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ASSAY FOR CODEINE, MORPHINE AND TEN POTENTIAL URINARY METABOLITES BY GAS CHROMATOGRAPHY—MASS FRAGMENTOGRAPHY

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SUMMARY

A mass fragmentography (MF) assay is described for ten potential, minor urinary metabolites of codeine (C) and morphine (M). Samples were hydrolyzed, extracted, derivatized with Tri-Sil Z and analyzed by methane chemical ionization (CI)-MF. The method is sensitive to ca. 0.01 μg/ml for all compounds with the exception of normorphine (NM) which was difficult to extract with chloroform. The sensitivity of the MF assay for NM was only ca. 0.10 μg/ml. Various solvent systems were investigated for optimization of extraction efficiency of all metabolites. A separate method for the extraction of NM is reported which utilizes a solid buffer—solvent combination, i.e., potassium carbonate—isopropanol. This latter method provided the best overall recovery of NM (39.0 ± 3.4%). Gas chromatographic (GC) retention times of C, M and metabolites are reported for three liquid phases (3%) on Gas-Chrom Q (100—120 mesh). Resolution of metabolites (as trisilyl derivatives) was best on Silar-5CP and this phase was used in metabolic studies of C and M. GC resolution was not complete for all compounds; however, selection of specific ions for monitoring by MF provided the required specificity for all compounds except the 6α- and 6β-hydroxy isomers. CI spectra for all metabolites are reported. The MF assay was used for urinary analysis of samples from guinea pigs that received single doses of C (15 mg/kg) or M (8 mg/kg). Following C administration 6α- and 6β-hydrocodol, 6α,β-hydromorphol (undifferentiated), HM and M were measured. Following M administration only 6α,β-hydromorphol was found. The amount of total metabolite as percent dose for each component was calculated as < 1%.

INTRODUCTION

Codeine (C) and morphine (M) have been used extensively throughout the world for many years for the relief of moderate to severe pain. Additionally, C is the opiate of choice for relief of cough since it has less dependence liability than M. Despite their long and continued widespread use, metabolic and dispositional studies on these compounds continue to yield new information. Several new metabolites of M [1, 2] and C [3] have recently been identified.

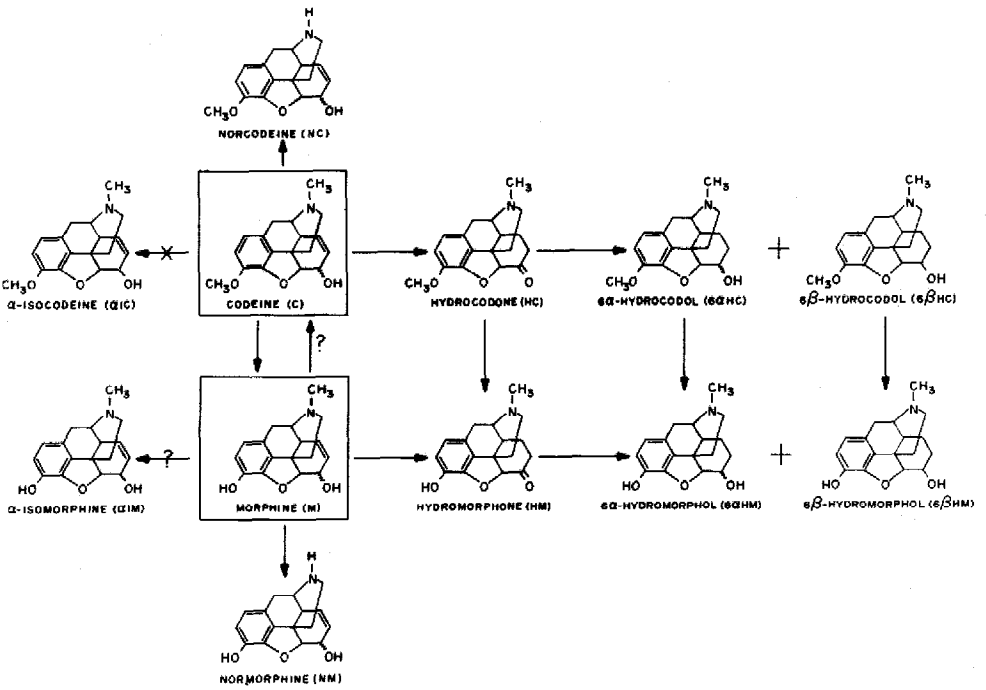

Fig. 1. Biotransformation of codeine and morphine.

