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HPLC with Enzymatic Detection as a Candidate Reference Method for Serum Creatinine

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We present a candidate Reference Method for determining the concentration of serum creatinine. The method is based on HPLC combined with enzymatic determination. Creatinine plus ^{14}[C]creatinine is extracted by cation-exchange chromatography, subjected to reversed-phase HPLC, and finally quantified enzymatically. Enzymatic measurement ensures no interference from co-eluting compounds, which has been a problem for some reported HPLC assays relying on ultraviolet detection. The average corrected recovery was 100.1% (SEM = 1.1%; n = 15). The accuracy was verified by assaying five sera with target values determined by isotope dilution mass spectrometry. The total interassay CV was ≤2.5%. We applied the method to study the specificity of HPLC–ultraviolet detection, using 72 plasma samples from hospitalized patients; no interference was noted. Thus, HPLC–ultraviolet detection appears to be specific, provided that sample cleanup is based on cation-exchange chromatography. Our diode-array detector studies of peak homogeneity supported this conclusion. Still, combined HPLC–enzymatic determination ensures even greater accuracy, ranking close to that by isotope dilution mass spectrometry.

Additional Keyphrases: *chromatography, reversed-phase isotope dilution mass spectrometry compared*

It is well known that the analytical principle used commonly for determining creatinine in plasma or serum with some version of the alkaline picrate method is subject to interference from numerous endogeneous and exogeneous substances (*1*). Some plasma compounds may also interfere with enzymatic methods, which are more specific (*1, 2*). Thus, an even more specific methodology is needed for establishing target creatinine concentrations in serum Reference Material that is intended for control of accuracy. Because of its specificity, high-performance liquid chromatography (HPLC) might be considered suitable for this (*3*), and >30 HPLC methods for creatinine have been published in recent years (*1, 4*), most involving reversed-phase systems with standard C_{18} columns. Although some authors have ruled out interference from numerous known endogeneous and exogenous compounds by adding these to serum samples and assaying (*3, 5–7*), others have noted that serum extracts from uremic patients may contain unknown ultraviolet-absorbing substances that could constitute a problem (*8–11*). Potential interference in HPLC assays with ultraviolet detection can be circumvented by introducing a more selective detection principle, e.g., enzymatic determination. Here, we present an HPLC method in which the creatinine fraction is collected and quantified enzymatically. We demonstrate that the method is without bias, indicating its status as a candidate Reference Method for creatinine. Further, we used this method to systematically evaluate the specificity of HPLC with ultraviolet detection.

Among the numerous HPLC methods published, we selected that of Lim et al. (*12*) as a basis for part of our method. The sample pretreatment involves cation-exchange chromatography, and the liquid-chromatography is carried out isocratically on a C_{18} column. We supplemented the method by adding 1-[^{14}C]creatinine as an internal standard for correction of recovery (*13*). The enzymatic step is based on the creatinine amidohydrolase (creatininase; see below) and *p*-aminophenazone system described by Fossati et al. (*14*), in a more recent version (*2*). To optimize calibration, we studied the precision profile and carried out a weighted regression analysis to estimate the calibration function.

Materials and Methods

Apparatus

A Model 1090 M liquid chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with an ultraviolet diode-array detector and a Foxy fraction collector was used. The column (300 × 4 mm) was packed with Nucleosil C_{18} (5-μm particles) from Mackerey-Nagel, Düren, F.R.G. For the absorbance measurements, we used a Pye Unicam (Cambridge, U.K.) SP 1750 spectrophotometer. Radioactive creatinine was measured with a Model 2000 CA liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, IL). The lyophilization apparatus was from Heto Lab Equipment A/S, Birkerod, Denmark. Creatinine calibration solutions were prepared with Class A volumetric pipettes.

Reagents

Aqueous calibrator solutions were prepared immediately before use from creatinine, Standard Reference Material 914a, Clinical Standard, from the National Institute of Standards and Technology (NIST), Gaith-

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ersburg, MD 20899. We obtained 1-[^{14}C]creatinine, 444 GBq/mol (12 Ci/mol), from Amersham International plc, Amersham, Bucks., U.K. before we purified the isotope by HPLC. The liquid scintillation solution, Insta-Gel, was from Packard. Extraction columns consisted of disposable plastic Econo-columns filled with the strong cation-exchange resin AG50W-X12(H^+) (200–400 mesh; Bio-Rad Labs., Richmond, CA).

