Journal of Chromatography, 230 (1982) 57—67
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1249

DETECTION AND MEASUREMENT OF OPIUM ALKALOIDS AND METABOLITES IN URINE OF OPIUM EATERS BY METHANE CHEMICAL IONIZATION MASS FRAGMENTOGRAPHY

EDWARD J. CONE*, CHARLES W. GORODETZKY, SHU YUAN YEH, WILLIAM D. DARWIN and WILLIAM F. BUCHWALD

National Institute on Drug Abuse, Division of Research, Addiction Research Center, P.O. Box 12390, Lexington, KY 40583 (U.S.A.)

(First received August 11th, 1981; revised manuscript received February 4th, 1982)

SUMMARY

A gas chromatographic—mass spectrometric assay for eight opium alkaloids in human urine following opium ingestion is described. The compounds were extracted from urine with methylene chloride—isopropanol (7:3, v/v) at pH 9.5, evaporated, derivatized with Tri-Sil Z and analyzed by methane chemical ionization mass fragmentography. The method is sensitive to ca. 0.01 μg/ml for morphine and codeine and ca. 0.05 μg/ml for the other compounds. Adsorption problems on the gas chromatography column prevented obtaining reproducible results for the measurement of noscapine. Extraction efficiencies over the pH range of 8—11 for the eight compounds are reported. Retention times of the opium alkaloids were determined using five different liquid phases (3%) on Gas-Chrom Q (100—120 mesh) and two column lengths (36 cm and 183 cm). The 36-cm column packed with OV-210 was selected for use in the assay. Ions were selected for monitoring for each component from their methane chemical ionization spectrum to provide the needed sensitivity and specificity for analysis of a multi-component mixture. The assay was used for the analysis of an "opium eater's" urine. Morphine, codeine, nomorphine, norcodeine and noscapine were detected; however, no evidence was obtained for thebaine, papaverine or oripavine. Unconjugated morphine (0.64 μg/ml) was present at nearly twice the concentration of codeine (0.37 μg/ml) and normorphine and norcodeine were present in equal amounts (ca. 0.15 μg/ml).

INTRODUCTION

Opium, the dried exudate of the poppy plant *Papaver somniferum*, contains some twenty-five or more alkaloids which are responsible for its pharmacological activity. The major alkaloids, morphine (MOR), codeine (COD), thebaine (THE), papaverine (PAP) and noscapine (NOS), account for 0.3—10% of dry weight with the remaining alkaloids occurring in trace amounts [1].

Numerous assays have been developed for measurement of these compounds in opium preparations by gas chromatography (GC) [2—5] and high-performance liquid chromatography [6—11]. Combined gas chromatography—mass spectrometry (GC—MS) has been used for the forensic identification of opium constituents [12]. A single report has appeared on the urinary excretion of MOR and COD following the consumption of opium in which the compounds were separated and measured on thin-layer chromatography (TLC) [13]. However, none of the more sensitive and specific assays for the opium alkaloids have been applied to the study of the urinary excretion profiles of "opium eaters". This report describes the use of methane chemical ionization (CI) mass fragmentography (MF) for the detection and measurement of opium alkaloids and metabolites (See Fig. 1) in human urine following opium ingestion.

