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 β 2-microglobulin (β 2-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, aL-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 μ l) 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. β 2-MG, LZM, α 1-acid glycoprotein (α 1-AGP) and HSA were used as standard proteins. The minimum detectable amount of each protein was 0.025 μ g for β 2-MG, 0.05 μ g for LZM, 0.25 μ g for α 1-AGP and 0.2 μ g 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 β 2-MG was good ($r = 0.99$, $p < 0.01$). The two HSA assays also correlated well ($r = 0.99$, $p < 0.01$). 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. β 2-MG, α 1-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

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