Reagents for the amidohydrolase enzymatic method were supplied as the test combination Creatinine PAP (Boehringer Mannheim, Mannheim, F.R.G.). Other chemicals were reagent grade. For control of accuracy, we used five lyophilized sera with creatinine concentrations determined by isotope dilution mass spectrometry (ID-MS). One serum (Standard Reference Material 909) was purchased from NIST (*15*), and the other four sera (pools A–D) were distributed by the Community Bureau of Reference under the Commission of the European Communities in an intercomparison study. Measurements of the latter sera by ID-MS were performed by Professor L. Siekmann of the Institute of Clinical Biochemistry, University of Bonn, F.R.G. (*16*).

Analytical Procedure

Extraction. To 2000 µL of serum, plasma, or aqueous calibrator solution we added the internal standard, 1-[^{14}C]creatinine, in an amount corresponding to 1670 counts/s (10^5 counts/min). After mixing for 30 s, we allowed the isotope to equilibrate for ≥2 h at 20–25 °C. Then we diluted the samples with 10 mL of adsorption buffer (per liter, 40 mmol of citric acid and 20 mmol of Na_2HPO_4, pH adjusted to 3.00). We filled the plastic extraction columns with 300 mg of resin dissolved in 2 mL of 2.5 mol/L NaOH reagent. We washed the columns with 2 mL of H_2O, followed by 4 mL of adsorption buffer. The sample dilutions were then poured carefully onto the columns. Having washed the columns twice with 4 mL of H_2O, we eluted the creatinine with eluting buffer (2 mL added three times), 0.15 mol/L ammonium acetate. The eluate was lyophilized.

HPLC. We dissolved the eluates in 500 µL of H_2O, of which duplicate 100-µL aliquots were injected onto the column. The chromatography was performed with a mobile phase of 0.01 mol/L ammonium acetate and methanol (98/2, by vol) at a flow rate of 1 mL/min. The retention time of creatinine was determined by monitoring absorbance at 236 nm, and the fraction collector was adjusted accordingly. The creatinine fraction collected was lyophilized. The diode-array detector also recorded the whole ultraviolet spectrum across the creatinine peak for evaluation of peak homogeneity. Chromatograms of an aqueous solution of creatinine and of plasma samples with creatinine concentrations within the reference range and at an increased concentration are displayed in Figure 1.

Enzymatic determination and measurement of radioactivity. The creatinine fractions were dissolved in 1000 µL of H_2O, of which 500 µL was transferred to tubes with 10 mL of Insta-Gel for measurement of radioactivity for 10 min. Depending on whether the

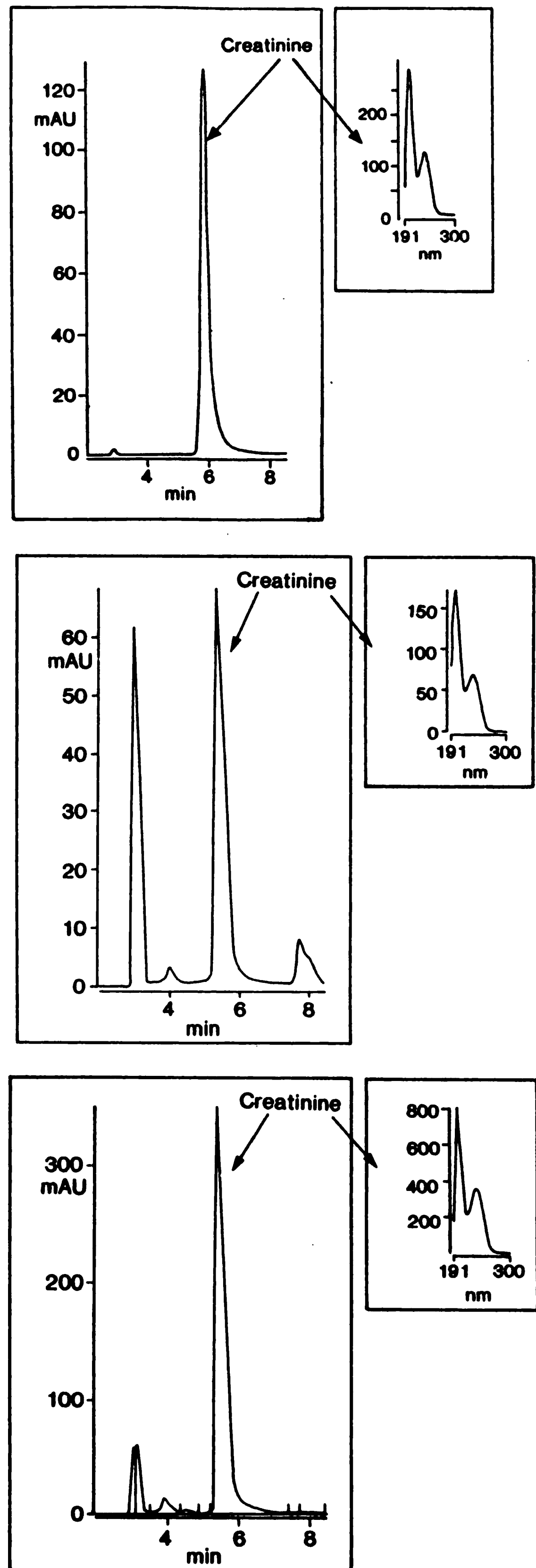
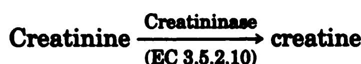
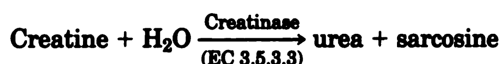