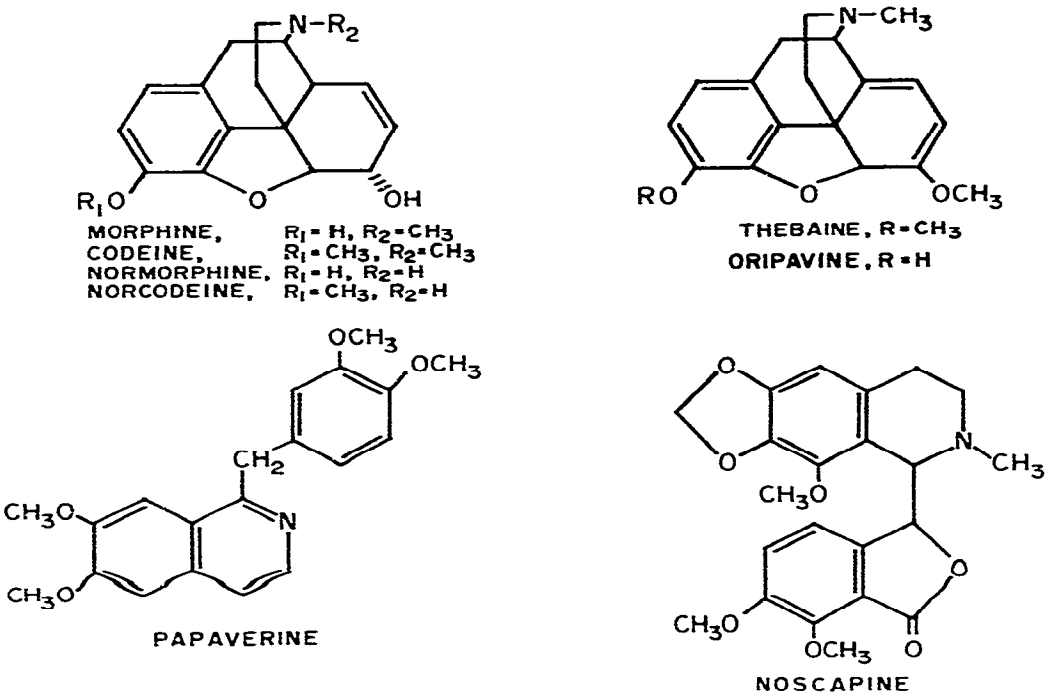

Fig. 1. Structures of opium alkaloids.

EXPERIMENTAL

Drug standards

MOR, COD and NOS were purchased from Mallinckrodt (St. Louis, MO, U.S.A.). Normorphine (NOM) and norcodeine (NOC) were purchased from Merck (Rahway, NJ, U.S.A.). PAP was purchased from Sigma (St. Louis, MO, U.S.A.). THE and α-isocodeine (internal standard, IS) were obtained from the Research Technology Branch, Division of Research, National Institute on Drug Abuse (Rockville, MD, U.S.A.). Oripavine (ORI) was a gift from McFarlan Smith (Edinburgh, Great Britain). All compounds were analyzed by TLC and GC—MS for purity and structural verification.

Instrumentation

Gas chromatography. Analyses were performed on a Varian Model 2700 gas chromatograph equipped with a flame ionization detector. Glass columns (0.36 m or 1.83 m × 2 mm I.D.) were packed with Gas-Chrom Q (100—120 mesh) coated with 3% liquid phase. The injector and detector were maintained at 190°C and 275°C, respectively. Nitrogen was used as the carrier gas at a flow-rate of 20 ml/min. The column temperature was programmed as indicated in Table I. The reported retention times represent the mean of triplicate determinations.

Gas chromatography—mass spectrometry. Mass spectra were obtained with a Finnigan Model 4021 Automated GC/MS/DS system operating in the CI mode. The gas chromatograph consisted of a glass column (0.36 m × 2 mm I.D.) packed with 3% OV-210 on Gas-Chrom Q (100—120 mesh). The temperature of the injector and source were 190°C and 260°C, respectively. The GC oven was programmed as follows: 1-min hold at 170°C; program 170°C to 260°C at 10°C/min; 3-min hold at 260°C. A venting valve was opened for 30 sec following sample injection to allow volatiles and solvent to be diverted from the GC—MS source. Methane was used as carrier and reagent gas at a flow-rate of 20 ml/min. The electron energy was set at 70 eV and the multiplier voltage at 1.4 kV. Total ion scans were collected over the range of 80—600 a.m.u.

