

Direct Quantification of Coproporphyrins and Uroporphyrins in Urine by Derivative Synchronous Fluorescence Spectroscopy

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The second-derivative synchronous fluorescence spectroscopic technique is applied to the simultaneous and direct assay of coproporphyrins and uroporphyrins in human urine. This technique resolves the overlapping conventional spectra, obviating the need for pre-analysis sample separation techniques, and measurements can be made in a single scan. The amplitudes of the derivative peaks are linearly related to uroporphyrin (0.4 to 300 $\mu\text{g/L}$) and coproporphyrin (0.4 to 250 $\mu\text{g/L}$) concentrations. The detection limit for both porphyrins is 0.1 $\mu\text{g/L}$. Analytical recoveries range between 98 and 101%. Within- and between-assay CVs are reported. Results for 24-h urine specimens correlated well with those obtained by an extraction-ion-exchange chromatography combined method. The proposed method is inexpensive and requires no sophisticated detection equipment.

Additional Keyphrases: porphyrins • fluorometry • ion-exchange chromatography compared • screening • reference interval

Estimates of porphyrin concentrations are a central feature of diagnosis of various disorders resulting from altered porphyrin metabolism (1). The total porphyrin content of urine, as well as the relative concentrations of the individual components, especially uroporphyrin and coproporphyrin, must be determined. The methods of analysis available for porphyrins have recently been reviewed by Moore (2) and Bissel (3). These methods are usually based on isolating the porphyrins by acidification and extraction into an organic solvent; quantitative separation of the uroporphyrins and coproporphyrins is carried out by selective solvent extraction, thin-layer chromatography, or "high-performance" liquid chromatography, fluorometry being used for quantification. Owing to the wide polarity range of uroporphyrin and coproporphyrin, their complete extraction from urine and separation and quantification pose problems and are rather costly (4). In addition, the separation procedures are tedious and time-consuming, making them unsuitable for routine analytical work.

Improved selectivity of the measurements and the data-processing steps in an analytical method lessens the need for physical separations or chemical reactions, leading to simpler and faster procedures (5). One approach to improve the selectivity of analytical methods—one that has been scarcely dealt with so far—involves the use of derivative synchronous fluorescence spectroscopy, a technique originally introduced by John and Soutar (6), a combination of the synchronous (7) and derivative (8) fluorescence spectroscopy techniques.

Synchronous spectra are obtained by locking the excitation and emission monochromator drives together at fixed increments of wavelength ($\Delta\lambda$) (7) or energy ($\Delta\nu$) (9). The

resulting spectra are narrower and hence simpler than conventional fluorescence spectra, which aids the identification and quantification of multiple components. In addition, these spectra can be obtained with any straightforward spectrofluorometer capable of simultaneously scanning both monochromators. The principles behind this technique have been comprehensively described (10) and the applications reviewed (11). Derivative spectroscopy is a straightforward approach for improving selectivity in clinical chemistry (12). Secondary, weak spectral features such as slopes and peak shoulders are enhanced and thus made easier to identify and interpret. The selectivity of fluorometric analysis is greatly increased by the use of both techniques in conjunction and, although few applications to resolve problems in clinical chemistry have been described thus far (13-15), the results confirm that derivative synchronous fluorescence spectroscopy is well suited to the resolution of mixtures of compounds with similar spectra.

We have used this technique to develop and evaluate a method for the direct and simultaneous quantification of uroporphyrins and coproporphyrins in urine. Dilution of the urine samples with hydrochloric acid until they appear colorless only negligibly quenches fluorescence and generates no interference from nonspecific fluorescent species (16). Using the second-derivative synchronous spectrum of the mixture of uroporphyrin and coproporphyrin, we can complete their analysis in urine in only one scan, without resorting to separation techniques. The quantitative results agree well with those obtained after purification by other, more tedious, procedures.

