Detection of 1-Hydroxypyrene as a Urine Biomarker of Human PAH Exposure Determined by Fluorescence and Solid-Matrix Luminescence Spectroscopy

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A new solid-matrix luminescence method was developed for human biomonitoring that employs room-temperature fluorescence and phosphorescence for the detection and identification of 1-hydroxypyrene (1-OH-PY). Several high-performance liquid chromatography (HPLC) fractions from human urine samples were characterized by both solution fluorescence and solid-matrix luminescence. The solution fluorescence data and the solid-matrix luminescence data complemented each other. For example, in one of the fractions, the solution fluorescence showed the presence of tetrahydrotetrols of benzo[*a*]pyrene. However, in this fraction, 1-OH-PY was readily detected by solid-matrix luminescence. In addition, solution fluorescence showed that 1-OH-PY was not in some of the fractions, but solid-matrix luminescence showed that 1-OH-PY was present. Solid-matrix phosphorescence was very effective in detecting 1-OH-PY in the fractions where solution fluorescence and solid-matrix fluorescence indicated that 1-OH-PY was not present. The limits of detection were in the pmol/mL range by solution fluorescence and in the subpicomole range with solid-matrix luminescence.

Index Headings: **Solid-matrix fluorescence/phosphorescence; 1-Hydroxypyrene; Biomarkers.**

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a very important class of compounds that are carcinogenic in laboratory animals. They are the products of the combustion of vegetable matter and are found in coal tar pitch, tobacco smoke, and charbroiled food.[1] The characterization and determination of the hydroxylated metabolites of PAHs are important for assessing human exposure and uptake of PAHs. One important approach in assessing human exposure to PAHs is to measure PAH metabolites excreted in urine.[2] In particular, 1-hydroxypyrene (1-OH-PY) has been used as a biomarker in the measurement of human exposure to PAHs.[3–6] A variety of analytical methods have been developed for the measurement and characterization of 1-OH-PY and related products. Strickland et al.[2] identified 1-OH-PY glucuronide as a major pyrene metabolite in human urine by synchronous fluorescence spectroscopy and gas chromatography–mass spectrometry. Jongeneelen et al.[7] determined hydroxylated metabolites of PAHs, including 1-OH-PY, in urine by high-performance liquid chromatography (HPLC). A coupled-column HPLC system was developed for the determination of free and conjugated 1-OH-PY in urine.[8] Also, an HPLC/fluorescence method for the quantitation of 1-OH-PY has been reported.[9]

Solution room-temperature fluorescence (RTF) is a widely used spectroscopic technique in the trace analysis of organic compounds. Most aromatic compounds in solution show RTF, and this makes RTF a versatile analytical tool. Also, numerous aromatic compounds, when adsorbed on solid matrices such as sodium acetate, filter paper, cyclodextrin–salt mixtures, and trehalose, emit, in addition to RTF, strong solid-matrix room-temperature phosphorescence (RTP).[10,11] Filter paper is one of the most widely employed solid-matrices for obtaining RTF and RTP from adsorbed lumiphors due to its simplicity, ease of sample application, and low limits of detection. Recently, Whatman 1PS filter paper was shown to be a better alternative to Whatman no. 1 filter paper in obtaining solid-matrix RTF and RTP.[12] A heat-cured coating of silicone and a proprietary tin complex in the 1PS filter paper imparts hydrophobic character to the paper.[14]

In this work, a small volume of water and methanol, containing 1 ng or less of the lumiphor, formed a small droplet when spotted onto the 1PS filter paper without spreading on the filter paper surface. As the solvent evaporated, the lumiphor was adsorbed in a localized area of the filter paper, and this led to an increase in the sensitivity of the solid-matrix RTF and RTP. Also, thallium nitrate was added to 1PS filter paper to enhance the RTP of the adsorbed compounds. In the heavy-atom effect, the probability of intersystem crossing from the excited singlet state to the excited triplet state increases, and this, in turn, enhances the probability of phosphorescence.[14]

This report illustrates that solution RTF and solid-matrix RTF and RTP spectroscopic techniques are useful in characterizing and identifying trace quantities of 1-OH-PY but that solid-matrix RTP was very selective in detecting 1-OH-PY in the HPLC fractions of urine samples of coal tar-treated psoriasis patients. Because r-7,t-8,t-9,c-10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BP-tetrol I-1) could possibly appear in the HPLC fractions, this metabolite was also investigated.

