversed-phase HPLC with a new column for analysis of urinary proteins. The chromatographic system consisted of a Hitachi 655A-11 liquid chromatograph, an L-5000LC controller, A-655A valuable wavelength UV monitor and a D-2000 chroma-tointegrator. We used IPG PACK ODS column (Hitachi, Ltd.) packed with polyporous glass, mainly made of SiO2, Al2O3, B2O3. The urine samples were centrifuged at 4°C and stored at -20 °C until use. Urine samples (10–100 µl) were injected into the chromatographic system without any further precolumn procedure. A linear gradient consisting of 1.0 ml trifluoroacetic acid per liter of water and 0.7 ml trifluoroacetic acid per liter of acetonitrile was used at a flow rate of 1.0 ml/min. The detection wavelength was 210 nm. As a result, the linearity of the calibration curves for standard proteins and the sensitivity of this method were excellent. β2-MG, LZM, α-acid glycoprotein (α-AGP) and HSA were used as standard proteins. The minimum detectable amount of each protein was 0.025 µg for β2-MG, 0.05 µg for LZM, 0.25 µg for α-AGP and 0.2 µg for HSA. Reproducibility was high, the coefficients of variation for standard proteins being from 1.8 to 7.5%. The correlation between the RIA and the HPLC assay of β2-MG was good (r = 0.99, p < O.O1). The two HSA assays also correlated well (r = 0.99, p < O.O1). Figure 1 shows some representative chromatograms. Figure 1A shows the pattern of standard proteins, figure 1B shows the pattern of urinary proteins from healthy subjects. The pattern of urinary proteins from patients with nephrotic syndrome and Lowe’s syndrome are shown in figure 1C and 1D, respectively. β2-MG, α-AGP and HSA were eluted as sharp peaks at 16.39–16.86 min, 21.95–22.38 min and 30.51–31.87 min, respectively. The analysis of urinary proteins
in renal diseases has recently been carried out by various HPLC methods. Gel permeation chromatography, through which proteins are separated according to their molecular weights, was used for the separation of urinary proteins [6]. However, this method is not so sensitive, particularly for the separation of low-molecular-weight proteins. Ion-exchange chromatography requires, as a first step, removal of low-molecular-weight substance before analysis [5,7]. The reversed-phase HPLC with a C₁₈-column [8] provided a fast and good separation of urinary proteins, which makes it possible to detect microalbuminuria. Nevertheless, the sensitivity of this method was inadequate for detecting β₂-MG in human urine compared with our method with the new column. This new IPG PACK ODS column has the advantage of being mechanically strong, resistant to strong acid and heat stable till 600 °C. The pore size of this material is almost unchangeable against mechanical pressure or chemicals. By using reversed-phase HPLC with this new column, we could obtain sharper peaks of a chromatogram and easily identify urinary proteins by their retention times. Moreover, both β₂-MG/HSA and α₁-AGP/HSA ratio which were calculated by comparing each peak area in the chromatograms may be good indicators in differentiating the proteinuria of various renal diseases. Furthermore, before analysis, samples need centrifugation only once. With regard to simplicity, fastness and sensitivity, this method is suitable for the use in routine screening and quantification of urinary proteins of various renal diseases.

Reversed-Phase HPLC for Analysis of Urinary Proteins

Fig. 1. Chromatograms of standard proteins (A); normal urine (B); urine from patients with nephrotic syndrome (C) and Lowe’s syndrome (D). The numbers indicate the time of elution. Peaks: 1, β₂-MG; 2, α₁-AGP; 3, transferrin; 4, HSA.

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References


