Simple, Rapid, and Micro High-Pressure Liquid Chromatographic Determination of Endogenous "True" Creatinine in Plasma, Serum, and Urine

WIN L. CHIOU ˣ, **M. A. F. GADALLA**, and **GEOFFREY W. PENG**

Received February 4, 1977, from the *Clinical Pharmacokinetics Laboratory and Department of Pharmacy, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612.* Accepted for publication June 1, 1977.

Abstract □ Previously published methods for endogenous creatinine levels in plasma, serum, or urine lack specificity or are subject to interferences from endogenous or exogenous substances. The developed simple, rapid, and specific high-pressure liquid chromatographic method includes the novel deproteinization and extraction of 1 volume of plasma or serum with 2.5 volumes of acetonitrile and also of 1 volume of urine with 40 volumes of a 20% water-80% acetonitrile solution. An aliquot of the supernate is then injected directly into the chromatograph. A cation-exchange column and acidified (0.02% of 85% phosphoric acid) 0.1 *M* ammonium phosphate solution as the mobile phase, with a flow rate of 2 ml/min, were used. Creatinine, with a retention time of 3.8 min, was monitored *via* its UV absorption at 215 nm. Both peak height and integrated area methods of quantitation yielded the same results. Several methods were employed to show that the "suspected" creatinine peak from plasma samples was due entirely to the "true" creatinine. No interference was found in samples obtained from normal and renal patients. The day-to-day variation in the detector response was small. Each assay requires only about 5 min for completion. Ten microliters of plasma or serum or 1 μl of urine is sufficient for analysis.

Keyphrases □ Creatinine—high-pressure liquid chromatographic analysis in biological fluids □ High-pressure liquid chromatography—analysis, creatinine in biological fluids

Accurate measurements of endogenous creatinine levels in plasma, serum, or urine are important in the monitoring of renal function and dosage regimen modifications in patients with renal insufficiency (1–16). Although determinations of creatinine levels have been performed routinely in hospitals and clinics for decades, attempts to improve the assay have appeared continuously since the introduction of the Jaffe alkaline picrate reaction (17).

BACKGROUND

The Jaffe alkaline picrate method, with various modifications, is still the most widely used procedure for creatinine quantitation (18–23). Its nonspecificity and interferences by endogenous and exogenous noncreatinine chromogens have been well documented (24–27). It has been recommended that a factor of 0.7 be used to calculate the measured "apparent" creatinine value so as to obtain the "true" endogenous creatinine value (1, 2, 28). The degree of such interferences by noncreatinine chromogens was reported to be decreased as the creatinine level increased (29).

In attempting to measure directly the true creatinine in plasma or serum and to avoid interferences by other chromogens, Yatzidis (27, 30) recently developed a method based on the absorbance difference measured at 500 nm of plasma or serum samples after reaction with picrate reagents buffered at pH 9.65 and 11.50. The sample required for the assay is 0.1 or 0.2 ml. One obvious drawback is that the reaction with the picrate reagents alone takes 45–60 min. The author's claim of noninterferences, however, could not be substantiated in this laboratory since absorbances or recoveries of the spiked creatinine in 5% (w/v) human albumin solution and normal plasma were lower than the predicted values based on the standard curve constructed from aqueous creatinine solutions (31). Further studies in this laboratory on plasma samples from eight renal patients showed that the interferences ranged from 20 to 44%.

Another method involves the use of a cation-exchange membrane to remove proteins and interfering substances from serum samples and the subsequent reaction of creatinine desorbed from the membrane with the alkaline picrate (32). This method requires 0.4 ml of serum and would probably take about 30 min for each assay. Acetoacetate and possibly some other interfering substances are not removed. Therefore, interferences occur at least in samples from ketotic patients (32).

A reaction kinetic assay for serum and urine creatinine, using the Jaffe reaction but without deproteinization, was reported to be simple and reliable and to require only 0.5–2 min (33). The serum sample required per test is 0.2 ml. One drawback is that the interference from compounds with a similar or identical reaction rate cannot be detected. Furthermore, accuracy for samples from renal patients has not been demonstrated.

A kinetic enzymatic method for the determination of serum creatinine was recently reported (34). It requires 0.25 ml of serum and takes about 10 min for each assay. It was claimed to be subject to few or no interferences. Preparation and stability (34) of the reagent, which consisted of nine components, appear to be the disadvantages. The applicability of the method to plasma and urine samples was not studied. Furthermore, the accuracy and reproducibility of measurements for concentrations below 1 mg % were not reported. Serum creatinine levels in normal infants often might be as low as 0.5 mg %.

Apparently, none of these cited investigations presented convincing experimental evidence that the absorbances measured after reaction with various reagents were indeed entirely due to the true creatinine. Presence of interfering substances, endogenous or exogenous, can hardly be detected by the specific assays employed during analysis.

Accuracy and precision in measurements of creatinine levels in plasma, serum, and/or urine are critical to the correlation between renal function and renal clearance or biological drug half-life. It is reasonable to speculate that the poor or unpredictable correlation of certain drugs (35, 36) may be caused partly by the creatinine assay problem.

This paper reports a simple, rapid, specific, reliable, and micro high-pressure liquid chromatographic (HPLC) method developed for the determination of the endogenous "true" creatinine in plasma, serum, and urine. The method also is likely to be subject to fewer interferences by other noncreatinine substances than any other creatinine assay published previously. Evidence that the concentration measured was due entirely to the true creatinine also will be presented.