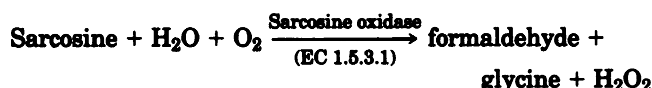
Fig. 1. Chromatograms of (*top*) an aqueous solution of creatinine (100 µmol/L), (*middle*) a patient's plasma sample with creatinine concentration of 65 µmol/L, and (*bottom*) a patient's sample with creatinine concentration of 1280 µmol/L

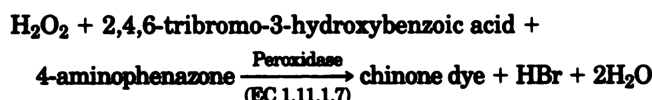
mAU, milli-absorbance units; *inserts* show the ultraviolet spectrum at the apex of the peaks. Note the difference in *y*-axis scales

peak area was less or greater than that of the 400 µmol/L calibrator, we transferred respectively 400 or 100 µL of the rest of the fraction to tubes for enzymatic determination and lyophilization. The enzymatic reactions are as follows:









We redissolved the aliquots in 1000 µL of modified Creatinine-PAP reagent solution containing the listed enzymes and cofactors. The modification consisted of a doubling of the Tris · HCl buffer strength from 0.15 to 0.30 mol/L. To further ensure an appropriate pH (9.0) for the enzymatic reactions, we added 60 µL of 0.01 mol/L NaOH per milliliter of mobile phase transferred for the enzymatic measurement. The tubes were incubated at room temperature for 20 min, and the absorbance of the chinone dye was read at 510 nm. In line with the duplicate runs of calibrators/samples, we measured two reagent blank solutions for each series of samples. The coefficient of variation of the blank measurements was 0.9% (n = 15 duplicate measurements). The difference (h_i) between sample and the average blank absorbance was recorded.

Calculation of results. A linear calibration curve was estimated from a set of seven aqueous calibrators with concentrations (x_i) 25, 50, 100, 200, 400, 600, and 1000 µmol/L. The response y_i is the net absorbance (h_i) corrected for recovery. If we denote the fractional recovery of the isotope as g_i, then y_i is equal to (h_i-g_i) or ($4h_i/g_i$), depending on whether 400 or 100 µL was transferred for enzymatic measurement. In conjunction with the HPLC purification of the isotope, we determined the molar activity, expressed as counts/min per mole. Accordingly, a correction for the amount of added isotope (~1.5 nmol per 2000 µL of sample or calibrator) could be made. A linear calibration function was estimated by a weighted least-squares regression procedure (*17*) (see *Appendix*).

