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Forensic Analysis of a Confiscated Illicit Heroin Sample

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Abstract

Different forensic analytical methods involving physical and microscopical examination, color tests, Thin Layer Chromatography (TLC) and Gas Chromatography/ Mass Spectrometry (GC/MS) were applied for the identification and characterization of an illicit heroin sample confiscated in Egypt. These methods confirmed that the heroin sample under investigation is a poorly manufactured sample, prepared by acetylation with acetic anhydride, badly stored, adulterated and it is of the South West Asian type and suggested to be abused by inhalation through smoking.

Keywords: Chromatography-Mass Spectrometry (GC-MS); Confiscated illicit; Heroin; opium alkaloids; TLC

Introduction

Heroin (Diacetylmorphine) is a semi-synthetic analogue of morphine, prepared by acetylating with acetyl chloride or acetic anhydride. It belongs to the internationally controlled narcotic analgesics with morphine, codeine and other synthetic drugs [1,2]. It was some seventy years after the first isolation of morphine from opium before the synthesis of diacetylmorphine was first reported in 1874. Commercial production by the Bayer Company, who named this new drug heroin, began in 1898. By the beginning of the twentieth century, heroin was widely accepted by the medical profession, and was typically used as a substitute for codeine and morphine in tuberculosis and other respiratory diseases. It was also about this time that heroin first appeared in China. A few years after the 1925 International Convention on Narcotics, international controls began to limit the supply of heroin, and the clandestine manufacture of heroin began [1-3]. No one could have suspected this would become one of the most notorious drugs of our time. The global illicit production of opium (from which heroin is processed) becomes increasingly concentrated in Afghanistan which has been producing three-quarters of the world's illicit opium [4].

Analysis of illicit heroin samples in criminal cases to identify their main active principles, diluents, adulterants and impurities that may adversely affect the abuser's heath is important for judicial purposes, identification of the source of a sample, tracing of distribution routes and identifying new production processes and ascertains whether two or more exhibits came from an identical source [3,5]. Different forensic analytical methods are concerned with heroin analysis these methods include: presumptive color tests3, [6-10]; Thin Layer Chromatography [3,7,11-19]. Without question the gas chromatography interfaced Mass Spectrometer (GC/MS) is one of the most useful tools available to the forensic drug chemist. It is able to provide highly specific spectral data on individual compounds in a complex mixture of compounds, without prior separation of these components [3]. Many GC/MS methods were published for illicit heroin preparations analysis [20-27]. In this report, the analyses of heroin, its impurities and adulterants using color and precipitation tests, TLC, GC/MS are described with the aim of providing information about the origin, the trafficking and synthetic routes of illicit heroin samples.

Experimental

Material and Reagent

Drugs

Confiscated heroin sample: It was obtained from seizure number 978 / 1988, Suez- Egypt.

Reference Materials

Opiate alkaloids

- Morphine sulfate ampoules (20 mg/ml) from Misr Co. for Pharmaceutical industry, Egypt.
- Codeine phosphate powder from Supreme organization of Drugs and Medical Requirements, Cairo (Czechoslovakia).
- Papaverine hydrochloride powder from BDH chemical, Ltd. England
- Noscapine: It was isolated from Noscapine Syrup (Agropharm, Buckingham House, church Road, Pann, Bucks. HP10 8LN).
- Heroin (diacetylmorphine) and O_3-Monoacetylmorphine were prepared in our laboratory from morphine.
- O6-monoacetylmorphine hydrochloride: United Nations UNIES Vienna international center. Chief, scientific section, DOA/ UNDDP, Howard Street.
- Acetylcodeine: It was prepared in our laboratory from codeine.

The identity of the prepared compounds was confirmed by comparison of their spectral data (UV, IR and EI-MS) with the previously published ones [2,3,7,10,26,28-30]

Apparatus

- Leitz Wetzlar microscope GMBH fitted with camera Lucida (Germany).
- Precoated silica gel G60F254 (20 x 20 cm x 0.2 mm thick) on aluminum (E-Merck and Machery-Nagel, Germany), Ultra-violet lamp operating at λ 254, 366 nm (Desaga, Germany) for location of TLC spots, Glass jars of different sizes, micro-pipettes for spotting.
- GC/MS was Carried out at National Research Center, Dokki, Cairo, Egypt on GC/MS Finnigan mat SSQ7000 chromatograph with Digital DEC 3000 Work station. Helium was used as carrier gas at a flow rate of 1.6 ml/min and column head pressure was 13 Psi. The gas chromatograph was coupled with mass detector (MS) at 70 eV in EI ionization mode.

Chemicals and Reagents

The following reagents were used during the course of this work:

- Derivatizing reagents: N-methyl-Trimethyl Silyltrifluoro acetamide (MSTFA) reagent for silylation was purchased from Sigma USA.
- All reagents and solvents for TLC separation were of analytical grade and purchased from (Adwik, Egypt), while those for GC/MS analysis were of spectroscopic grades.
- Color reagents: Marquis, Froehde, Meck,s reagent, Chromatographic Spray reagents: Dragendroff,s and acidified Potassium iodoplatinnate were prepared according to United Nations publications [3].

General Procedure

Microscopic Characters, Color, Solubility and Precipitation (Anion) Tests

Sample Preparation

- **Microscopic characters:** About 0.5 g of the heroin sample was dissolved by gentle shaking in 10 ml of distilled water. The obtained solution was centrifuged, the clear supernatant was separated, transferred to a clean test tube and reserved for carrying out the color tests (solution 1), and the residue was subjected to exhaustive washing with water to be suitable for microscopic examination [3]. Several mounts of vegetable debris were prepared in water, chloral hydrate, and phloroglucinol and conc. HCl and examined under the microscope.
- **For color tests:** The previously reserved clear supernatant of the heroin sample was used (solution 1). Other portions of heroin were dissolved in methanol [1mg in 0.5ml] for Oliver test8 and [10 mg in 1ml] for Murexide test31 then centrifuged. With a disposable pipette, the supernatant was drawn into a clean test tube (solution 2&3).
- **For solubility test:** About 100 mg of illicit heroin sample was dissolved in 0.5ml distilled water (to test its solubility in water). The same steps were repeated but water was replaced by ethanol (to test the presence of carbohydrates) [2,7].
- **For precipitation (Anion) test:** About 1g of the illicit heroin sample was dissolved in approximately 5ml distilled water and centrifuged. The supernatant was removed to a clean test tube (solution 4) for precipitation test [2,3,7].

Color and Precipitation (Anion) Tests Procedures

The procedures for presumptive color and precipitation (Anion) tests are cited in (Table 1).

Test Name	Method	Use	Ref
Presumptive color tests:			
Marquis, Froehde, Meck›s and nitric acid test	One drop of heroin solution (solution 1) was placed in each well in a spot tile followed by adding 3 drops of each Marquis, Froehde, Meck›s and nitric acid reagent.	Heroin and opium alkaloid detection	[2,3,6-8]
Oliver test:	A trace of copper sulfate solution (1% in water) was added by means of a glass rod wetted with copper sulfate to solution 2. The solution was stirred, and 1ml of 3% hydrogen peroxide and 1ml of conc. ammonium hydroxide were added and shaken.	Heroin detection	[6,8]
Murexide test	One ml of 10% HCl was mixed with 1.0 ml of solution 3 to which 0.1g. Potassium chlorate was added in white porcelain dish and evaporated to dryness on a water bath. The residue was exposed separately to amm. vapor.	Caffeine detection	[31]
Test for anions			
Silver nitrate test	A portion of solution 4 was added to few drops of silver nitrate 5.0% w/v solution.		[2]
	Chloride precipitate is insoluble in conc. Nitric, soluble in dilute ammonia solution, from which it can be precipitated by addition of nitric acid.	Chloride detection	
	Tartarate precipitate is soluble in nitric acid.	Tartarate detection	
	Citrate precipitate is soluble in nitric acid.	Citrate detection	
Acetic anhydride test	A small amount of illicit heroin sample was heated with 0.5 ml acetic anhydride at 80 °C for 10 minutes.	Citrate detection	
Barium chloride test	Another portion of solution 4 was added to few drops of barium chloride 10% w/v solution. Sulfate precipitate is insoluble in HCl.	Sulfate anion detection	[2,3]

Table 1a: Procedures for presumptive color and precipitation tests of the heroin sample.

Thin Layer Chromatography (TLC)

Developing solvents

System A: Chloroform-n-hexane-triethylamine (9:9:4 v/v)16

System B: Chloroform-Methanol (9:1 v/v)7

Sample and standard solutions preparation

Five mgs heroin sample were dissolved in 1 ml methanol and centrifuged. The supernatant was separated into a clean vial, from which 3µl were spotted to the TLC plate. Morphine sulfate, codeine phosphate, papaverine hydrochloride, O6-mono-acetylmorphine hydrochloride, acetyl codeine, heroin and caffeine were made at conc. 5 mg / ml methanol. Noscapine was made at the same concentration but in chloroform [3].