EXPERIMENTAL

Apparatus. Spex Fluorolog and Perkin Elmer LS50B spectrofluorimeters were used to obtain room-temperature solid-matrix RTF and RTP and solution RTF spectra of the fractionated urine samples. The spectra obtained were not corrected for changes in source output with wavelength, detector response, and monochromator functions.

Reagents. Reference standards of 1-OH-PY of 98% purity (Aldrich, Milwaukee, WI) and BP-tetrol I-1 (NCI Chemical Carcinogen Repository, Midwest Research In-

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stitute, Kansas City, MO) and solvents of HPLC-grade methanol and water (J. T. Baker, Phillipsburg, NJ) were used as received. Whatman No. 1 and 1PS filter paper circles were developed in ethanol to collect impurities at one end.

Monoclonal Antibody. The monoclonal antibody 8E11 was originally raised against benzo[*a*]pyrene-diol-epoxide-deoxyguanosine bound to keyhole limpet heamoncyanin (KLH). This antibody has a relatively broad specificity for PAHs and their metabolites. It has a relatively low affinity, which makes it a poor diagnostic probe. (It was originally intended to be used in an enzyme-linked immunosorbent assay. The broad specificity and low affinity made it a relatively worthless reagent for this purpose.) However, urine is a very complex medium, and preparative technologies are useful prior to analytical HPLC. The monoclonal antibody 8E11 is a fine preparative reagent because it binds to the compounds of interest (PAHs and their metabolites) while ignoring "garbage" (urophores and bilirubin and other protein degradation products). The low affinity allows easy removal of the products of interest (an antibody with high affinity could possibly bind to the PAHs of interest irreversibly).

HPLC Urine Fractions. Psoriasis patients receiving Goekerman [coal tar/ultraviolet (UV)] therapy at Columbia Presbyterian Hospital, New York, were recruited into a biological markers of PAH exposure study. Institutional Review Board stipulations were observed. Study subjects administered topical coal tar preparations to themselves and donated a single sample of urine at the end of the treatment period. Urine samples were subjected to immunoaffinity chromatography (anti-BP-dG) as previously described.[2] Immunoaffinity-purified materials were subsequently separated by reversed-phase HPLC and analyzed spectrofluorometrically. A reversed-phase (5-μm ODS) column 4.6 × 250 mm, Vydac, Hesperia, CA) was eluted with a water methanol gradient (30–100% at 1 mL/min). One-milliliter fractions were collected.

A total of 40 HPLC fractions of urine samples from coal tar-treated psoriasis patients were made available for the identification of 1-OH-PY and BP-tetrol I-1. The first set of 14 samples of CT 103 were labeled from 6 to 10 and from 14 to 22; the second set of 14 samples of CT 106 were numbered from 6 to 10 and from 14 to 22. The third set of 12 samples of CT 119 were labeled from 6 to 10 and from 16 to 22. The abbreviation, "CT", stands for coal tar, and the numbers are patient numbers. The numbers used are arbitrary. The sample set chosen was a convenient size.

Solution RTF. A portion (~250 μL) of each of the HPLC fractions and the same volume of reference standard materials (1-OH-PY and BP-tetrol I-1) were individually added to a quartz micro cell (700 μL) and used to obtain RTF excitation and emission spectra and also room-temperature synchronous fluorescence spectra of the samples.

Solid-Matrix RTF. A portion (250 μL) of each of the 40 HPLC fractions was individually concentrated fivefold by heating them to dryness at 110 °C and then redissolving the dried material in water (50 μL). Aliquots (5 μL) of the concentrated neutral fractions and the reference standards were spotted separately on the top of four Whatman No. 1 or 1PS filter paper circles (1/4 in. diameter). The filter paper samples were held in a triangular Delrin sample holder. The spotted solution stayed on the 1PS filter paper as a small droplet, but the droplet spread somewhat in the case of Whatman No. 1 filter paper. The filter paper samples were dried at 110 °C for 45 min. Samples adsorbed on 10% α-cyclodextrin–sodium chloride mixture were prepared as reported earlier.[15] A stream of nitrogen gas that was oxygen and moisture free was passed into the sample compartment while the solid-matrix RTF spectra were acquired.

