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DETERMINATION OF DEXTROMETHORPHAN AND METABOLITES IN HUMAN PLASMA AND URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

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SUMMARY

Sensitive methods were developed for the analysis of dextromethorphan (I) and two metabolites, (+)-17-methyl-morphinan-3-ol (II) and (+)-morphinan-3-ol (III), in plasma as well as dextromethorphan and three metabolites II, III and (+)-3-methoxymorphinan (IV) in urine using high-performance liquid chromatography followed by detection with a fluorometer. Dextromethorphan and its metabolites were extracted from plasma and urine and separated in the reversed-phase mode. The practical lower limits of determination for I, II, and III in plasma were 0.5, 5, and 5 ng/ml, respectively; for I, II, III, and IV in urine, the limits were 20 ng/ml, 0.6 μg/ml, 0.5 μg/ml, and 15 ng/ml, respectively. The linearity of the calibration graphs was excellent (r varied from 0.9994 to 0.9999) over concentration ranges of two orders of magnitude.

INTRODUCTION

Dextromethorphan (I), (+)-3-methoxy-17-methylmorphinan, is a widely used synthetic antitussive agent. Analytical methods for its determination in plasma include radioimmunoassay [1], gas—liquid chromatography (GLC) following extraction and conversion to an electron-capturing derivative with trichloroethyl chloroformate [2], and GLC following extraction using a nitrogen-selective detector [3, 4]. Two of these methods have been used to determine it in urine [2, 4]. It has also been determined in urine using high-performance liquid chromatography (HPLC) with ultraviolet detection [5].

Methods for the determination of dextrorphan (II), (+)-17-methylmorphinan-3-ol, the O-demethylated metabolite of dextromethorphan, and its glucuronide and sulfate ester conjugates in plasma and urine have been reported. After enzymatic hydrolysis of the esters and extraction, total

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dextrorphan has been determined in plasma by direct fluorescence [6] and HPLC with fluorescence detection [7], in plasma and urine by GLC using a nitrogen-selective detector [4], and in urine by GLC using a flame-ionization detector [8], as well as with HPLC using ultraviolet detection [5].

Metabolites (+)-morphinan-3-ol (III), after either enzymatic or acid hydrolysis of its glucuronide and sulfate ester conjugates, and (+)-3-methoxymorphinan (IV) have been determined in human urine by GLC with a nitrogen-selective detector [4] and a flame-ionization detector [8], as well as by HPLC using ultraviolet detection [5].

This report describes HPLC methods with fluorescence detection for determination of I, II and III in plasma, as well as I, II, III, and IV in urine. Since II and III exist as glucuronide and sulfate ester conjugates in plasma and urine, samples were subjected to enzymatic hydrolysis prior to extraction so that total II and III could be determined. Plasma profiles and urinary excretion data following single doses of 60 mg dextromethorphan hydrobromide are also presented.

EXPERIMENTAL

Apparatus

The HPLC system was assembled from a Waters Assoc. (Milford, MA, U.S.A.) Model 6000A solvent delivery system, a Waters Assoc. Model 710B WISP automatic sample processor, a 25 cm × 4.6 mm I.D. stainless-steel column packed with RP-18 (5-μm Ultrasphere; Altex, Berkeley, CA, U.S.A.) for analysis of II and III in plasma, RP-18 (5-μm Hypersil; Chromanetics, Baltimore, MD, U.S.A.) for analysis of I in plasma, or RP-Phenyl (5-μm Spherisorb; Chromanetics) for analysis of I, II, III, and IV in urine, a 6.0 cm × 4.6 mm I.D. stainless-steel pre-column packed with RP-18 (10-μm Bondapak C_{18}; Waters Assoc.) for analysis of I, II, and III in plasma, or a pre-column packed with RP-Phenyl (37—50 μm Bondapak; Waters Assoc.) for analysis of I—IV in urine, a Schoeffel Instrument (Westwood, NJ, U.S.A.) Model FS970 spectrofluorometer operated at λ_{ex} of 200 nm and no emission cut-off filter for analysis of I, II, and III in plasma as well as I and IV in urine and λ_{ex} of 200 nm with a Corning (Medfield, MA, U.S.A.) Model 7-54 220—400 nm band-pass emission filter for analysis of II and III in urine. The liquid chromatograph was connected to a Spectra Physics (Santa Clara, CA, U.S.A.) Model 4100 integrator calculator.

Reagents

Dextromethorphan hydrobromide and levallorphan tartrate were obtained from the United States Pharmacopeial Convention, (Rockville, MD, U.S.A.); II · tartrate and III · HBr were supplied by Hoffmann-La Roche (Nutley, NJ, U.S.A.); IV · HCl was obtained from Pennwalt (Rochester, NY, U.S.A.); viloxizine base was supplied by the Stuart Pharmaceuticals Division of ICI Americas (Wilmington, DE, U.S.A.); β-glucuronidase type H-1 (containing 300 000—400 000 U β-glucuronidase and 15 000—40 000 μmol U of arylsulfatase activity per g) was obtained from Sigma (St. Louis, MO, U.S.A.); sequanol-grade triethylamine came from Pierce (Rockford, IL, U.S.A.); 1-octanesulfonate as the sodium salt was supplied by Regis (Morton Grove, IL, U.S.A.); *n*-nonylamine was from Aldrich (Milwaukee, WI, U.S.A.); acetonitrile was

analytical-reagent grade from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.); all other reagents were reagent grade.