Results

Evaluation of the HPLC–Enzymatic Procedure

Calibration curve. The average calibration points for 10 analytical series are shown in Figure 2, together with an estimated regression line. As is apparent from the precision profile (Figure 3), the SD of the response increases largely in proportion to the concentration when the latter exceeds 100 µmol/L, but tends to approach a constant value at lower concentrations. Therefore, we estimated the calibration line for each analytical series by using a weighted least-squares regression procedure with weights inversely proportional to the variances (squared standard deviations) as explained in the *Appendix*. For the example given, with a ratio of about 10 between the maximum and minimum SD of the response, the weighted approach is about twice as precise as the unweighted regression procedure commonly used (*Appendix*). Linearity of the average calibration curve was confirmed by a variance ratio test (F = 1.12; P >0.3; ν_1 = 63, ν_2 = 5) (*17*). We reanalyzed

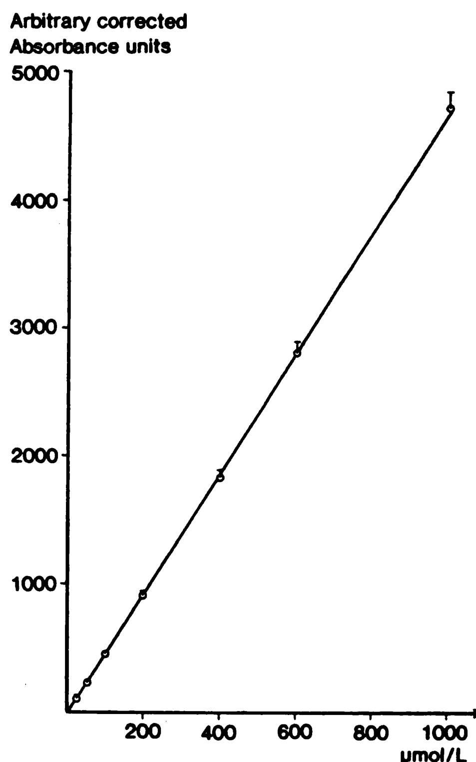


Fig. 2. Average responses of the HPLC–enzymatic method for 10 calibration series with the regression line
Bars indicate 1 SD

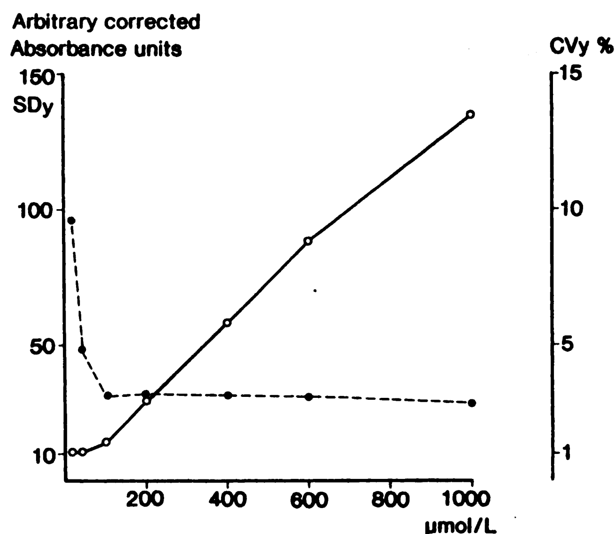


Fig. 3. SD of the HPLC–enzymatic response, SD_y (—) and CV_y (- - -), as functions of the creatinine concentration

serum samples with concentrations >1000 µmol/L by using 500 instead of 2000 µL of serum.

Accuracy. The recovery of the added isotope in the creatinine fraction by HPLC averaged 83.0% (SD = 4.0%, n = 62). When creatinine was added to serum samples in amounts from 25 to 1000 µmol/L and analyzed by the HPLC–enzymatic procedure, in which extraction losses are taken into account by the isotope, the average recovery was 100.1% (SEM = 1.1%; n = 15). The accuracy of the method (Table 1) was further validated by analyzing the five sera with target values known from ID-MS measurements (*15, 16*). We observed a good agreement between the results by both methods. The deviations from the means of the HPLC–enzymatic measurements from the target values ranged from −2.8% to +1.0%.

Precision. The total interassay CV was estimated to be ≤2.5% for plasma pools prepared from patients' samples (Table 2). An intra-assay CV of 1.5% was recorded for 15 patients' samples analyzed in duplicate in the same series, i.e., true duplicates carried through the whole procedure. Although the HPLC–enzymatic procedure has relatively many steps, the imprecision was kept low by using a tracer as an internal standard and by optimizing the conditions for the enzymatic step. The tracer added corresponded to 10^5 counts/min, of which about one-tenth was finally counted, i.e., about 10^4 counts/min. A counting time of 10 min yields 10^5 counts, so that the CV due to random variation of isotope decay is only $(\sqrt{10^5}/10^5)\,100\% = 0.3\%$. When the enzymatic procedure is applied directly to 50 µL of serum, the relative imprecision is at a minimum at creatinine concentrations >300 µmol/L, corresponding to 15 nmol in the test tube (*2*). Starting with extraction of 2 mL of serum, of which either 4/50 or 1/50 (depending on the creatinine concentration) is finally measured enzymatically, we obtain ≥15 nmol in the test tube for creatinine concentrations >100 µmol/L in serum. Additionally, because creatinine is separated from creatine by the HPLC step (Figure 4), it is not necessary to correct for the creatine content, as is done for serum samples measured directly. This also improves the precision of the assay when applied to HPLC fractions. Finally, the optimized weighted-estimation procedure for the calibration function increases precision.