Mass fragmentography. Quantitative analyses were performed by MF with the same conditions as those for GC—MS. The ions selected for monitoring and confirmation of each substance were as follows: MOR, *m/e* 340 and 414; COD, *m/e* 282 and 372; NOM, *m/e* 326 and 400; NOC, *m/e* 268 and 358; THE, *m/e* 312 and 340; ORI, *m/e* 370 and 354; PAP, *m/e* 340 and 368; NOS, *m/e* 220 and 195; IS, *m/e* 372. Standard curves were prepared for MOR (0—20 μg/ml), COD (0—10 μg/ml) and the remainder of the compounds (0—2 μg/ml). Linear relationships of peak intensity of drug to IS vs. concentration were obtained for all compounds with the exception of NOS. The response for NOS was variable and appeared to be related to the age of the column.

Extraction procedure

An aliquot (5 ml) of each urine specimen was mixed with IS (30 μg) and the pH was adjusted to 9.5 with 2 *N* sodium hydroxide. Sodium chloride (1.0 g) and 15 ml of methylene chloride—isopropanol (7:3, v/v) solution were added and the contents were shaken for 20 min. Following centrifugation, the aqueous layer was discarded and 13 ml of the organic phase were transferred and evaporated to dryness under nitrogen. Tri-Sil Z (Pierce, Rockford, IL, U.S.A.) (0.1 ml) was added and the tube was sealed and incubated at room temperature for 1 h. From 1—3 μl were removed for analysis by GC—MS.

Extraction efficiency and pH studies

Standard buffered solutions of the opiate alkaloids (10 μg/ml) were prepared in the pH range of 8—11. Aliquots were extracted in triplicate as described in the extraction procedure with the IS added in the evaporation step. Following derivatization, the extracts were analyzed by GC (36 cm × 2 mm column packed with 3% OV-210 on Gas-Chrom Q). Comparison of peak height ratios of extracted standards to those of unextracted standards provided percent recoveries.

TABLE I

INFLUENCE OF LIQUID PHASE AND COLUMN LENGTH ON THE GC RETENTION TIMES OF EIGHT OPIUM ALKALOIDS

The values represent relative retention times and are the mean of triplicate determinations. Values in brackets represent uncorrected retention time in min.

Compound	GC column phases and length*									
	OV-101		OV-17		OV-210		OV-225		Silar-5CP	
	36 cm	183 cm	36 cm	183 cm	36 cm	183 cm	36 cm	183 cm	36 cm	183 cm
MOR-TMS$_2$	1.00 (6.8)	1.00 (8.0)	1.00 (5.3)	1.00 (18.8)	1.00 (2.5)	1.00 (2.8)	1.00 (5.3)	1.00 (16.3)	1.00 (4.4)	1.00 (9.2)
COD-TMS	0.91	0.87	1.01	1.02	1.01	1.01	1.09	1.05	1.20	1.50
NOM-TMS$_2$	1.00	1.00	1.07	1.05	1.16	1.17	1.13	1.08	1.27	1.31
NOC-TMS	0.90	0.90	1.07	1.07	1.18	1.20	1.23	1.15	1.45	1.77
ORI-TMS	0.99	1.05	1.18	1.18	1.14	1.24	1.22	1.25	1.53	1.10
THE	0.99	0.97	1.34	1.30	1.43	1.48	1.54	1.46	1.93	1.19
PAP	1.34	1.66	1.90	2.15	2.76	2.91	2.42	2.68	3.03	—
NOS	1.70	2.89	2.91	—	4.17	6.83	5.47	—	—	—

*Retention times were determined on the 36-cm columns with a temperature program of 170°C to 260°C at 10°C/min and on the 183-cm columns with a temperature program of 220°C to 260°C at 10°C/min.

RESULTS AND DISCUSSION

Extraction of opium alkaloids from urine

A single, one-step extraction procedure was adopted for the recovery of the opium alkaloids from urine. Further sample clean-up by back extraction with dilute mineral acid resulted in significant losses of THE and PAP. With the exception of NOM, all compounds were efficiently extracted with methylene chloride—isopropanol (7:3, v/v) from solution in the pH range of 8.5—10.5 (Fig. 2). At pH 9.5 recoveries ranged from 83% to 91%. Recovery for NOM at pH 9.5 was 39%.