Solutions

A β-glucuronidase solution containing approximately 3000 U/ml β-glucuronidase and 230 μmol U of arylsulfatase activity was prepared by dissolving β-glucuronidase type H-1 in 0.1 *M* sodium citrate, pH 5.0.

The following mobile phases (as well as the stationary phases) were selected to give optimal resolution between the compounds of interest and matrix components.

The mobile phase for the determination of I in plasma was composed of 0.022 *M* sodium acetate—0.094 *M* perchloric acid—0.0196 *M* *n*-nonylamine, pH 4.3 (using sodium hydroxide)—40% acetonitrile. The volumetric flow-rate of the mobile phase was 0.7 ml/min.

For the determination of II and III in plasma, the mobile phase was composed of 0.022 *M* sodium acetate—0.094 *M* perchloric acid—0.0072 *M* triethylamine, pH 4.3 (using sodium hydroxide)—27% acetonitrile. The volumetric flow-rate of the mobile phase was 1.0 ml/min.

The mobile phase for the determination of I and IV in urine was composed of 0.01 *M* potassium dihydrogen phosphate, pH 3.0 (using phosphoric acid), and 30% acetonitrile. The volumetric flow-rate of the mobile phase was 0.7 ml/min.

For the determination of II and III in urine, the mobile phase was composed of 0.01 *M* potassium dihydrogen phosphate—0.01 *M* 1-octanesulfonate sodium salt, pH 3.0 (using phosphoric acid)—30% acetonitrile. The volumetric flow-rate of the mobile phase was 1.5 ml/min.

Stock solutions of I · HBr, II · tartrate, III · HBr, IV · HCl, and internal standards levallorphan tartrate and viloxizine base (0.1 mg/ml in water) were prepared and stored at 4°C. Standard curves were generated by spiking blank plasma or urine with varying amounts of I, II, III or IV and a constant amount of either internal standard. Microliter aliquots of the stock solutions or dilutions of the stock solutions in water were added to the plasma or urine. The internal standard concentrations in plasma were 15 ng/ml levallorphan for analysis of I and 200 ng/ml viloxizine for analysis of II and III. The internal standard concentrations in urine were 800 ng/ml levallorphan for analysis of I and IV and 17 μg/ml levallorphan for analysis of II and III.

For each compound, separate standard curves were prepared daily. Concentrations of the drug and metabolite standards in plasma and urine were as follows: I: 0.5—24 ng/ml in plasma, I: 20—4900 ng/ml in urine; II: 5—496 ng/ml in plasma, II: 0.6—56 μg/ml in urine; III: 5—495 ng/ml in plasma, III: 0.5—50 μg/ml in urine; and IV: 15—3590 ng/ml in urine. These samples were treated according to the extraction procedures described.

Enzymatic hydrolysis

The quantity of β-glucuronidase plus arylsulfatase and the incubation time required for complete hydrolysis of the conjugated metabolites were determined. Aliquots of plasma containing the equivalent of more than 496 ng/ml II and III, and urine containing 56 μg/ml of II and III were hydrolyzed

under a variety of conditions. An incubation time of 1.0 h at 37°C using 1 ml of enzyme solution per ml of plasma and 30 min at 37°C using 0.5 ml of enzyme solution per 0.1 ml of urine gave complete hydrolysis. To insure complete hydrolysis, a 2.0-h incubation time was used for plasma and a 1.0-h incubation time for urine.

Analytical procedure

The following procedures were selected because they were found to give optimal recoveries of I—IV while minimizing coextraction of sample matrix components.

Determination of I in plasma. Plasma samples (2 ml) were placed into 40-ml PTFE-stoppered silanized-glass centrifuge tubes, followed by the addition of internal standard, levallorphan tartrate (15 ng/ml of plasma). They were made alkaline by the addition of 0.5 ml of saturated sodium carbonate in water, followed by extraction with hexane (20 ml containing 0.1%, v/v, triethylamine). The tubes were gently shaken on a horizontal shaker for 20 min. After centrifugation (≥ 1000 *g*) for 10 min, the upper organic layer was transferred to a second centrifuge tube followed by evaporation to dryness under nitrogen in a 50°C water bath. The residues were then dissolved in mobile phase (300 μl), and mixed in a Vortex mixer. Aliquots (150 μl) of these solutions were then injected into the HPLC system.

Determination of total II and III in plasma. Internal standard viloxizine (200 ng/ml plasma) was added to plasma samples (1 ml) in 15-ml PTFE-stoppered silanized-glass centrifuge tubes. β-Glucuronidase type H-1 (1.0 ml of 3000 U/ml solution in 0.1 *M* sodium citrate buffer, pH 5.0) was added, followed by incubation for 2 h at 37°C. Saturated sodium carbonate in water (0.5 ml) and 10% *n*-butanol in *n*-butyl chloride (5 ml) were then added and the tubes gently shaken on a horizontal shaker for 20 min. After centrifugation (≥ 1000 *g*) for 5 min, the upper organic layer was transferred to a 40-ml PTFE-stoppered silanized-glass centrifuge tube. The remaining aqueous layer was re-extracted with 10% *n*-butanol in *n*-butyl chloride (5 ml) in the same manner. After centrifugation (≥ 1000 *g*) for 5 min, the upper organic layer was combined with the first organic extracts. The combined organic layers were extracted with 1% acetic acid (500 μl) by vortex-mixing for 1 min. After centrifugation (≥ 1000 *g*) for 5 min, aliquots (50 μl) of the lower aqueous phase were injected into the HPLC system.