The metabolic profiles for C and M (Fig. 1) which have evolved include numerous metabolites with equal or greater analgesic potency than that of the parent compound. The relationship of these metabolites to the pharmacological activity of M and C remains in question; however, measurement of these compounds in urine and blood should contribute to the understanding of their actions. Numerous assays have been described for the detection and measurement of M and C in biological fluids. These methods include assay by mass fragmentography (MF) [4–6], gas chromatography (GC) with electron-capture detection [7–10], GC with flame ionization detection [11, 12], radioisotopic techniques [13, 14], radioimmunoassay [15, 16] and high-performance liquid chromatography with electrochemical detection [17]. However, there have been no reports describing the simultaneous assay of M, C and as many as ten possible metabolites. This report describes an MF assay for the opiates of Fig. 1 in urine following the administration of M or C. Additional data are presented for the solvent extraction and GC separation of these compounds. These methods were used to demonstrate the presence of several minor metabolites in the urine of guinea pigs following a single dose of M or C.

MATERIALS AND METHODS

Chemicals

Sources for the narcotic standards were as follows: C and M (Mallinckrodt, St. Louis, MO, U.S.A.); norcodeine (NC) and normorphine (NM) (Merck, Rahway, NJ, U.S.A.); hydrocodone (HC) (Merrell National, Cincinnati, OH,

U.S.A.); hydromorphone (HM) (Knoll, Whippany, NJ, U.S.A.); cyclazocine (CY) (Sterling Winthrop, Rensselaer, NY, U.S.A.); α-isocodeine (αIC), 6β-hydrocodol (6βHC), 6α-hydromorphol (6αHM), and 6β-hydromorphol (6βHM) (Drug Addiction Laboratory, University of Virginia, Richmond, VA, U.S.A.). 6α-Hydrocodol (6αHC) and norhydrocodone (NHC) were generous gifts from Dr. Everette May, Medical College of Virginia, Richmond, VA, U.S.A. and Dr. J.W. Barnhart, Dow Chemical Co., Midland, MI, U.S.A., respectively. All compounds were analyzed by gas chromatography—mass spectrometry (GC—MS) before use.

Solvents and chemicals were of reagent grade quality. Chloroform (1% v/v ethanol) was purchased from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.) and was treated with calcium hydroxide for 12 h prior to use in order to eliminate phosgene and related impurities.

Gas chromatography

The analyses were performed on a Varian gas chromatograph Model 2700 equipped with a flame ionization detector. Glass columns (1.83 m × 2 mm) were used. Injector and detector temperatures were maintained at 275°C. Column temperature was maintained isothermally at the indicated temperatures. The air, hydrogen and nitrogen (carrier) gas flow-rates were 300, 30 and 50 ml/min, respectively. Column packings tested were 3% Silar-5CP, 3% Silar-10C, 3% OV-225 and 3% OV-17 on 100—120 mesh Gas-Chrom Q. The reported retention times represent an average of triplicate determinations.

Gas chromatography—mass spectrometry

Mass spectral data were obtained on a Finnigan Model 3300 quadrupole gas chromatograph—mass spectrometer operating in the methane chemical ionization (CI) mode. The gas chromatograph—mass spectrometer was equipped with a Finnigan Model 6000 Interactive Data System. The gas chromatograph consisted of a glass column (1.52 m × 2 mm) packed with 3% Silar-5CP on 100—120 mesh Gas-Chrom Q and was coupled to the mass spectrometer by a glass-lined stainless-steel tube and a venting valve. The electron energy was 80 eV. Methane (flow-rate 20 ml/min) was used as the carrier and reagent gas.

The temperatures of the injector, column and ion source were 250°C, 250°C and 100°C, respectively. After sample injection, the venting valve was opened for 20 sec, allowing solvent and highly volatile substances to escape without entering the ion source. MF recordings were performed. The ions selected for monitoring for each compound at its respective retention (min) were as follows: C, *m/e* 372, 2.78; M, *m/e* 430, 1.76; NC, *m/e* 268, 4.29; NM, *m/e* 326, 2.78; HC, *m/e* 372, 2.46; 6αHC, *m/e* 374, 1.72; 6βHC, *m/e* 374, 1.83; NHC, *m/e* 358, 3.79; αIC, *m/e* 372, 2.04; HM, *m/e* 430, 1.48; 6αHM, *m/e* 432, 1.02, 6βHM, *m/e* 432, 1.02; αIM, *m/e* 430, 1.26.