Solid-Matrix RTP. Aliquots (3 μL) of aqueous thallium nitrate (0.3 M) and aqueous sodium hydroxide (1.0 M, 3 μL) were spotted consecutively onto the 1PS filter paper circle, followed by each of the concentrated CT samples (5 μL) and then methanol (3 μL). The methanol was spotted on the 1PS filter paper because the solvent for the standards contained methanol. Samples were dried (110 °C, 45 min). Reference standards in methanol–water (50:50) were treated similarly with the 1PS filter paper matrix. The solid-matrix RTP spectra were also obtained in a dry and oxygen-free nitrogen atmosphere. Blank spectra were subtracted from the solution RTF and solid-matrix RTF and RTP spectra of the samples prior to the characterization of 1-OH-PY and BP-tetrol I-1.

RESULTS AND DISCUSSION

Methods Development. It was important to develop a method that would permit the distinction between 1-OH-PY and BP-tetrol I-1, because both compounds could appear in a given HPLC fraction. Figure 1 shows the RTF excitation and emission spectra of 1-OH-PY and BP-tetrol I-1. In neutral solution, they have different features that permit their distinction. The excitation spectrum of BP-tetrol I-1 in solution is characterized by the presence of four major peaks at 267, 278, 328, and 343 nm. The excitation spectrum for 1-OH-PY shares the peaks at 267 and 277, has a second peak at 346, and has a shoulder at 335 nm. The emission spectrum of BP-tetrol I-1 in solution is also clearly distinct from the emission spectrum of the 1-OH-PY in solution. For BP-tetrol I-1, there are two sharp peaks at 378 and 398 nm and a small peak at 389 nm. This result contrasts with the emission spectrum for 1-OH-PY, which has two peaks at 387 and 409 nm. In addition, BP-tetrol I-1 has a band at 420 nm, but for 1-OH-PY this band is shifted to 430 nm.

The solid-matrix RTF excitation and emission spectra for these two compounds exhibit the same characteristic features when adsorbed on Whatman No. 1 or 1PS filter paper as they do in solution (Fig. 2). However, 1-OH-PY showed major spectral changes in the presence of sodium hydroxide (0.1 M) for solution RTF and for solid-matrix RTF and RTP spectra obtained under alkaline conditions. In sodium hydroxide solution, 1-OH-PY is in the anionic form. The major peaks in the excitation spectrum of neutral 1-OH-PY are red shifted for the anion, with two prominent peaks occurring at 280 and 400 nm. The solution emission spectrum of the anion of 1-OH-PY shows a broad band at 448 nm, in the place

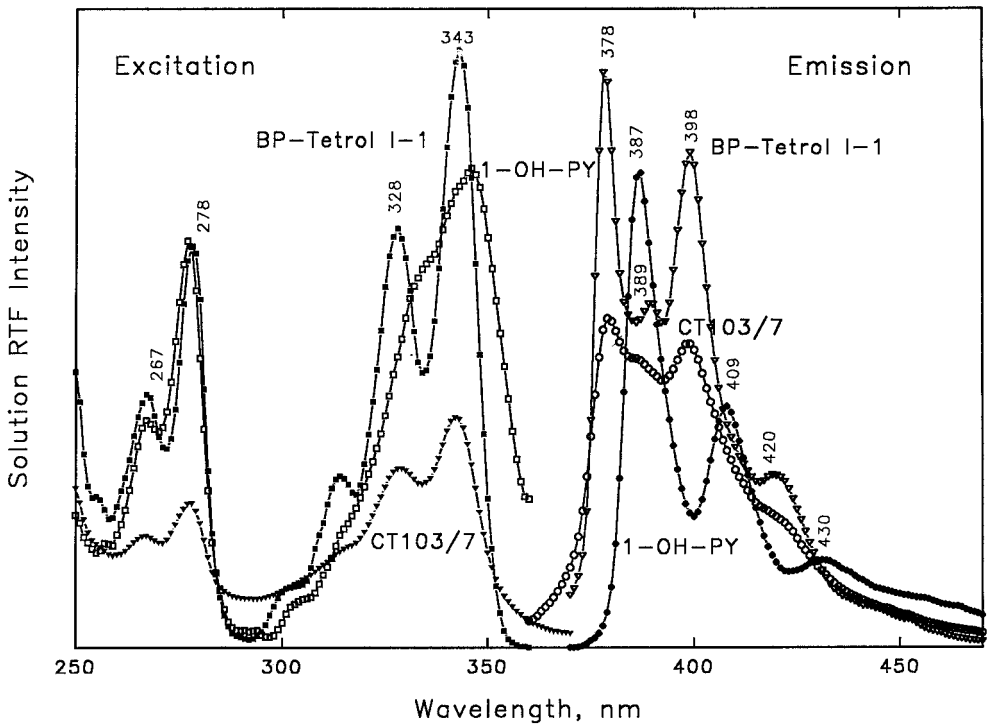

FIG. 1. Solution RTF excitation spectra of BP-tetrol I-1 (-■-) (50 ng/mL) in methanol–water (50:50), 1-hydroxypyrene (-□-) (50 ng/mL) in methanol–water (50:50), and a urine sample CT 103/7 (-▼-) and solution emission spectra of BP-tetrol I-1 (-▽-), 1-hydroxypyrene (-●-), and CT 103/7 (-○-). These spectra were obtained with different bandwidths, and the intensities were not comparable to one another.