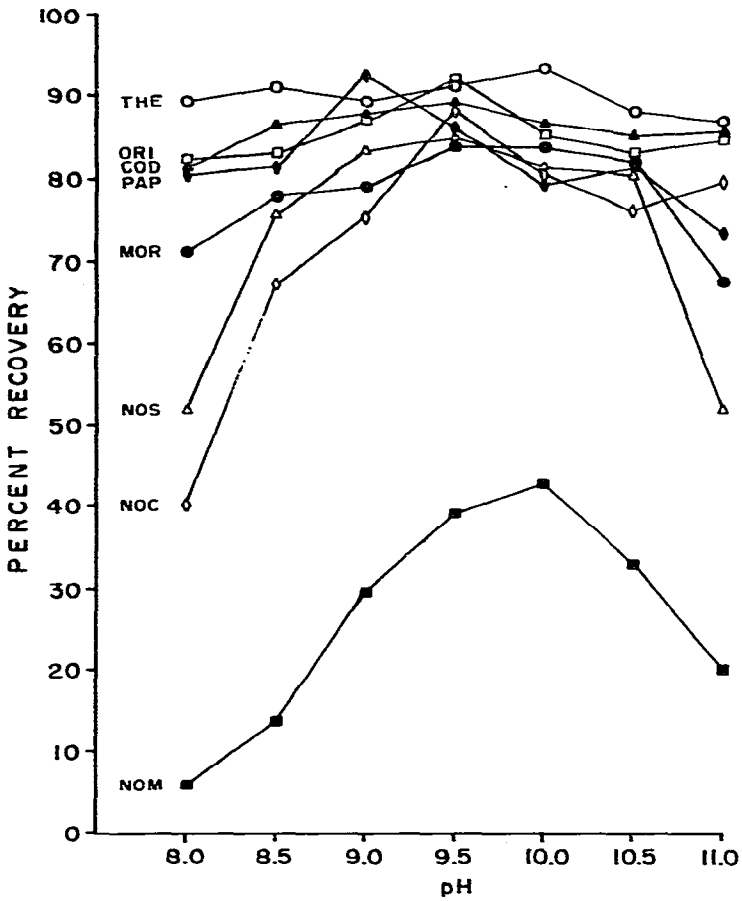

Fig. 2. Efficiencies of methylene chloride—isopropanol (7:3, v/v) extraction of opium alkaloids at pH 8—11.

GC separation of opium alkaloids

Resolution of the opium alkaloids [trimethylsilyl (TMS) derivatives] was attempted by GC with five different liquid phases (3% by weight on Gas-Chrom Q, 100—120 mesh). Two column lengths were employed; a short column (36 cm) and a longer column (183 cm) were packed with each of the liquid phases noted in Table I (listed in order of increasing polarity). The most complete

separation of the components was obtained on Silar-5CP, the most polar liquid phase tested. Unfortunately, NOS did not elute from either the short or long column with this packing, nor did it elute from the long columns packed with OV-17 or OV-225, making these columns unacceptable for further use. Resolution of all components was not complete on any of the other columns examined; however, since complete chromatographic resolution was not required for MF assay, considerations in selection of column length were given to reduction of assay time and background bleed response. The 36-cm column provided the shortest retention times in all cases as well as substantial reductions in bleed rate. Also, resolution on the 36-cm column was almost equal to the 183-cm column, hence the short columns were used in all further assay work.

Selection of the liquid phase was made on the basis of assay time and resolution. The most acceptable phase was OV-210, intermediate in polarity of the phases tested. The GC tracings for the opium components and IS on OV-210 are shown in Fig. 3. The same sample was injected on both columns using the indicated temperature programs. All other instrumental settings were the same. On both columns, MOR and COD were unresolved and eluted as one peak; likewise NOM, NOC and ORI were unresolved and eluted following the MOR/COD peak. IS, THE, PAP and NOS were well resolved. The reduction in retention times and bleed rates as well as an enhanced response for NOS on the short column are apparent.