Although the number of ions that could be monitored simultaneously was limited to 4, by appropriate selection, all of the compounds in Fig. 1 could be determined in two separate runs. Standard curves were prepared by adding known amounts of drug and metabolites (0—4 μg/ml) to predrug control urine containing internal standard, αIC (5 μg/ml). The standard samples were

TABLE I

SOLVENT EXTRACTION EFFICIENCIES OF CODEINE (C), MORPHINE (M) AND DERIVATIVES

Samples were extracted in triplicate by Method A (Materials and Methods) and the mean ± S.E. is reported. Corrections were made for sample aliquots.

Compound	*n*-butyl chloride	Chloroform	Chloroform—isopropyl alcohol					Isopropyl alcohol—solid buffer
			95:5	90:10	85:15	80:20	70:30	
C	52.3 ± 6.4	75.9 ± 1.4	74.8 ± 3.0	71.2 ± 2.1	61.3 ± 1.2	59.5 ± 1.7	46.4 ± 1.9	77.4 ± 2.0
M	1.1 ± 0.3	36.4 ± 2.1	71.4 ± 4.3	78.7 ± 4.0	83.0 ± 4.1	81.2 ± 4.9	68.0 ± 1.2	80.6 ± 2.1
NC	0	52.0 ± 5.1	45.6 ± 0.5	57.2 ± 4.6	59.6 ± 4.3	45.0 ± 2.2	31.4 ± 0.8	ND*
NM	0	7.2 ± 0.4	7.2 ± 0.6	7.2 ± 1.0	10.5 ± 1.6	11.9 ± 0.8	13.2 ± 3.8	39.0 ± 3.4
HC	77.8 ± 7.5	59.2 ± 0.7	60.0 ± 1.6	53.0 ± 0.6	49.8 ± 1.3	46.8 ± 1.1	23.6 ± 2.1	ND
6αHC	59.3 ± 3.1	71.5 ± 0.6	66.9 ± 1.1	65.1 ± 1.6	58.5 ± 1.4	53.9 ± 0.8	43.1**	ND
6βHC	54.4 ± 6.2	86.7 ± 0.9	88.9 ± 0.8	83.3 ± 0.5	78.3 ± 0.3	72.6 ± 0.7	61.5**	ND
NHC	32.3 ± 0.7	76.2 ± 4.9	60.8 ± 3.4	53.1 ± 5.5	32.0 ± 3.8	16.6 ± 3.6	19.9 ± 0.3	ND
HM	1.9 ± 0.3	85.3 ± 6.7	88.8 ± 6.2	92.5 ± 4.7	87.4 ± 3.0	78.5 ± 4.9	62.6 ± 6.2	74.5 ± 2.2
6αHM	0	39.1 ± 2.0	77.0 ± 4.1	88.4 ± 4.9	93.7 ± 5.4	94.5 ± 6.7	73.8 ± 1.0	94.3 ± 2.8
6βHM	0	20.1 ± 3.4	64.7 ± 2.4	83.6 ± 4.2	95.7 ± 6.3	98.1 ± 6.5	76.7 ± 0.9	93.7 ± 0.5
αIM	0	17.8 ± 1.4	55.8 ± 2.5	69.6 ± 2.2	77.8 ± 0.6	79.5 ± 0.8	75.6 ± 0.6	88.4 ± 1.3

*ND = not determined.

**Only single determinations were made.

processed in similar fashion to the drug samples. A daily standard curve was constructed. Linear relationships of peak height ratios of drug or metabolite to internal standard were observed throughout the concentration range.

Extraction methods

Two general procedures (Methods A and B) were employed for the extraction of C, M and metabolites. Method A is essentially the same as that described for the analysis of metabolites of naltrexone [18]. Using this method, urine samples (10 ml) were acid-hydrolyzed (10% concentrated hydrochloric acid, v/v), pH adjusted to 10.0 ± 0.1 and buffered with 2 ml of phosphate buffer (40% K_2HPO_4, w/v). Sodium chloride was added (1 g per 10 ml), and the solution was extracted with 15 ml of an organic solvent. The extract was transferred to a tube containing 3 ml of 2 *N* hydrochloric acid and the contents were shaken for 10 min. The organic layer was removed and the pH of the solution adjusted to 10.0 ± 1 with 2 *N* sodium hydroxide solution. The solution was buffered, 1 g of sodium chloride added, and extracted with 15 ml of organic solvent. The extract was removed and evaporated to dryness at 60°C under a nitrogen atmosphere. The residue was dissolved in methanol (1 ml), transferred to acylation tubes (Regis, Morton Grove, IL, U.S.A.) and the solvent evaporated to dryness. Tri-Sil Z (Pierce Chemical, Rockford, IL, U.S.A.) (100 μl) was added. The tube was sealed and heated at 95–100°C for 2 h; after cooling, 2–5 μl were analyzed by GC–MS.