of two peaks at 387 and 409 nm in the neutral species. No such spectral changes, on the other hand, were observed for BP-tetrol I-1 in a sodium hydroxide solution (0.1 M) or for the alkaline solution spotted on filter paper. Primarily on the basis of these results, it was possible to develop a method for the identification of both 1-OH-PY and BP-tetrol I-1 in the urine fractions.

The spectral differences between the neutral and an-

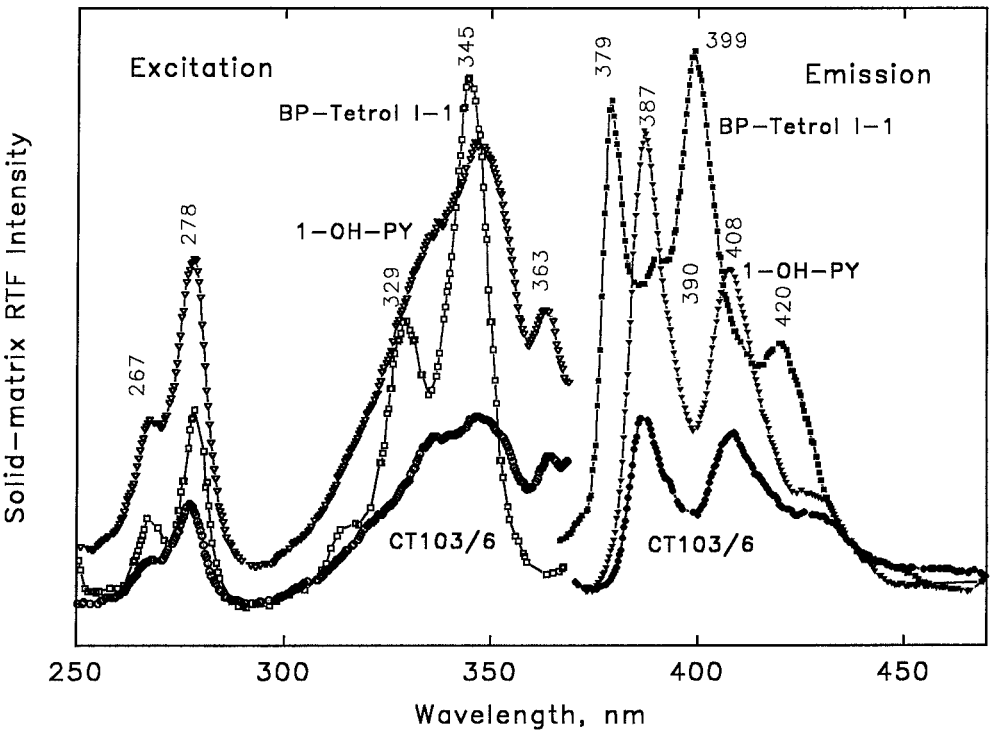

FIG. 2. Solid-matrix RTF excitation spectra of neutral BP-tetrol I-1 (-□-) (10 ng/spot), neutral 1-hydroxypyrene (-▽-) (15 ng/spot), and 5 μL concentrated neutral urine sample CT 103/6 (-○-) and solid-matrix RTF emission spectra of BP-tetrol I-1 (-■-), 1-hydroxypyrene (-▼-), and CT 103/6 (-●-) adsorbed on Whatman 1PS filter paper. Intensities of these spectra were not comparable to one another.