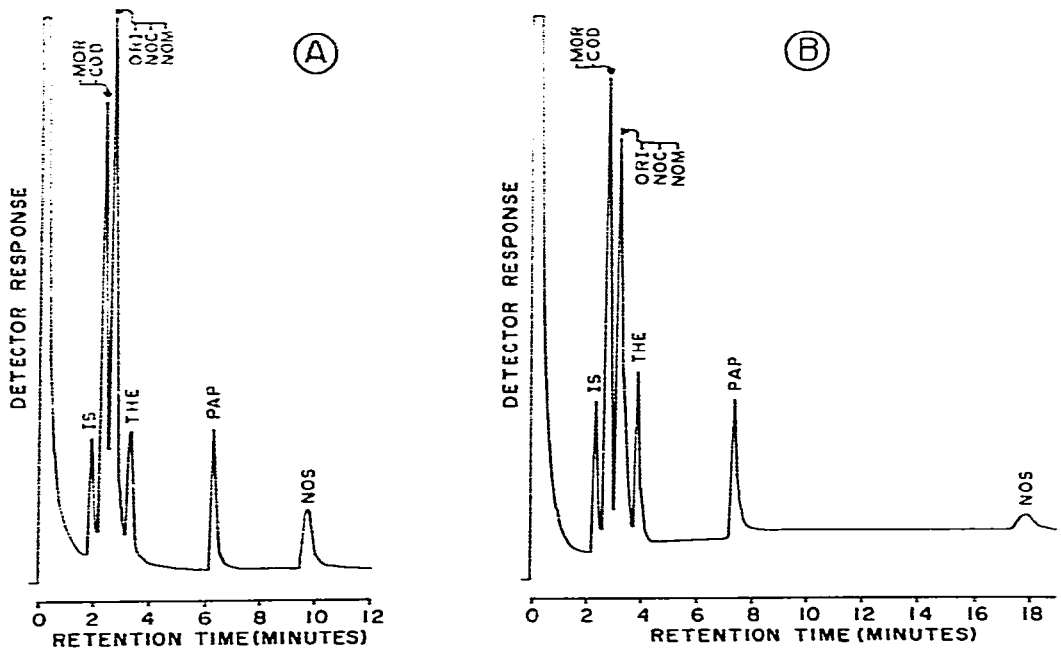

Fig. 3. GC separation of eight opium alkaloids and internal standard on 3% OV-210. (A) 36-cm column with a temperature program of 170°C to 260°C at 10°C/min, (B) 183-cm column with a temperature program of 220°C to 260°C at 10°C/min. All other instrumental settings were the same for both columns.

Methane CI—MS of opium alkaloids

Following derivatization the eight alkaloids and IS were analyzed by GC—MS under CI conditions with methane as carrier and reagent gas. The spectra are summarized in Table II. Six of the nine compounds formed TMS derivatives. All of the components, with the exception of NOS, displayed a strong $(M+1)^+$ ion and $(M+29)^+$ ion. The most abundant ion for NOS was *m/e* 220 indicating loss of the isobenzofuranone ring from the parent compound. Characteristically, the most abundant ions for MOR-TMS_2 were represented by the $(M-15)^+$ ions, whereas those for COD-TMS and NOC-TMS were represented by the $(MH-90)^+$ ions [loss of $HOSi(CH_3)_3$]. The $(M+1)^+$ ion was the most abundant ion in the spectra of ORI-TMS, THE, PAP and IS-TMS.

MF of opium alkaloids

In the development of an assay for opium constituents in urine it was presumed that any of the eight compounds could be present in urine in varying amounts. Since the chromatographic columns tested did not resolve all components, the specificity and sensitivity of the MF assay depended on the ions selected for monitoring. Ideally the selected ions should be present in high relative abundance and be unique for each compound.

