Quantitative Determination of Dextromethorphan and Three Metabolites in Urine by Reverse-Phase High-Performance Liquid Chromatography

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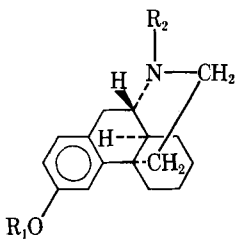
Abstract □ A high-performance liquid chromatographic (HPLC) method for the quantitation of dextromethorphan (I) and its three metabolites, dextrorphan (II), 3-hydroxy-9α,13α,14α-morphinan (III), and 3-methoxy-9α,13α,14α-morphinan (IV), in urine was developed. For the analysis of nonconjugated compounds, urine samples at pH 11–11.5, containing 3-methoxy-17-methyl-10-oxo-9α,13α,14α-morphinan as an internal standard, were applied to an extraction column, and the compounds were eluted with 10% *n*-butyl alcohol–hexane. The organic eluant was extracted with 0.1 M HCl, and an aliquot of the acidic extract was analyzed by HPLC utilizing a 5-μm phenyl column (25 × 0.46-cm i.d.) with a mobile phase of 10 mM potassium phosphate–acetonitrile (45:55, pH 4.0); the column effluent was monitored by UV detection at 280 nm. Free and conjugated metabolites in the enzyme-treated urine were analyzed by selective extraction of I and IV with hexane from urine samples at pH > 12 and extraction of II and III with 10% *n*-butyl alcohol–hexane from urine samples at pH 11–11.5. The minimum quantifiable levels of I–IV ranged from 0.017 to 0.09 μg of base/mL and from 0.11 to 0.21 μg of base/mL in nonhydrolyzed and hydrolyzed urine, respectively.

Keyphrases □ Dextromethorphan—quantitation with metabolites by high-performance liquid chromatography, human urine □ Dextrorphan—quantitation in human urine after administration of dextromethorphan, high-performance liquid chromatography □ Cough suppressants—dextromethorphan, dextrorphan, and metabolites, quantitation in human urine by high-performance liquid chromatography

Dextromethorphan (3-methoxy-17-methyl-9α,13α,-14α-morphinan, I), a widely used nonnarcotic antitussive agent, has been judged to be safe and effective, with a reported activity of about one-half the potency of codeine (1) against citric acid-induced cough. Considerable intersubject variations in plasma levels of I have been reported by Barnhart and Massad (2). O'Brien *et al.* described the measurement of I by GC with nitrogen detection and also reported variable human plasma levels of I following single and multiple doses (3). Hinsvark *et al.* suggested that these differences are attributable to genetic polymorphism (4). Therefore, a study to determine if differences in the elimination rates of I were related to different biotransformation routes was initiated in our laboratories. The initial phase required the development of a procedure capable of measuring I and its known metabolites in urine.



I: $R_1 = R_2 = CH_3$
II: $R_1 = H$, $R_2 = CH_3$
III: $R_1 = R_2 = H$
IV: $R_1 = CH_3$, $R_2 = H$

Recently, Barnhart described a GC procedure to measure the amount of I and three of its metabolites [dextrorphan (II), 3-hydroxy-9α,13α,14α-morphinan (III) and 3-methoxy-9α,13α,14α-morphinan (IV)] in urine (5). It suffers the usual problems associated with derivatization. The approach utilized in this paper uses high-performance liquid chromatography (HPLC) to simultaneously measure I and its metabolites in urine. The procedure is presented and data are provided which describe the accuracy and precision of the method. Examples of chromatograms from assay of 24-h urine samples of subjects who had received 60 mg of dextromethorphan hydrobromide orally are also presented.

EXPERIMENTAL

Materials—Dextromethorphan hydrobromide[1], dextrorphan tartrate[2], 3-hydroxy-9α,13α,14α-morphinan[2], and thebaine[3] were available commercially. The 3-methoxy-9α,13α,14α-morphinan hydrochloride was synthesized by *N*-demethylation of dextromethorphan (6). The internal standard, 3-methoxy-17-methyl-10-oxo-9α,13α,14α-morphinan[4] was prepared by a previously described method (7).

All reagents were analytical grade. The chromatographic solvents used in the chromatographic separation were HPLC grade. Membrane filters[5] were used for filtration of the mobile phase. Disposable extraction columns[6] were utilized for extraction. β-Glucuronidase–sulfatase[7] was used for hydrolysis of conjugated metabolites for the assay of total metabolites.

Instrumentation—A modular chromatograph consisting of a pump[8], an autosample injector[9], a fixed-wavelength absorbance detector[10], and a recorder[11] was used. A reverse-phase phenyl column[12] (25 × 0.46-cm i.d.) packed at 8000 psi was used for all analyses. A laboratory data system[13] was used for quantitation and identification of chromatographic peaks.

Chromatographic Conditions—The mobile phase was 10 mM potassium phosphate (monobasic)–acetonitrile (45:55, v/v) adjusted to pH 4.0 with 8.5% phosphoric acid. The flow rate was 1.2 mL/min. Following preparation, the mobile phase was filtered through a membrane filter and deaerated under vacuum. The detector was set at 280 nm with a sensitivity of 0.05 AUFS.