Determination of I and IV in urine. Internal standard levallorphan tartrate (800 ng/ml of urine) was added to urine samples (1 ml) in 40-ml PTFE-stoppered silanized-glass centrifuge tubes. Saturated sodium carbonate in water (0.5 ml) and hexane (20 ml containing 0.1% triethylamine) were added to the samples, and the tubes gently shaken on a horizontal shaker for 20 min. After centrifugation (≥ 1000 *g*) for 5 min, the upper organic layer was transferred to a second centrifuge tube followed by evaporation to dryness under nitrogen in a 50°C water bath. The residues were dissolved in acetonitrile—1% acetic acid (300 μl, 1:1, v/v), and mixed on a Vortex mixer. Aliquots (60 μl) of these solutions were injected into the HPLC system.

Determination of total II and III in urine. Internal standard levallorphan (17 μg/ml urine) was added to urine samples (100 μl) in 15-ml PTFE-stoppered

silanized-glass centrifuge tubes. β-Glucuronidase type H-1 (0.5 ml of a 1500 U per 0.5 ml solution in 0.1 *M* sodium citrate buffer, pH 5.0) was added, followed by incubation at 37°C for 1 h. Saturated sodium carbonate in water (0.5 ml) and 10% *n*-butanol in *n*-butyl chloride (10 ml) were then added and the tubes gently shaken on a horizontonal shaker for 20 min. After centrifugation (⩾ 1000 *g*) for 5 min, the upper organic layer was transferred to a second centrifuge tube after first freezing the aqueous layer in a dry ice—acetone bath, followed by evaporation to dryness under nitrogen in a 50°C water bath. The residues were then dissolved in acetonitrile—1% acetic acid (2 ml, 1:1, v/v) and mixed on a Vortex mixer. Aliquots (50 μl) of these solutions were injected into the HPLC system.

Extraction efficiency

The extraction efficiency of each compound from plasma and urine was determined. Plasma and urine samples were spiked with I—IV (except IV in

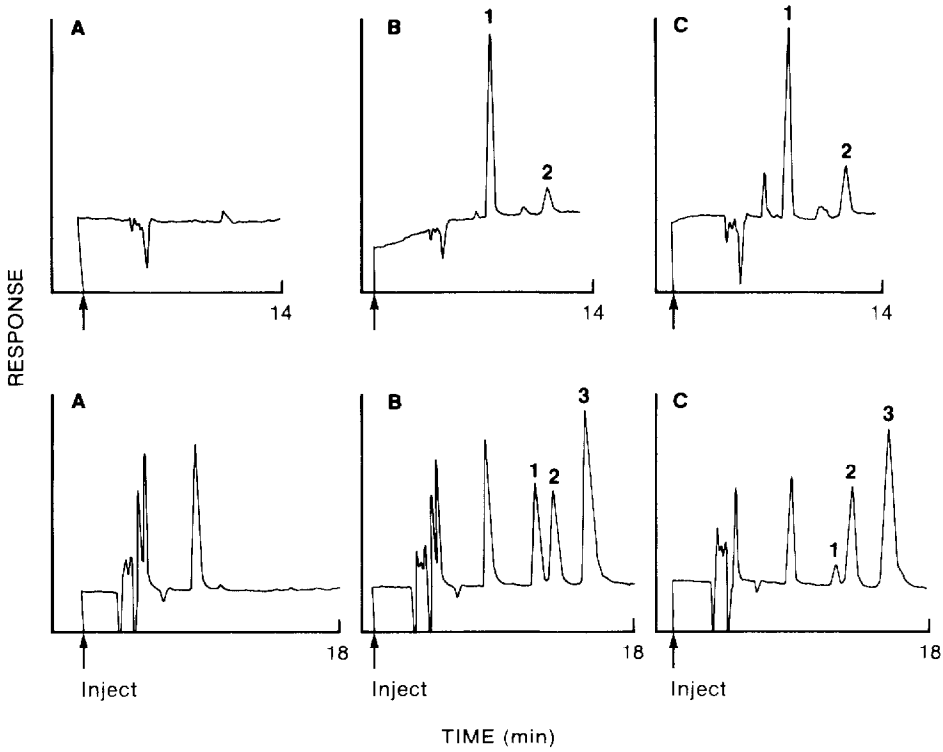

Fig. 1. (Top) Chromatograms of an analysis of (A) a plasma blank; (B) a plasma sample with added dextromethorphan (2.55 ng/ml) and internal standard, levallorphan (15 ng/ml); and (C) a plasma sample from a subject dosed with 60 mg dextromethorphan hydrobromide with added internal standard (4.69 and 15 ng/ml, respectively). Peaks: 1 = internal standard, levallorphan (retention time = 7.87 min); 2 = dextromethorphan (retention time = 11.77 min). (Bottom) Chromatograms of an analysis of (A) a plasma blank; (B) a plasma sample with added dextrorphan (48.5 ng/ml, (+)-morphinan-3-ol (47.3 ng/ml), and internal standard, viloxizine (200 ng/ml); and (C) a plasma sample from a subject dosed with 60 mg dextromethorphan hydrobromide with added internal standard (11.49, 10.86, and 200 ng/ml, respectively). Peaks: 1 = (+)-morphinan-3-ol (retention time = 11.3 min); 2 = dextrorphan (retention time = 12.49 min); 3 = internal standard viloxizine (retention time = 14.81 min).

plasma) and carried through the entire analysis procedure. The recovery of each compound was determined by comparing peak areas of extracted standards with those of unextracted standards. The unextracted standards were prepared by the addition of I—IV to water to give concentrations equivalent to those of the extracted standards.