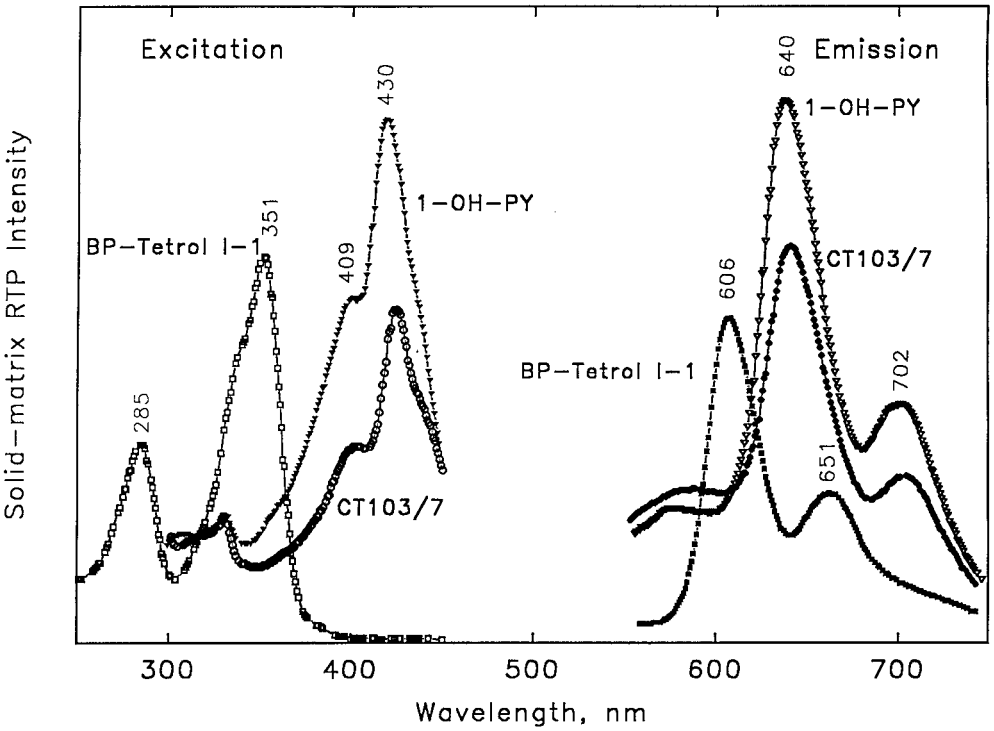

FIG. 3. Solid-matrix RTP excitation spectra of BP-tetrol I-1 (-□-) (25 ng/spot), 1-hydroxypyrene (-▼-) (15 ng/spot), and 5 μL concentrated urine sample CT 103/7 (-○-) and solid-matrix RTP emission spectra of BP-tetrol I-1 (-■-), 1-hydroxypyrene (-▽-), and CT 103/7 (-●-) adsorbed on Whatman 1PS filter paper. Spotted solutions on the filter paper contained sodium hydroxide and thallium nitrate. Intensities of these spectra were not comparable to one another.

ionic forms of 1-OH-PY were clearly manifest in their solid-matrix RTP excitation and emission spectra. A broad band of the anion at 430 nm in the excitation spectrum of 1-OH-PY contrasted to a weak band at 300 nm for the neutral form. A strong RTP emission peak appeared at 630 nm for the anion, and a weak peak appeared at 616 nm for the neutral form of 1-OH-PY. BP-tetrol I-1 showed no such spectral shifts in its solid-matrix RTP excitation and emission spectra under basic conditions. Only a weak band was present at 606 nm in the RTP emission spectrum, which was far removed from the 630-nm peak of the anion of 1-OH-PY (Fig. 3). Thus, the spectral shifts in the solid-matrix RTP spectra, due to the formation on the anion of 1-OH-PY, were used to distinguish 1-OH-PY from BP-tetrol I-1 in the urine fractions.

TABLE I. Excitation and emission wavelengths for 1-OH-PY and BP-tetrol I-1.

Compound	Solution excitation wavelengths (nm)
1-OH-PY	267, 277, 355(s),[a] 346,
BP-tetrol I-1	267, 278, 328, 343
	Solution emission wavelengths (nm)
1-OH-PY	387, 409, 430,
BP-tetrol I-1	378, 389, 398, 420
	Solid-matrix RTF excitation wavelengths (nm)
1-OH-PY	267, 278, 337, 346, 363,
BP-tetrol I-1	267, 278, 329, 345
	Solid-matrix RTF emission wavelengths (nm)
1-OH-PY	387, 408, 426,
BP-tetrol I-1	379, 390, 399, 420
	Solid-matrix RTP excitation wavelengths (nm)
1-OH-PY (anion)[b]	409, 430,
BP-tetrol I-1	285, 351
	Solid-matrix RTP emission wavelengths (nm)
1-OH-PY (anion)[b]	640, 702,
BP-tetrol I-1	606, 651

[a] A shoulder for the spectrum is indicated by "s".
[b] Anion of 1-OH-PY.