Preparation of Validation Samples and Standards—Validation samples, analyzed under single-blind conditions, were prepared in control human urine by spiking with combinations of I, II, III, and IV at various concentrations. One set of samples was analyzed on preparation; the other set was frozen for 2 weeks, thawed, and analyzed. Duplicate standards were prepared on each day of analysis of the validation samples by adding

[1] USP reference standard.
[2] Hoffmann-La Roche, Inc., Nutley, N.J.
[3] Mallinckrodt Chemical Co., St. Louis, Mo.
[4] Synthesized by Dr. Metro Fedorchuk in the Department of Organic Chemistry.
[5] 0.45-μm; Rainin Instrument Co., Woburn, Mass.
[6] Clin-Elut; Analytichem Internation, Inc., Lawndale, Calif.
[7] Glusulase; Endo Laboratories, Garden City, N.Y.
[8] Model M-45; Waters Associates, Milford, Mass.
[9] Model WISP-710B; Waters Associates, Milford, Mass.
[10] Model 440; Waters Associates, Milford, Mass.
[11] Omni-Scribe B-5000 Strip Chart Recorder; Houston Instrument, Austin, Texas.
[12] Spherisorb phenyl, 5-μm; Deeside Ind. Est., Clwyd. UK, Hauppauge, NY 11787.
[13] Model 3353E; Hewlett-Packard, Avondale, Pa.

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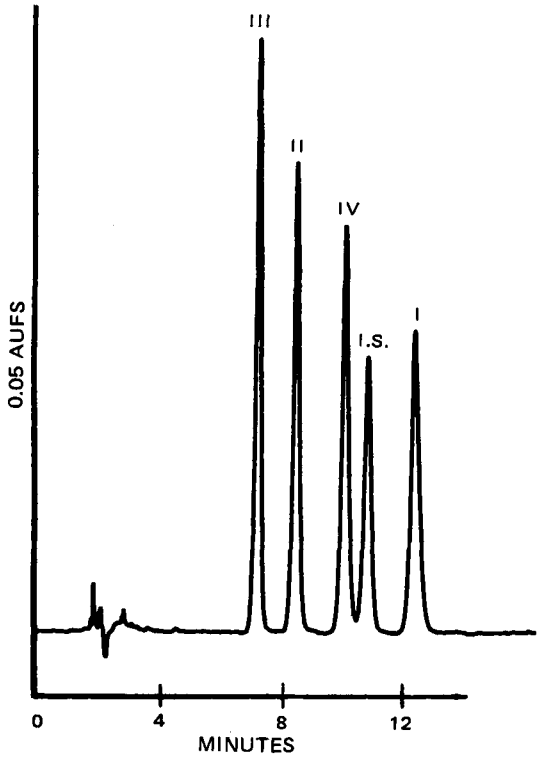

Figure 1—*Chromatogram at 280 nm of dextromethorphan and three metabolites with an internal standard (3 methoxy-17-methyl-10-oxo-9α,13α,14α-morphinan) using a 5-μm phenyl 25 × 0.46-cm column with 10 mM potassium phosphate–acetonitrile (45:55, pH 4.0) at 1.2 mL/min. Key: (I) dextromethorphan; (II) dextrorphan; (III) 3-hydroxy-9α,13α,14α-morphinan; (IV) 3-methoxy-9α,13α,14α-morphinan.*

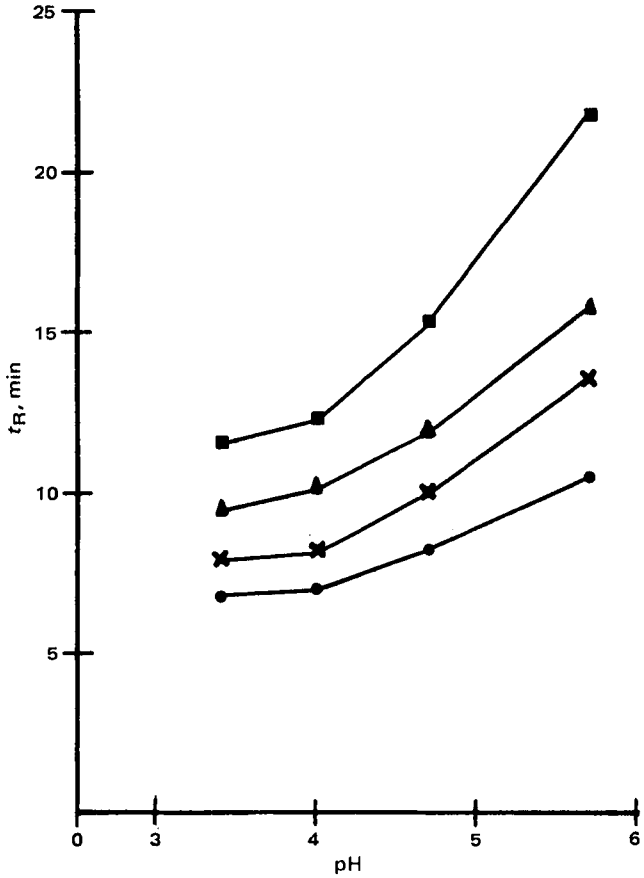

Figure 2—*Effect of pH of the mobile phase (10 mM potassium phosphate–acetonitrile, 45:55, pH 4.0) on the retention times (t_R) of dextromethorphan (■), dextrorphan (x), 3-hydroxy-9α,13α,14α-morphinan (●), and 3-methoxy-9α,13α,14α-morphinan (▲).*

100 μL of stock solutions to control human urine (2 mL) to give final concentrations of 0.00, 0.05, 0.10, 0.25, 1.25, 2.50, 5.00, and 10.00 μg of base/mL for all four compounds.