Method B utilized a solid buffer system in which the urine samples (10 ml) were acid-hydrolyzed as in Method A, followed by the careful addition of 7.5 g of anhydrous K_2CO_3. The solution was extracted with 6 ml of isopropanol and the extract transferred to a new tube and evaporated to dryness under nitrogen. Chloroform (7 ml) and 3 ml of 2 *N* hydrochloric acid were added and the contents were shaken for 10 min. The organic phase was aspirated and 1.8 g of anhydrous K_2CO_3 were added, followed by 15 ml of isopropanol. The contents were shaken for 10 min and the extract was transferred to an acylation tube and evaporated to dryness. The residue was derivatized and analyzed as in Method A.

RESULTS AND DISCUSSION

Extraction of opiates from urine

Prior to extraction, all urine samples were acid-hydrolyzed by treatment with 10% (v/v) concentrated hydrochloric acid and heating in an autoclave (115°C and 15 p.s.i.) for 30 min. This procedure is effective for the cleavage of glucuronide conjugates of morphine and related compounds. The pH values of the resulting solutions were adjusted to 10.0 ± 0.1, buffered with phosphate buffer and extracted with an organic solvent. The organic phase was back extracted into 2 *N* hydrochloric acid and the aqueous phase removed, pH adjusted to 10.0 ± 0.1, buffered and extracted with organic solvent (Method A). Analysis of the first organic extract without further clean-up was attempted but proved difficult since sensitivity for the minor metabolites was reduced because of the high endogenous background from urine. Back-extraction into acid followed by re-extraction effectively reduced background interferences to acceptable levels.

Percent recoveries of M, C and various congeners from urine were determined with several organic solvents and are listed in Table I. *n*-Butyl chloride provided lower recoveries than did chloroform for all compounds except HC. Increasing the polarity of chloroform by the addition of isopropyl alcohol generally improved recoveries for compounds with a free phenolic group, eg., M, NM, 6αHM, 6βHM, αIM and lowered recoveries for compounds with a 3-O-methyl group, e.g., C, NC, HC, 6αHC, 6βHC, NHC.

An alternative extraction method (Method B) was developed for optimization of recovery of NM, a highly polar water-soluble metabolite which was poorly extracted by Method A. Method B utilized a solid buffer, potassium carbonate, to saturate the urine sample. The buffered sample was extracted with pure isopropanol which was removed and extracted with 2 *N* hydrochloric acid. The aqueous phase was removed, carefully saturated with solid potassium carbonate and extracted with isopropanol. This extraction procedure provided the best overall recovery of NM (39.0 ± 3.4%) together with high recoveries of other opiates. Occasional problems did arise with this procedure due to carry-over of salt into the final extract. Although derivatization in the presence of salt was successful, the presence of excess bulk material in sample tubes made sampling for analysis difficult. Despite these problems, Method B was the method of choice for the detection of NM because of the improved recovery.

GC separation of M, C and metabolites

Resolution of M, C and metabolites and their trimethylsilyl (TMS) derivatives by GC was attempted on four different liquid phases (3% on Gas-Chrom Q, 100—120 mesh, 1.8 m glass column). Retention times are listed in Table II for three of the phases. The fourth phase tested, Silar-10C, was

TABLE II

RELATIVE RETENTION TIMES (RRT) OF CODEINE (C), MORPHINE (M) AND DERIVATIVES

RRT values reported are the mean of triplicate determinations. Values in brackets represent uncorrected retention times.