Materials and Methods

Apparatus. For spectrofluorometric measurements we used a fluorescence spectrophotometer (Model MPF-43A; Perkin-Elmer Corp., Norwalk CT 06856) fitted with 1-cm cells and a xenon-arc source. The temperature of the spectrofluorometer cell compartment was kept at 25 °C by circulating water through it. The spectral band-pass settings for the excitation and emission slits were 5 and 15 nm, respectively. For synchronous fluorescence measurements, both excitation and emission monochromators were interlocked and scanned simultaneously at 4 nm/s. Derivative spectra were obtained by electronic differentiation of the signal from a Perkin-Elmer derivative accessory (Model H 200-0507). Of the six differential time constants available from the mode switch, we used position 6 for all measurements. The spectrofluorometer response (time constant) was set at 3 s. We adjusted the spectrofluorometer daily, using a series of six fluorescence polymer samples (Perkin-Elmer), to compensate for changes in the source intensity (17). We made all absorbance measurements with a Model Lambda 1 C632-0001 spectrophotometer (Perkin Elmer, Oak Brook, IL 60521).

Reagents. Uroporphyrin I dihydrochloride and coproporphyrin I dihydrochloride were purchased in dry standardized vials from Sigma Chemical Co., St. Louis, MO 63178.

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Stock 40 mg/L solutions of each porphyrin were prepared in 6 mol/L hydrochloric acid and stored in the dark in a refrigerator (4 °C). Each month we diluted aliquots of the stock standards with 1.5 mol/L hydrochloric acid to prepare fresh working standard solutions. Prepacked ion-exchange columns containing an anion-exchange resin (cat. no. 11108) were used as supplied by Biosystems (Atom S.A., Barcelona, Spain). All other chemicals used were AR grade, from Merck, Darmstadt, F.R.G. All water used was doubly distilled in glass.

Sample treatment. Urine specimens were collected from ostensibly normal volunteers over 24 h.

To oxidize the porphyrinogen to porphyrin, we used two methods: (a) adding sodium carbonate to a final concentration of 10 g of sodium carbonate per liter of 24-h urine, according to the instructions of Biosystems' porphyrins kit (these urine samples were kept in a darkened container at room temperature for at least one day until analysis); and (b) adding, in sequence, to a 0.5-mL aliquot of 24-h urine, 2.5 mL of 3 mol/L hydrochloric acid solution, 0.1 mL of a 50 mmol/L disodium EDTA solution, and one drop of 300 g/L hydrogen peroxide solution, then diluting to 5 mL with doubly distilled water (4).

These samples were kept at room temperature for at least 30 min until analysis.

Fluorometric analysis. Preparation of the samples for quantitative fluorometric analysis varies according to the sample treatment used.

For method *a*, dilute aliquots (0.2–0.8 mL) of the urine samples with 2.5 mL of 3 mol/L hydrochloric acid and doubly distilled water to give a final volume of 5 mL.

For method *b*, make spectral measurements of the prepared sample without additional treatment.

Record second-derivative synchronous fluorescence spectra by scanning both monochromators simultaneously at a 210-nm constant difference. Scan the excitation monochromator from 330 to 500 nm and the emission monochromator from 540 to 710 nm. Measure the amplitude of the second-derivative spectra from peak to peak (12), i.e., measure the difference between the derivative signals at two wavelengths corresponding to an adjacent maximum and minimum, to determine relative fluorescence intensity, expressed as ΔI (Figure 1). Determine the concentrations of uroporphyrin and coproporphyrin by interpolation on the working curves prepared from analyses of standard solutions of uroporphyrin (final concentration, 0.4 to 300 $\mu\text{g/L}$) and coproporphyrin (final concentration, 0.4 to 250 $\mu\text{g/L}$) in 1.5 mol/L hydrochloric acid. The derivative signal obtained between the fluorescence minimum at 605 nm and the maximum at 612 nm ($\Delta I_{605-612}$) is directly related to the coproporphyrin concentration. The measurement between 645 and 685 nm ($\Delta I_{645-685}$) corresponds linearly to the contribution of both porphyrins. The difference between the total signal at $\Delta I_{645-685}$ and the corresponding signal attributed to the action of coproporphyrin at this ΔI value (determined by a prior calibration of $\Delta I_{645-685}$ vs coproporphyrin concentration) is directly related to the concentration of uroporphyrin.