Extraction Procedures—*Dextromethorphan and Unconjugated Metabolites*—The urine sample (2 mL) was adjusted to pH 11–11.5 with concentrated ammonium hydroxide, and 50 μL of a solution of 3-methoxy-17-methyl-10-oxo-9α,13α,14α-morphinan (1 μg of base/50 μL in 0.01 M HCl) was added as the internal standard. The mixture was gently vortexed and transferred to an extraction column with a pipet. After 5 min, the column was eluted with 10% *n*-butyl alcohol–hexane (10 mL). The collected eluant (~7 mL) was extracted with 0.1 M HCl (0.4 mL) by roto-mixing for 40 min and centrifuged. The organic layer was aspirated and discarded. The acid extract (40 μL) was analyzed by HPLC.

Dextromethorphan and Total (Free and Conjugated) Metabolites—The urine sample (2 mL) was adjusted to pH 5–5.5 with 50 mM sodium acetate buffer (pH 3.5), and the β-glucuronidase–sulfatase mixture (0.2 mL) was added. After incubation at 37°C for 18 h, the mixture was adjusted to pH 12–12.5 with 1 M NaOH, and 50 μL of a solution of 3-methoxy-17-methyl-10-oxo-9α,13α,14α-morphinan (1 μg of base/50 μL in 0.01 M HCl) was added as the internal standard. The mixture was extracted with hexane (6 mL) by roto-mixing for 40 min and centrifuged. The aqueous layer was frozen in a dry ice–acetone bath. The hexane extract was decanted into a centrifuge tube and back-extracted with 0.1 M HCl (0.2 mL) by roto-mixing for 30 min. The acid extract (25 μL) was analyzed by HPLC for I and IV. The thawed aqueous layer was adjusted to pH 11–11.5 with 1 M HCl. After addition of 50 μL of a solution of thebaine, the internal standard (1 μg of base/50 μL in 0.01 M HCl), the mixture was extracted by the aforementioned method for the determination of free metabolites. The acid extract (25 μL) was analyzed for total II and III.

Extraction Efficiency—The extraction efficiency of each compound from urine was investigated at pH ranging from 2 to 13. The pH-adjusted urine samples (2 mL) were spiked with 100 μL of a mixture of I, II, III, and IV in solution and processed through the entire extraction procedure and chromatographic analysis for the determination of both free and total metabolites. The recovery of each compound was determined by comparing the peak heights of extracted standards with those of unextracted standards. The unextracted standards were prepared by the addition of stock solutions of I, II, III, and IV to 0.1 M HCl to give concentrations equivalent to that of extracted standards.

Quantitation—The duplicate fresh standards at seven different concentrations ranging from 0.05 to 10.0 μg of base/mL of I–IV were analyzed together with validation samples. From the standards, the peak height ratios of each compound to the internal standard were obtained with the aid of a laboratory data system. From linear regression of the peak height ratios of each compound with respect to its concentration in the standards, the concentrations of each compound in the validation samples were determined by inverse prediction.

RESULTS AND DISCUSSION

HPLC of Dextromethorphan and Metabolites—Figure 1 is a representative chromatogram of I, II, III, IV, and the internal standard on a phenyl column. The phenyl column was chosen after evaluation of octadecylsilanyl and cyano columns. Unlike the others, the phenyl column gave both symmetric peaks and baseline separations of compounds of diverse polarity.

The effect of pH and acetonitrile composition on retention times of the four compounds was investigated. The retention times of all four compounds increased as the pH of the mobile phase increased (Fig. 2). Similarly, the retention times of the compounds increased with decreasing concentrations of acetonitrile in the 10 mM potassium phosphate buffer at pH 4.0 (Fig. 3). These studies suggest that the chromatographic characteristics of dextromethorphan and its related alkaloids are dependent on both the pH and composition of the mobile phase. A mobile phase of 10 mM potassium phosphate buffer–acetonitrile (45:55) at pH 4.0 was chosen because it permitted the baseline separation of dextromethorphan and its three metabolites with reasonable analysis time and sensitivity.