TLC separation

The methanolic solution of heroin sample and standard solutions were examined by TLC on pre-coated silica gel plates using solvent systems A and B. The developed plates were air dried and visualized under UV at 254 nm followed by spraying separately with Dragendroff,s and acidified potassium iodoplatinnate reagents.

Gas Chromatography Interfaced Mass Spectrometer (GC/MS)

Sample Preparation

Illicit heroin sample was subjected to several sample preparations either as total or neutral fraction for full sample characterization

A) Total illicit heroin sample

1) Direct analysis: Five mgs of illicit heroin sample were dissolved in 1 ml methanol- chloroform (4:1 v/v), sonicated for 10 min. and 2 µl of this solution were injected into HP-5 column.

2) Analysis after derivatization: Five mgs of illicit heroin sample were subjected to silylation with 150 µl of N-methyl N-Tri Methyl Silyl Tri Fluro-Acetamide (MSTFA) in 1.2 ml chloroform- pyridine (5:1 v/v) for 10 minutes at 70 ˚C. After 1 hour at room temperature, 2µl of this mixture were injected into HP-5 column and 1µl into DB-1 column.

B) Neutral fraction

For the determination of the neutral components of the illicit heroin sample, 30 mgs of illicit heroin sample were dissolved in

10 ml of 0.5 N sulfuric acid, extracted by shaking with ether (2x, 5 ml each), and centrifuged. The ethereal layer was dried over anhydrous sodium sulfate, evaporated to dryness using a rotary evaporator, the residue was dissolved in 75 µl of chloroform and 2µl were injected directly into HP-5 column [7,27,32]. Silylation of the neutral fraction is not recommended as reported by [32,27]. The identification of the components was achieved by comparing the fragmentation pattern of the resulting mass spectra with those recorded in mass library spectral database and published data.

Operating conditions for GC [2,3,7,33](Table 1b)

Column (HP-5)	Fused silica capillary column (5% phenyl) Methylsiloxane (HP-5) (0.25) I.D, 30 m. length
Volume of injected sample	2 µl
Injector temperature	250 ˚C
Column temperature	Start at 150 ˚C, Increase at 6 ˚C/ minute to 280 ˚C, isothermal for 10 minute, Increase at 9 ˚C/ minute to 300 ˚C, isothermal for 15 minute, end of program
Column (DB-1)	Fused silica capillary column Dimethyl-polysiloxane (DB-1) (0.32) I.D, 30 m. length
Volume of injected sample	1 µl
Injector temperature	250 ˚C
Column temperature	Starts at 150 ˚C, Increases by 9 ˚C/ minute to 300 ˚C, isothermal for 10 minute, end of program

Results and discussion

Results

Physical Characters

The physical characters of illicit heroin sample are summarized in (Table 2).

Seizure shape	Cylindrical pieces with rounded ends, wrapped externally with green adhesive tape and internally with another wrapping of yellowish-white to light brown paper from which the sample was obtained (chunks) measuring 0.5-1.5 cm D. Figure (1 A&B)
Sample shape	Small granular pieces (chunks) of about 0.5 to 1.5 cm in diameter (Figure 1 C)
Sample weight	50 g
Sample color	Dark brown to nearly black.
Sample touch	Rough
Sample odor	Strong vinegar- like odor.
5-Solubility	Soluble in water producing brownish turbid solution containing vegetable debris.

Table 2: Physical characters of illicit heroin sample.

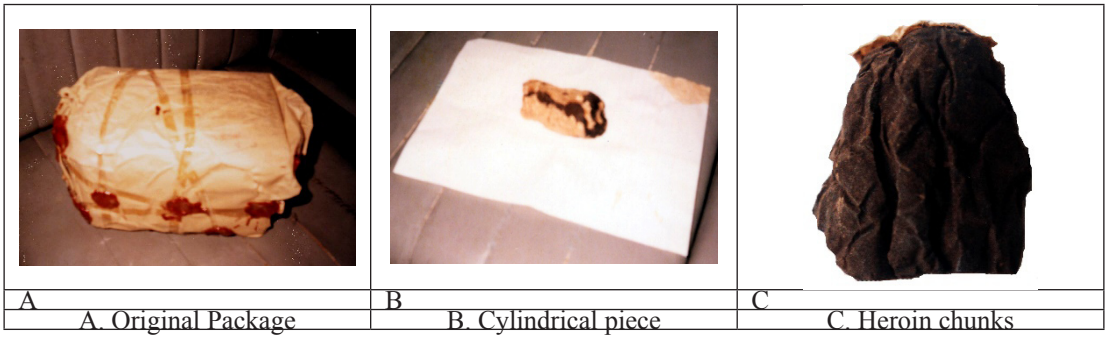


Figure 1: Confiscated heroin (seizure number 978/1988, Suez-Egypt).

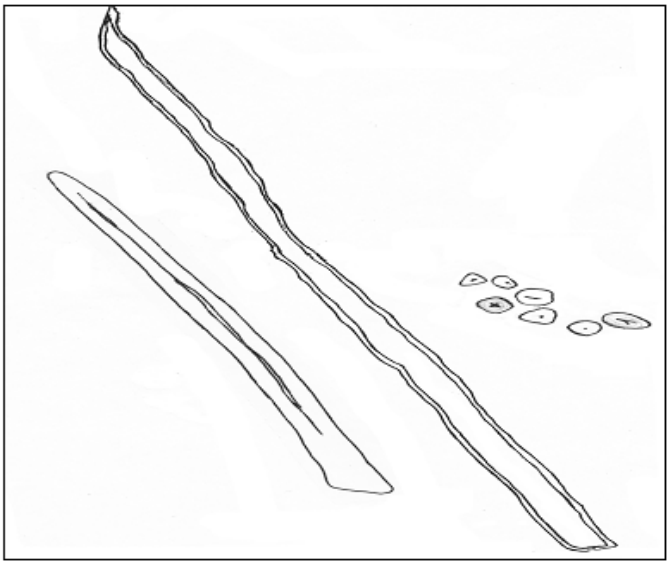
Microscopic Examination

The following fragments (Figure 2) were detected

1-Abundant starch granules: simple, small rounded granules, with centric split or cleft hilum and faint concentric striations which are more visible in larger granules and measuring 11-12-15 µ. (D.).

2-Long fibers: with thin non-lignified wall, wide lumen and acute apex measuring 225-456-825 µ. (L.) and 15-24-30 µ. (W.)

3-Short fibers: with thick non-lignified wall, narrow lumen and bluntly pointed apex measuring 122-211-237 µ. (L.) and 17-20-23 µ. (W.).



A-Starch granules (X= 284), B- Long fiber with wide lumen (X= 169), C- Short fiber with narrow lumen (X= 284).

Figure 2: Vegetable debris of the heroin sample.

Color, Solubility and Precipitation Tests

The results are shown in (Table 3)

Test Name	Result	Indication
Presumptive color tests		
Marquis	A violet / reddish purple color	Presence of morphine, codeine or heroin.
Froehde	A purple / green color	Presence of morphine, codeine or heroin.
Meck	A blue / green color	Presence of morphine, codeine or heroin.
Nitric acid	A red-orange color was obtained at first which was gradually changed to a bright green color	Presence of heroin.
Oliver	A persistent pink to red color	Presence of heroin
Murexide	Purple color after exposure to ammonia vapor.	Presence of caffeine or other xanthine alkaloids
Solubility tests		
Solubility in water	Soluble in water leaving vegetable debris	Presence of heroin salt
Solubility in ethanol	Soluble in ethanol, leaving vegetable residue	Presence of starch
Precipitation (Anion) test		
Silver nitrate	No white precipitate	Absence of chloride ion.
Barium chloride	White precipitate	Presence of sulfate ion.

Table 3: Results of the presumptive color and precipitation tests for the heroin sample.