If uroporphyrin concentrations exceed those of coproporphyrin, this gives rise to a bathochromic shift of the signal $\Delta I_{605-612}$ resulting from the effect of uroporphyrin on this signal, so that the coproporphyrin concentration of the sample cannot be related to it. In this case, it is necessary to run several calibration plots of the signal $\Delta I_{605-612}$ vs uroporphyrin concentration at different fixed amounts of

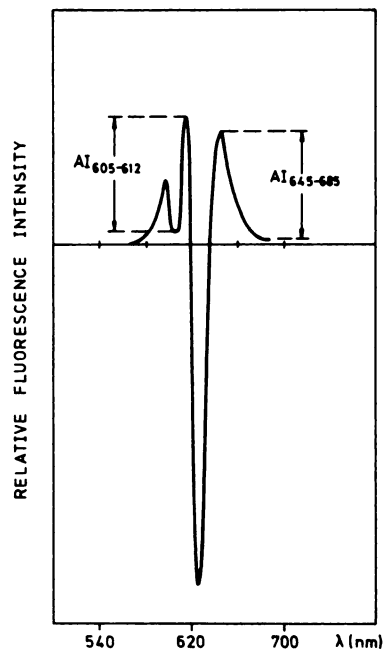


Fig. 1. Second-derivative synchronous fluorescence spectrum of a mixture of coproporphyrin (30 $\mu\text{g/L}$) and uroporphyrin (15 $\mu\text{g/L}$) in 1.5 mol/L hydrochloric acid

coproporphyrin. The slopes of these graphs are plotted as a function of the corresponding coproporphyrin concentration. Increasing volumes of uroporphyrin solution are added to several aliquots of urine sample (0.2–0.8 mL) and the coproporphyrin concentration is obtained by interpolation of the slope of the above-mentioned calibration plot.

Results and Discussion

Interpretation of Spectra

Because the conventional excitation and fluorescence maxima of copro- and uro-type porphyrins differ by only 4 nm or less, their separate analysis by conventional spectrofluorometry is unfeasible without the use of separation techniques (Figure 2, left). The more significant synchronous fluorescence spectra, obtained over a range of $\Delta\lambda$ from 150 to 300 nm, are shown in Figure 2 (right). From this, we observe the following:

- Synchronous spectrofluorometry offers improved selectivity over conventional spectrofluorometry thanks to the narrowing of the spectral bandwidth, which in turn depends on the $\Delta\lambda$ value.
- The synchronous fluorescence spectra show one band and a small shoulder for $\Delta\lambda$ lesser than 280 nm.
- Fluorescence intensity is maximum when the wavelength increment corresponds to that between absorption and emission maxima (i.e., 190 and 250 nm for both porphyrins); increments that differ from those optimum values will result in a lower contribution from each component to the overall spectrum.
- The maxima undergo bathochromic shifts with increasing $\Delta\lambda$.

These effects can be accounted for as follows: for a given point in a synchronously excited emission spectrum, the observed fluorescence signal will be a product of the relative response at λ_{em} in the fluorescence emission spectrum and the relative response at λ_{ex} in the excitation spectrum, and this offers an opportunity to study selectively a certain

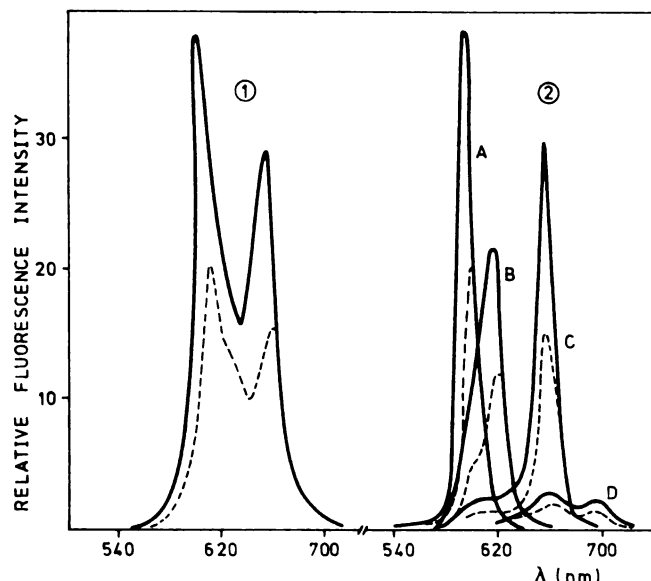


Fig. 2. Fluorescence emission spectra excited at 400 nm (1) and synchronous (2) spectra, at different $\Delta\lambda$ values, of uroporphyrin (-----) and coproporphyrin (—). $\Delta\lambda$ values: (A) 190 nm, (B) 210 nm, (C) 250 nm, (D) 290 nm. Uroporphyrin and coproporphyrin concentrations as in Fig. 1