EXPERIMENTAL

Materials—Creatinine[1], ammonium phosphate[1], and phosphoric acid (85%) were reagent grade and were used without further purification. Glass-distilled acetonitrile[2], methanol[2], and distilled water were used.

Preliminary Spectrophotometric Studies—To study the feasibility of using the UV detector directly for creatinine quantitation by the HPLC method, the UV spectra of creatinine in possible mobile phases such as water and acidified 0.1 *M* ammonium phosphate (pH 2.6) containing 0.02% (v/v) phosphoric acid were obtained with a UV-visible recording spectrophotometer[3].

HPLC System—The system consisted of a solvent delivery pump[4], a sample injection valve[5], a variable wavelength detector[6], a variable span potentiometric 12.7-cm (5-in.) recorder[7], and an electronic integrator[8]. Unless otherwise specified, a 30-cm cation-exchange column[9], acidified 0.1 *M* ammonium phosphate as the mobile phase, a wavelength of 215 nm for the absorbance monitoring, and a chart paper speed of 30.5 cm

[1] Fisher Scientific Co., Fair Lawn, N.J.
[2] Burdick and Jackson Laboratories, Muskegon, Mich.
[3] Model 200, Perkin-Elmer Corp., Norwalk, Conn.
[4] Model M-6000 A, Waters Associates, Milford, Mass.
[5] U6K injector, Waters Associates, Milford, Mass.
[6] Model LC-55, Perkin-Elmer Corp., Norwalk, Conn.
[7] Laboratory Data Control, Riviera Beach, Fla.
[8] Autolab Minigrator, Spectra-Physics, Santa Clara, Calif.
[9] Partisil PXS 10/25 SCX, Whatman Inc., Clinton, N.J.

(12 in.)/hr were used. Short sections of the narrow diameter stainless steel tubing and Swageloks connected the column to the injection valve and the detector. The flow rate was set at 2 ml/min, and the study was conducted at ambient temperature.

Peak heights and relative areas under the curves obtained from the digital integrator were used to construct standard curves and to measure creatinine concentrations in biological samples. Degassing of the mobile phases was unnecessary. The retention times of creatinine from aqueous creatinine solutions and plasma or urine samples spiked with creatinine after treatment with acetonitrile (to be described later) were all identical (3.8 min). Unspiked plasma, serum, and urine samples from human subjects also yielded creatinine peaks with the identical retention time.

Standard Curves of Creatinine in Water—Because of the large difference of creatinine levels in plasma (or in serum) and urine, slightly different methods were used to prepare samples for the HPLC study. To study the recovery of the spiked creatinine in plasma and urine, it was necessary to establish standard curves of creatinine in water with the same procedures used for the plasma and urine creatinine analysis.

For the plasma creatinine study, the following standard solutions of creatinine in water were prepared: 0.5, 1.0, 2.0, 4, 6, 8, 10, 15, and 20 mg %. In a 13 × 100-mm culture tube, 0.2 ml of the standard solution was mixed with 0.5 ml of acetonitrile, and 50 μl of the mixture (solution) was then injected directly into the chromatograph.

For the urine creatinine study, the following standard creatinine solutions in water were prepared: 50, 100, 200, 300, and 400 mg %. In the same type of culture tube, 0.1 ml of the standard solution was mixed with 4 ml of 20% (v/v) water–80% acetonitrile solution in a vortex mixer[10]; 10 μl of the resultant solution was then injected directly into the chromatograph for creatinine determination.

Standard Curves of Creatinine in Spiked Plasma—Aliquots of 0.2 ml of plasma[11] were pipetted into 13 × 100-mm culture tubes and spiked with concentrated aqueous creatinine stock solutions (100, 200, and 1000 mg %) to yield 2, 4, 6, 8, 10, 15, and 20 mg % of the spiked creatinine in the plasma. To each tube, 0.5 ml of acetonitrile was added (mainly for deproteinization). Then the mixture was vortexed for a few seconds and centrifuged at 2000 rpm for about 1 min. Fifty microliters of the supernate was injected directly into the chromatograph for creatinine analysis. A plasma sample without the spiking of additional creatinine also was analyzed similarly as a control.

Standard Curves of Creatinine in Spiked Urine—Aliquots of 0.1 ml of freshly voided urine from a normal adult were pipetted into 13 × 100-mm culture tubes and spiked with various volumes of the aqueous creatinine stock solution (1 g %) to obtain concentrations of 50, 100, 200, 300, and 400 mg % of the spiked creatinine. To each tube, 4 ml of the 20% water–80% acetonitrile solution was added. Then the mixture was vortexed for a few seconds and centrifuged, and 10 μl of the supernate was injected into the chromatograph. One urine sample without spiking of additional creatinine also was studied as a control.

Evidence of Creatinine in Plasma as Measured by HPLC Assay—Several tests were performed to show that the HPLC peak with a retention time of 3.8 min obtained from human plasma samples was attributed to creatinine alone: (*a*) comparison of HPLC peak characteristics obtained from the aqueous creatinine solution and plasma sample using a reversed-phase-type column[12] and various combinations of methanol–water as the mobile phase, (*b*) comparison of HPLC peak characteristics obtained from the aqueous creatinine solution and plasma sample using the cation-exchange column[9] and acidified 0.1 *M* ammonium phosphate as the mobile phase, and (*c*) comparison of HPLC peak heights and areas obtained at 215 and 233 nm from the aqueous creatinine solution and plasma sample using the cation-exchange column and acidified 0.1 *M* ammonium phosphate as the mobile phase.