RESULTS AND DISCUSSION

Figs. 1 and 2 show typical chromatograms for dextromethorphan and its metabolites in spiked human plasma and urine, respectively, as well as plasma

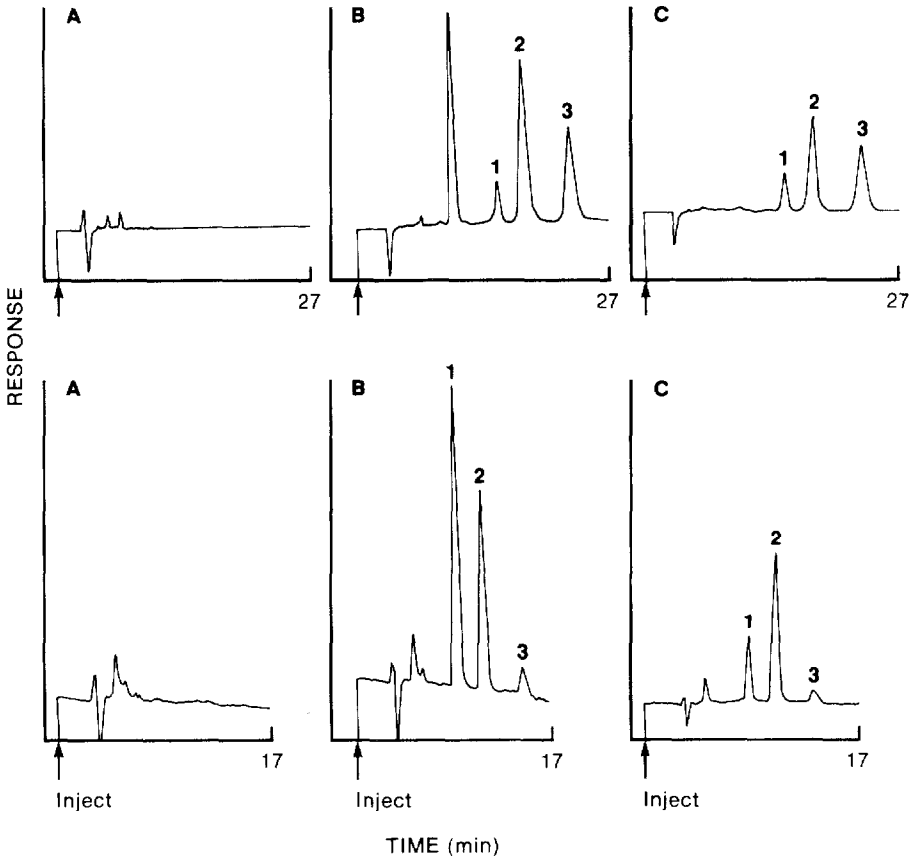

Fig. 2. (Top) Chromatograms for an analysis of (A) a urine blank; (B) a urine sample with added dextromethorphan (481.95 ng/ml), (+)-3-methoxymorphinan (473.2 ng/ml), and internal standard, levallorphan (800 ng/ml); and (C) a urine sample from a subject dosed with 60 mg dextromethorphan hydrobromide with added internal standard (312.5, 335.6 and 800 ng/ml, respectively). Peaks: 1 = internal standard, levallorphan (retention time = 14.89 min); 2 = (+)-3-methoxymorphinan (retention time = 17.84 min); 3 = dextromethorphan (retention time = 22.9 min). (Bottom) Chromatograms of an analysis of (A) a urine blank; (B) a urine sample with added dextrorphan (13.99 μg/ml), (+)-morphinan-3-ol (14.79 μg/ml), and internal standard, levallorphan (17 μg/ml); and (C) a urine sample from a subject dosed with 60 mg dextromethorphan hydrobromide with added internal standard (23.75, 6.95, and 17 μg/ml, respectively). Peaks: 1 = (+)-morphinan-3-ol (retention time = 8.16 min); 2 = dextrorphan (retention time = 10.17 min); 3 = internal standard levallorphan (retention time = 13.32 min).