Extraction and Recovery—In the development of this assay, several problems were encountered. The first was attaining an efficient simultaneous extraction of all four compounds from urine. The second was concentrating the compounds to give sufficient sensitivity for the analysis

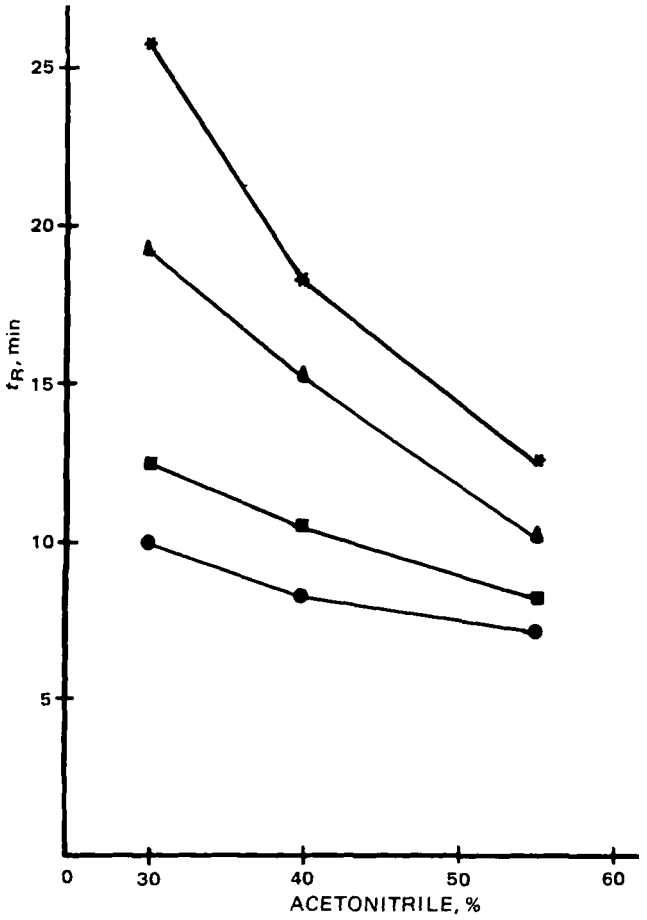

Figure 3—*Effect of acetonitrile percentage in 10 mM potassium phosphate buffer at pH 4.0 on the retention time (t_R) of dextromethorphan (*), dextrorphan (■), 3-hydroxy-9α,13α,14α-morphinan (●), and 3-methoxy-9α,13α,14α-morphinan (▲).*

of the free compounds in nonhydrolyzed urine, while using a solvent that gave little background interference from hydrolyzed and nonhydrolyzed urine samples. Thirdly, an unexpected problem arose in that the hydrochloride salts of I and IV were found to be fairly soluble in relatively polar organic solvents, resulting in a poor extraction efficiency of the amines from the organic phase into the acidic phase in the back-extraction procedure. A fourth problem was the concentration difference of I

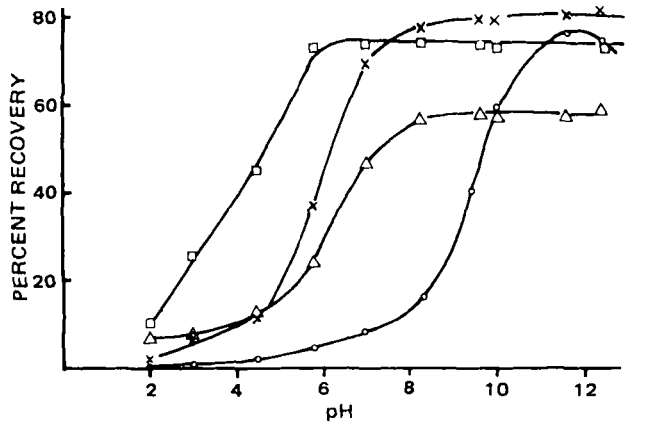

Figure 4—*Extraction efficiency* versus *pH value of urine samples. Two milliliters of urine containing a mixture of I–IV was extracted with 10% n-butyl alcohol–hexane at pH 11–11.5 by an extraction column. About 7 mL of the eluant was extracted with 0.4 mL of 0.1 M HCl. Each point represents the average of three determinations at four different concentrations. Key: (□) dextromethorphan (I); (x) dextrorphan (II); (○) 3-hydroxy-9α,13α,14α-morphinan (III); (△) 3-methoxy-9α,13α,-14α-morphinan (IV).*

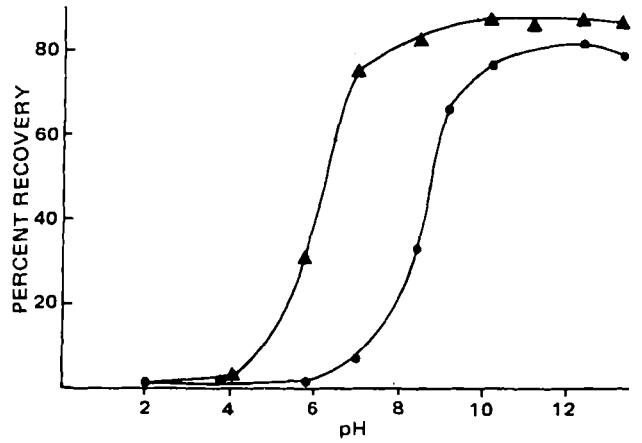

Figure 5—*Extraction efficiency* versus *pH value of urine samples. Two milliliters of enzyme-treated urine containing a mixture of I–IV was extracted with 6 mL of hexane at pH 12–12.5 by liquid–liquid extraction. The separated hexane extract was extracted with 0.2 mL of 0.1 M HCl. Each point represents the average of three determinations at three different concentrations. Key: (▲) dextromethorphan (I); (●) 3-methoxy-9α,13α,14α-morphinan (IV).*

and IV compared with II and III observed in hydrolyzed urine samples of humans who had received doses of dextromethorphan.