component in a mixture when the traditional approach with fixed excitation would be unsuccessful. However, when the conventional emission spectra corresponding to the mixture's components strongly overlap, as is the case with uroporphyrin and coproporphyrin, synchronous fluorescence spectroscopy requires the additional resolution capability offered by derivative fluorescence spectroscopy for the simultaneous determination of the different individual components. It has been checked that the peak height for coproporphyrins (Figure 1, $\Delta I_{605-612}$) does not vary in a systematic manner with the concentration of uroporphyrins and that the additivity of the signal yielded by both porphyrins ($\Delta I_{645-685}$) is retained throughout the whole concentration range where the method was applied.

Optimization

The instrumental, chemical, and physico-chemical variables were optimized for greater resolution and signal enhancement. The instrumental variables ($\Delta\lambda$, wavelength scanning speed, differentiation constant, slit widths, and spectrofluorometer time constant) affect both the resolution and the signal, because they modify the wavelength ranges and the number and relative intensity of peaks of the second-derivative synchronous fluorescence spectrum. The chemical and physico-chemical variables (hydrochloric acid concentration, ionic strength, dielectric constant, and temperature) influence only ΔI and therefore affect only the sensitivity of the method.

The effect of the different variables was evaluated at three coproporphyrin/uroporphyrin ratios (1:1, 2:1, and 10:1). Coproporphyrin was added in greater amounts than uroporphyrin according to the normal proportions found in urine. For selection of the appropriate $\Delta\lambda$ value, we studied its effect on the second-derivative synchronous fluorescence spectra, at values between 150 and 300 nm. The spectral distribution is a function of the wavelength increment, and the same general considerations on the influence of $\Delta\lambda$ on the synchronous spectra made in the above section are applicable here. There are three types of spectrum in the $\Delta\lambda$

interval tested (Figure 3), as can be readily inferred from Figure 2 (right). The best resolution of the mixture is obtained when the $\Delta\lambda$ value is between 200 and 250 nm. In this range, a small shoulder appears in the synchronous spectrum which results in the appearance of a new peak in the derivative synchronous spectrum. This fact makes it possible to correlate the signal $\Delta I_{605-612}$ with the coproporphyrin concentration and the total concentration of porphyrins can be derived from the signal $\Delta I_{645-685}$. The second-derivative synchronous fluorescence spectra of both porphyrins overlap appreciably outside of this interval. A $\Delta\lambda$ value of 210 nm was chosen because it yielded lower errors.

Generally, decreasing wavelength-scanning speeds and the differentiation constant result in increased spectral resolution (number of peaks) and decreased peak amplitude. This is so because when these parameters decrease, so does the signal differentiation over a given time interval and therefore, in addition to a decreased amplitude, there is a greater possibility of the fine structural features being sharpened, and the resolution of overlapping bands is, in principle, improved. In the second-derivative synchronous fluorescence spectra of both porphyrins, the peak amplitude shows this behavior, but the number of peaks does not change with changes in these variables. This is a result of the number of peaks also depending on the spectrum shape. When the spectral bands are well-defined—i.e., they have few shoulders, as is the case with the synchronous spectra of both porphyrins—the number of peaks is scarcely modified. The optimum speed is 4 nm/s; above this, $\Delta I_{605-612}$ does not correlate with the coproporphyrin concentration, and below it there is a loss of additivity in $\Delta I_{645-685}$. The additivity of coproporphyrin and uroporphyrin in the signal $\Delta I_{645-685}$ degrades as the spectrofluorometer time-constant decreases. Thus, we chose a value of 3 s despite the decrease in relative intensity of all peaks resulting from increasing time constants, owing to the high level of smoothing applied.