Detection limit. We used as the lower limit of detection 3 SD for measurements of a low-concentration sample.

Table 1. Accuracy of the HPLC–Enzymatic Procedure

Sample	No. of measurements	Mean, µmol/L	ID-MS value, µmol/L	Deviation from ID-MS value	
				µmol/L	%
SRM 909	10	153.9	152.4	+1.5	+1.0
Pool A	3	328.4	332.1	−3.7	−1.1
Pool B	3	154.2	155.5	−1.3	−0.8
Pool C	3	120.5	124.0	−3.5	−2.8
Pool D	3	289.8	291.0	−1.2	−0.4

SRM, Standard Reference Material.

Table 2. Precision of the HPLC–Enzymatic Procedure

Sample	No. of measurements	Mean, µmol/L	Total interassay		Intra-assay CV, %
			SD, µmol/L	CV, %	
SRM 909	10	154	2.68	1.7	
Pool 1	5	40.1	0.88	2.2	
Pool 2	10	99.4	2.08	2.1	
Pool 5	9	870	22.1	2.5	
Patients' samples in duplicate	15				1.5

Assuming that the analytical SD is approximately constant at concentrations <50 µmol/L, as is the SD of the detector response (Figure 3). The limit of detection is thus 3 × 0.88 µmol/L = 2.6 µmol/L. Of more interest is the lower limit of quantification, defined as 10-fold the analytical SD, i.e., 8.8 µmol/L. At this concentration, the CV is 10%.

Interference. Potential interferants to the HPLC enzymatic procedure are primarily the endogenous and exogenous compounds known to disturb the set of enzymatic reactions we used (*2*). To interfere, a compound must be extracted and eluted in the same fraction as creatinine on the HPLC column. Ascorbate and bilirubins, both known to inhibit the peroxidase step, are not extracted from serum, as confirmed by analyzing extracts of serum from icteric patients or enriched with ascorbate, 500 µmol/L (the bilirubins were assayed by a diazo method and ascorbate by HPLC). Measurements of the creatinine concentrations of these sera showed interference <0.2%.

Creatine and sarcosine are intermediates of the enzymatic reactions and increase the results for creatinine. Creatine is extracted (about 50%) with creatinine, but elutes before creatinine (Figure 4). Creatinine measurements of a serum pool enriched with 500 µmol of creatine per liter indicated an interference of <1%. Sarcosine is not extracted, and addition of 500 µmol of sarcosine per liter to a serum pool resulted in an interference of <0.2%. The drug calcium dobesilate,

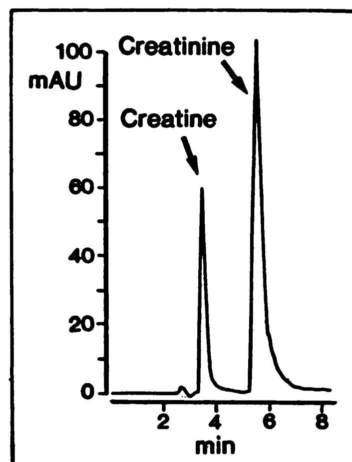


Fig. 4. Chromatogram of an aqueous solution of creatine (250 µmol/L) and creatinine (250 µmol/L) with detection at 210 nm

which has a quinone structure (*2*), interferes with the detection step but is not extracted. Its interference was <0.2%, as judged from measurements of a pool enriched with 500 μmol/L of calcium dobesilate.

Comparison of HPLC–Enzymatic and HPLC–UV Measurements

To evaluate systematically whether interference with HPLC–ultraviolet detection of creatinine in patients' samples is a problem, as suggested by some authors (*9–11*), we analyzed a series of samples by both ultraviolet and enzymatic detection methods. The samples originated from five hospitalized patients whose creatinine values were within the reference interval (44–133 μmol/L) and from 67 patients with renal diseases and increased concentrations, most of whom were undergoing hemodialysis. The HPLC–ultraviolet results were obtained from an extra assay with an injection volume of only 25 μL, which is more optimal for ultraviolet detection. The relation between the response (the HPLC peak area at 236 nm divided by the fractional recovery of the isotope) and the concentration was linear to at least 1000 μmol/L ($F = 1.05$; $P > 0.3$; $\upsilon_1 = 49$; $\upsilon_2 = 5$) (Figure 5). Again, the calibration line was estimated by weighted least-squares regression analysis.