The ions selected for monitoring for the opium alkaloids were the two most abundant ions in the spectrum for each component. Monitoring of these ions for THE, PAP, NOS and IS at their respective retention times offered the required sensitivity and specificity since the OV-210 column adequately resolved these from the other compounds of interest. The remaining substances eluted on OV-210 as two clusters of unresolved compounds (Fig. 3), one containing MOR and COD and the other ORI, NOC and NOM. Consequently, it was necessary to assess the potential interference of each member of the cluster of compounds on the remaining members of that cluster. By injecting each individual compound singly followed by MF analysis of all selected ions of interest, it was possible to determine the relative potential interference for each component at its corresponding scan number (SN, analogous to retention time). These data are shown in Table III. For example, interference in the measurement of COD-TMS at SN = 42 using the ion at *m/e* 372 could occur from MOR-TMS_2 at SN = 43. Although the percent relative abundance (%RA) of this interference from MOR-TMS_2 is only 6%, at low concentrations of COD-TMS and high concentrations of MOR-TMS_2 the interference could be very significant since these two compounds are unresolved. On the other hand, contributions to this ion from the remaining compounds at SN = 32, 48 and 52 are not potential interferences since they are well resolved from COD-TMS. Selection of the ion at *m/e* 282 for monitoring of COD-TMS is an even better choice since there is no interference from MOR-TMS_2. The ions of choice for each compound are underlined in Table III. No serious interferences were present for any of the compounds. The secondary ions were used for confirmation.

A typical MF scan of opium standards extracted from normal urine is shown in Fig. 4A. Peak intensity ratios of drug to IS were measured in the range of 0—20 μg/ml for MOR, 0—10 μg/ml for COD and 0—2 μg/ml for the other compounds. Lower limits of sensitivity for MOR and COD were ca. 0.01 μg/ml and

'T/ 3LE II

METHANE CHEMICAL IONIZATION SPECTRA OF EIGHT OPIUM ALKALOIDS AND INTERNAL STANDARD

Compound	Mol. wt.	Methane CI spectra*			
		$(M+29)^+$	$(M+1)^+$	M^+	Prominent fragment ions
MOR-TMS$_2$	429	458 (19)	430 (70)	429 (55)	416 (15), 415 (34), 414 (100), 371 (18), 341 (22), 340 (91)
COD-TMS	371	400 (16)	372 (51)	371 (36)	356 (31), 313 (19), 283 (20), 282 (100)
NOM-TMS$_2$	415	444 (18)	416 (74)	415 (49)	401 (32), 400 (100), 371 (16), 326 (90), 222 (15)
NOC-TMS	357	386 (14)	358 (50)	357 (28)	342 (34), 313 (13), 269 (19), 268 (100), 164 (11)
ORI-TMS	369	398 (17)	370 (100)	369 (41)	355 (24), 354 (82), 353 (11), 108 (24)
THE	311	340 (13)	312 (100)	311 (47)	—
PAP	339	368 (22)	340 (100)	339 (11)	—
NOS	413	442 (1)	414 (6) 413 (0)	221 (14)	220 (100), 195 (13), 137 (8)
IS-TMS	371	400 (17)	372 (100)	371 (39)	357 (11), 356 (43), 282 (34)

*m/e (percent abundance). The ions underlined are those slected for monitoring in the MF assay.

