Determining of “True” Serum Creatinine by High-Performance Liquid Chromatography Combined with a Continuous-Flow Microanalyzer

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We coupled a high-performance liquid chromatograph with a continuous-flow microanalyzer to produce a system for specifically determining “true” creatinine in urine and serum specimens. A selective microbore pellicular cation-exchange column and a single eluting sodium citrate buffer are used to separate the noncreatinine but Jaffé-positive constituents from the creatinine in normal and experimental specimens. The effluent is analyzed continuously, on-line, the alkaline picrate complex being developed and measured in the microanalyzer. Physiological samples, reference standards, and internal control specimens are assayed in 6-min intervals subsequent to the initial injection. The relationship between concentration and peak area is linear for creatinine standards between 5 and 10 mg/liter. Specimen volumes ranging from 1 to 25 μl, and containing creatinine in amounts exceeding 5 ng per injected sample, can be assayed with this system.

Additional Keyphrases: creatinine in urine · non-creatinine Jaffé-positive constituents

Measurement of serum creatinine has been assuming a steadily increasing role in assessing the progress of the patient with reduced renal function (1–3), but current methods are not specific, and so investigators have sought to develop simpler and more specific procedures for measuring this constituent in physiological fluids.

Encouraged by several recent developments in instrumentation (4, 5), we describe a simple and unique procedure for determining “true” creatinine in serum and urine. By coupling a pump intended for high-performance liquid chromatography to a miniaturized continuous-flow analyzer, we can separate and measure creatinine in untreated serum and urine specimens accurately and reproducibly.

Any interfering Jaffé-positive compounds in the sample are removed by the cation-exchange column before the creatinine is eluted. With this new technique, one now can detect subtle metabolic differences in the clinical profile of the patient with reduced renal function.

Materials and Methods

A Varian LC series 4100 positive-displacement syringe pump (Varian Aerograph, Walnut Creek, Calif. 94598),3 coupled directly to a miniaturized continuous-flow analyzer, was used in this study. The miniaturized analyzer described by Neeley and coworkers (6–8) was originally designed to perform various types of routine analyses at an accelerated rate and reduced cost per analysis. In the design of this new system, we focused on improving specificity for creatinine.

The layout of the system, shown diagrammatically in Figure 1, consists of the following components:

A 1.0 meter by 2.1 mm (i.d.) stainless-steel column, dry-packed with HS-Pellionex-SCX, a pellicular strong cation exchanger (Whatman, Clifton, N. J. 07014) that can be used at temperatures up to 80 °C. Cross-linked polystyrene and sulfonic acid groups form the active stationary and ionogenic phases of the exchanger, respectively. The resin’s capacity is about 9 microequiv- alents per gram of packing material. This particular type of exchanger is used in special cases where speed of analysis is of primary concern.

A 37 °C heating bath, containing a 1.14 mm (i.d.), 25-turn, polyethylene tube wrapped on aluminum rod, 12 mm in diameter, the modification by Neeley et
al. (4) of a heating bath from Acculab, Division of Precision Technology Inc., Norwood, N. J. 07648.

An AutoAnalyzer proportioning pump, geared to half speed [as modified by Neely et al. (4); Technicon Instruments Corp., Tarrytown, N. Y. 10591]. A Model 20, analog-to-digital multiplex converter (Terrasyn, Inc., Longmont, Colo. 80501).

A Model 9810 programmable calculator (Hewlett-Packard, Loveland, Colo. 80537).

A Model 9865A cassette recorder (Hewlett-Packard).

A Model 9862A X-Y plotter (Hewlett-Packard).


Reagents

Pure reference standards of creatinine (SRM No. 914, National Bureau of Standards, Washington, D. C. 20760) were used to prepare all working standards. Analytical-grade sodium pyruvate, glucose, oxaloacetic acid, α-ketoglutaric acid, ascorbic acid, and acetoacetic acid were purchased from Sigma Chemical Co., St. Louis, Mo. 63178. “Monitrol II” control serum was obtained from Scientific Products, Columbia, Md. 21045.

Saturated picric acid (Technicon) and 0.5 mol/liter NaOH (Fisher Scientific Co., Pittsburgh, Pa. 15219) were used to prepare the alkaline picrate reagent. The sodium citrate buffer (50 mmol of solution Na+ per liter) was prepared by adjusting a 50 mmol/liter solution of NaOH to pH 4.25 with monohydrated citric acid (Mallinckrodt, Inc., St. Louis, Mo. 63147).

Procedure

The dry-packed column contents were converted to the sodium form with the citrate buffer. Degassed sodium citrate buffer was used as the mobile phase, at a flow rate of 0.33 ml/min. All separations were done at ambient temperatures, at column pressures ranging between 30 and 33 kg/cm². The column effluent flowed directly into a mini-creatinine manifold, where it was mixed with saturated picric acid and sodium hydroxide in a volume ratio of 1.8/1/1 (0.33 ml/0.18 ml/0.18 ml).

Total analysis time is 6 min per sample. No regeneration of the column is required between samples. The lower detection limit of the system is 3 mg of creatinine per liter. An on-line data acquisition system is preprogrammed to record and store the numerical values for the peaks onto a tape cassette. Peak areas of creatinine and the other Jaffé-positive compounds separated are quantitated off-line, along with replotting of original data.

Results and Discussion

We analyzed and quantified a series of pure reference standards, containing creatinine in concentrations ranging from 5 to 100 mg/liter. Sample concentration and absorbance were related linearly. Coefficients of variation for these multiple analyses ranged from 0.9 to 1.8%.

Data on within-run precision were obtained by analyzing 20 specimens of a pooled serum and 20 Monitrol II control specimens. The concentration of creatinine in pooled serum was established at 9.8 ± 0.250 (SD)
mg/liter (CV, 2.09%). Monitrol II values were 39.0 ± 0.210 (SD) mg/liter (CV, 0.66%). Day-to-day precision data, collected with use of 20 pooled serum and 20 Monitrol II specimens, as evaluated during five days, were 9.8 ± 0.230 (SD) mg/liter (CV, 1.95%) and 39.0 ± 0.310 (SD) mg/liter (CV, 0.72%), respectively.

To study the effects of interfering substances on serum creatinine, we separate and quantified a series of Jaffé-positive compounds mixed with samples of Monitrol II. Figure 2 shows the results for the seven different separations. In each case, the concentrations of the various Jaffé-positive compounds added to Monitrol II was much higher than that encountered in normal serum samples. Except for the acetoacetic acid/Monitrol II, the observed concentration of creatinine remained constant. Even in this case, the carryover of the impurity into creatinine did not cause an error of more than 8%.

Comparing our system to that of the SMA 6/60 (Technicon) continuous-flow analyzer, we analyzed 10 serum samples in duplicate. The average difference was 10%; our system usually gave lower values. One specimen gave a 150% difference. As was true here, such large discrepancy is usually accompanied by a non-creatinine Jaffé-positive prepeak that is usually high. This indicates that some of the interfering substances are not completely removed by the dialyzer in the SMA 6/60 system.

The system we describe in this report is not restricted solely to creatinine determination. We plan to evaluate its application to other common problems encountered in the clinical laboratory.

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References