To carry out a simultaneous extraction of a mixture of compounds that have both acidic and basic functions, a pH profile is important. Using 10% *n*-butyl alcohol–hexane as the extracting solvent, the recoveries of I, II, and IV were constant over the pH range of 9–13. However, the recovery of III was markedly dependent on the pH, with the maximum occurring at pH 11–11.5 (Fig. 4).

Having optimized the pH, the next problem was efficient and clean extraction of dextromethorphan and its metabolites from urine, followed by back-extraction into a small volume of acid to effect removal of neutral materials and concentration of the compounds. A variety of solvents and solvent mixtures were investigated. Solvents such as hexane and toluene effectively extracted I and IV, but gave poor recovery of the more polar II and III. Relatively polar solvents such as 10 to 20% *n*-butyl alcohol in

Table I—Analysis of Dextromethorphan (I) in Nonhydrolyzed Validation Urine Samples

Nominal Value, μg/mL	Analyzed Value in Fresh Samples, μg/mL	Analyzed Value in Frozen Samples, μg/mL
0	<0.05	<0.09
	<0.05	<0.09
	<0.05	<0.09
0.5	0.50	0.52
	0.51	0.51
	0.49	0.50
Mean	0.50	0.51
SD (%)[a]	0.01 (2.0)	0.01 (1.9)
Mean % difference[b]	0.0	2.0
1.0	0.97	1.10
	1.03	1.05
	0.98	1.12
Mean	0.99	1.09
SD (%)[a]	0.03 (3.2)	0.03 (3.3)
Mean % difference[b]	−0.7	9.0
3.0	2.65	2.69
	2.64	2.70
	2.62	2.67
Mean	2.64	2.69
SD (%)[a]	0.01 (0.6)	0.01 (0.6)
Mean % difference[b]	−12.1	−10.4
5.0	4.57	4.71
	4.68	4.74
	4.44	4.57
Mean	4.56	4.67
SD (%)	0.12 (2.6)	0.09 (1.9)
Mean % difference[b]	−8.7	−6.5

[a] (%) = relative standard deviation = *SD*/mean × 100. [b] Mean % difference = (Mean − Nominal/Nominal) × 100.