Denoting the results by HPLC–enzymatic analysis as e_i and the HPLC-UV results as u_i, we plotted percent paired differences $\{[u_i - e_i]/[(e_i + u_i)/2]\} \times 100\%$ against the average result $[(e_i + u_i)/2]$, as shown in Figure 6 (*18*). We evaluated percent (relative) differences because both measurements are subject to proportional random errors. The average of the percent differences, −0.73%, was only slightly different from zero ($P < 0.02$), showing that there is at most a negligible systematic difference between the measurements. The presence of co-eluting ultraviolet-absorbing compounds in a plasma extract would be termed a positive outlier of the relative differences. In Figure 6, only three observations are located slightly beyond the limits ±2 SD from 0; two are below the lower limit, and one exceeds the upper limit. Of 72 observations, we would expect about three observations beyond the ±2 SD limits, simply from random variation. Thus, we consider that no cases of ultraviolet interference were disclosed for this series of samples, which should be representative in a clinical context.

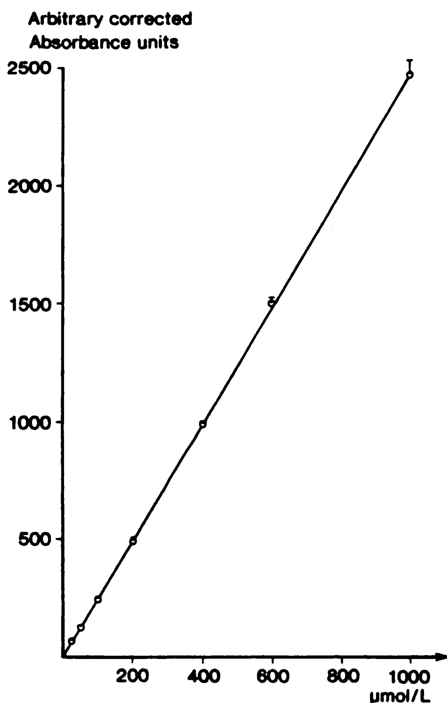


Fig. 5. The average responses of the HPLC–ultraviolet method for eight calibration series and the regression line
Bars indicate 1 SD

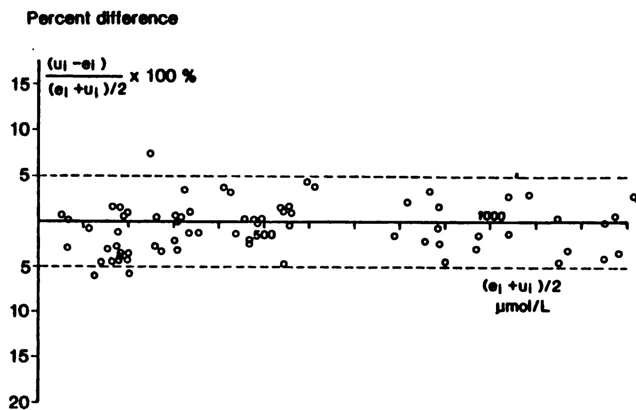


Fig. 6. The percent paired differences between HPLC–ultraviolet (u_i) and HPLC–enzymatic (e_i) determination of creatinine plotted against the average of the determinations
Dotted lines mark ±2 SD from zero

A supplementary way to study the problem of ultraviolet interference is with the diode-array detector. The peak homogeneity is evaluated by a comparison of the spectra recorded up- and down-slope on the peak. A correlation coefficient between the spectra of ≥0.95 is regarded as a sign of homogeneity (*19*). The average for the spectra in our method was $r = 0.995$ (range 0.950–1.000). Thus, this investigation supported the conclusion of no ultraviolet interference.

Discussion

Generally, ID-MS is considered the most accurate and matrix-independent principle of analysis for low-molecular-mass compounds in clinical chemistry (*20*). The equipment, however, is expensive, and special isotopes and expertise are needed for handling this methodology. ID-MS methods for creatinine measurement have been established in only a few laboratories (*15, 16, 21*). HPLC, which is a less resource-demanding technology, is thus more suitable as a reference methodology for regional quality-control programs (*3*).