Reproducibility Studies—Plasma samples from one normal and two renal patients and urine samples from three normal subjects were assayed for creatinine concentrations in six duplicates at the same time, using 0.2 ml of plasma and 0.1 ml of urine. A plasma sample also was analyzed seven times during 3 weeks. The sample was frozen between analyses.

RESULTS AND DISCUSSION

In all previously published methods, creatinine levels in biological samples, with or without further isolation and purification, were not measured directly by spectrophotometry. Such a quantitation method is obviously the simplest one in any HPLC method. The UV spectrum of 2 mg % creatinine in water was run (Fig. 1), and peak absorption wavelengths at 234 and 201 nm were found.

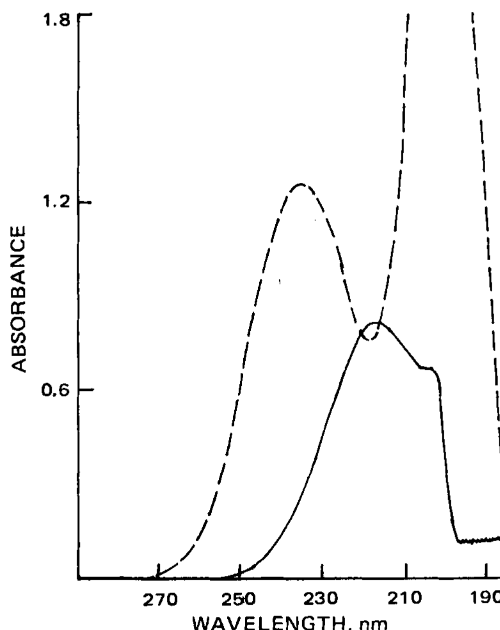


Figure 1—*UV spectra of 2 mg % creatinine in water (- - -) and in acidified 0.1* M *ammonium phosphate (—).*

In the preliminary studies using the reversed-phase column[12] and various combinations of the methanol–water system as the mobile phase, injections of aqueous creatinine solutions with concentrations clinically encountered all resulted in distinct peaks on chromatograms when monitored at 234 nm. Unfortunately, the resolution was not satisfactory with the plasma samples deproteinized with acetonitrile as described under *Experimental*. The best resolution was obtained when 50% methanol–50% water was used as a mobile phase. A typical chromatogram is shown in Fig. 2. It is possible that other solvent systems or other types of the reversed-phase column may yield satisfactory resolution.

Since creatinine is a weak base with a K_b value of 3.57×10^{-11} at 40° (37), it seems logical to use a cation-exchange column[9]. A pH of 2.6 was chosen for the aqueous mobile phase since most creatinine would be in the ionic form. As expected, water acidified with phosphoric acid [about 0.02% (v/v)] as a mobile phase resulted in an almost total retention of creatinine. Addition of ammonium phosphate to the acidified mobile phase reduced the creatinine retention time. Acidified 0.1 *M* ammonium phosphate produced satisfactory resolution with a reasonably short retention time (3.8 min). Chromatograms from the spiked and unspiked plasma of a normal subject and from unspiked plasma of two renal patients are shown in Fig. 3.

Creatinine is very soluble in water and insoluble in most water-immiscible organic solvents (37). Therefore, purification and isolation by the classical extraction method appear to be impossible. The use of the water-miscible acetonitrile with an acetonitrile–plasma or serum ratio of 2.5, as employed in the present study, serves three major functions: (*a*) solubilization of creatinine in the supernate, (*b*) deproteinization of plasma or serum samples, and (*c*) salting out of inorganic or organic salts from plasma or serum samples.

Although creatinine is not soluble in pure acetonitrile, creatinine from concentrated aqueous solutions would not precipitate after mixing with 2.5 volumes of acetonitrile. The efficiency of deproteinization was tested by mixing the supernate with equal or larger volumes of 15% trichloroacetic acid. In no case was precipitation observed, indicating the completeness of the deproteinizing process.

To study the possible precipitation problem that might take place in the column and subsequently damage or block the column (38), 100 μl of the supernate was injected into the mobile phase in beakers. Again, no precipitation was visible, indicating the decreased likelihood of precipitation and column clogging after prolonged use. In fact, the resolution and retention time of creatinine remained unchanged after more than 1000 injections. The postulation of possible salting out or precipitation of organic or inorganic salts from biological samples after treatment with acetonitrile is supported by the fact that precipitation occurred after

[10] Vortex Genie, Fisher Scientific Co., Fair Lawn, N.J.
[11] Obtained from the University Hospital Blood Bank.
[12] ODS Sil-X-1, Perkin-Elmer Corp., Norwalk, Conn.

Table I—Linearity of Creatinine in Plasma Based on Peak Area and Peak Height Methods

Number[a]	Spiked Creatinine Concentration, mg %	Peak Area (Arbitrary Units)	Response Factor[b]	Peak Height, cm	Response Factor[c]
1	0	2.438	—	1.30	—
2	0.5	3.443	2.01	1.90	1.20
3	1.0	4.464	2.03	2.45	1.15
4	1.5	5.341	1.94	3.00	1.13
5	2.0	6.171	1.88	3.59	1.15
6	0	1.757	—	1.05	—
7	2.0	5.588	1.92	3.35	1.15
8	4.0	10.315	2.14	5.65	1.15
9	6.0	14.022	2.04	7.35	1.05
10	8.0	17.616	1.98	10.25	1.15
11	10.0	21.702	2.00	13.00	1.19
12	15.0	33.472	2.01	19.25	1.21
13	20.0	41.907	2.01	24.10	1.15
		Average	2.00		1.15
		SD	0.07		0.04
		CV	3.44%		3.70%

[a] Two sources of plasma samples were used for Numbers 1–5 and 6–13, respectively. [b] Response factor = (peak area from spiked samples − peak area from unspiked samples)/(spiked creatinine concentration). [c] Response factor = (peak height from spiked samples − peak height from unspiked samples)/(spiked creatinine concentration).

mixing the normal phosphate buffer solution (pH 7.4) with the same ratio of acetonitrile. Trichloroacetic acid is probably the most widely used deproteinizing agent. After the addition of the concentrated aqueous solution (10–30%) to the plasma or serum sample, the mixture is usually centrifuged or filtered and the supernate or filtrate is then neutralized with a certain amount of base. Plasma or serum samples also are commonly deproteinized by the addition of sodium tungstate solution and subsequent neutralization with acid.