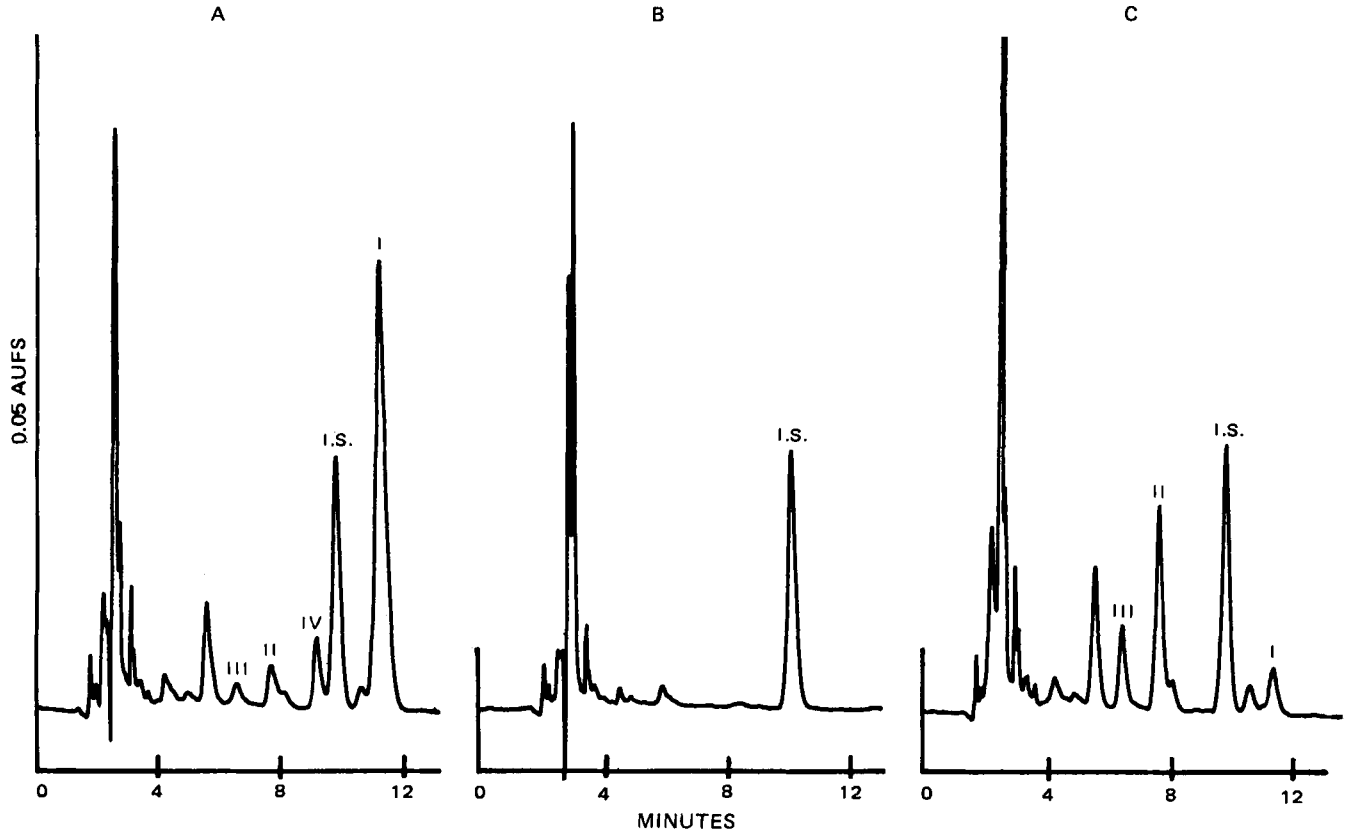

Figure 6—*Chromatograms of extracts of 2 mL of nonhydrolyzed 24-h urine samples from humans who were given 60 mg of dextromethorphan hydrobromide orally. Key: (A) sample determined to contain 0.04 μg of III, 0.26 μg of II, 0.66 μg of IV, and 4.83 μg of I/mL; (B) extracted control human urine samples containing only internal standard, 3-methoxy-17-methyl-10-oxo-9α,13α,14α-morphinan (0.5 μg/mL); (C) sample determined to contain 0.42 μg of III, 1.28 μg of II, and 0.49 μg of I/mL.*

either toluene or ethyl acetate gave high recoveries of I–IV, but the high solubility of their hydrochloride salts in the organic solvents gave poor extraction into the small volume of acidic phase needed for concentration and cleanup. The solvent chosen to satisfy all the criteria for the efficient simultaneous transfer of I–IV from the urine sample to a small volume of acid with low background was 10% *n*-butyl alcohol–hexane at pH 11–11.5. The recovery was ~80% for I, II, and III and 60% for IV.

A further problem that developed on analysis of hydrolyzed human

Table II—Analysis of Dextrorphan (II) in Nonhydrolyzed Validation Urine Samples

Nominal Value, μg/mL	Analyzed Value in Fresh Samples, μg/mL	Analyzed Value in Frozen Samples, μg/mL
0	<0.05	<0.033
	<0.05	<0.033
	<0.05	<0.033
0.2	0.20	0.19
	0.19	0.20
	0.20	0.21
Mean	0.20	0.20
SD (%)[a]	0.01 (2.9)	0.01 (5.0)
Mean % difference[a]	−1.7	0.0
1.0	1.03	0.96
	1.00	0.98
	1.02	0.96
Mean	1.02	0.97
SD (%)[a]	0.01 (1.5)	0.01 (1.2)
Mean % difference[a]	1.7	−3.3
3.0	2.90	3.04
	3.01	2.82
	2.88	3.05
Mean	2.93	2.97
SD (%)[a]	0.07 (2.4)	0.13 (4.4)
Mean % difference[a]	−2.3	−1.0
5.0	4.87	4.64
	4.83	4.82
	4.76	4.72
Mean	4.82	4.73
SD (%)[a]	0.06 (1.5)	0.09 (1.9)
Mean % difference[a]	−3.6	−5.5

[a] Defined in Table I.

Table III—Analysis of 3-Hydroxy-9α,13α,14α-morphinan (III) in Nonhydrolyzed Validation Samples

Nominal Value, μg/mL	Analyzed Value in Fresh Samples, μg/mL	Analyzed Value in Frozen Samples, μg/mL
0	<0.03	<0.017
	<0.03	<0.017
	<0.03	<0.017
0.2	0.21	0.21
	0.21	0.19
	0.21	0.22
Mean	0.21	0.21
SD (%)[a]	0.00 (0.0)	0.02 (7.4)
Mean % difference[a]	+5.0	+3.3
0.5	0.52	0.46
	0.52	0.47
	0.49	0.47
Mean	0.51	0.47
SD (%)[a]	0.02 (3.4)	0.01 (1.2)
Mean % difference[a]	+2.0	−6.7
2.0	2.06	1.81
	2.07	1.92
	1.93	1.95
Mean	2.02	1.89
SD (%)[a]	0.08 (3.8)	0.07 (3.9)
Mean % difference[a]	+1.0	−5.3
4.0	4.09	3.78
	4.03	4.21
	4.06	4.02
Mean	4.06	4.00
SD (%)[a]	0.03 (0.7)	0.21 (5.4)
Mean % difference[a]	+1.5	+0.08

[a] Defined in Table I.

Table IV—Analysis of 3-Methoxy-9α,13α,14α-morphinan (IV) in Nonhydrolyzed Validation Samples

Nominal Value, μg/mL	Analyzed Value in Fresh Samples, μg/mL	Analyzed Value in Frozen Samples, μg/mL
0	<0.05	<0.09
	<0.05	<0.09
	<0.05	<0.09
0.1	0.11	0.11
	0.10	0.11
	0.11	0.08
Mean	0.11	0.10
SD (%)[a]	0.01 (5.4)	0.02 (17.3)
Mean % difference[a]	6.7	0.0
0.5	0.51	0.52
	0.51	0.51
	0.50	0.52
Mean	0.51	0.52
SD (%)[a]	0.01 (1.1)	0.01 (1.1)
Mean % difference[a]	1.3	3.3
1.0	0.98	1.05
	1.01	1.03
	1.00	1.01
Mean	1.00	1.03
SD (%)[a]	0.01 (1.5)	0.02 (1.9)
Mean % difference[a]	−0.3	3.0
2.5	2.40	2.43
	2.40	2.54
	2.41	2.42
Mean	2.40	2.46
SD (%)[a]	0.01 (0.2)	0.07 (2.7)
Mean % difference[a]	−3.9	−1.5