TABLE III

POTENTIAL INTERFERENCES IN THE MASS FRAGMENTOGRAPHIC ASSAY OF UNRESOLVED MIXTURES OF OPIUM ALKALOIDS

Compound (SN)*	Selected ions (RA)**	Percent relative abundance of interfering ion from other compounds (SN)***						
		MOR-TMS$_1$ (43)	COD-TMS (42)	NOM-TMS$_2$ (52)	NOC-TMS (52)	ORI-TMS (48)	THE (66)	IS-TMS (32)
MOR-TMS$_2$	340 (91)	91	0	4	2	1	13	0
(43)	414 (100)	100	0	11	0	0	0	0
COD-TMS	282 (100)	0	100	0	0	0	0	34
(42)	372 (51)	6	51	4	1	9	0	100
NOM-TMS$_2$	326 (90)	0	0	90	0	2	0	0
(52)	400 (100)	2	16	100	0	2	0	17
NOC-TMS	268 (100)	0	0	0	100	0	0	0
(52)	358 (50)	0	2	0	50	0	0	5
ORI-TMS	370 (100)	0	15	0	0	100	0	25
(48)	354 (82)	0	0	4	0	82	0	0
THE	312 (100)	0	0	3	0	2	100	0
(66)	340 (13)	91	0	4	2	1	13	0
IS-TMS	372 (100)	6	51	4	1	9	0	100
(32)	282 (34)	0	100	0	0	0	0	34

*Scan number (corresponding to retention time) at which the compound is monitored.
**Percent relative abundance of ions selected for monitoring. Ions used for quantitation are underlined.
***Scan number at which the interference will occur.