TABLE I

ANALYSIS OF DEXTROMETHORPHAN IN PLASMA SAMPLES

Nominal value (ng/ml)	Determined value (ng/ml)	Mean	S.D.	R.S.D. (%)
0.48	0.57	0.48	0.08	16.8
	0.38			
	0.51			
	0.46			
2.40	2.51	2.40	0.12	4.8
	2.29			
	2.49			
	2.31			
4.8	5.23	4.80	0.3	6.2
	4.68			
	4.74			
	4.55			
24.0	24.96	24.0	0.87	3.6
	23.76			
	24.35			
	22.92			

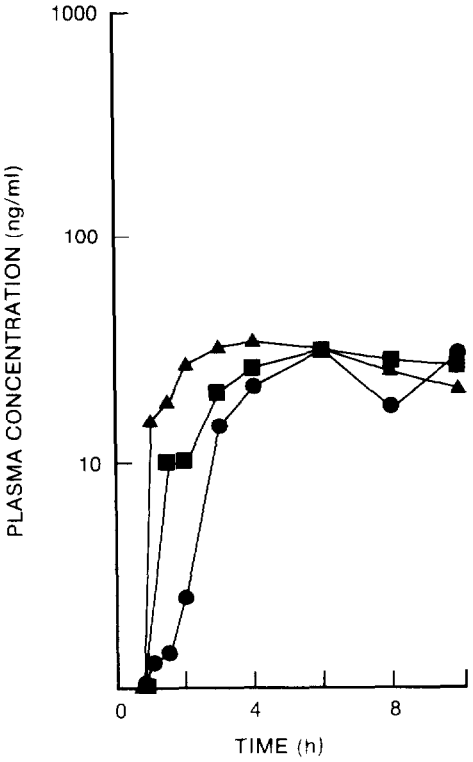

Fig. 3. Plasma concentrations of dextromethorphan (●), dextrorphan (▲), and (+)-morphinan-3-ol (■) in subject 1 following a single oral dose of 60 mg dextromethorphan hydrobromide.

and urine obtained from a human subject after oral ingestion of 60 mg dextromethorphan hydrobromide. Analysis of pre-dose plasma and urine samples from eighteen human subjects presented no chromatographic peaks that would interfere with either dextromethorphan and its metabolites or internal standards. Plots of peak area ratios (I, II, III or IV to internal standard) against I, II, III or IV concentrations were linear. Typical standard curves for plasma and urine had correlation coefficients of 0.9994—0.9999 over concentration ranges of two orders of magnitude.

The recoveries of I, II, and III from plasma were approximately 96%, 87%, and 90%, respectively. The recoveries from urine were approximately 90% for I, II, and III, and 67% for IV. Recoveries from plasma and urine of internal standards levallorphan and viloxizine were approximately 66% and 69%, respectively. These values for recoveries of I—IV agree with those recently reported [5].

TABLE II

ANALYSIS OF DEXTRORPHAN AND (+)-MORPHINAN-3-OL IN PLASMA SAMPLES

Dextrorphan					(+)-Morphinan-3-ol				
Nominal value (ng/ml)	Determined value (ng/ml)	Mean	S.D.	R.S.D. (%)	Nominal value (ng/ml)	Determined value (ng/ml)	Mean	S.D.	R.S.D. (%)
4.96	4.75	4.96	0.28	5.7	4.95	4.59	4.95	0.68	13.7
	5.30					5.93			
	4.88					4.90			
	5.21					5.21			
	4.66					4.13			
9.92	9.60	9.92	0.42	4.2	9.90	9.43	9.90	0.77	7.8
	9.97					9.96			
	9.72					11.11			
	9.68					9.09			
	10.63					9.93			
24.8	25.26	24.81	0.49	2.0	24.75	24.69	24.74	0.90	3.6
	25.14					25.38			
	24.39					25.22			
	24.18					23.20			
	25.07					25.22			
99.2	97.10	99.20	1.48	1.5	99.0	95.50	99.01	3.64	3.7
	99.98					103.23			
	100.37					101.21			
	100.36					100.16			
	98.19					94.93			
248	247.87	248.03	4.77	1.9	247.5	244.32	247.46	6.60	2.7
	254.22					256.47			
	251.11					251.43			
	242.59					239.46			
	244.35					245.63			
496	459.67	495.95	28.85	5.8	495.0	461.68	495.11	27.74	5.6
	487.34					488.70			
	518.74					514.29			
	482.77					531.30			
	531.25					479.57			

TABLE III

ANALYSIS OF DEXTROMETHORPHAN AND (+)-3-METHOXYMORPHINAN IN URINE SAMPLES

Dextromethorphan					(+)-3-Methoxymorphinan				
Nominal value (ng/ml)	Determined value (ng/ml)	Mean	S.D.	R.S.D. (%)	Nominal value (ng/ml)	Determined value (ng/ml)	Mean	S.D.	R.S.D. (%)
19.6	19.77	19.60	0.64	3.3	14.4	12.24	14.40	1.56	10.8
	18.55					15.81			
	19.57					14.36			
	19.86					15.19			
	20.26								
98.0	103.02	98.0	5.24	5.3	71.8	72.04	71.80	0.79	1.1
	99.96					71.17			
	92.81					73.07			
	102.22					71.51			
	91.98					71.21			
490.0	461.43	490.0	22.2	4.5	359.0	365.44	361.29	4.46	1.2
	523.08					356.57			
	494.33					357.37			
	487.13					364.23			
	484.25					362.85			
2450.0	2236.0	2449.6	191.6	7.8	1793.0	1817.0	1793.6	29.7	1.7
	2321.0					1769.0			
	2696.0					1819.0			
	2596.0					17.54.0			
	2399.0					1809.0			
4899.0	4634.0	4899.2	550.4	11.2	3588.0	3544.6	3588.6	154.6	4.3
	4480.0					3410.0			
	4470.0					3522.0			
	5172.0					3646.0			
	5740.0					3821.0			