[a] Defined in Table I.

urine samples from subjects given dextromethorphan was that the concentrations of I and IV were much lower than those of II and III. To increase sensitivity and decrease background interference for the analysis of I and IV, a sequential extraction procedure was designed. Compounds I and IV, which do not have a phenolic hydroxyl group, were selectively extracted from the enzyme-treated urine at pH 12–12.5 with hexane and back-extracted into 0.1 M HCl from the separated hexane extract. Compounds II and III, which remained in the aqueous layer, were extracted with 10% *n*-butyl alcohol–hexane at pH 11–11.5 as previously described for the simultaneous extraction of I–IV.

Two internal standards were required in this procedure: 3-methoxy-17-methyl-10-oxo-9α,13α,14α-morphinan was found to behave chemically and physically like I and IV, while thebaine was used for II and III. By this specific extraction procedure, the recoveries of I and IV were increased to 90–95% and 80–85%, respectively (Fig. 5), and the sensitivity for each compound was doubled, since the acid volume was reduced to one-half in the acid back-extraction. The recoveries of II and III were ~80%.

Table V—Analysis of Dextromethorphan (I) and 3-Methoxy-9α,13α,14α-morphinan (IV) in Enzyme-Treated Validation Samples

Dextromethorphan		3-Methoxy-9α,13α,14α-morphinan	
Nominal Value, μg/mL	Analyzed[a] Value, μg/mL	Nominal Value, μg/mL	Analyzed[a] Value, μg/mL
0.1	<0.15	0.1	<0.15
	<0.15		<0.15
	<0.15		<0.15
0.5	0.51	0.5	0.43
	0.49		0.40
	0.50		0.40
Mean	0.50		0.41
SD (%)[b]	0.01 (2.0)		0.02 (4.2)
Mean % difference[b]	0.0		−18.0
1.0	1.02	1.0	0.86
	1.03		0.85
	1.02		0.89
Mean	1.02		0.87
SD (%)[b]	0.01 (0.5)		0.02 (2.4)
Mean % difference[b]	2.3		−13.3
4.0	4.35	3.0	3.00
	3.85		2.91
	3.79		3.00
Mean	4.00		2.97
SD (%)[b]	0.30 (7.7)		0.05 (1.7)
Mean % difference[b]	−0.08		−1.0

[a] Analyzed immediately after preparation. [b] Defined in Table I.

Table VI—Analysis of Dextrorphan (II) and 3-Hydroxy-9α,13α,14α-morphinan (III) in Enzyme-Treated Validation Samples

Dextrorphan		3-Hydroxy-9α,13α,14α-morphinan	
Nominal Value, μg/mL	Analyzed[a] Value, μg/mL	Nominal Value, μg/mL	Analyzed[a] Value, μg/mL
0	<0.11	0.05	<0.21
	<0.11		<0.21
	<0.11		<0.21
0.5	0.53	0.2	0.31
	0.53		0.22
	0.53		0.23
Mean	0.53		0.25
SD (%)[b]	0.00 (0.0)		0.05 (19.5)
Mean % difference[b]	6.0		26.5
1.0	1.03	0.5	0.60
	1.00		0.47
	0.97		0.50
Mean	1.00		0.52
SD (%)[b]	0.03 (3.0)		0.07 (13.0)
Mean % difference[b]	0.0		4.7
3.0	2.99	2.0	2.21
	2.97		1.84
	2.97		2.05
Mean	2.98		2.03
SD (%)[b]	0.01 (0.4)		0.18 (9.1)
Mean % difference[b]	−0.8		1.7
5.0	4.88	4.0	4.37
	4.92		3.52
	5.13		3.85
Mean	4.98		3.91
SD (%)[b]	0.13 (2.7)		0.43 (10.9)
Mean % difference[b]	−0.47		−2.2

[a] Analyzed immediately after preparation. [b] Defined in Table I.

Assay Sensitivity and Precision—The standard curves for I–IV in nonhydrolyzed urine samples fit a linear model ($r > 0.997$) over the range of 0.05–5.0 μg of base/mL. Similarly, the standard curves for I–IV in hydrolyzed urine samples were linear ($r > 0.995$) over the range of 0.10–10.0 μg of base/mL. The range of minimum quantifiable levels, determined as that concentration whose lower 80% confidence limit just encompasses zero (8), was 0.017–0.09 μg of base/mL and 0.11–0.21 μg of base/mL for I–IV in nonhydrolyzed and hydrolyzed urine samples, respectively.

The accuracy of the assays, expressed as the mean percent difference, ranged from −12.1% to 9.0% over all concentrations of I–IV in nonhydrolyzed urine (Tables I–IV) and −18.0% to 26.5% in hydrolyzed urine (Tables V and VI). The precision of the assay, expressed as the mean % *SD*, was 2.93 ± 3.1% ($n = 32$) for all concentrations of I–IV in nonhydrolyzed urine samples and 5.51 ± 5.77% ($n = 14$) for all concentrations in hydrolyzed urine. Quantitation of each compound assayed immediately after preparation and after freezing (Tables I–IV) showed that there was no loss of drugs due to freezing the urine samples.