Solution RTF Spectra of Samples. In the following discussion for the excitation and emission wavelengths for the urine fractions, it would be appropriate to compare these wavelengths with the excitation and emission wavelengths given in Table I for the solution excitation and emission wavelengths for the standard samples. Also, the spectra given for standards in Fig. 1 should be considered.

CT 103 Samples. The urine sample CT 103/7 gave stronger RTF signals than the urine samples CT 103/6, 103/8, and 103/9. In these four samples, the RTF excitation and emission spectra showed peaks corresponding to those of standard BP-tetrol I-1 in methanol–water. For example, Fig. 1 shows that the RTF excitation peak at 277 nm was stronger than the one at 267 nm, the peak at 328 nm was well defined, and the peak at 342 nm was the strongest. Also, as Fig. 1 shows, there were three emission peaks at 379, 386, and 398 nm, in addition to a shoulder at 418 nm. The three main emission bands and the shoulder at 418 nm were a clear indication of the presence of BP-tetrol I-1. The samples CT 103/10 and 103/14–22 showed no peaks corresponding to either the standard 1-OH-PY or BP-tetrol I-1 in methanol–water.

CT 106 and CT 119 Samples. The urine sample CT 106/6 gave a stronger intensity than the urine samples CT 106/7–10. Also, the urine sample CT 119/7 gave a stronger intensity than the urine samples CT 119/6, 8, and 9. The excitation and emission spectra of these samples showed the following peaks corresponding to those in the standard 1-OH-PY in methanol–water solvent: (1) The RTF excitation spectra showed two peaks at 275 and 341 nm and two shoulders at 266 and 330 nm. The shoulder at 330 nm, along with other spectral bands, indicated the presence of 1-OH-PY. (2) The RTF emission spectra of the urine samples CT 106 and 119 showed two peaks at 387 and 409 nm and a shoulder at 422 nm, and the middle peak at 389 nm between the two peaks at 378 and 398 nm of BP-tetrol I-1 was missing in these samples. The urine samples CT 106/14–22 and CT 106/10 and 16–22 showed no peaks corresponding to either the standard 1-OH-PY or BP-tetrol I-1 in methanol–water. Essentially the same results were obtained for the urine sample CT 119, but the RTF excitation spectra showed two peaks at 279 and 342 nm and two shoulders at 267 and 330 nm.

Solid-Matrix RTF Spectra of Standards. Figure 2 gives the solid-matrix RTF excitation and emission spectra of the standards and spectra from a typical urine fraction. The excitation spectrum of standard neutral 1-OH-PY adsorbed on 1PS filter paper showed five peaks at 267, 278, 337, 346, and 363 nm. Of these, the 278-nm peak was sharp, and the peak at 346 nm was the most intense peak. Only a shoulder appeared at 337 nm for 1-OH-PY, whereas a sharp band appeared at 329 nm for standard BP-tetrol I-1 adsorbed on FP. For 1-OH-PY adsorbed on filter paper under alkaline conditions, a broad peak at approximately 400 nm appeared in place of the peak at 346 nm for neutral 1-OH-PY adsorbed on filter paper. As indicated in Fig. 2, the excitation spectrum of neutral standard BP-tetrol I-1 adsorbed on 1PS filter paper showed peaks at 267, 278, 329, and 345 nm. The peaks at 278, 329, and 345 nm were sharp, and the peak at 345 nm was more intense than the peaks at 278 and 329 nm. Another distinctive feature between the two spectra is that the peak at 329 nm in BP-tetrol I-1 was sharp compared to a shoulder at 337 nm in 1-OH-PY adsorbed on filter paper. An alkaline solution of BP-tetrol I-1 showed no shift in peak wavelengths compared to the neutral species when adsorbed on 1PS filter paper.

The emission spectrum of neutral standard 1-OH-PY on 1PS filter paper showed two sharp peaks at 387 and 408 nm and a relatively broad band at 426 nm (Fig. 2). The peak at 387 nm was stronger in intensity than the peak at 408 nm. An alkaline sample of 1-OH-PY adsorbed on filter paper showed a peak at approximately 434 nm in the place of two sharp peaks at 387 and 408 nm for the neutral species adsorbed on filter paper. The emission spectrum of neutral BP-tetrol I-1 on 1PS filter paper showed three peaks at 379, 399, and 420 nm and a small peak at 390 nm. The peak at 399 nm was stronger in intensity than the peaks at 379 and 420 nm (Fig. 2). The alkaline sample of BP-tetrol I-1 adsorbed on Whatman No. 1 filter paper showed two sharp peaks at 379 and 399 nm and a small peak at 419 nm, with a small peak at 390 nm. This spectrum was essentially the same as the neutral species in solution.