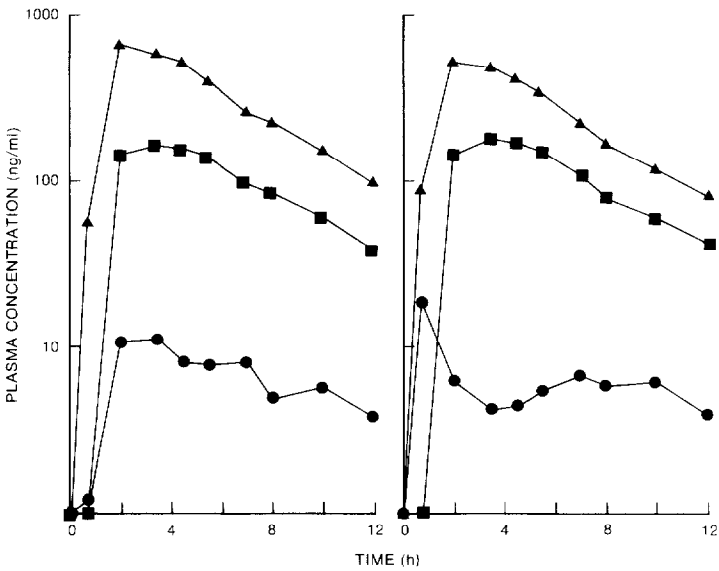

Fig. 4. Plasma concentrations of dextromethorphan (•), dextrorphan (▲), and (+)-morphinan-3-ol (■) in subject 2 (left) and 3 (right) following a single oral dose of 60 mg dextromethorphan hydrobromide.

TABLE IV

ANALYSIS OF DEXTRORPHAN AND (+)-MORPHINAN-3-OL IN URINE SAMPLES

Dextrorphan					(+)-Morphinan-3-ol				
Nominal value (μg/ml)	Determined value (μg/ml)	Mean	S.D.	R.S.D. (%)	Nominal value (μg/ml)	Determined value (μg/ml)	Mean	S.D.	R.S.D. (%)
0.58	0.569	0.58	0.009	1.6	0.45	0.479	0.446	0.06	13.1
	0.593					0.346			
	0.585					0.479			
	0.576					0.484			
	0.577					0.440			
1.45	1.448	1.43	0.03	2.1	1.25	1.252	1.251	0.05	4.1
	1.442					1.294			
	1.435					1.163			
	1.437					1.275			
	1.371					1.272			
5.64	5.674	5.64	0.12	2.2	4.42	4.445	4.340	0.10	2.3
	5.663					4.429			
	5.450					4.233			
	5.786					4.500			
	5.626					4.391			
28.2	26.94	28.20	1.39	4.9	24.6	23.42	24.60	1.03	4.2
	26.65					23.95			
	28.29					25.68			
	29.49					24.29			
	29.61					25.68			
56.4	53.16	56.40	2.00	3.5	49.2	46.59	49.21	3.81	7.7
	56.47					54.40			
	58.66					44.51			
	56.69					50.26			
	57.01					50.27			

The precision of the assay methods, expressed as the relative standard deviation, ranged from 16.8% to 3.6%, 5.8% to 1.5%, and 13.7% to 2.7% for I, II, and III in plasma, respectively. Precision ranged from 11.2% to 3.3%, 4.9% to 2.1%, 13.1% to 2.3%, and 10.8% to 1.1% for I, II, III, and IV in urine, respectively (Tables I—IV).

The practical lower limits of determination (where chromatographic peaks have a signal-to-noise ratio of $\geqslant$ 5:1) for I, II, and III in plasma were 0.5, 5, and 5 ng/ml, respectively. The practical lower limits of determination for I, II, III, and IV in urine were 20 ng/ml, 0.6 μg/ml, 0.5 μg/ml, and 15 ng/ml, respectively.

To demonstrate the usefulness of these methods, results from the analysis of plasma samples obtained from four healthy subjects who had ingested 60 mg dextromethorphan hydrobromide in water are shown in Figs. 3—5. Cumulative urinary excretion of dextromethorphan and its metabolites are shown in Figs. 6—8. Urinary recovery of dextromethorphan and its metabolites, expressed as dextromethorphan base, was 20.8%, 61.9%, 48.0%, and 64.5% for subjects 1—4, respectively.

Earlier reports [1—11] have suggested that dextromethorphan is rapidly and extensively metabolized via first-pass hepatic metabolism, and that metab-

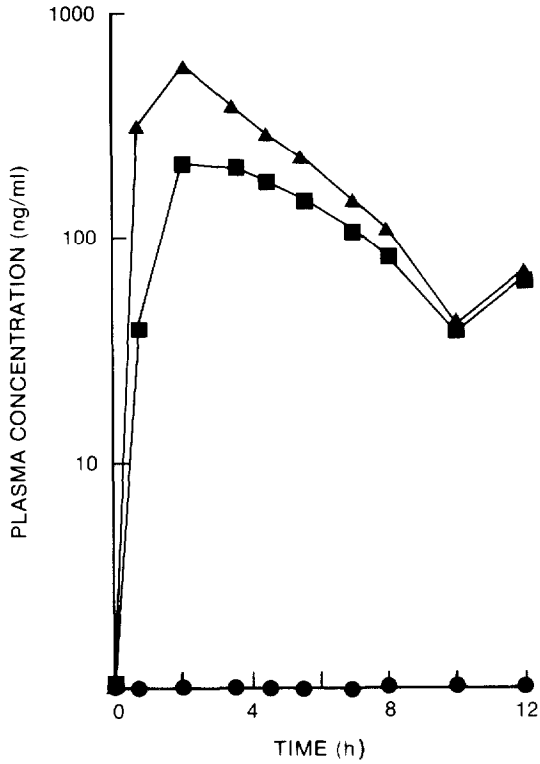

Fig. 5. Plasma concentrations of dextromethorphan (●), dextrorphan (▲), and (+)-morphinan-3-ol (■) in subject 4 following a single oral dose of 60 mg dextromethorphan.