Most HPLC methods for creatinine are based on the reversed-phase principle with standard C_{18} columns (*4*); only a few involve cation-exchange columns (*3, 4, 22, 23*). A major difference between reversed-phase systems is the sample pretreatment: protein precipitation with trichloroacetic acid or acetonitrile, ultrafiltration, or cation-exchange chromatography (*4*). The first two unselective procedures yield chromatograms with various peaks close to creatinine, especially in extracts from

uremic patients (*8–10*). Cation-exchange chromatography is a more selective cleanup procedure, but interference is still possible and can be excluded only by a systematic evaluation. Commonly, suspected interferants are added to serum samples to study whether the measured creatinine concentration is affected (*3–7, 22*). However, that type of study discloses interference only from well-characterized endogenous and exogenous compounds, but does not preclude interference from drug metabolites and unknown metabolic products that might accumulate in plasma of patients with renal disease. A better way to study specificity is to assess whether the supposed creatinine peak disappears after treatment with an enzyme that converts creatinine to some other compound. Because interfering compounds may be present in serum of relatively few patients, a reasonably large number of serum samples should be studied to exclude interferences in general. So far, only a few samples have been subjected to enzymatic treatment (*8, 22*). Further, the presence of a small residual peak after enzymatic treatment may leave the question unsettled about whether the enzymatic breakdown was completed or whether another compound was present. We consider the comparison between the HPLC–ultraviolet and HPLC–enzymatic measurements of a reasonable number of patients' samples as a better way to study the problem. Another possibility would be to compare measurements by HPLC–ultraviolet detection and ID-MS, which so far has been done for only a few plasma pools (*3, 24*). The comparison performed here suggests that HPLC–ultraviolet detection, given cation-exchange cleanup of plasma samples, is a specific measurement principle, and that the somewhat laborious HPLC–enzymatic combination is not necessary. The latter, however, is very specific, and its accuracy should rank very close to that of ID-MS. A compromise might be to use HPLC–ultraviolet detection, then check peak homogeneity with a diode-array detector, and reserve the HPLC–enzymatic combination for samples with signs of peak impurity.

Quite recently, a cation-exchange HPLC method combined with an unselective sample pretreatment procedure has been proposed as a Reference Method for serum creatinine determination (*3*). This combination is expected to provide the same degree of specificity as cation-exchange chromatography for sample cleanup combined with a reversed-phase HPLC system. The accuracy of the method seems good, as judged from comparison with ID-MS measurements on four serum pools. The precision, however, was only moderate, with interassay CVs ranging from 3% to 11%. This may be due to the lack of an internal standard in the measurement procedure.

Appendix: Estimation of the Calibration Function by Weighted Least-Squares Regression (*17*)

From the set of k paired values of calibrator concentrations (x_i) and absorbances corrected for recovery loss (y_i), we estimate the equation

$$y_i = a + b(x_i - \bar{x}_w) = a_o + b \times x_i \quad [a_o = a - b \times \bar{x}_w]$$

where $a = \bar{y}_w$ and

$$b = \sum_{i=1}^{k} w_i \times n_i \times (x_i - \bar{x}_w) \times \bar{y}_i / \sum_{i=1}^{k} w_i \times n_i \times (x_i - \bar{x}_w)^2$$

$\bar{x}_w$ and $\bar{y}_w$ are the weighted averages

$$\bar{x}_w = \sum_{i=1}^{k} w_i \times n_i \times x_i / \sum_{i=1}^{k} w_i \times n_i$$

$$\bar{y}_w = \sum_{i=1}^{k} w_i \times n_i \times \bar{y}_i / \sum_{i=1}^{k} w_i \times n_i,$$

and $\bar{y}_i = \sum_{j=1}^{n_i} y_{ij}$.

n_i is the number of absorbance readings per calibrator, usually 2 [y_{i1} and y_{i2} corresponding to the duplicated HPLC run; $\bar{y}_i = (y_{i1} + y_{i2})/2$]. Assuming a proportional random measurement error, the weights $w_i = 1/x_i^2$ [exception: for $x_i \leq 100$ μmol/L, $w_i = 1/100^2$ because the proportional relationship is truncated at 100 μmol/L (Figure 3)].