The slit width was studied in both monochromators for an

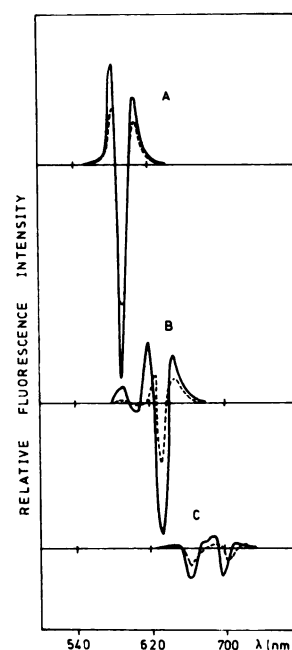


Fig. 3. Effect of $\Delta\lambda$ on the second-derivative synchronous fluorescence spectrum of coproporphyrin (—) and uroporphyrin (-----). $\Delta\lambda$ values: (A) 180 nm, (B) 220 nm, (C) 290 nm. Uroporphyrin and coproporphyrin concentrations as in Fig. 1

emission/excitation ratio between 1 and 10. A ratio of 3 gave the lowest percent error.

Various solvents with different dielectric constants and several salts at different concentrations were tested for their ability to enhance the Stokes shift and in order to determine the effect of the ionic strength, respectively, on the second-derivative synchronous fluorescence spectra, but our results show that these variables have no effect on these systems. The effect of changes in the acidity of the samples on the fluorescence of these analytes was studied by addition of hydrochloric acid to give final concentrations between 1.3 and 2.5 mol/L. The amplitude of the peaks of interest only decreased slightly for concentrations of hydrochloric acid above 2 mol/L. The spectra were practically independent of the temperature in the range 15–50 °C and hence this is not a critical factor in choosing the experimental conditions.

Linearity and Precision

The relation between the derivative amplitude (ΔI) and the analyte concentration was linear in the ranges 0.4 to 300 μg of uroporphyrin and 0.4 to 250 μg of coproporphyrin per liter, as shown in Table 1. Standard errors of estimate and correlation coefficients suggest very good linearity. The lower limit of detection for both porphyrins, as this is defined by IUPAC, is 0.1 $\mu\text{g/L}$ (18).

To test the precision of the method, we assayed a series of samples with different concentrations. The mean within-assay and between-assay coefficients of variation (19) found are listed in Table 2. Although it is surprising that the within-assay CVs exceed the between-assay CVs, similar results were reported in earlier papers (14, 15). A possible explanation for this is based on the enhancement of high-frequency noise components in the signals from which the derivative spectra are generated. This increased noise decreases the precision of derivative measurement and the within-assay CVs are relatively high. However, there is good agreement within the mean values obtained between series, from which the between-assay CVs, relatively low, were obtained.

Urine Samples

Uro- and coproporphyrins were determined in samples of 24-h urine specimens from apparently normal individuals. The second-derivative synchronous fluorescence spectra obtained from these samples were similar to those from pure

standards in the region of interest. The analytical recoveries achieved for three different concentrations of uro- (6, 20, and 30 $\mu\text{g/L}$) and coproporphyrins (10, 20, and 40 $\mu\text{g/L}$), added separately to urine, ranged from about 98 to 101% (mean of five determinations for each concentration of each porphyrin). The oxidation of porphyrinogen to porphyrin was carried out by two methods as described in *Materials and Methods*. The addition of hydrogen peroxide resulted in a fast oxidation, although slight but increasing fluorescence development was noted until about 30 min. We determined uro- and coproporphyrin concentrations in 24-h urines from 10 normal adults by the two oxidation methods, using different sample volumes (between 0.1 and 1 mL) in order to select the best possible conditions. The results were compared with those obtained by the combination of two different methods: (a) Total porphyrins were extracted from urine onto anion-exchange resin, supplied by Biosystems, eluted with hydrochloric acid (3 mol/L), and quantified by conventional fluorometry. (b) Coproporphyrins were estimated by extraction into ethyl acetate and then into hydrochloric acid (1.5 mol/L) (20), discarding uro-type porphyrins. Quantification was photometric. Uroporphyrins were estimated by difference in both methods. Uro- and coproporphyrin values found by methods a and b were corrected for their respective analytical recovery percentage (approximately 90%). Table 3 list some carefully selected results, summarized as linear least-squares statistics. This table shows only the results obtained with use of sodium carbonate medium, because the oxidation of porphyrinogen in this medium gives substantially better values (the slopes are closer to unity and the correlation coefficients suggest better linearity) than in the presence of hydrogen peroxide. The overall conclusions that can be drawn from this study are the following:

- Results for sodium carbonate agree well with those obtained by extraction–chromatography, taking into account the inherent methodological differences: two different separation (extraction, chromatography) and instrumental (conventional fluorometry, photometry) techniques are used.

- Use of urine volumes >0.8 mL generally results in smaller measured uro- and coproporphyrin concentrations, because the dilution is not sufficient to yield an almost colorless solution.

- Urine volumes <0.2 mL result in low precision. Sodium carbonate and urine volumes between 0.2 and 0.8 mL were optimum for the oxidation of porphyrinogen.

Typical results from 20 samples obtained under these optimum conditions show good correlation with extraction–chromatography method for urinary coproporphyrin ($r = 0.993$) and uroporphyrin ($r = 0.981$), for 24-h excretions ranging between 60 and 210 μg of coproporphyrin and 15 to 50 μg of uroporphyrin.

Urine samples from two patients suffering from porphyria cutanea tarda were also analyzed. In our method, when the uroporphyrin concentration exceeds the coproporphyrin concentration, as is the case with this disease, the signal for $\Delta I_{605-612}$ is not directly related to the coproporphyrin concentration because uroporphyrin causes a bathochromic shift of this signal at a uro/copro ratio >1.5 . For a urine sample in which the uro/copro ratio is unknown, this can be estimated by comparing the sample spectrum with that of a standard coproporphyrin solution. If the bathochromic shift is observed, uroporphyrin exceeds coproporphyrin. The results obtained for both urine samples for 24-h excretions with our method—(a) 1200 μg of coproporphyrin and 5680

Table 1. Quantitative Performance of the Proposed Second-Derivative Synchronous Fluorescence Method

Concn range, μg/L	Slope (and SD)	Intercept (and SD)	SEE	<i>r</i>
		μg/L		
<i>Uroporphyrin</i>				
0.4– 15 ^b	5.037 (0.371)	−0.04 (0.62)	0.86	0.990
15 – 50 ^c	0.454 (0.009)	−0.33 (0.28)	0.34	0.9990
50 –300 ^d	0.065 (0.001)	+0.31 (0.39)	0.22	0.9991
<i>Coproporphyrin</i>				
0.4– 15 ^b	1.340 (0.008)	−0.10 (0.06)	0.10	0.99993
15 – 50 ^c	0.371 (0.002)	−0.07 (0.06)	0.07	0.99991
50 –250 ^d	0.119 (0.002)	+0.17 (0.42)	0.35	0.9997
<i>Coproporphyrin</i> ^a				
0.4– 15 ^b	1.853 (0.020)	+0.24 (0.15)	0.25	0.9998
15 – 50 ^c	0.476 (0.017)	+0.35 (0.53)	0.63	0.998
50 –250 ^d	0.154 (0.002)	−0.57 (0.46)	0.38	0.9996

^a Results determined from $\Delta I_{605-612}$; all others from $\Delta I_{645-685}$. Instrumental sensitivity: ^b 10, ^c 3, ^d 0.3

Table 2. Precision (CV) of the Proposed Second-Derivative Synchronous Fluorescence Method