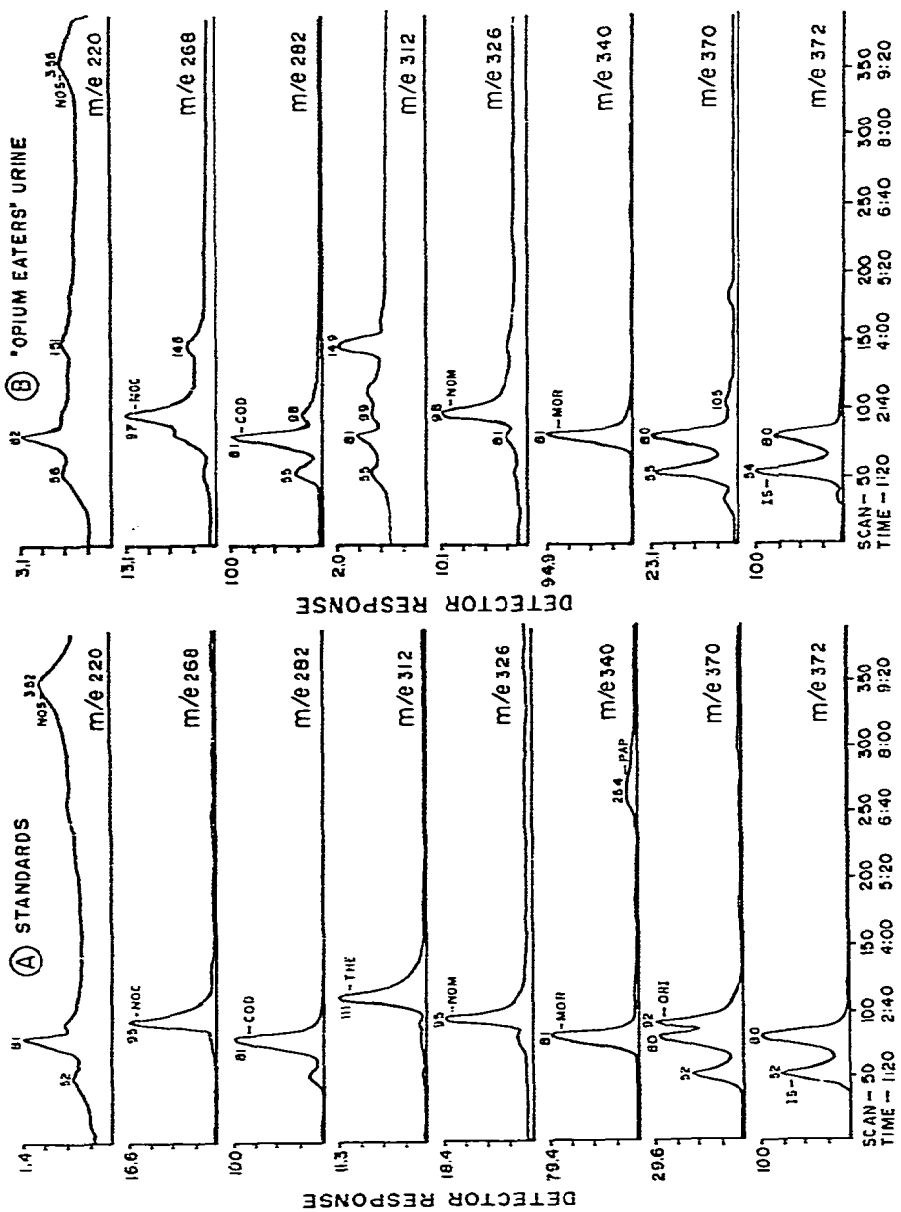

Fig. 4. Selected ion recordings of urinary extracts of standards (A) and "opium eater's" urine (B).

ca. 0.05 μg/ml for the remainder of the compounds. Plots of peak intensity ratios versus concentration were linear with correlation coefficients (r) ⩾ 0.97. An exception to this was NOS which gave variable responses which seemed to be related to the age of the GC column. The strongest responses were obtained with freshly packed columns. After several weeks of column use the response for NOS often became erratic and weaker, an indication of probable adsorption on active sites of the column. Retention times also varied from column to column as indicated by the SN for the IS (see Table III and Fig. 4). This was not a problem, however, since relative retention times were reproducible.

MF analysis of an "opium eater's" urine

Urine was obtained from a male "opium eater" hospitalized for treatment of cancer of the esophagus. The patient was ingesting approximately 1 g per day of a dark resinous material which he identified as dross from the opium pipe or "sukhteh" [14]. The urine samples were extracted as described and analyzed by MF. Fig. 4B shows the response from an unhydrolyzed urine extract. MOR, COD, NOM, NOC and NOS were detected; however, no evidence was obtained for the presence of THE, PAP or ORI. The concentration of MOR (0.64 μg/ml) was approximately twice that of COD (0.37 μg/ml), whereas NOM and NOC were present in equal amounts (ca. 0.15 μg/ml). Treatment of the urine sample with β-glucuronidase enzyme produced a greater than tenfold increase in concentration for the four compounds, a finding consistent with the extensive conjugation normally observed in opiate metabolic studies.

It is anticipated that this method could serve as a useful assay for further metabolic studies on "opium eaters", a large population of drug users which have received very little attention.

REFERENCES

1 A.K. Reynolds and L.O. Randall, Morphine and Allied Drugs, University of Toronto Press, Toronto, 1957, p. 3.
2 G. Fisher and R. Gillard, J. Pharm. Sci., 66 (1977) 421.
3 D. Furmanec, J. Chromatogr., 89 (1974) 76.
4 K. Norayanaswami, H.C. Golani and R.D. Dua, Forensic Sci. Int., 14 (1979) 181.
5 G.R. Nakamura and T.T. Noguchi, J. Forensic Sci. Soc., 14 (1974) 347.
6 C.Y. Wu and J.J. Wittick, Anal. Chem., 49 (1977) 359.
7 C. Olieman, L. Maat, K. Waliszewski and H.C. Beyerman, J. Chromatogr., 133 (1977) 382.
8 H.W. Ziegler, T.H. Beasley, Sr. and D.W. Smith, J. Ass. Offic. Anal. Chem., 58 (1975) 888.
9 J.H. Knox and J. Jurand, J. Chromatogr., 82 (1973) 398.
10 C.-Y. Wu, S. Siggia, T. Robinson and R.D. Waskiewicz, Anal. Chim. Acta, 63 (1973) 393.
11 T.H. Beasley, D.W. Smith, H.W. Ziegler and R.L. Charles, J. Ass. Offic. Anal. Chem., 57 (1974) 85.
12 R.M. Smith, J. Forensic Sci., 18 (1973) 327.
13 L.H. Yong and N.T. Lik, Bull. Narcotics, 29 (1977) 45.
14 T. Hewer, E. Rose, P. Ghadirian, M. Castegnaro, C. Malaveille, H. Bartsch and N. Day, Lancet, ii (1978) 494.