Compound	RRT on Silar-5CP (min)		RRT on OV-225 (min)		RRT on OV-17 (min)	
	Underivatized compound (250°C)	Silyl derivative (250°C)	Underivatized compound (240°C)	Silyl derivative (210°C)	Underivatized compound (240°C)	Silyl derivative (240°C)
C	2.30 (6.45)	5.07 (3.80)	1.87 (3.31)	4.85 (9.95)	2.14 (5.16)	2.96 (4.00)
M	—*	3.13 (2.35)	4.16 (7.36)	3.78 (7.75)	2.69 (6.46)	2.74 (3.70)
NC	3.64 (10.20)	8.07 (6.05)	2.54 (4.49)	6.95 (14.25)	2.52 (6.06)	3.44 (4.65)
NM	—	4.93 (3.70)	6.20 (10.98)	5.46 (11.20)	3.26 (7.83)	3.26 (4.40)
HC	4.11 (11.50)	4.60 (3.45)	3.09 (5.47)	4.39 (9.00)	2.94 (7.05)	3.07 (4.15)
6αHC	2.04 (5.70)	3.27 (2.45)	1.71 (3.03)	3.00 (6.15)	2.07 (4.96)	2.26 (3.05)
6βHC	2.64 (7.40)	3.07 (2.30)	2.11 (3.74)	2.98 (6.10)	2.28 (5.47)	2.37 (3.20)
NHC	6.11 (17.10)	7.00 (5.25)	4.41 (7.80)	6.17 (12.65)	3.48 (8.35)	3.56 (4.80)
HM	—	2.73 (2.05)	5.07 (8.98)	3.15 (6.45)	3.28 (7.87)	2.70 (3.65)
6αHM	—	1.87 (1.40)	—	2.05 (4.20)	2.49 (5.85)	1.89 (2.55)
6βHM	—	1.87 (1.40)	—	2.17 (4.45)	2.60 (6.10)	2.07 (2.80)
Cy	1.00 (2.80)	1.00 (0.75)	1.00 (1.77)	1.00 (2.05)	1.00 (2.40)	1.00 (1.35)

*Indicates unsatisfactory results for GC analysis.

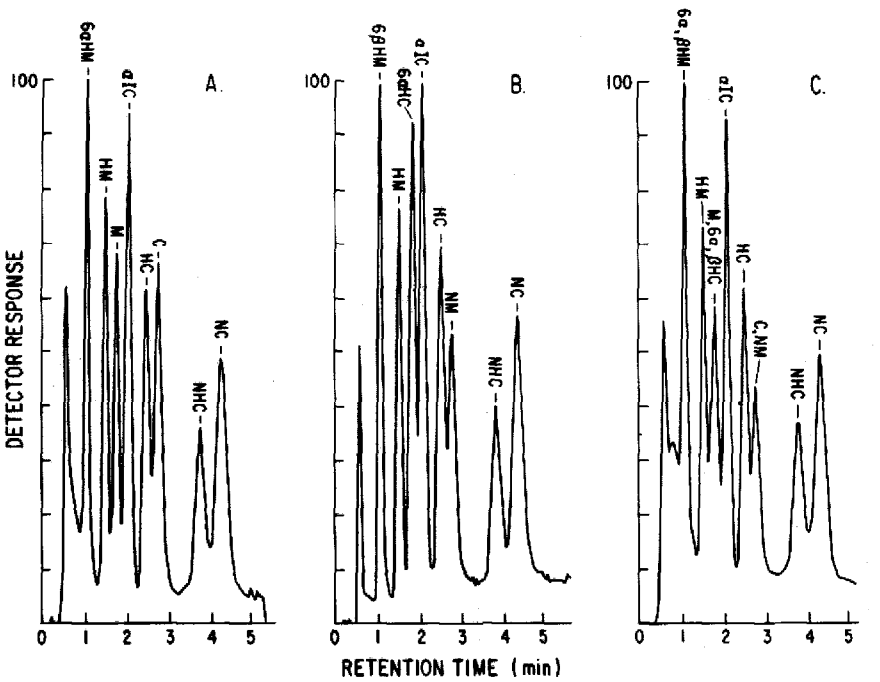

Fig. 2. GC separation of trimethylsilyl derivatives of codeine, morphine and metabolites on 3% Silar-5CP. (A) Codeine (C), morphine (M) and six derivatives; (B) eight derivatives; (C) all compounds in Fig. 1.

unsuitable for analysis of either free or TMS derivatives because of the poor chromatographic response of these compounds on this liquid phase. Silar-5CP also was not suitable for analysis of M and related compounds with free phenolic groups (underivatized); however, the analogous compounds in the C series (3-O-methyl) demonstrated good chromatographic characteristics on this phase.

Derivatization consistently improved peak shape and shortened retention times for the three phases in Table II. Resolution of all compounds as TMS derivatives was somewhat better on the more polar phases (Silar-5CP and OV-225) than on OV-17; however, all compounds in Fig. 1 were not resolvable on either of the three systems.