Compared with these two common methods, the acetonitrile method is much simpler and faster. Acetonitrile is chemically much more inert and is also expected to cause less interferences in quantitation by the various analytical detection methods. Furthermore, plasma or serum samples after treatment with trichloroacetic acid or tungstate and acid were very turbid. Centrifugation at 2000 rpm for 1 min in both methods failed to produce a clear supernate. On the other hand, addition of acetonitrile and subsequent vortexing for a few seconds resulted in a clear supernate, with precipitates settling in the bottom of the tube and forming a hard cake. Centrifugation for 1 min would ensure the supernate to be free of particles, and the supernate could then easily be poured to another tube if required.

Therefore, it is concluded that acetonitrile is an ideal deproteinizing agent. Its use in deproteinization for the direct HPLC study appears not to have been previously reported. Such a method of deproteinization was also successful for assays of gentamicin and other drugs in plasma or serum samples. Results of extensive studies will be reported later.

The linearity of typical standard curves based on peak height and integrated area measurements of creatinine spiked in human plasma samples is summarized in Table I. The concentrations of spiked creatinine, ranging from 0 to 20 mg %, were investigated using plasma samples from two different sources. The excellent linearity of the standard curves was indicated by the consistency of the response factors obtained at all concentrations in both sources of plasma samples.

The response factors were obtained as the ratios of the corrected responses divided by the spiked creatinine concentrations. The corrected responses were the peak heights or peak areas from spiked plasma samples minus the blank values of the plasma from the same source. The blank values were due to the creatinine already present in the plasma samples. In using the peak heights for quantitation, it is recommended

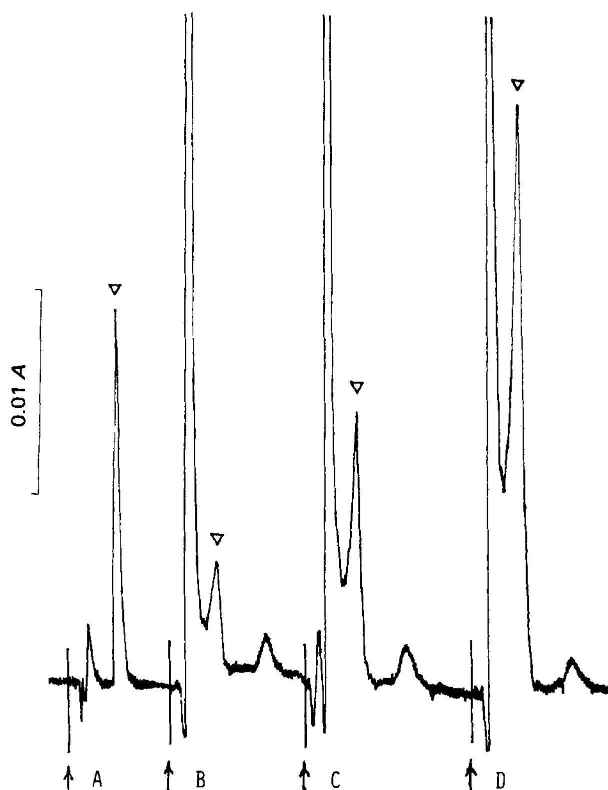


Figure 2—*Chromatograms of creatinine in water (A), creatinine in plasma (B), 2 mg % creatinine spiked in plasma (C), and 4 mg % creatinine spiked in plasma (D). The (∇) and (↑) indicate the peak of creatinine and the point of injection, respectively. Column = reversed phase (ODS Sil-X-1). Mobile phase = water–methanol (1:1, v/v). Sensitivity = 0.05* A *(full-scale deflection). Creatinine retention time = 3 min.*

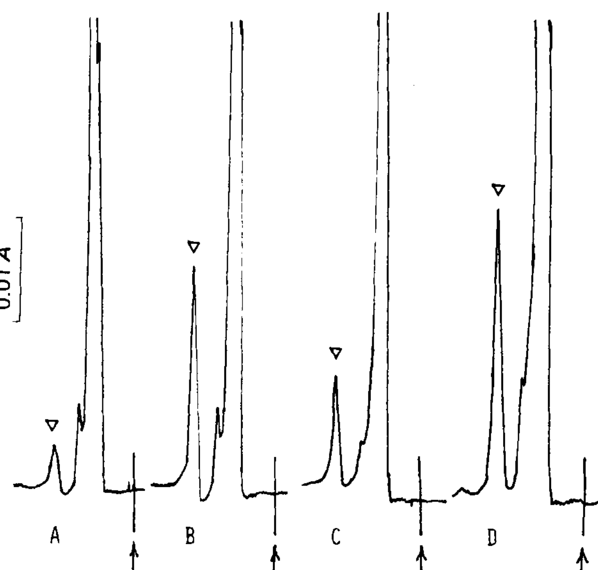


Figure 3—*Chromatograms of creatinine from normal subject plasma (A), the same plasma spiked with 4 mg % creatinine (B), and plasma from two renal patients (C and D). The (∇) and (↑) indicate the peak of creatinine and the point of injection, respectively. Column = cation exchange (Partisil SCX). Sensitivity = 0.05* A *(full-scale deflection). Creatinine retention time = 3.8 min.*