TLC

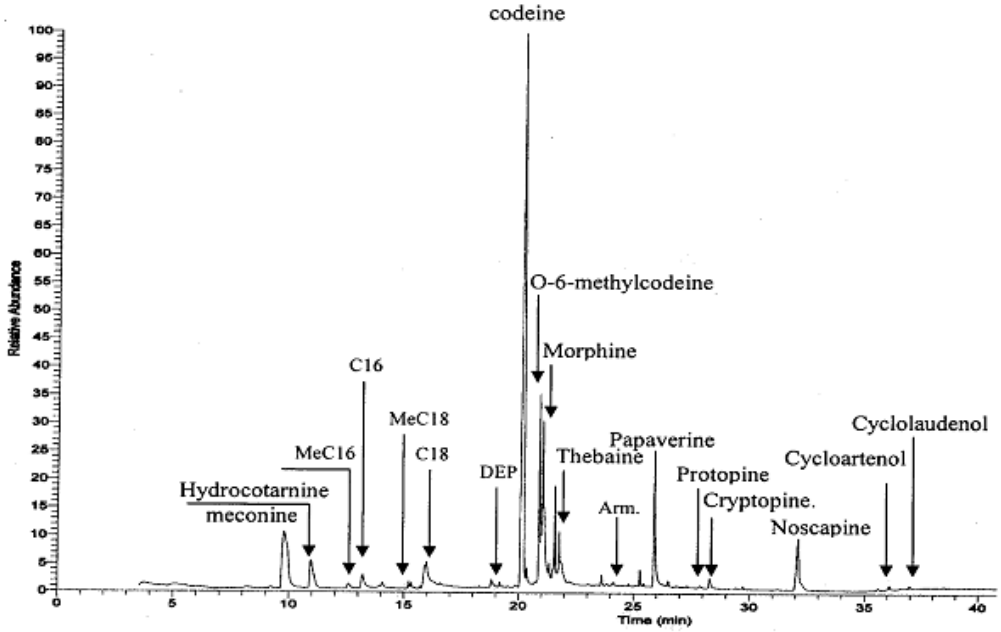
The results are shown in (Table 4)

Reference	R_f x100		Heroin sample
	System A	System B	
Morphine	**16.9**	**14.5**	**(+)**
Codeine	**36.9**	**28.9**	**(+)**
Caffeine	**40.9**	**72.6**	**(+)**
O^6- monoacetylmorphine	**66.15**	**27.6**	**(+)**
Papaverine	**68.7**	**78.9**	**(+)**
Heroin	**69.2**	**63.16**	**(+)**
Acetylcodeine	**75.8**	**55.3**	**(+)**
Noscapine	**91.0**	**89.5**	**(+)**

Table 4: Results of TLC investigation of the heroin sample.

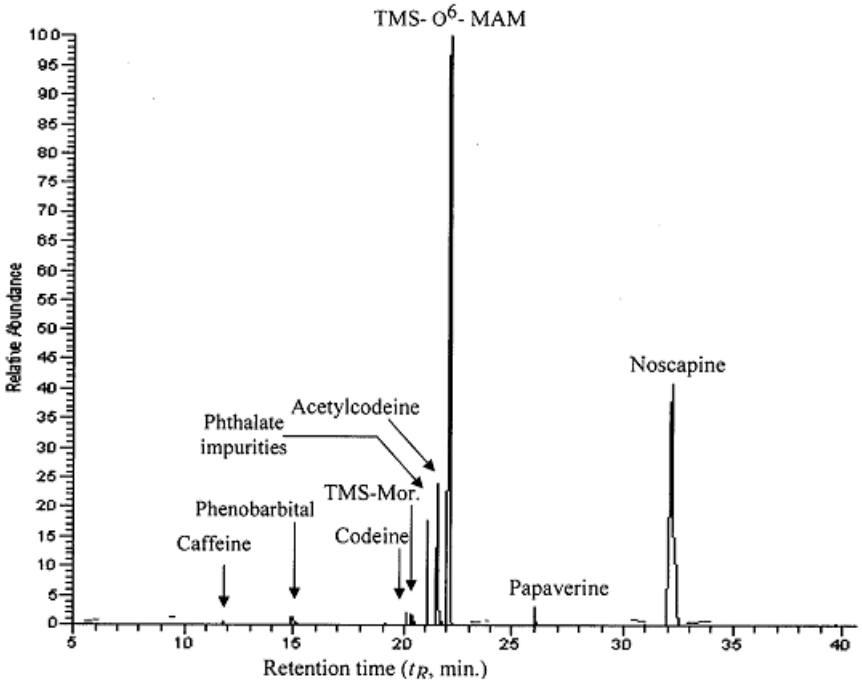
GC/MS

The GC chromatograms are shown in (Figures 3-6), while the results of GC/MS are cited in (Table 5,6) and illustrated in (Figures 7). The identified components of the heroin sample are listed in (Table 7) and shown in (Figures 8).



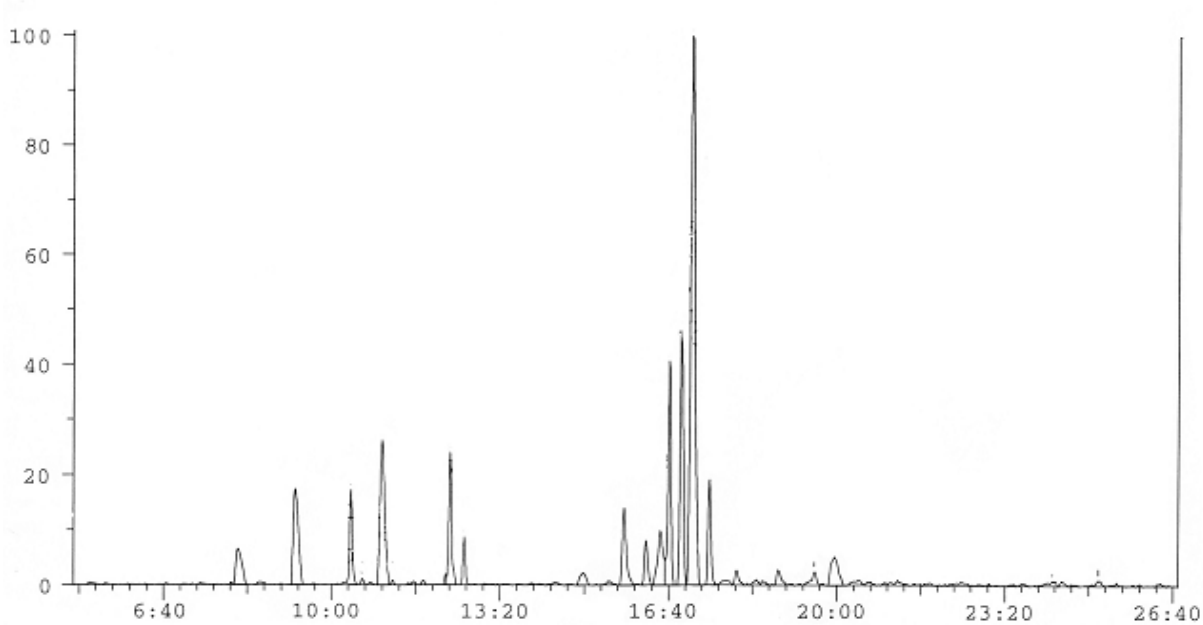
MePh: Mephobarbital; MeOL: Methyl oleate (9-Octadecenoic acid methyl ester); MeSt: Methyl stearate (Octadecanoic acid methyl ester); DEP: 3,6-dimethoxy-4,5 epoxy-phenanthrene; Acl&L.: May be a mixture of N-Acetylnorlaudanosine and laudanosine.

Figure 3: GC of the non silylated heroin sample on (HP-5) column.



TMS-mor: Trimethylsilylmorphine; TMS-O6- MAM: Trimethylsilyl- O6 monoacetyl-morphine.

Figure 4: GC of the silylated heroin sample (MSTAF) on (HP-5) column.



Me C16: Methyl palmitate (hexadecanoic acid methyl ester); MeOl: Methyl oleate (9-Octadecenoic acid methyl ester); MeSt: Methyl stearate (Octadecanoic acid methyl ester); DEP: 3,6 Dimethoxy- 4,5 epoxy-phenanthrene; TMS-Mor.: Timethylsilyl-morphine; TMS- O6-MAM: Timethylsilyl -O6 monoacetyl-morphine; AN: N-Acetylanhydronornarceine

Figure 5: GC of the silylated heroin sample (MSTAF) on (DB-1) column.