Concn, μg/L	Within assay (n = 6)						Between assay (n = 5)					
	ΔI ₆₄₅₋₆₈₅			ΔI ₆₀₅₋₆₁₂			ΔI ₆₄₅₋₆₈₅			ΔI ₆₀₅₋₆₁₂		
	\bar{x}	SD	CV, %	\bar{x}	SD	CV, %	\bar{x}	SD	CV, %	\bar{x}	SD	CV, %
<i>Coproporphyrin</i>												
5	5.0	0.405	8.1	5.0	0.315	6.3	5.0	0.016	0.32	5.0	0.017	0.35
50	50.1	1.303	2.6	50.2	1.205	2.4	50.1	0.075	0.15	50.2	0.050	0.10
200	199.9	6.597	3.3	199.9	4.997	2.5	199.9	0.279	0.14	199.9	0.099	0.05
<i>Uroporphyrin</i>												
1	1.0	0.08	8.0				1.0	0.016	1.58			
20	20.0	0.202	1.0				20.0	0.100	0.50			
100	100.1	2.102	2.1				100.1	0.190	0.19			

Table 3. Representative Least-Squares Statistics for Comparison of Results by Second-Derivative Synchronous Fluorometry (y) and by Extraction and Ion-Exchange Chromatography (x)

		Mean intercept (and SD)		SEE	
Urine vol, mL	Mean slope (and SD)	μg/L			r
<i>Coproporphyrin</i>					
0.2	0.98 (0.001)	+1.13 (1.3)		5.7	0.995
0.4	0.95 (0.07)	+1.21 (1.7)		6.9	0.991
1.0	0.93 (0.08)	-1.30 (1.8)		7.1	0.962
<i>Uroporphyrin</i>					
0.2	1.07 (0.06)	-1.50 (1.9)		7.5	0.980
0.4	1.10 (0.07)	-3.01 (1.5)		6.4	0.982
1.0	0.92 (0.07)	+0.08 (2.0)		9.9	0.950

μg of uroporphyrin; and (b) 455 μg of coproporphyrin and 795 μg of uroporphyrin—correlate well with those found by the extraction-chromatography method: (a) 1248 μg of coproporphyrin and 5614 μg of uroporphyrin; and (b) 420 μg of coproporphyrin and 820 μg of uroporphyrin.

Comparison with Other Methods

Second-derivative ultraviolet-visible absorption spectroscopy has been previously used to quantify urinary porphyrins (4) in a fast, straightforward method where the amplitude of the second-derivative signal is linearly related to the porphyrin concentration, and the wavelength of the central peak provides an estimate of the ratio of uroporphyrin to coproporphyrin (4). However, because wavelength calibration at the recording of second-derivative spectra is clearly critical (a 0.5-nm error will produce a 5 to 20% error in the uroporphyrin/coproporphyrin ratios), wavelength accuracy must be carefully confirmed. The use of synchronous fluorescence spectroscopy increases assay selectivity by narrowing the spectral bandwidth, as is characteristic of this technique, and second-derivative synchronous fluorescence spectra show a single peak, the amplitude of which, as has been shown, is linearly related to the coproporphyrin concentration. The enhanced spectral resolution results in simpler, more precise measurements than the derivative absorption spectroscopy method (4). In addition, the detection limit obtained in our method is 1/60th of that achieved by derivative absorption spectroscopy.

Derivative fluorescence spectra of aqueous solutions of porphyrins have been reported (21), but no actual clinical methods based on derivative fluorescence measurements have been described.

With regard to separation techniques, although solvent-extraction methods can be used in investigating porphyrins,

"high-pressure" liquid-partition chromatography with a reversed-phase column is preferable. However, the chromatographic procedure is rather time consuming for routine assays in clinical chemistry and requires special and expensive apparatus.

The method proposed here for determination of urinary porphyrins illustrates the advantages of derivative synchronous fluorescence measurements. This technique opens up a wide range of possibilities for further improvement in the selectivity of clinical analyses and may be used where simplicity, rapidity, and cost-effectiveness are sought. The main advantage of the recommended procedure is that no pre-analysis separation is needed. This inexpensive method is also suitable for routine screening of a large number of samples as only one scan and no highly specialized equipment are required for the simultaneous assay of both porphyrins. Any commercial spectrofluorometer in which the excitation and emission monochromators can be interlocked is usable, and derivative spectra can be obtained by using a low-cost electronic differentiator that is readily fitted to the spectrofluorometer. Finally, because of their high analytical recoveries and precision, the assays can be validly performed without an internal standard.

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