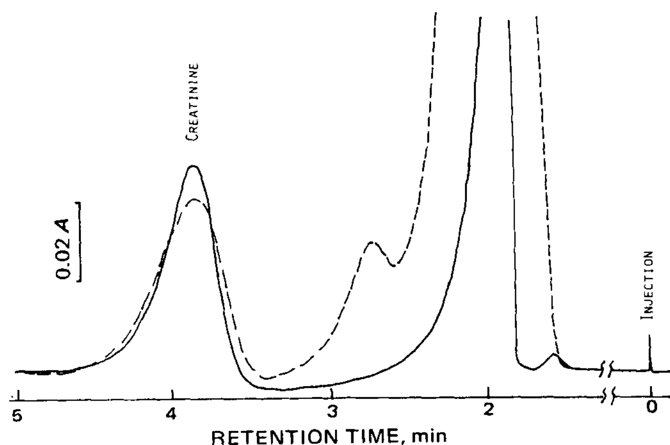


Figure 4—*Chromatograms of creatinine in water (—) and plasma (- - -). Column = cation exchange (Partisil SCX). Sensitivity = 0.1* A *(full-scale deflection). Chart speed = 5.1 cm (2 in.)/min.*

that a 25.4-cm (10-in.) [instead of 12.7-cm (5-in.)] recorder be used for easier and more accurate measurement.

The standard curves based on the peak area measurements of aqueous creatinine solutions also showed excellent linearity, with the slopes identical to those based on peak area measurement of creatinine-spiked plasma samples. This result indicates the complete recovery of the spiked creatinine in the deproteinized supernate and shows that aqueous creatinine solutions can be used to establish the standard curves based on the area method for plasma creatinine assays and also for the routine calibration of the instrument. However, when peak height measurements were used, the standard curves from aqueous creatinine solutions had steeper slopes than those from spiked plasma samples despite excellent linearity in both cases. At the same concentration of creatinine in water and in plasma, the peak heights from the aqueous solutions were higher (16.4%) than those obtained from the plasma samples, but the integrated peak areas were the same for the aqueous solutions and the plasma samples.

The lower peak height with the same integrated area under the curve from the spiked plasma can be explained by the widening of the peak. The peak-widening phenomenon was clearly demonstrated when the two types of samples with similar creatinine concentration were studied using a faster recorder chart speed of 5.1 cm (2 in.)/min. The chromatograms obtained are shown in Fig. 4. The creatinine retention times from both samples were essentially the same.

The exact cause of such peak widening is unknown. The pH factor was ruled out since creatinine dissolved in pH 7.4 phosphate buffer did not

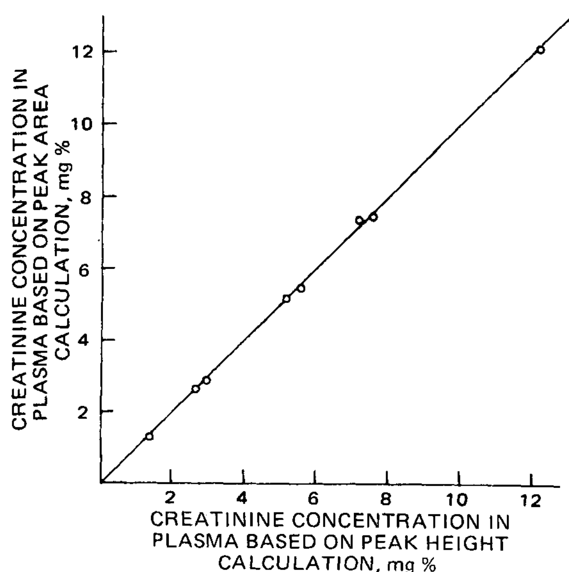


Figure 5—*Correlation between peak height and peak area calculations for creatinine levels in plasma in eight subjects. The line has a slope of one.*

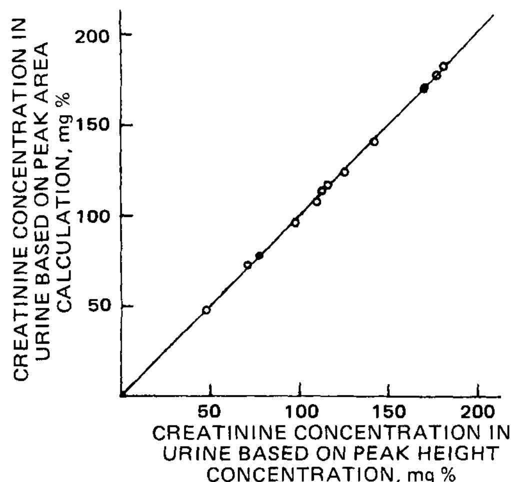


Figure 6—*Correlation between peak height and peak area calculations for creatinine in urine in 12 subjects. The line has a slope of one.*

manifest such a property. Some basic organic compounds present in plasma might interfere with the interaction between the creatinine cation and the cation-exchange column. Since the slopes from the aqueous creatinine and the spiked plasma samples were different when the peak height method was employed, the slope from the spiked plasma study should be used to estimate creatinine levels of unknown plasma samples.