Silar-5CP was selected for use in continued metabolic studies on opiates since it provided equal or better separation of the compounds in Fig. 1 than did the other phases. Urine extracts were analyzed on Silar-5CP as the TMS derivatives in most cases. Under these conditions up to eight of the compounds could be resolved to near baseline resolution in a single run (Fig. 2A and B); however, when all components were present, clusters of two to three compounds eluted simultaneously. The 6α- and 6β-hydroxy derivatives of HC and HM proved the most difficult to separate by GC methods. 6αHC and 6βHC could be separated on Silar-5CP in the underivatized form, whereas 6αHM and 6βHM did not elute on this system and were not separable on OV-225 or OV-17. Prior separation of these isomers by thin-layer chromatography [18] will provide the specificity for separate determination.

TABLE III

METHANE CHEMICAL IONIZATION SPECTRA OF CODEINE (C), MORPHINE (M), AND DERIVATIVES

Compound	t_R (min)*	Mol. wt.	Methane CI spectra**			
			$(M+29)^+$	$(M+1)^+$	M^+	Prominent fragment ions
C-TMS	2.78	371	400 (14)	372 (44)	371 (29)	370 (15), 356 (20), 283 (26), 282 (100)
M-TMS_2	1.76	429	458 (19)	430 (78)	429 (55)	431 (32), 415 (37), 414 (92), 371 (16), 341 (33), 340 (100)
NC-TMS	4.36	357	386 (12)	358 (45)	357 (24)	342 (25), 269 (27), 268 (100)
NM-TMS_2	2.78	415	444 (17)	416 (74)	415 (47)	417 (38), 401 (36), 400 (93), 371 (21), 327 (36), 326 (100)
HC-TMS	2.49	371	400 (17)	372 (100)	371 (38)	373 (37), 370 (21), 356 (32)
6αHC-TMS	1.83	373	402 (13)	374 (100)	373 (56)	375 (32), 372 (28), 358 (26), 315 (19), 284 (20)
6βHC-TMS	1.72	373	402 (18)	374 (100)	373 (55)	375 (43), 372 (30), 358 (43), 315 (19), 284 (47), 285 (18)
NHC-TMS	3.83	357	386 (16)	358 (100)	357 (40)	359 (38), 356 (15), 342 (31)
HM-TMS_2	1.48	429	458 (17)	430 (100)	429 (46)	432 (21), 431 (44), 415 (33), 414 (76)
6αHM-TMS_2	1.02	431	460 (13)	432 (100)	431 (72)	433 (54), 430 (27), 417 (38), 416 (74), 373 (20), 342 (23)
6βHM-TMS_2	1.02	431	460 (19)	432 (96)	431 (76)	433 (61), 430 (33), 417 (46), 416 (100), 373 (22), 343 (33), 342 (82)
αIM-TMS_2	1.22	429	458 (25)	430 (100)	429 (49)	431 (39), 428 (22), 415 (28), 414 (82), 340 (37)
αIC-TMS	2.04	371	400 (17)	372 (100)	371 (43)	373 (48), 370 (22), 356 (29), 282 (38)

*t_R (min) were determined by GC—MS with a 1.5-m glass column packed with 3% Silar-5CP on Gas-Chrom Q (100—120 mesh). Methane was the carrier gas at a flow-rate of 30 ml/min. The column was operated isothermally at 245°C.

**m/e (relative abundance). Only ions ≥ 15% relative abundance are reported. The ions selected for monitoring by MF are underlined.

Methane CI-MS spectra of M, C and metabolites

The compounds in Fig. 1 were analyzed as TMS derivatives by GC—MS under CI conditions with methane as carrier and reagent gas. The spectra and retention times (t_R) are listed in Table III. All of the compounds displayed strong $(M+1)^+$ and $(M+29)^+$ ions. The $(M+1)^+$ ion was the most abundant ion for eight of the compounds and the $(M-89)^+$ ion (loss of TMSOH) was the most abundant ion for four compounds. The four compounds which readily eliminated TMSOH contain a C7—C8 carbon—carbon double bond and α-hydroxy configuration which apparently serves to facilitate this decomposition pathway. Only one compound, 6βHM, had an $(M-15)^+$ ion as the most abundant ion.