Chromatograms of I and free (unconjugated) II–IV in 24-h urine samples of humans who were dosed with 60 mg of dextromethorphan hydrobromide orally are presented in Fig. 6. Compared with Fig. 6C, Fig. 6A shows relatively high concentrations of I (4.83 μg of base/mL) and IV (0.66 μg of base/mL) and low concentrations of the polar metabolites, III (0.04 μg of base/mL) and II (0.26 μg of base/mL). In Fig. 6C, the concentrations of I, II, and III were 0.49, 1.28, and 0.42 μg of base/mL, respectively; IV was not detected. Figure 6B is a chromatogram of the extract of a control human urine sample containing the internal standard. Results of the assay of urine samples from clinical studies will be reported separately.

The simultaneous quantitation of dextromethorphan and its urinary metabolites provided several challenges in the development of both extraction and chromatographic methods. The methods presented are

precise, accurate, specific, and sensitive for the assay of dextromethorphan and its urinary metabolites and have been used to quantitate these compounds in urine from humans who received a therapeutic dose of dextromethorphan hydrobromide.

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Effect of Renal Failure and Bis(2-ethylhexyl) Phthalate Pretreatment on the Disposition and Metabolism of Antipyrine in the Rat

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Abstract □ Renal failure patients undergoing hemodialysis are regularly exposed to phthalate plasticizers leached from dialysis tubings. Previous studies have shown that antipyrine is eliminated more rapidly in chronic renal failure patients compared with normal individuals. Therefore, the effect of bis(2-ethylhexyl) phthalate on the metabolism of antipyrine was investigated in normal and renal failure rats. In normal animals, the elimination kinetics of an intravenous dose of antipyrine (20 mg/kg) was determined before and after 14 days of peroral treatment with 2 mL/kg/d of bis(2-ethylhexyl) phthalate. The plasma clearance of antipyrine increased markedly after bis(2-ethylhexyl) phthalate treatment. There was a corresponding decrease in the elimination half-life of antipyrine, whereas the apparent volume of distribution was not affected. Both liver weight and hepatic cytochrome P_{450} content increased following exposure to bis(2-ethylhexyl) phthalate, indicating the induction of hepatic microsomal enzymes. The fractional urinary recovery of the *N*-demethyl, 4-hydroxy, and 3-hydroxymethyl metabolites of antipyrine was not altered, suggesting that all three oxidative pathways were induced to the same extent. Renal failure alone did not affect the elimination kinetics of antipyrine. However, antipyrine clearance was induced to a greater extent by bis(2-ethylhexyl) phthalate treatment in the renal failure rats as compared with the control animals. The potential for phthalate plasticizers to alter hepatic drug metabolism in hemodialysis patients should be considered.

Keyphrases □ Antipyrine—disposition, pharmacokinetics, effect of bis(2-ethylhexyl) phthalate pretreatment and renal failure, rats □ Bis(2-ethylhexyl) phthalate—pretreatment, effect on antipyrine disposition and pharmacokinetics, renal failure, rats □ Renal failure—effect on antipyrine disposition and pharmacokinetics, rats, bis(2-ethylhexyl) phthalate pretreatment

There is increasing evidence suggesting that the metabolism of some drugs may be altered in renal failure (1). In general, an inhibition of drug metabolism is observed; *e.g.*, the oxidative metabolism of propranolol (2) and propoxyphene (3) and the acetylation of drugs such as sulfisoxazole (4) and aminosalicylic acid (5) are apparently inhibited in uremia. Phenytoin (6, 7) and antipyrine (8–10) are apparent exceptions to this rule, as the metabolic clearance of these compounds has been reported to be increased in patients with renal insufficiency.

The increased metabolic clearance of phenytoin in patients with renal failure is attributed to reduced protein binding of this drug in uremic serum (11). Antipyrine, however, is only slightly bound to serum and tissue proteins (12), and a decrease in serum protein binding would not be expected to significantly alter the metabolic clearance of this drug. Alternative explanations for this phenomenon, therefore, should be considered.

It is known that plasticizers can be leached from plastic medical devices such as blood transfusion bags and hemodialysis tubing into blood and certain intravenous fluids. The most common plasticizer used in the production of medical-grade plastics, bis(2-ethylhexyl) phthalate (I), has been identified as a contaminant in blood that was stored in transfusion bags (13, 14) or passed through plastic tubing (15). Clinical studies have shown that patients undergoing maintenance hemodialysis are regularly exposed to I (16, 17). Serum concentrations of I in the microgram/milliliter range were observed in renal failure patients during hemodialysis. It is possible that impaired renal function and repeated dialysis may lead to significant accumulation of I and its metabolites in hemodialysis patients (18).

Animal studies have shown that chronic exposure to I can induce changes in the *in vitro* activities of a number of hepatic drug-metabolizing enzymes (19–23). Both inhibition and induction of hepatic enzymatic activities have been observed. The reason for these conflicting results between studies may be due to variations in the selection of drug substrate, treatment schedule, and route of ad-

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