Plasma creatinine levels from one normal subject and seven renal patients were estimated by both the peak height and area methods. The results are plotted in Fig. 5. All data points are on or close to the line with a slope of one, indicating that both methods can be used for quantitation.

No assay published to date has shown that the creatinine levels measured were due entirely to creatinine; interference from other endogenous or exogenous (such as drugs or diet) substances on the assay accuracy could not be eliminated entirely, nor could it be detected with assurance during the assay. The suspected creatinine peak from the plasma samples in the present HPLC study is concluded to be caused entirely by the true creatinine, and such a conclusion is supported by the following findings.

The retention times of the suspected creatinine peak from the plasma, spiked plasma, and aqueous creatinine solution in the reversed-phase column (methanol-water mobile phase system) and the cation-exchange column (acidified ammonium phosphate mobile phase system) were all identical for each particular system studied. Some typical chromatograms are shown in Figs. 2 and 3. These results indicate that the suspected creatinine has partition or solubility and adsorption properties identical to those of the pure creatinine. The results from the cation-exchange column studies also indicate that both suspected and pure creatinines have identical ionic dissociation properties. The identical peak height or area ratio (3.3 in both cases) between 215 and 233 nm from both aqueous creatinine and renal patient plasma samples also indicates that both suspected and pure creatinines have the identical UV absorption property at these two wavelengths and that the suspected peak is entirely from the true creatinine. The same UV absorption property also was found in the methanol–water system.

One important advantage of the present HPLC method over all the previously published methods is that the presence of an interfering substance in plasma can most likely be detected by its influence on the sharpness and resolution of the creatinine peak. The excellence of the present HPLC method also is demonstrated by the fact that no interference was found in the analyses of plasma samples from seven renal patients who were maintained on various kinds of drugs.

The excellent linearity of typical standard curves based on peak area and height measurements of creatinine-spiked urine samples, in concentrations ranging from 0 to 400 mg %, is summarized in Table II. When comparing these standard curves with those from an aqueous creatinine solution, a pattern similar to that found in the plasma analysis was observed; the slopes of standard curves from urine samples and from aqueous solutions were identical using the peak area method, but the aqueous solutions gave steeper slopes using the peak height method. The peak height from the urine sample for the same spiked creatinine concentration was only 10.8% lower than that from the aqueous creatinine

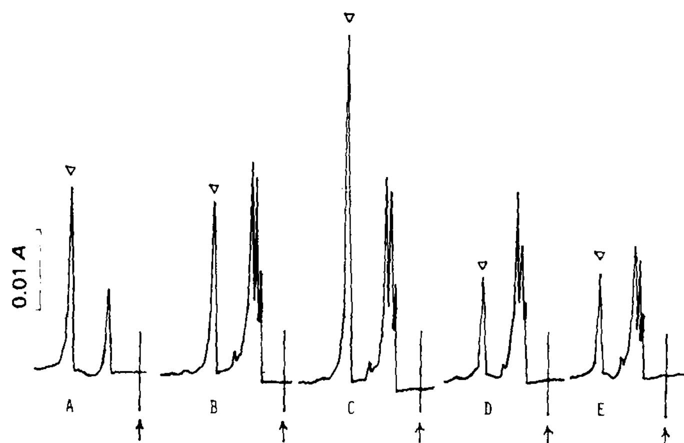


Figure 7—*Chromatograms of creatinine from water (A), urine of a normal subject (B), the same urine sample spiked with creatinine (C), and urine of two normal subjects (D and E). The (∇) and (↑) indicate the peak of creatinine and the point of injection, respectively. Column = cation exchange (Partisil SCX). Sensitivity = 0.05 A (full-scale deflection). Creatinine retention time = 3.8 min.*

solution. This result might be explained by the greater dilution used in the urine study (41 *versus* 3.5 times).

Creatinine concentrations of urine samples from 12 normal subjects analyzed by both the peak height and area methods are plotted in Fig. 6. The two methods of calculation gave essentially the same results. In spite of the large amount of complex substances present in the urine samples, chromatograms from all urine samples were remarkably well resolved and no interference with the creatinine peak was found. Typical chromatograms from urine samples of three normal subjects are shown in Fig. 7.

Reproducibility is important in any assay developed. Results of six duplicate studies on three plasma and three urine samples are summarized in Table III. Excellent reproducibility of the proposed HPLC method is clearly demonstrated by the low coefficients of variation. The excellent day-to-day reproducibility also is demonstrated by the results of the plasma study on one normal subject in which the same plasma sample was analyzed seven times during 3 weeks. These analyses gave an average creatinine concentration of 1.28 mg % with a standard deviation of 0.03 mg % and a coefficient of variation of 2.34%. Since the sample preparation is extremely simple and the day-to-day instrumental variation is minimal, it is concluded that no internal standard is necessary for the proposed method.

The ratio of 2.5 between the volume of acetonitrile and the volume of plasma or serum was satisfactory. It is likely that other ratios also may give satisfactory results. In the urine analysis also, other reagent to urine ratios or other strengths of acetonitrile (80% in the present study) probably could be used. With the same acetonitrile ratios used in the present study, 10 μl of the plasma or serum sample and 1 μl of the urine sample could be analyzed for creatinine with the same degree of accuracy. The study for the plasma or serum sample, in this case, can be conducted in a tapered tube, and only 25 μl of the deproteinized supernate must be injected into the chromatograph. Use of the different initial volumes of

Table II—Linearity of Creatinine in Urine Samples Based on Peak Area and Peak Height Methods

Spiked Creatinine Concentration, mg %	Peak Area (Arbitrary Units)	Response Factor[a]	Peak Height, cm	Response Factor[b]
0	57.27	—	4.45	—
50	73.92	3.33	5.45	2.00
100	88.59	3.13	6.45	2.00
200	121.11	3.14	8.50	2.03
300	153.82	3.22	10.60	2.05
400	186.53	3.23	12.55	2.03
	Average	3.21		2.02
	SD	0.08		0.02
	CV	2.52%		1.00%

[a] Response factor = (peak area from spiked samples − peak area from unspiked samples) × 10/(spiked creatinine concentration). [b] Response factor = (peak height from spiked samples − peak height from unspiked samples) × 100/(spiked creatinine concentration).