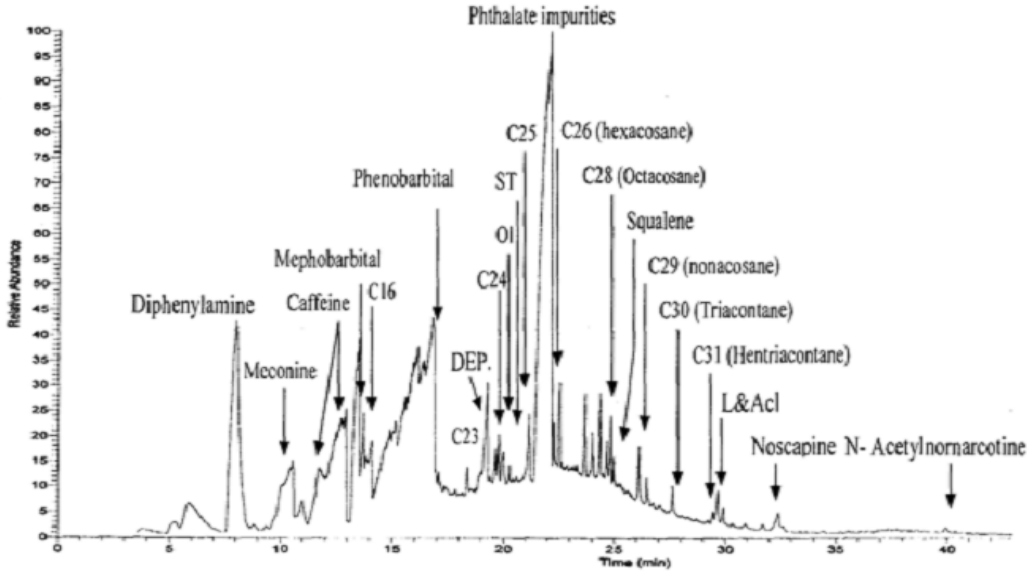
Component	t_R, min.			Relative %			$[M]^+$	Base Peak	Fragment ions m/z (relative intensity)	Reference
	Before HP-5	After		Before HP-5	After					
		HP-5	DB-1		HP-5	DB-1				
Nicotinamide	4.75	–	–	1.39	–	–	122	122	78 (63), 106 (57), 51 (10), 123 (7)	34
Isomeconine	–	–	8.08	–	–	2.83	194	194	165 (87), 147 (56), 50 (36), 63 (33), 176 (29), 77 (27)	
Meconine	9.82	–	–	8.42	–	–	194 (97)	165	147 (77), 176 (47), 118 (30), 119 (30), 77(27)	32
Hydrocotamine	10.96	–	–	1.94	–	–	221 (6)	220	178 (74), 205 (21), 163 (20), 177 (19)	35,36
Caffeine	11.43	11.8	9.15	7.13	0.17	6.34	194	194	109 (46), 55 (25), 67 (20), 82 (17), 193 (14), 165 (7), 137 (6)	34
Methyl palmitate	–	–	10.2	–	–	3.44	270 (17)	74	87 (70), 143 (21), 55 (20), 227 (15)	37
Methyl phenobarbital (Mephobarbital)	12.67	–	–	1.03	–	–	246 (10)	218	117 (33),146 (20), 118 (16), 114 (15)	34
Phenobarbital	14.03	14.9	10.6	5.02	0.77	8.85	232 (20)	204	117 (27),161 (16), 146 (12), 205 (11), 77 (10)	34
Methyl oleate	15.33	–	12.2	0.08	–	4.9	296 (10)	55	83 (39), 69 (36), 74 (34), 264 (32), 96 (24), 222 (13), 110 (11)	34,36
Methyl stearate	15.73	–	12.4	0.09	–	1.55	298 (21)	74	87 (70), 55 (25), 143 (26), 75 (24), 255 (16), 199 (11)	34,37
DEP	18.82	–	14.6	0.69	–	0.98	252 (85)	237	194 (21), 138 (15)	38
Codeine	20.10	20.1	15.5	6.41	0.37	4.03	299	299	162 (36), 229 (22), 300 (21), 124 (19), 282 (8), 242 (10)	39
TMS- Morphine	–	20.3	16.1	–	0.36	1.96	429 (52)	73	236 (28), 146 (18), 196 (17), 414(16)	2
Morphine	21.09	–	–	12.79	–	–	285	285	162 (35), 215 (23), 286 (18), 124 (16), 268 (15), 228 (7)	34,40
Acetylcodeine	21.69	21.6	16.6	7.72	5.38	10.6	341	341	282 (55), 229 (33), 204 (22), 124 (15), 59 (15) 162 (14), 115 (12)	2
O^6-MAM	22.06	–	17.1	27.20	–	30.7	327	327	268 (74), 215 (27), 146 (20), 328 (18), 162 (15), 204 (14), 124 (13), 81 (12)	26
TMS- O^6MAM	–	22.1	17.3	–	41.4	4.0	399	399	73 (72), 340 (61), 287 (40), 204 (33), 324 (28)	2
Heroin	23.22	–	18.0	0.54	–	0.72	369 (56)	327	268 (52), 310 (45), 204 (36), 215 (32), 328 (20), 81 (19), 162 (18)	
Papaverine	25.91	26.0	19.6	2.88	0.99	2.8	338	338	324 (97), 339 (90), 308 (20), 325 (15), 154 (14)	
Cryptopine	28.33	–	–	0.07	–	–	369 (4)	148	179 (20), 149 (10), 190 (7), 91 (6)	41

Table 5: Results of GC-MS of identified components of the heroin sample before and after silylation (MSTAF) using HP-5 and DB-1 columns.

Component	t_R, min			Relative %			$[M]^+$	Base Peak	Fragment ions m/z (relative intensity)	Reference
	Before HP-5	After		Before HP-5	After					
		HP-5	DB-1		HP-5	DB-1				
Mixture of Laudanosine & N-Acetylnorlaudanosine	29.66	–	–	0.06	–	–				
Laudanosine							357 (3)	192		42
N-Acetylnorlaudanosine							385	234	235 (12), 151 (10), 107 (6)	32
Noscapine	32.28	32.2	25.3	13.7	34.7	Trace	413	220	221 (14), 205 (11)	2
May be: N-Acetylanhydronornarceine	–	–	25.5	–	–	Trace	455	193		32

Methyl palmitate: (hexadecanoic acid methyl ester); Methyl oleate: (9-Octadecenoic acid methyl ester); Methyl stearate (Octadecanoic acid methyl ester); DEP: 3,6 Dimethoxy- 4,5 epoxyphenanthrene; TMS- Morphine: trimethylsilyl morphine; O^6-MAM: O^6 monoacetylmorphine; TMS- O^6 MAM: Trimethylsilyl-O^6 monoacetylmorphine

Table (5): Cont.



C16: Palmitic acid (Hexadecanoic acid); C23: n- Tricosane; DEP: 3,6 Dimethoxy- 4,5 epoxyphenan-threne; C24: n-Tetracosane; Ol: Oleoamide (9-Octadecenamide); ST: Stearamide (Octadecanamide); C25: Pentacosane; L&Acl: mixture of N-Acetylnorlaudanosine and laudanosine

Figure 6: GC of non silylated neutral fraction of the heroin sample using HP-5 column.

Component	t_R, min.	Relative %	$[M]^+$	Base Peak	Fragment ions m/z (relative intensity)	Reference
Diphenylamine	8.02	11.1	169	169	168 (60), 167 (34), 83 (23), 170 (15), 51 (10), 77 (10)	34
Meconine	10.5	4.24	194 (97)	165	147 (77), 176 (47), 118 (30), 119 (30), 77(27)	32
Caffeine	12.9	9.84	194	194	109 (46), 55 (25), 67 (20), 82 (17), 193 (14), 165 (7), 137 (6)	34
Methl phenobarbital (Mephobarbital)	13.7	2.81	246 (10)	218	117 (33),146 (20), 118 (16), 114 (15)	
Palmitic acid (Hexadecanoic acid)	14.1	1.21	256 (55)	73	60 (82), 57 (76), 55 (60), 129 (55), 72 (46), 69 (36), 85 (34), 83 (32), 213 (31), 97 (30), 157 (25)	34, 36
Phenobarbital	16.8	12.2	232 (20)	204	117 (27),161 (16), 146 (12), 205 (11), 77 (10)	34
n-Tricosane	18.4	0.42	324	57	71 (77), 85 (56), 99 (21), 113 (15), 127 (10),141 (7)	
DEP	19.3	2.68	252 (85)	237	194 (21), 138 (15)	38
n- Tetracosane	19.8	0.59	338 (6)	57	71 (77), 85 (56), 99 (21), 113 (15), 127 (11), 141 (7), 155 (5)	34
Oleoamide (9-Octadecenamide)	19.9	0.61	281 (6)	59	72 (67), 126 (16), 83 (15), 97 (12), 112 (11)	36
Stearamide (Octadecanamide)	20.3	0.19	283 (5)	59	72 (44), 58 (15), 128 (12), 58 (10), 240 (7), 86 (6)	34
Pentacosane	21.2	1.23	352 (7)	57	71 (76), 85 (59), 99 (21), 113 (16), 127 (10), 141 (7)	
Hexacosane	22.5	0.07	366 (7)	57	71 (76), 85 (60), 99 (24), 113 (16), 239 (11)	34
Octacosane	24.8	0.7	394 (5)	57	71 (78), 85 (60), 99 (25), 113 (16), 127 (11), 141 (7)	
Squalene	25.0	0.22	410 (1)	69	81 (55), 149 (41), 137 (16), 121 (15)	
Nonacosane	26.1	0.74	408 (6)	57	71 (80), 85 (62), 99 (25), 113 (17), 338 (15)	
Triacontane	27.6	0.4	422 (2)	57	71 (80), 85 (61), 99 (25), 113 (16), 127 (12), 141 (10)	
Hentriacontane	29.5	0.18	436	57	71 (76), 85 (60), 99 (25), 113 (17), 127 (12), 141 (10)	
mixture of : laudanosine and N-Acetylnorlaudanosine	29.9	0.28				
Laudanosine			357 (3)	192		42
N-Acetylnorlauanosine			385	234	235 (12), 151 (10), 107 (6)	32
Noscapine	32.4	0.51	413	220	221 (14), 205 (11)	2
N-Acetylnonarcotine	39.9	0.17	411	248	206 (69), 191 (15), 207 (10), 133 (6)	32,36

