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 %.

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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

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levels[1]. A boiling alkaline picrate method claimed for the determination of "true" creatinine has also been shown to give an average 32.4 ± 27.2% (*SD*) overestimation, with the highest overestimation being 102% (7).

We did not advocate that our method be used routinely for all creatinine determinations in patients. Since plasma or serum samples as small as 10 μl are sufficient in our method, we suggested that the method would be "particularly valuable to the monitoring of the renal function in premature and mature infants, children, and adults with collapsed vascular veins. In these patients, the sample required can be obtained easily from the fingertip or the heel in the case of tiny, premature infants."

Plăce misunderstood our paper regarding the analysis time for each sample. We stated in the Abstract that: "Each assay required only about 5 min for completion." This statement was mistaken by Place to mean 5 min per chromatographic time. In fact, we meant that the time from the receipt of a plasma or serum sample to the reporting of the creatinine level of the sample is ~5 min. Less time is required for multiple-sample analyses. This time compares favorably with the membrane method, which requires ~90 min for emergency cases. The proposed HPLC method can certainly be modified and automated. The potential clinical application of the HPLC method for creatinine assay was examined in two recent papers (5, 6).

Regarding Place's (2) comment on the work of Moss *et al.* (8), it should be pointed out that the lowest creatinine concentration shown in their standard curve is 2 mg %, although the use of a 1 mg % solution was mentioned in the text. Certainly, it is not most desirable to perform the reproducibility study using the creatinine level below the range used for the standard curve.

Although the data on reproducibility of measurements of concentrations <1 mg % were not presented explicitly in our papers (1, 7), they were clearly implied. Excellent reproducible results were always obtained for low creatinine levels in our many standard curve and plasma level studies (1, 7). Using a modified mobile phase and a 254-nm fixed wavelength detector, the coefficient of variation for 0.5 mg % serum sample is only about 2% (9). Our comment (1) on the interferences in samples from ketotic patients should have been directed to the colorimetric method of Heinegård and Tiderström (10) and not to the ion-exchange membrane method.

It is important to point out that in using our HPLC method (1) for creatinine, the filter device[2] to separate plasma or serum from blood cells should not be used; chemicals leached out from the device will interfere with the assay. The possibility of such interference in other HPLC methods should be studied prior to their use.

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[1] Unpublished data.
[2] Filter sampler blood serum filter, Glassrock Products, Fairburn, GA 30212.

Effect of Sodium Sulfate on Acetaminophen Elimination by Rats

Keyphrases □ Sodium sulfate—effect on acetaminophen elimination, protection against acetaminophen toxicity, rats □ Acetaminophen—toxicity, prevention by sodium sulfate, effect on elimination □ Analgesics—acetaminophen, toxicity, prevention by sodium sulfate

To the Editor:

Accidental or intentional ingestion of large acetaminophen overdoses can cause hepatotoxicity and damage to other vital organs, sometimes with lethal outcome (1, 2). This toxic effect is produced by a reactive metabolite, which is formed in parallel with acetaminophen glucuronide and acetaminophen sulfate, the two major drug metabolites (3). Pharmacokinetic analysis of acetaminophen disposition in acutely intoxicated patients and certain other evidence from studies in humans indicate that the reactive acetaminophen metabolite is formed by apparent first-order kinetics while the two major conjugated metabolites are formed by capacity-limited kinetics (4, 5). Results of acetaminophen conjugation interaction studies in humans suggest that the availability of sulfate is rate limiting in the formation of acetaminophen sulfate (6, 7). If the formation of acetaminophen sulfate can be accelerated by administration of sodium sulfate or another suitable sulfate source, then the amount of reactive metabolite formed and, therefore, the acetaminophen toxicity should be decreased.

Preliminary studies in mice showed that intraperitoneal administration of sodium sulfate significantly increased the median lethal dose (*i.e.*, decreased the toxicity) of acetaminophen (8), but the effect of sodium sulfate on acetaminophen disposition was not determined. There is some indication that acetaminophen sulfate formation by rats is capacity limited and that treatment with sodium sulfate increases the urinary excretion of the conjugate (9, 10). However, the reported data do not establish definitively the effect of sodium sulfate on acetaminophen elimination kinetics since acetaminophen concentrations in plasma were not determined and urine collections were terminated before the excretion of drug and metabolites was completed.

In view of the potential clinical utility of sodium sulfate

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