CI-MF analysis of M, C and metabolites

Although MF analysis was limited to the scanning of four ions per run, selection of appropriate ions allowed the measurement of all compounds and internal standard in two runs. Since the 6α- and 6β-hydroxy metabolites were not resolved on OV-225 as TMS derivatives, they were measured as combinations, i.e., 6α,βHC and 6α,βHM. The ions which were scanned were selected from Table III on the basis of their relative abundance, selectivity in the presence of components with similar retention times and commonality with

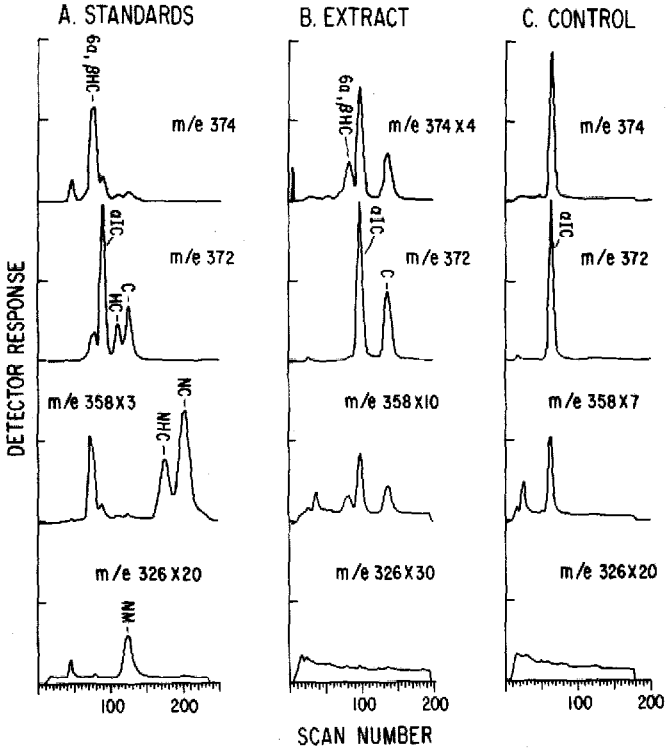

Fig. 3. Mass fragmentograms of extracts (trimethylsilyl derivatives) of guinea pig urine. (A) Control urine with added standards; (B) 24-h sample following subcutaneous administration of codeine (15 mg/kg); (C) control urine collected prior to drug administration. Each run was restricted to four ions as shown.

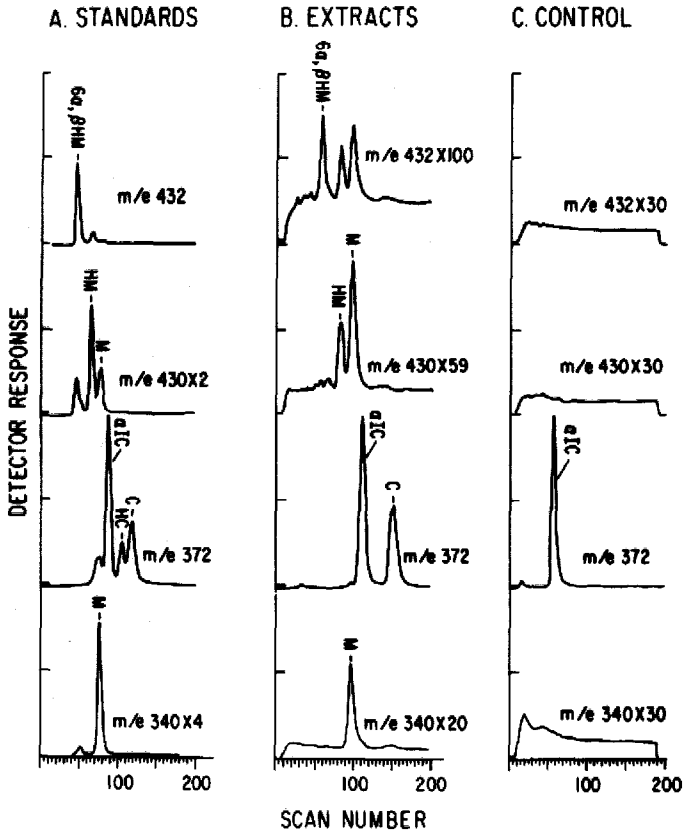

Fig. 4. Mass fragmentograms of extracts (trimethylsilyl derivatives) of guinea pig urine. (A) Control urine with added standards; (B) 24-h sample following subcutaneous administration of codeine (15 mg/kg) (C) control urine collected prior to drug administration. Each run was restricted to four ions as shown.

other resolvable compounds. For instance, ion 430 *m/e* was scanned for M (t_R = 1.76) and HM (t_R = 1.48) which were resolved chromatographically but were not common to the spectra of 6αHC (t_R = 1.83) and 6βHC (t_R = 1.72).