Table III—Reproducibility Studies in the Determinations of Creatinine Levels in Plasma and Urine of Three Subjects by the Integrated Area Method

	Plasma Creatinine Concentrations Found, mg %			Urine Creatinine Concentrations Found, mg %		
	Subject 1	Subject 2	Subject 3	Subject 1	Subject 2	Subject 3
	2.455	5.213	1.283	70.26	191.12	166.25
	2.599	5.344	1.217	72.38	185.03	172.18
	2.438	5.520	1.174	69.30	192.55	169.40
	2.460	5.532	1.276	74.93	186.25	172.25
	2.594	5.675	1.212	68.97	187.61	173.21
	2.369	5.457	1.228	73.27	184.37	171.45
Average	2.49	5.46	1.23	71.5	187.8	170.8
SD	0.09	0.16	0.04	2.38	3.33	2.56
CV	3.86%	2.95%	3.35%	3.33%	1.77%	1.50%

the same plasma sample such as 10, 30, 100, and 200 μl all resulted in the same creatinine value.

The proposed method for the true creatinine analysis is reliable, simple, and rapid. Each separate analysis of a sample takes only about 5 min. Another major advantage of this method is the ease of preparation and the economy of the mobile phase used. The microsize of the plasma or serum sample required for analysis is 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.

With the use of this novel assay for creatinine, it is possible that different findings or conclusions regarding the clinical pharmacokinetics of creatinine and its relationship with the pharmacokinetics of drugs and the renal function may be arrived at when they are reevaluated.

REFERENCES

(1) W. L. Chiou and F. H. Hsu, *Res. Commun. Chem. Pathol. Pharmacol.*, **10**, 315 (1975).

(2) W. L. Chiou and F. H. Hsu, *J. Clin. Pharmacol.*, **15**, 427 (1975).

(3) "Clinical Pharmacokinetics: A Symposium," G. Levy, Ed., APhA Academy of Pharmaceutical Sciences, Washington, D.C., 1974, p. 1.

(4) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y., 1975.

(5) M. Gibaldi and G. Levy, *J. Am. Med. Assoc.*, **235**, 1864 (1976).

(6) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence Publications, Hamilton, Ill., 1976.

(7) S. Riegelman and W. Sadee, in "Clinical Pharmacokinetics: A Symposium," G. Levy, Ed., APhA Academy of Pharmaceutical Sciences, Washington, D.C., 1974, p. 169.

(8) R. E. Notari, "Biopharmaceutics and Pharmacokinetics: An Introduction," 2nd ed., Dekker, New York, N.Y., 1975.

(9) R. W. Jelliffe, *Am. J. Med.*, **57**, 63 (1974).

(10) P. G. Welling, W. A. Craig, and C. M. Kunin, *Clin. Pharmacol. Ther.*, **18**, 45 (1975).

(11) W. M. Bennett, I. Singer, and C. H. Coggins, *J. Am. Med. Assoc.*, **223**, 991 (1973).

(12) M. Kristensen, J. M. Hansen, J. Kampmann, B. Lumholtz, and K. S. Nielsen, *J. Clin. Pharmacol.*, **14**, 307 (1974).

(13) T. N. Tozer, *J. Pharmacokinet. Biopharm.*, **2**, 13 (1974).

(14) D. L. Giusti and L. Hayton, *Drug Intell. Clin. Pharmacol.*, **7**, 382 (1973).

(15) J. R. Koup, W. J. Jusko, C. M. Elwood, and R. K. Kohli, *Clin. Pharmacol. Ther.*, **18**, 9 (1975).

(16) "The Effect of Disease States on Drug Pharmacokinetics," L. Z. Benet, Ed., American Pharmaceutical Association, Washington, D.C., 1976.

(17) O. Folin, *J. Biol. Chem.*, **17**, 475 (1914).

(18) O. M. Edwards, R. I. S. Bayliss, and S. Millen, *Lancet*, **2**, 1165 (1969).

(19) J. Brochner-Mortensen and P. Rodbro, *Scand. J. Clin. Lab. Invest.*, **36**, 35 (1976).

(20) M. C. Crim, D. H. Calloway, and S. Margen, *J. Nutr.*, **105**, 428 (1975).

(21) W. J. Turner and S. Cohn, *Clin. Pharmacol. Ther.*, **18**, 405 (1975).

