Comparison of High-Pressure Liquid Chromatographic and Ion-Exchange Membrane Methods for Creatinine

Keyphrases □ High-pressure liquid chromatography—analysis of creatinine, membrane method □ Creatinine—high-pressure liquid chromatography analysis, membrane method

To the Editor:

Chiou *et al.* (1) described a high-pressure liquid chromatographic (HPLC) method for creatinine and stated that previously published methods "lack specificity" or are "subject to interferences." In particular, they stated that in the cation-exchange membrane method of Vedsø *et al.* (2), the membrane is used "to remove proteins and interfering substances from serum samples . . . . Acetoacetate and possibly some other interfering substances are not removed. Therefore, interferences occur at least in samples from ketotic patients."

In fact, the cation-exchange membrane is used to *remove creatininium ions* from diluted serum at pH 3.1. Acetoacetate, acetone, glucose, and pyruvate are not removed (3); therefore, interference by these substances does *not* occur. (This is clearly stated in the original article.)

Chiou *et al.* (1) also stated that the membrane method "would probably take about 30 min for each assay," while their own method requires "only about 5 min for completion." In fact, an assay with the membrane method takes about 1.5 hr (the original article clearly stated that ion exchange takes 1 hr and desorption requires 20 min). Since the membrane method is a batch technique, a routine workload of 250 determinations (representing 100 sample duplicates) can be accomplished by one technician in 2.5 hr. This quantity is in contrast to the method of Chiou *et al.* (1) in which the throughput appears to be about 12 determinations/chromatograph hr, which is hardly suitable for routine workloads.

The membrane method has been improved since publication in 1974. The picrate reagent now contains 9.8 mmoles of picric acid/liter and 82 mmoles of sodium hydroxide/liter. The reagent blank is now about 0.030*A*, and the slope of the standard curve is about 2.0*A* mole^{-1} liter $\times 10^3$. With the improved method, the coefficient of variation at 50 μmoles/liter (0.57 mg %) is about 5%; in the normal range, it is about 4%. These values are in sharp contrast to those of Moss *et al.* (4), who found a coefficient of variation of 14% for creatinine values of 67 μmoles/liter (0.76 mg %).

Chiou *et al.* (1) erroneously stated that Moss *et al.* (4) did not report on the reproducibility of measurements of concentrations below 1 mg %. In fact, the article of Moss *et al.* is one of the few that *does* give this information. However, Chiou *et al.* do not report the reproducibility of their method below 1 mg %.

(1) W. L. Chiou, M. A. F. Gadalla, and G. W. Peng, *J. Pharm. Sci.*, **67**, 182 (1978).

(2) S. Vedsø, C. Rud, and J. F. Place, *Scand. J. Clin. Lab. Invest.*, **34**, 275 (1974).

(3) A. C. Teger-Nilsson, *ibid.*, **13**, 326 (1961).

(4) G. A. Moss, R. J. L. Bondar, and D. M. Buzzelli, *Clin. Chem.*, **21**, 1422 (1975).

John F. Place
Department of Clinical Chemistry
Vejle Hospital
7100 Vejle, Denmark

Received September 26, 1978.
Accepted for publication November 22, 1978.

Creatinine IX: Specificity and Sensitivity of High-Performance Liquid Chromatographic and Ion-Exchange Membrane Methods for Determination of Endogenous Creatinine

Keyphrases □ Creatinine—analysis, high-performance liquid chromatography, ion-exchange membrane assay □ High-performance liquid chromatography—analysis, creatinine

To the Editor:

In commenting on our extensive studies (1) on the development of a simple, rapid, and micro high-pressure liquid chromatographic (HPLC) method for the determination of endogenous "true" creatinine, it appears that Place (2) has misunderstood the content of our paper. Our paper clearly stated that in the ion-exchange membrane method by Vedsø *et al.* (3), "the subsequent reaction of creatinine desorbed from the membrane with the alkaline picrate." Although the ion-exchange membrane method was claimed to be specific (3), it can only be considered more specific than some other published methods using the alkaline picrate reagent. This is obvious since there is no guarantee that other endogenous substance(s) not tested by them or other workers cannot react with the alkaline picrate in their method. In commenting on the kinetic method of Larsen (4), Vedsø *et al.* (3) erroneously implied that they achieved a *total specificity* in their membrane method.

The accuracy and specificity of the original membrane method (1) also can be questioned due to the unusually high absorbance for their blank sample. The blank absorbance, calculated by this author, is equivalent to 1.70 mg % of creatinine. Plasma or serum creatinine levels ranging from 0.4 to 0.8 mg % are quite commonly found in patients.

There should be no doubt that the HPLC methods (1, 5, 6) for the assay of creatinine in serum or plasma should be more specific than the other assays published to date. The automated analyzer method, generally considered to be quite specific, overestimated creatinine by as much as 45% in our studies and ~200% in others (5) in certain samples when compared with the HPLC methods. In our laboratory, a range of ~20–70% of overestimation also was recently found in many serum samples with low creatinine

0022-3549/79/0600-0802$01.00/0