DEP: 3,6 Dimethoxy- 4,5 epoxyphenanthrene; (–) Not detected

Table 6: Results of GC-MS of identified components of non silylated neutral fraction of the heroin sample using HP-5 column.

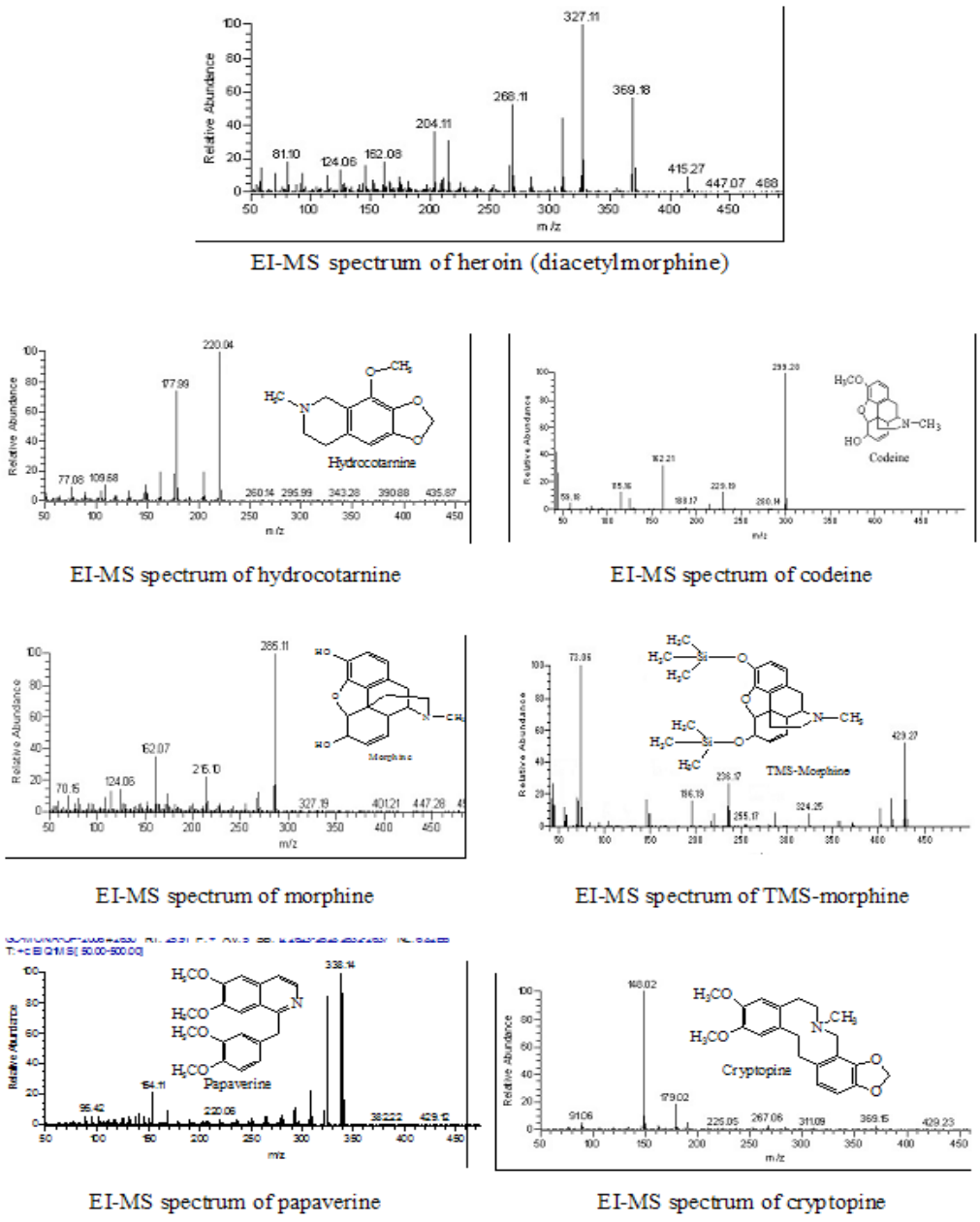
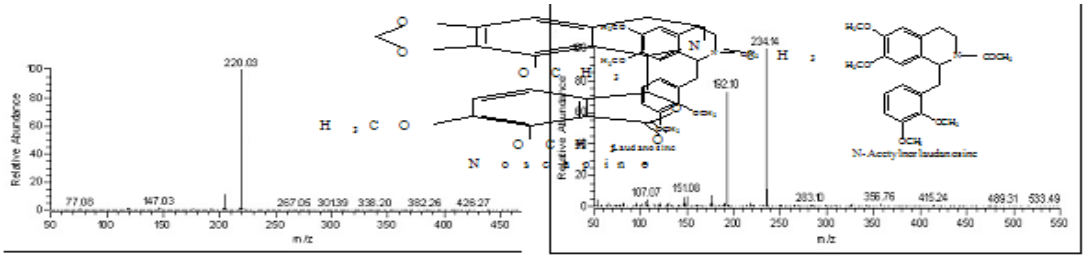


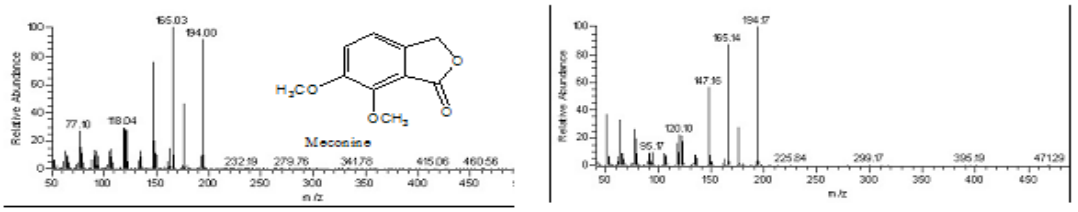
Figure (7): EI-MS spectrum of the detected components in heroin samples

Figure 7: EI-MS spectrum of the detected components in heroin samples.



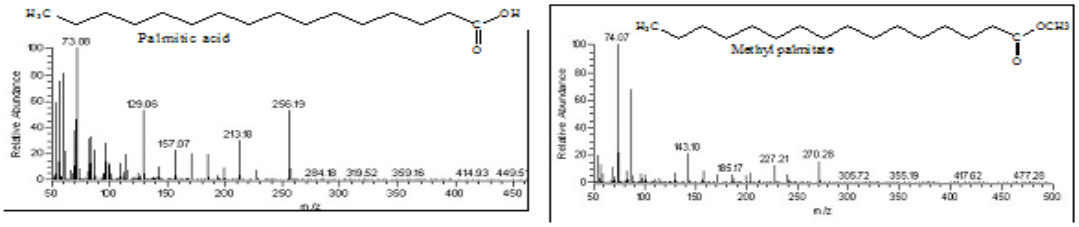
EI-MS spectrum of Noscapine

EI-MS spectrum of mixture of Laudanosine and N-Acetylnorlaudanosine



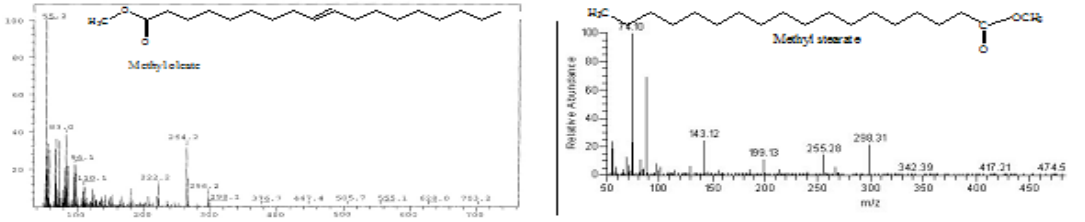
EI-MS spectrum of Meconine

EI-MS spectrum of iso-meconine



EI-MS spectrum of Palmitic acid (hexadecanoic acid)