Solid-Matrix RTF Spectra of Concentrated Samples. The HPLC fractions without concentration, adsorbed on Whatman No. 1 or 1PS filter paper, did not show any peaks for the RTF excitation and emission spectra. However, some of the concentrated samples indicated the presence of 1-OH-PY with the 1PS filter paper.

CT 103 Samples. The urine samples CT 103/ 6–10 showed peaks corresponding to neutral 1-OH-PY adsorbed on 1PS filter paper. The RTF excitation spectra showed two peaks at 278 and 348 nm and two shoulders at 267 and 330 nm, and the RTF emission peaks were at 387 and 408 nm, with a small peak at 428 nm.

CT 106 and CT 119 Samples. The urine samples CT 103/14–22, CT 106, and CT 119 showed no peaks corresponding to either 1-OH-PY or BP-tetrol I-1 adsorbed on filter paper.

The concentrated urine samples CT 106/6 and CT 119/7 adsorbed on 10% α-cyclodextrin/sodium chloride solid matrix showed weak RTF excitation and emission spectra with peaks corresponding to the standard 1-OH-PY adsorbed on filter paper. The solid-matrix excitation spectra showed two peaks at 331 and 345 nm, and the emission spectra showed two peaks at 386 and 407 nm, with a shoulder at 430 nm. It is likely that the concentration of 1-OH-PY was not sufficient to give strong spectra from the filter paper matrix. The use of 10% α-cyclodextrin/sodium chloride to obtain solid matrix fluorescence from adsorbed tetrols was reported earlier.[15,16]

Solid-Matrix RTP Spectra of Standards. Both 1-OH-PY and BP-tetrol I-1 adsorbed on Whatman No. 1 filter paper showed no RTP without a heavy-atom salt, and they showed moderate RTP signals on 1PS filter paper in the presence of thallium nitrate. With 1PS filter paper, 1-OH-PY showed RTP in the presence of sodium hydroxide and thallium nitrate, and BP-tetrol I-1 showed no change in RTP intensity in the presence of sodium hydroxide and thallium nitrate on 1PS filter paper. The standard solutions of 1-OH-PY and BP-tetrol I-1 and urine samples CT 103, CT 106, and CT 119 were prepared as described in the Experimental section. Figure 3 illustrates the solid-matrix RTP excitation and emission spectra of the standards. The excitation spectrum of alkaline standard 1-OH-PY adsorbed on 1PS filter paper with thallium nitrate showed a peak at 430 nm, with a shoulder at 409 nm. The excitation spectrum of alkaline standard BP-tetrol I-1 adsorbed on 1PS filter paper with thallium nitrate showed peaks at 285 and 351 nm. These peaks were sharp, and the peak at 351 nm was stronger in intensity than the peak at 285 nm.

The RTP emission spectrum of alkaline standard 1-OH-PY adsorbed on 1PS filter paper with thallium nitrate showed a peak at 640 nm and a peak at 702 nm. The emission spectrum of alkaline standard BP-tetrol I-1 adsorbed on 1PS filter paper with thallium nitrate showed a peak at 606 nm and a peak at 651 nm.

Solid-Matrix RTP Spectra of Concentrated Samples. The HPLC fractions with and without concentration, adsorbed on Whatman No. 1 or 1PS filter paper, did not show any peaks in the RTP excitation and emis-

sion spectra. However, some of the concentrated samples in the presence of sodium hydroxide and thallium nitrate gave positive results with the 1PS filter paper.

CT 103 Samples. The urine samples CT 103/6–10 showed peaks corresponding to the 1-OH-PY adsorbed on 1PS filter paper in the presence of sodium hydroxide and thallium nitrate. The average excitation peaks for the samples were at 400 nm and 425 nm, and the average emission peaks for the samples were at 638 nm and 704 nm (see Fig. 3). The urine samples CT 103/14–22 showed no peaks corresponding to either 1-OH-PY or BP-tetrol I-1 adsorbed on the solid matrix.