Standard curves were constructed for all components by plotting peak height ratios of compound divided by internal standard versus concentration (0—4 μg/ml for C and NC and 0–2 μg/ml for all other components). Correlation coefficients (r) for all compounds were consistently greater than 0.98. Minimal detectable quantities in urine were ca. 0.01 μg/ml for most compounds with the exception of NM which was ca. 0.10 μg/ml. Typical MF scans of standards extracted from normal urine are shown in Figs. 3A, 4A and 5A.

Urinary analyses for trace metabolites of M and C from guinea pig

Urine was collected from six guinea pigs (male, albino Hartley, weight 305—350 g) following the subcutaneous administration of C (15 mg/kg) or M (8 mg/kg). Samples were hydrolyzed, extracted and analyzed by MF as described. Daily standard curves were also prepared. Following C administration, 6α,βHC (Fig. 3B) was evident in the first analysis and 6α,βHM, HM and M (Fig. 4B) were present in the second run. Control urine from the same animals

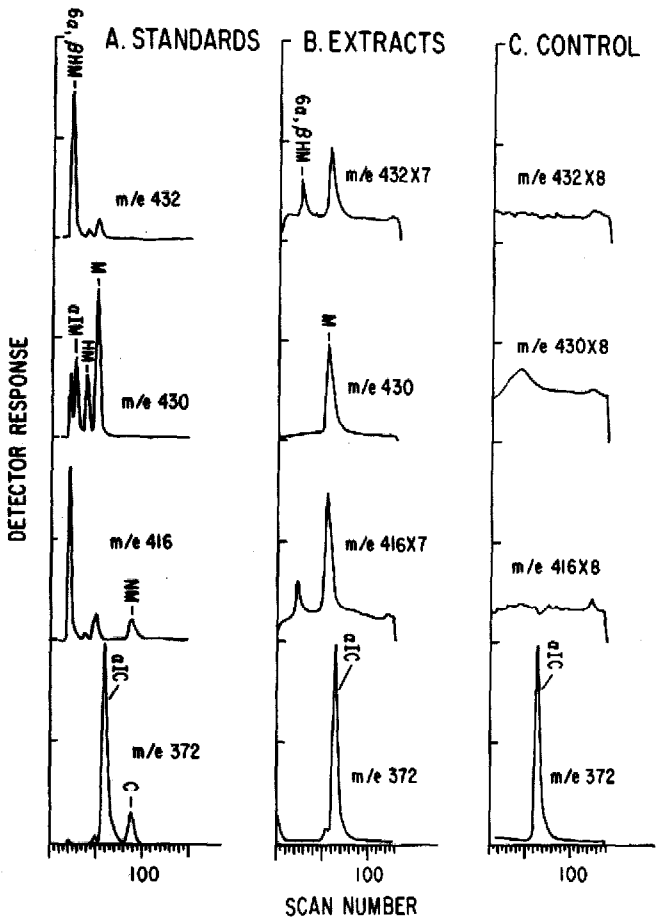

Fig. 5. Mass fragmentograms of extracts (trimethylsilyl derivatives of guinea pig urine. (A) Control urine with added standards; (B) 24-h sample following subcutaneous adminitration of morphine (8 mg/kg); (C) control urine collected prior to drug administration.

was free from interferences at the appropriate retention time (Figs. 3C and 4C). Further characterization of 6α,βHC by GC—MS on 3% Silar-5CP without derivatization revealed that 6αHC and 6βHC were present in nearly equal amounts. Following M administration only 6α,βHM was evident (Fig. 5B). The amount of total metabolite as percent dose for each component was calculated as less than 1%.

The presence of 6α,βHM in the urine of guinea pigs following M administration implies the formation of HM, in agreement with the report by Klutch [1] that HM is a minor metabolite of M. Although HM was not detected in samples of the present study it may be that the 6-keto-reductase activity of these animals is greater than those previously studied resulting in conversion of HM to 6αHM and/or 6βHM. Also, it should be noted that HM was detected in the urine of guinea pigs following C administration. Two sources are possible for production of this metabolite as shown in Fig. 1. C could be converted to HC in an analogous fashion to the transformation of M to HM, followed by O-demethylation of HC to HM. Alternately C could be first O-demethylated to

M followed by conversion of M to HM. The presence of M and 6αHC and/or 6βHC (reduction products of HC) suggests that both pathways are likely to be operative in the formation of HM from C.

The occurrence of these minor metabolites poses interesting pharmacological questions. Although most of these metabolites are equipotent or more potent than the parent drug, their abundance is sufficiently low to obviate significant pharmacological activity. Perhaps the occurrence of these compounds represents a fingerprint of the enzymatic capacity of individual species and perhaps even individual animals. The usefulness of these observations is under investigation.

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