EI-MS spectrum of methyl palmitate (hexadecanoic acid methyl ester)



EI-MS spectra of methyl oleate (9-octadecenoic acid methyl ester)

EI-MS spectrum of methyl stearate (Octadecanoic acid methyl ester)

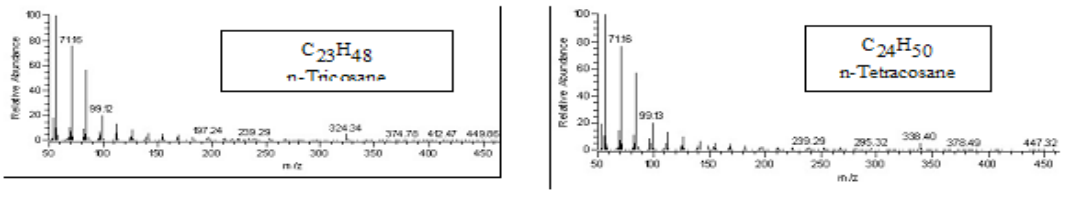
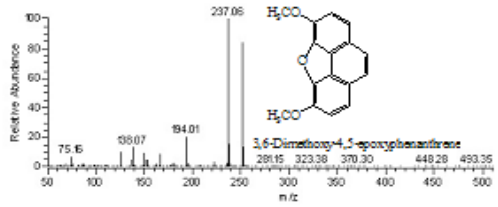
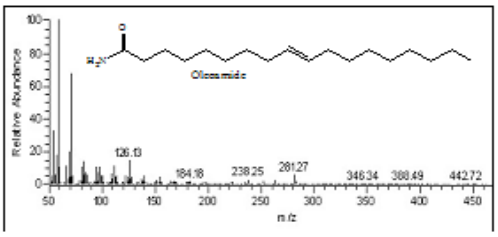


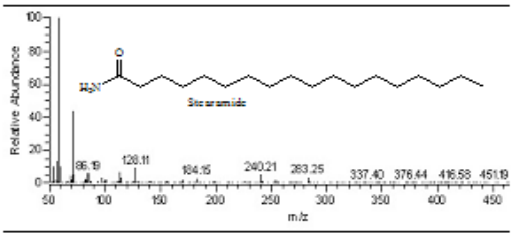
Figure (7): Cont.



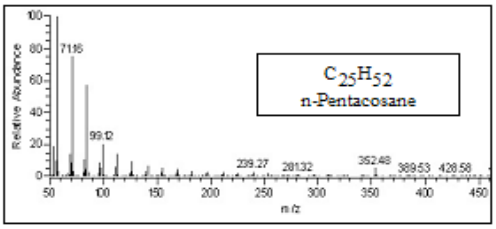
EI-MS spectrum of 3,6 Dimethoxy- 4,5 epoxy-phenanthrene



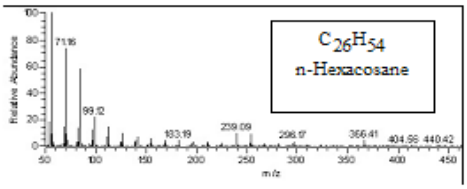
Mass spectra of Oleoamide (9-Octadecenamide)



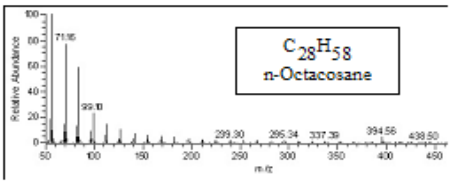
Mass spectra of Stearamide (Octadecanamide)



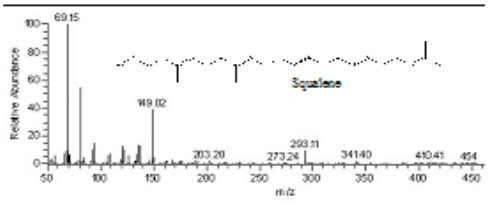
Mass spectra of n-Pentacosane



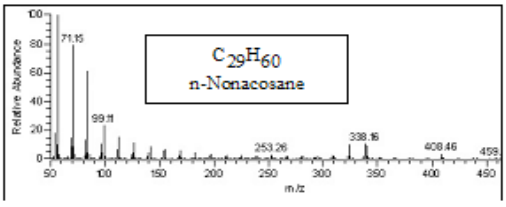
Mass spectra of n-Hexacosane



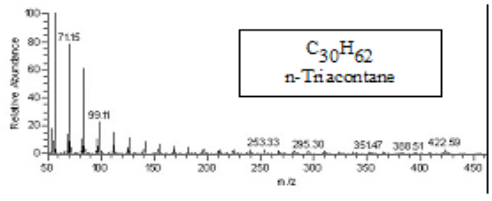
Mass spectra of n-Octacosane



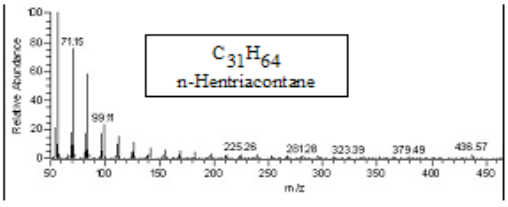
Mass spectra of squalene



Mass spectra of Nonacosane

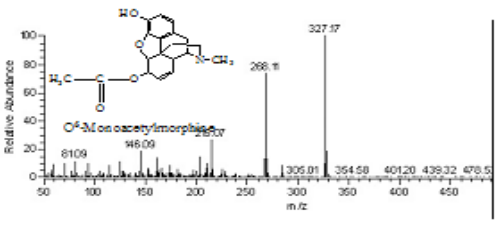


Mass spectra of Triacontane

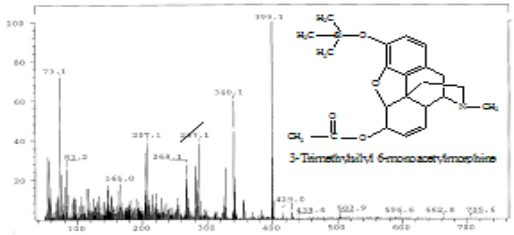


Mass spectra of n-Hentriacontane

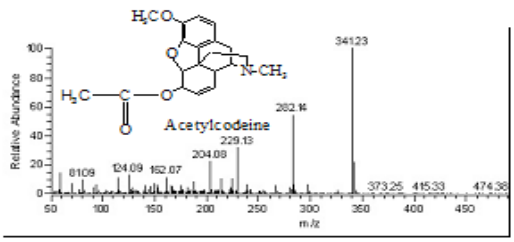
Figure (7): Cont.



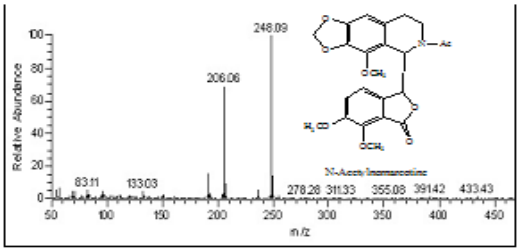
EI-MS spectrum of O^6-monoacetyl-morphine (O6-MAM)



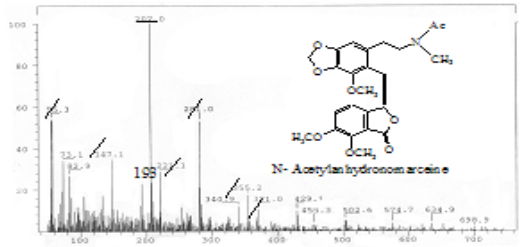
EI-MS spectrum of 3-Timethylsilyl-O^6 monoacetylmorphine (TMS-O^6-MAM).