(22) J. D. Jones and P. C. Burnett, *Clin. Chem.*, **20,** 1204 (1974).
(23) E. J. Sampson, D. D. Derck, and L. M. Demers, *ibid.*, **22,** 346 (1976).
(24) R. J. Henry, "Clinical Chemistry: Principles and Technics," Hoeber, New York, N.Y., 1964, p. 297.
(25) P. J. Watkins, *Clin. Chim. Acta,* **18,** 191 (1967).
(26) L. Sadilek, *ibid.*, **12,** 436 (1965).
(27) H. Yatzidis, *Clin. Chem.*, **20,** 1131 (1974).
(28) E. D. Frohlich, "Pathophysiology: Altered Regulatory Mechanisms in Disease," Lippincott, Philadelphia, Pa., 1972, p. 270.
(29) I. Davidsohn and J. B. Henry, "Clinical Diagnosis by Laboratory Methods," 15th ed., Saunders, Philadelphia, Pa., 1974, p. 596.
(30) H. Yatzidis, *Clin. Chem.*, **21,** 1848 (1975).
(31) F.-H. Hsu, MS thesis, University of Illinois, Chicago, Ill., 1976.
(32) S. Vedso, C. Rud, and J. F. Place, *Scand. J. Clin. Lab. Invest.*, **34,** 275 (1974).
(33) K. Larsen, *Clin. Chim. Acta,* **41,** 209 (1972).
(34) G. A. Moss, R. J. L. Bondar, and D. M. Buzzelli, *Clin. Chem.*, **21,** 1422 (1975).
(35) M. Barza, R. B. Brown, D. Sheu, M. Gibaldi, and L. Weinstein, *J. Infect. Dis.*, **132,** 165 (1975).
(36) G. H. McCracken, Jr., *Am. J. Dis. Child.*, **124,** 884 (1972).
(37) "The Merck Index," 9th ed., Merck & Co., Rahway, N.J., 1976, p. 334.
(38) G. W. Peng, V. Smith, A. Peng, and W. L. Chiou, *Res. Commun. Chem. Pathol. Pharmacol.*, **15,** 341 (1976).

ACKNOWLEDGMENTS

Supported in part by Special Project Grant PHS-05D 619-02 from the Department of Health, Education, and Welfare.

General Treatment of Linear Pharmacokinetics

PETER VENG PEDERSEN

Received December 20, 1976, from the *Department of Pharmacy, University of Sydney, N.S.W. 2006 Australia.* Accepted for publication April 28, 1977. Present address: Department of Pharmacy, University of California, San Francisco, CA 94143.

Abstract □ A general treatment of linear pharmacokinetics that enables equations to be obtained simply for all linear compartmental models, with input in one or more compartments, is presented. Two approaches are described: one based on a full Laplace transformation and one that avoids transformation of the input functions and the use of convolution integrals. The latter approach is of particular interest when dealing with complex input functions not having a simple Laplace transform. The concept of acceptor and donor subsystems is introduced. It is demonstrated that disposition in certain models may be simplified and analyzed in terms of disposition in subsystems of simpler composition. The treatment presented is illustrated with several examples.

Keyphrases □ Pharmacokinetics—general treatment for derivation of equations for all linear compartmental models □ Models, pharmacokinetic—general treatment for derivation of equations for all linear compartmental models

Mathematical modeling in pharmacokinetics is commonly based on linear models in which it is assumed that the rate of transfer from any compartment is proportional to the amount in that compartment (1–5). Benet (6) presented a general treatment of linear mammillary models that considers elimination from any compartment but allows input into the central compartment only. Other investigators (7, 8) extended Benet's approach to include input into a peripheral compartment, but they only considered mammillary models. This paper presents a general treatment of any linear pharmacokinetic model with input in one or more compartments.

Two approaches are presented: one requires a Laplace transformation of the input functions, and one avoids such a transformation. The concept of subsystems is introduced. It is demonstrated that certain models can be simplified and analyzed in terms of the disposition of the subsystems.

THEORY

Every possible model having n compartments is a subset of the dense n-compartmental system, defined as a system with reversible transfer between all n compartments and elimination and input in every compartment (*e.g.*, Scheme I). A description of the dense system will describe all compartmental models when the domains of the rate constants and input functions are defined as $k_{ij} \geq 0$ and $f_i(t) \geq 0$, $t > 0$, respectively.

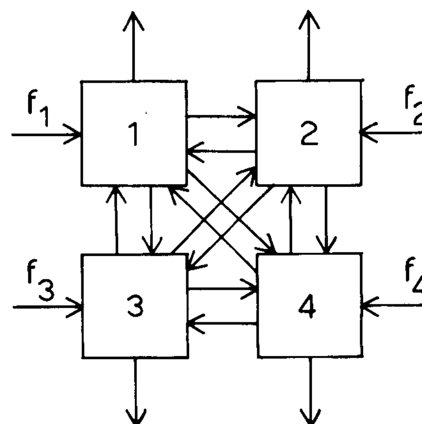
The linear differential equations that describe the kinetics in a dense system are given by[1]:

$$\mathbf{x}' = (\mathbf{K}^T - \mathbf{\Sigma})\mathbf{x} + \mathbf{f} \quad \text{(Eq. 1)}$$

$$\mathbf{\Sigma} = \text{diag}(E_1, E_2, \ldots, E_n) \quad \text{(Eq. 2)}$$

$$E_i = \sum_{j=0}^{n} k_{ij} \quad \text{(Eq. 3)}$$

The ith component of vector $\mathbf{x}$ is the amount in the ith compartment at time t. The ith diagonal element, E_i, of the diagonal matrix, $\mathbf{\Sigma}$, is the sum of the exit rate constants of the ith compartment; $\mathbf{K}^T$ is the transpose of the $n \times n$ matrix, $\mathbf{K} = k_{ij}$, which contains the intercompartmental rate constants. The diagonal elements of $\mathbf{K}$ and $\mathbf{K}^T$ are always zero.



Scheme I—Dense four-compartmental system with reversible transfer between all compartments and elimination and input in every compartment.

[1] Boldface capital letters denote $n \times n$ matrixes, and boldface lower case letters denote vectors of corresponding dimension.