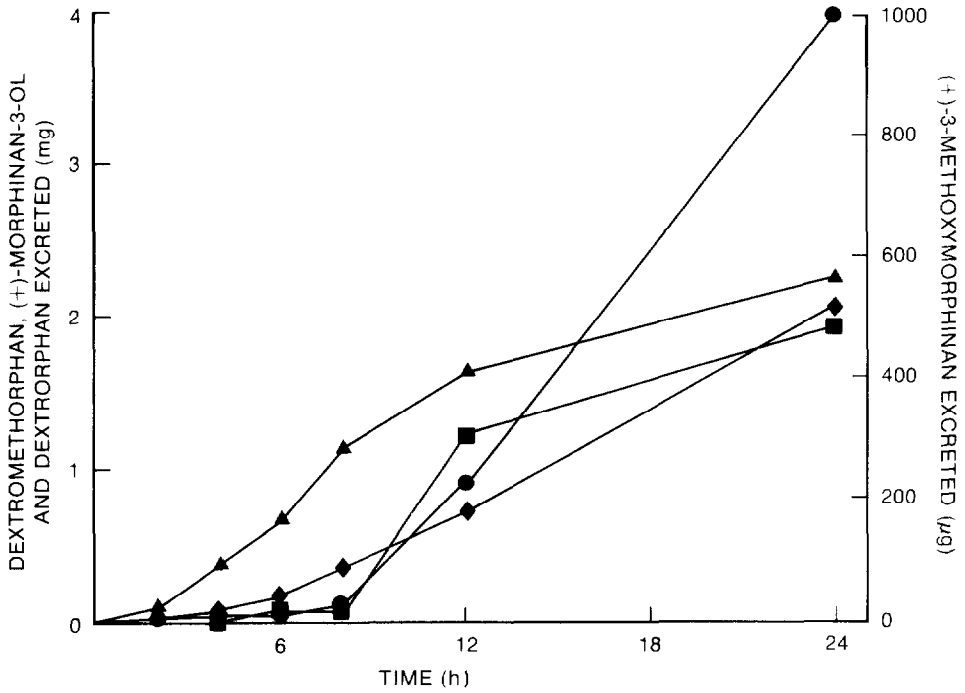

Fig. 6. Cumulative urinary excretion in subject 1 of dextromethorphan (●), dextrorphan (▲), and (+)-morphinan-3-ol (■) (left scale), and (+)-3-methoxymorphinan (♦) (right scale).