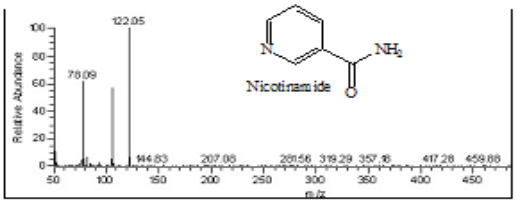
EI-MS spectrum of Acetylcodeine



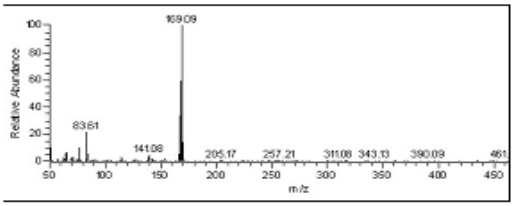
EI-MS spectrum of N- Acetylnornarcotine



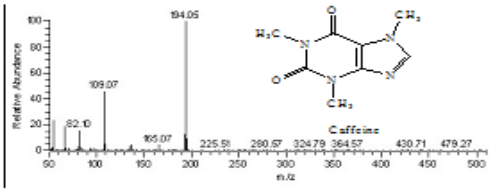
EI-MS spectrum of N-Acetylanhydronornarceine



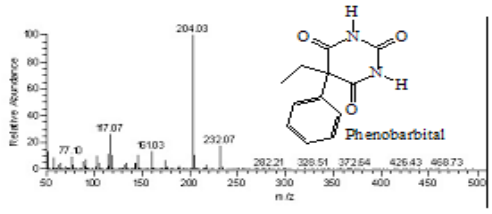
Mass spectra of Nicotinamide



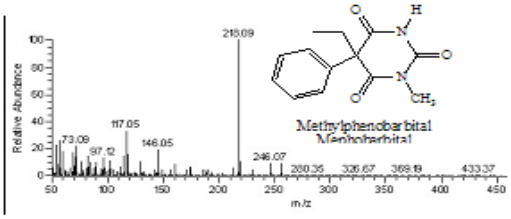
Mass spectra of Diphenylamine.



Mass spectra of Caffeine



Mass spectra of Phenobarbital



Mass spectra of Mephobarbital

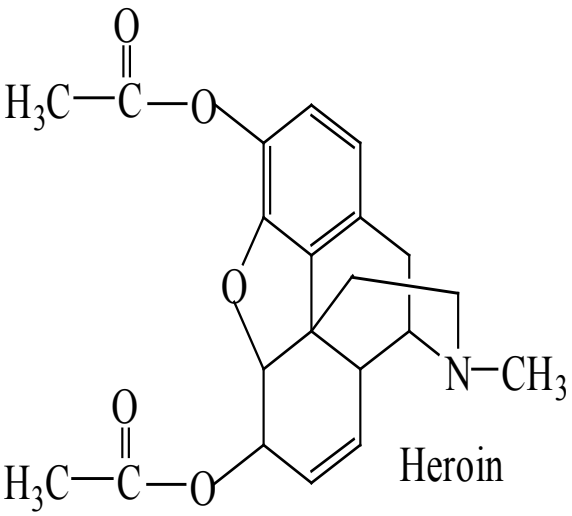
Figure 7: Cont.

Component	Molecular formula	Molecular weight	Mean relative %	Notes
Heroin	$C_{21}H_{23}NO_5$	369	0.6	
Natural impurities from opium:				
Hydrocotarnine	$C_{12}H_{15}NO_3$	221	1.94	
Codeine	$C_{18}H_{21}NO_3$	299	3.6	
Morphine	$C_{17}H_{19}NO_3$	285	5	
Papaverine	$C_{20}H_{21}NO_4$	339	2.2	
Cryptopine	$C_{21}H_{23}NO_5$	369	0.07	
Noscapine	$C_{22}H_{23}NO_7$	413	13.7	
laudanosine	$C_{21}H_{27}NO_4$	357	Trace	
Meconine	$C_{10}H_{10}O_4$	194	5.7	
Methyl palmitate (Hexadecanoic acid methyl ester)	$C_{17}H_{34}O_2$	270	3.4	
Palmitic acid (Hexadecanoic acid)	$C_{16}H_{32}O_2$	256	Trace	
Methyl oleate (9-Octadecenoic acid methyl ester)	$C_{19}H_{36}O_2$	296	2.5	
Methyl stearate (Octadecanoic acid methyl ester)	$C_{19}H_{38}O_2$	298	0.8	
n-Tricosane	$C_{23}H_{48}$	324	Trace	
3,6 Dimethoxy- 4,5 epoxy-phenanthrene	$C_{16}H_{12}O_3$	252	0.8	Thebaine + Ac_2O Decomposition product
n- Tetracosane	$C_{24}H_{50}$	338	Trace	
Oleoamide (9-Octadecenamide)	$C_{18}H_{35}NO$	281	Trace	
Stearamide (Octadecanamide)	$C_{18}H_{37}NO$	283	Trace	
Pentacosane	$C_{22}H_{52}$	352	Trace	
Hexacosane	$C_{26}H_{54}$	366	Trace	
Octacosane	$C_{28}H_{58}$	394	Trace	
Squalene	$C_{30}H_{50}$	410	Trace	
Nonacosane	$C2_9H_{60}$	408	Trace	
Triacontane	$C_{30}H_{62}$	422	Trace	
Hentriacontane	$C3_1H_{64}$	436	Trace	
Impurities from manufacturing process:				

O6 - Monoacetylmorphine	$C_{19}H_{21}NO_4$	327	34.4	Present in high concentration due to hydrolysis of heroin (Small quantities from morphine + Ac_2O)
Acetylcodeine	$C_{20}H_{23}NO_4$	341	7.9	
N- Acetylnorlaudanosine	$C_{22}H_{27}NO_5$	234	0.06	
N- Acetylnornarcotine	$C_{23}H_{23}NO_8$	441	Trace	Noscapine + O_2 + Ac_2O Decomposition product
N-Acetylanhydronornarceine	$C_{24}H_{25}NO_8$	455	Traces	Noscapine + Ac_2O Decomposition product
Adulterants:				
Nicotinamide	$C_6H_6N_2O$	122	1.39	
Diphenylamine	$C_{12}H_{11}N$	169	Trace	

Table 7: Identified components of the illicit heroin sample.

Heroin



Heroin

Natural impurities from opium

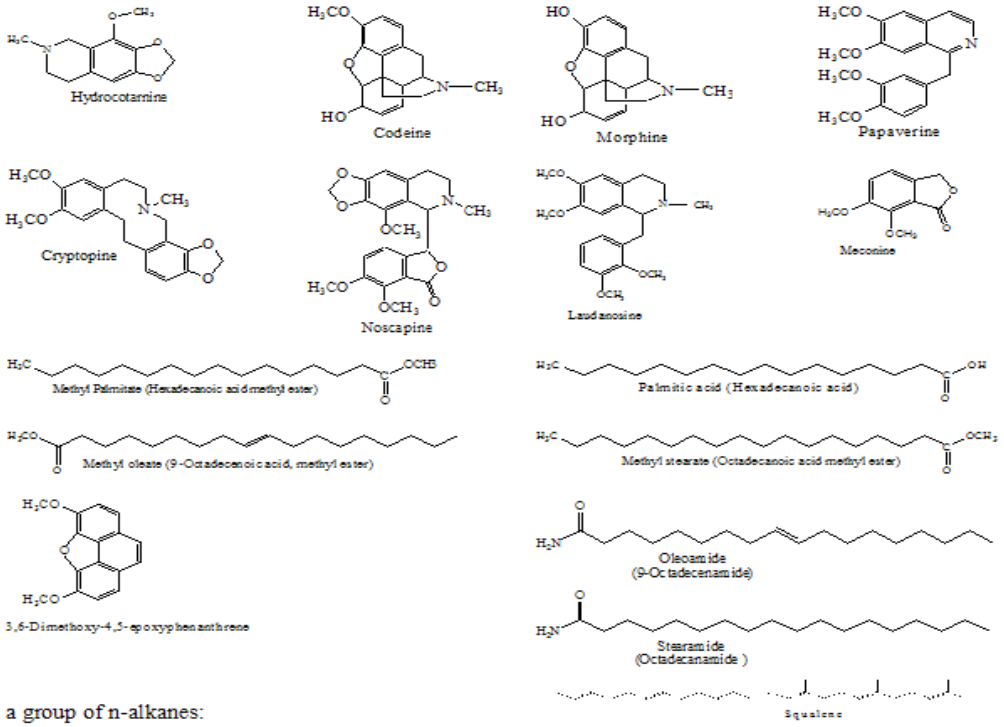
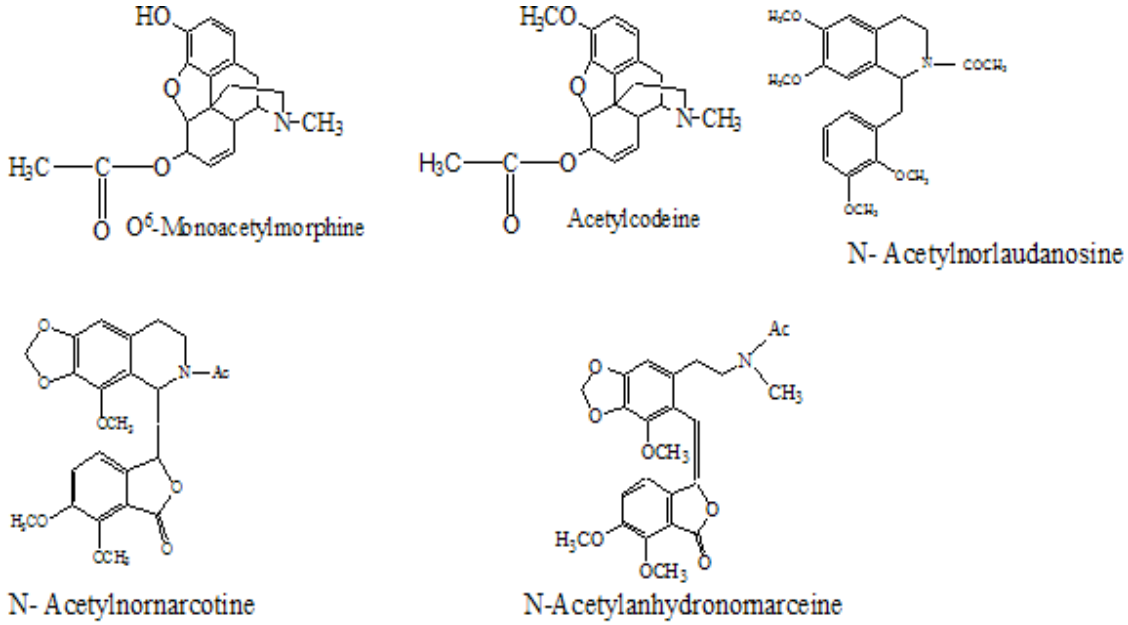