The principle in the weighted procedure is to assign more influence to absorbance readings at relatively low concentrations, where the random variation is smallest (Figure 3). Computer simulations show that for a calibration model with a ratio of 10 between the largest and smallest SD of the response, the variance of the slope is 50% smaller for the weighted than for the unweighted procedure.

References

1. Spencer K. Analytical reviews in clinical biochemistry: the estimation of creatinine. Ann Clin Biochem 1986;23:1–25.
2. Guder WG, Hoffmann GE, Hubbuch A, Poppe WA, Siedel J, Price CP. Multicentre evaluation of an enzymatic method for creatinine determination using a sensitive colour reagent. J Clin Chem Biochem 1986;24:889–902.
3. Rosano TG, Ambrose RT, Wu AHB, Swift TA, Yadegari P. Candidate reference method for determining creatinine in serum: method development and interlaboratory validation. Clin Chem 1990;36:1951–5.
4. Huang S-M, Huang Y-C. Chromatography and electrophoresis of creatinine and other guanidino compounds [Review]. J Chromatogr 1988;429:235–53.
5. Soldin SJ, Hill JG. Micromethod for determination of creatinine in biological fluids by high-performance liquid chromatography. Clin Chem 1978;24:747–50.
6. Okuda T, Oie T, Nishida M. Liquid-chromatographic measurement of creatinine in serum and urine. Clin Chem 1983;29:851–3.
7. Paroni R, Arcelloni C, Fermo I, Bonini PA. Determination of creatinine in serum and urine by a rapid liquid-chromatographic method. Clin Chem 1990;36:830–6.
8. Spierto FW, MacNeil ML, Culbreth P, Duncan I, Burtis CA. Development and validation of a liquid-chromatographic procedure method for serum creatinine. Clin Chem 1980;26:286–90.
9. Ginman RFA, Colliss JS. Modified liquid-chromatographic method for creatinine determinations [Letter]. Clin Chem

1985;31:331–2.
10. Bjerve KS, Egense J, Lampinen L-M, Masson P. Evaluation of several creatinine methods in search of a suitable secondary reference method: report from the subcommittee on reference method for creatinine, Nordic Society for Clinical Chemistry. Scand J Clin Lab Invest 1988;48:365–73.
11. Wiedemann E, Hägele E, Siedel J, Ziegenhorn J. Determination of creatinine in serum with HPLC and column switching. Fresenius Z Anal Chem 1986;324:333–4.
12. Lim CK, Richmond W, Robinson DP, Brown SS. Towards a definitive assay of creatinine in serum and urine: separation by high-performance liquid chromatography. J Chromatogr 1978;145:41–9.
13. Siekmann L, Siekmann A, Mackrodt D, Johnen C. Isotope dilution–high-performance liquid chromatography as a new technique for the development of reference methods. Fresenius Z Anal Chem 1986;324:279–80.
14. Fossati P, Prencipe L, Berti G. Enzymatic creatinine assay: a new colorimetric method based on hydrogen peroxide measurement. Clin Chem 1983;29:1494–6.
15. Welch MV, Cohen A, Hertz HS, et al. Determination of serum creatinine by isotope dilution mass spectrometry as a candidate definitive method. Anal Chem 1986;58:1681–5.
16. Siekmann L. Determination of creatinine in human serum by isotope dilution-mass spectrometry. J Clin Chem Clin Biochem 1985;23:137–44.
17. Hald A. Statistical theory with engineering applications. New York: Wiley, 1952.
18. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;i:307–10.
19. Tanabe K, Saëki S. Computer retrieval of infrared spectra by a correlation coefficient method. Anal Chem 1975;47:118–22.
20. De Leenheer AP, Lefevere MF, Lambert WE, Colinet ES. Isotope-dilution mass spectrometry in clinical chemistry. Adv Clin Chem 1985;24:111–61.
21. Björkhem I, Blomstrand R, Öhman G. Mass fragmentography of creatinine proposed as a reference method. Clin Chem 1977;23:2114–21.
22. Kågedal B, Olsson B. Determination of creatinine in serum by high-performance liquid chromatography: a comparison of three ion-exchange methods. J Chromatogr 1990;527:21–30.
23. Guy JM, Legg EF. An improved cation exchange HPLC method for the measurement of serum creatinine. Ann Clin Biochem 1990;27:223–6.
24. Werner G, Schneider V, Emmert J. Simultaneous determination of creatine, uric acid and creatinine by high-performance liquid chromatography with direct serum injection and multiwavelength detection. J Chromatogr 1990;525:265–75.