CT 106 Samples. The urine samples CT 106/6–10 and 19 showed peaks corresponding to the standard 1-OH-PY adsorbed on the filter paper, and the excitation peaks were in the range of 397–401 nm and 419–424 nm. None of the remaining urine samples (CT 106) showed peaks corresponding to either 1-OH-PY or BP-tetrol I-1 adsorbed on the filter paper.

CT 119 Samples. The urine samples CT 119/6–10, 16–18, and 20–22 showed peaks corresponding to the standard 1-OH-PY adsorbed on the filter paper, and the excitation peaks were in the range of 397–401 and 419–424 nm. The urine sample CT 119/19 showed no peaks corresponding to either 1-OH-PY or BP-tetrol I-1 adsorbed on the filter paper.

General Summary of Results. The solution RTF of the urine sample (CT 103/7) indicated the presence of BP-tetrol I-1, and the fluorescence emission showed a small band at 387 nm, which suggested the presence of 1-OH-PY. The solid-matrix RTF and RTP spectra showed the presence of 1-OH-PY in the CT 103/7 sample (Fig. 3) and in the CT 103/10 sample as well. However, the solid-matrix RTF and RTP signals of 1-OH-PY were about 20 and 30% stronger, respectively, than those of BP-tetrol I-1, and hence only the RTF and RTP of 1-OH-PY were observed with the solid-matrix luminescence. These results indicate that, for this sample, 1-OH-PY can be detected in the presence of BP-tetrol I-1.

The urine samples CT 106/6–10 and CT 119/6–9 were found to contain 1-OH-PY from the solution RTF spectra. The solid-matrix RTP excitation spectra of these samples and of the urine samples CT 106/19, 119/10, 16–18, and 20–22 confirmed the presence of 1-OH-PY. Solid-matrix RTF and RTP signals were not observed for the rest of the samples as the solid-matrix RTF and RTP signals were much weaker than the corresponding solution RTF signals. This result is supported by the fact that the entire unused portions of urine samples CT 106/6 and 119/7 adsorbed on 10% α-cyclodextrin/sodium chloride matrix showed weak RTF excitation and emission peaks corresponding to those of 1-OH-PY. These two fractions were chosen for the α-cyclodextrin/sodium chloride matrix study because they showed comparatively strong solution RTF. Urine samples CT 106/7–10, and 19 and CT 119/6, 8–18, and 20–22 also contained 1-OH-PY in amounts less than the limit of detection with RTF from the filter paper. On the other hand, the solid-matrix RTP excitation spectra revealed the presence of 1-OH-PY in all these fractions. These data demonstrate the effectiveness of RTP for detection of 1-OH-PY. Samples CT 103/14–22, CT 106/14–18, and CT 119/19 were negative for both BP-tetrol I-1 and 1-OH-PY by the solution RTF and solid-matrix RTF and RTP.

CONCLUSION

The sensitivity of solid-matrix luminescence techniques has been clearly illustrated for the identification of trace quantities of 1-OH-PY in human urine fractions. Solution RTF was not sensitive or selective enough to detect 1-OH-PY in several of the samples. The solid-matrix RTP technique was proven to be superior in detecting 1-OH-PY under suitable experimental conditions when compared to the solution and solid-matrix RTF spectroscopic techniques. The limits of detection for the solution fluorescence of 1-OH-PY and BP-tetrol I-1 were 4.6 pmol/mL and 18 pmol/mL, respectively. For solid-matrix fluorescence, the limits of detection were 0.14 pmol and 0.18 pmol, respectively, for 1-OH-PY and BP-tetrol I-1. With solid-matrix phosphorescence, the limits of detection were 0.69 pmol and 1.6 pmol, respectively, for 1-OH-PY and BP-tetrol I-1. It should be pointed out that the limits of detection were obtained with pure standards. Even though lower limits of detection were obtained with solid-matrix RTF, most likely quenching components or other species emitting fluorescence did not permit the unambiguous identification of 1-OH-PY and BP-tetrol I-1 by RTF. Also, scattered source radiation would be more of a problem with solid-matrix RTF. In addition, the limits of detection are based on single-intensity measurements, whereas the identification was based on the excitation and emission spectra.

The limits of detection obtained for the methodology developed in this work were comparable to or better than the earlier approaches for the characterization and determination of 1-OH-PY.[2,7–9] Also, solid-matrix RTP is very selective compared to previous methods, because both excitation and emission spectra can be obtained with very small quantities of samples. In addition, the RTP emission spectrum of 1-OH-PY appears at very long wavelengths where there would be less interference from other components.

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