Figure 8: Identified components of the illicit heroin sample.

Impurities from manufacture process



Adulterants

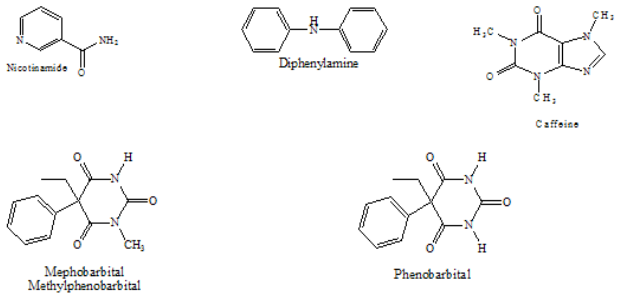


Figure 8: Cont.

Discussion

From the physical characters of illicit heroin, one may conclude the following:

1. It is crudely processed heroin sample as indicated by its dark brown or black color [43].

2. Its strong vinegar-like odor suggests the use of excess acetic anhydride as an acetylating agent or hydrolysis of heroin due to bad storage in humid atmosphere [2,3,7].

3. Microscopic examination: of the sample revealed the presence of abundant starch granules suggested that the heroin sample under investigation was adulterated or diluted with starch [12]. Color, Solubility and Precipitation (Anion) tests: From the data cited in (Table 3), one could conclude the following:

- Presumptive color tests indicated the presence of opium alkaloids, heroin and caffeine which is commonly used as heroin adulterant.
- Solubility and precipitation tests showed that the heroin sample is present in the sulfate salt form diluted with starch which is insoluble in water and ethanol [l].
- The TLC data cited in (Table 4), revealed the presence of diacetylmorphine (heroin), O6-monoacetylmorphine (O6-MAM), Acetylcodeine (AC), morphine, codeine, noscapine, papaverine and caffeine in the heroin sample under investigation. The presence of caffeine in the heroin sample could indicate that it is of the South West Asian type characterized by its cutting with caffeine [3,7].

GC/MS

GC/MS investigation of total heroin sample as well as neutral fraction revealed the presence of natural impurities from opium (24 components), impurities from the manufacturing process (5 components) and added adulterants (5 components). The identified components and their mean relative percentage are shown in (Table 7) and the structures of the identified components are illustrated in (Figure 8). From the fore mentioned data, one may conclude the following:

1.Heroin is present in a relatively low concentration (0.6%) and O6-monoacetyl-morphine (O_6- MAM) is the dominant component (34.4%) which could be attributed to the following:

- The use of excess H_2SO_4 during the manufacture process of heroin that result in the hydrolysis of heroin to yield high percentage of O_6-MAM and morphine (> 5%). These features are characteristics for poorly manufactured heroin which is the case of our sample [2].
- Partial hydrolysis of heroin to O_6-MAM and then to morphine upon storage under humid condition [2,24,27] and the liberation of acetic acid2 which is responsible for the strong vinegar-like odor of our sample.
- Partial hydrolysis of heroin to O_6-MAM during injection into the GC system and/or during the silylation process [27].

2. O_3-monoacetylmorphine (O_3-MAM) was not detected in our sample, as it is the product resulting from incomplete acetylation of morphine [2,24]. The absence of O_3-MAM and the presence of high percentage O_6-MAM (>10% relative to heroin) and morphine (>1% relative to heroin) confirms that post-processing hydrolysis have occurred to our sample [27].

3. Thebaine was never detected in our heroin sample as it is unstable towards acetylation conditions. However, 3,6 Dimethoxy-4,5 epoxyphenanthrene was detected as its decomposition product resulting from the reaction of thebaine with acetic anhydride [3,27,38].

4. Noscapine concentration in the sample was found to be about 13.7% (< 46%), which means that the present noscapine is a natural impurity from opium and is a non-intentionally added adulterant [21]. N-Acetylanhydronornarceine and N- Acetyl-nornarcotine were detected as the decomposition products of noscapine resulting from its reaction with acetic anhydride only or with acetic anhydride and oxygen, respectively [27].

5. The absence of 1-chloroheroin and 3-[1-(1-carboxymethoxyethyl)]-6-acetylmorphine (the 2 route specific markers for acetyl chloride and ethylene diacetate) as components in the confiscated heroin sample excludes the use of acetyl chloride and ethylene diacetate as acetylating agents and support the use of acetic anhydride as an acetylating agent in the manufacturing process (3-United Nations, 1998; 5-Odell et al., 2006).

6.The lipid fraction consisting of fatty acid and fatty acid methyl esters was well resolved only on DB-1 column due to the low polarity of DB-1 in comparison to HP-5. The presence of this lipid fraction is characteristic for low quality crude morphine produced in South West Asia [33].

7. It is important to note that, the fatty acid should be present in the original sample as volatile derivative with undetectable molecular ion peaks.

8. Polar compounds like morphine, monoacetylmorphine and Acetylcodeine were well resolved on polar stationary Phases HP-5 [44].

9. Caffeine and barbiturates (Phenobarbital and methyl phenobarbital) are characteristic as adulterants for South West Asian heroin. Caffeine was added to the heroin sample to enhance the amount of vaporized heroin without decomposition and improve the taste [45], while barbiturates were added as hypnotics [46].

Therefore, it could be concluded that the illicit heroin sample under investigation is a poorly manufactured sample, badly stored, adulterated and it is of the south West Asian type.

Conclusion

Application of physical and microscopical examination, color tests, Thin Layer Chromatography (TLC) and Gas Chromatography/ Mass Spectrometry (GC/MS) proved to be a very valuable tool for the characterization of the heroin sample under investigation, which has the following characteristics:

a. Physical characters: dark brown to nearly black, small granular pieces (chunks) having strong vinegar-like odor.

b. The absence of O_3-MAM and the presence of high percentage O_6-MAM content (>10% relative to heroin) and morphine (>1% relative to heroin) confirms that post-processing hydrolysis have occurred to our sample.

c. The presence of heroin, morphine and O_6-MAM at a concentration of about 37.0%; Noscapine (~ 13.7 %), papaverine (~ 2.2%) and Acetylcodeine (~7.9%) is characteristic for SWA type.

d. The relatively high concentration of noscapine (13.7%) relative to morphine (5%) confirms that the heroin sample is prepared via method 3 in which noscapine and morphine are in the ration 2:1 characteristic for crude morphine of SWA.

e. The presence of natural impurities such as meconine, noscapine, papaverine, cryptopine, laudanosine, fatty acids and fatty acid methyl esters confirms that it is a crudely processed heroin.

f. The presence of heroin as the sulfate salt.

g. The presence of abundant starch granules as adulterant.

h. The presence of caffeine and barbiturates (Phenobarbital and methylpheno-barbital) as adulterants is characteristic for SWA type.

Therefore, we can conclude that the heroin sample under investigation is: a poorly manufactured sample, prepared by acetylation with acetic anhydride, badly stored, adulterated, of the South West Asian type and suggested to be abused by inhalation through smoking.

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