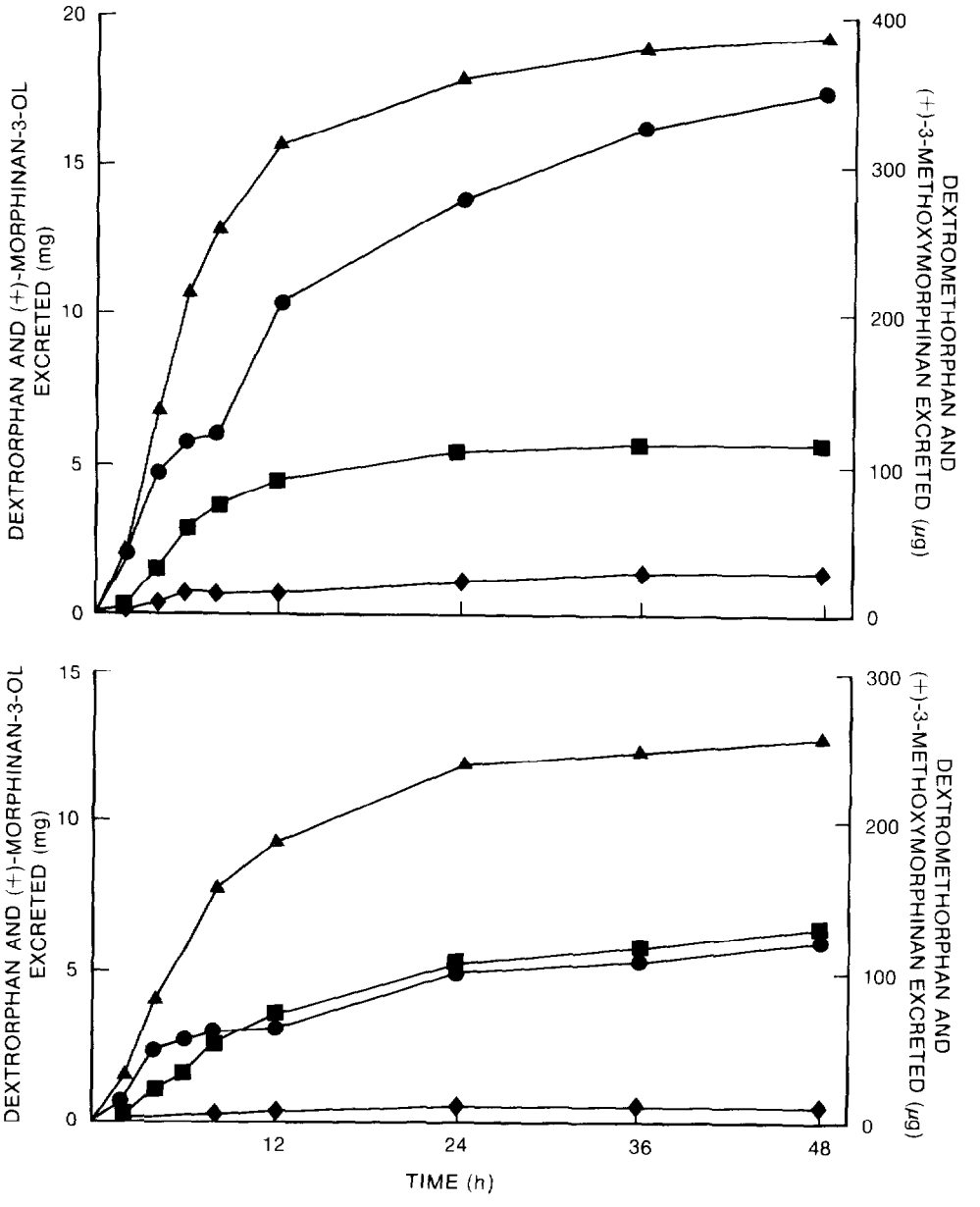

Fig. 7. Cumulative urinary excretion in subject 2 (top) and 3 (bottom) of dextrorphan (▲) and (+)-morphinan-3-ol (■) (left scale), and dextromethorphan (●), and (+)-3-methoxymorphinan (♦) (right scale).

olism is highly variable between subjects [2, 4, 8]. The antitussive activity of oral doses of I were attributed to the possible pharmacological activity of one or more of the metabolites [1, 2, 11]. One investigative group reported that subjects could be divided into three groups [4]. The plasma of one group contained primarily conjugated II, with no detectable I. Another group with plasma containing primarily conjugated II also contained levels of I that were

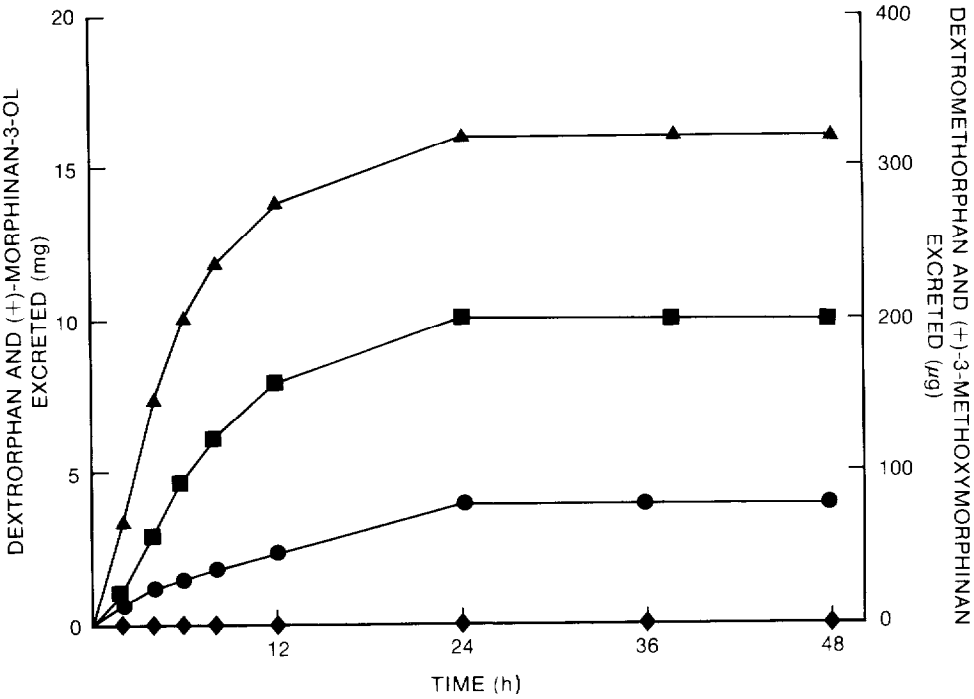

Fig. 8. Cumulative urinary excretion in subject 4 of dextrorphan (▲) and (+)-morphinan-3-ol (■) (left scale), and dextromethorphan (●) and (+)-3-methoxymorphinan (♦) (right scale).

approximately twenty times higher than those of the first group. Plasma from the third group contained only I. They determined that average urinary recoveries within 48 h were 86%, 74%, and 20% for the three groups, respectively, after a 25-mg oral dose of dextromethorphan hydrobromide. The plasma profiles and urinary recoveries seen in our four subjects, as well as those seen in subsequent studies with other subjects, support their findings.

An earlier report [7] noted the appearance of a chromatographic peak following hydrolysis of post-dose plasma samples. This peak did not appear when the hydrolysis step was omitted, and was thought to be another conjugated metabolite of dextromethorphan. Our findings suggest that this metabolite was (+)-morphinan-3-ol (III). Without prior enzymatic hydrolysis, no unconjugated III was detected in plasma using the method described.

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