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 acetylation 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 O3-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.

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 iodoplatinate 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).

**Chemicals and Reagents**

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

- Derivatizing reagents: N-methyl-Trimethyl Silyltribfluoro acetamide (MSTFA) reagent for silylation was purchased from Sigma USA.

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), Ultraviolet 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.
Presumptive color tests:

| Test Name                                      | Method                                                                 | Use                                      | Ref        |
|------------------------------------------------|------------------------------------------------------------------------|------------------------------------------|------------|
| 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 0.1 g. Potassium chloride 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. |                                |            |
|------------------------------------------------|----------------------------------------------------------------------------------|-----------------------------------|------------|
|                                                | 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                | [2]        |
|                                                | 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-monoacetylmorphine 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 Dragendorff’s and acidified potassium iodoplatinate 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 |

**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.**

**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.).

**Figure 1: Confiscated heroin (seizure number 978/1988, Suez-Egypt).**
**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                   | System A | System B | Heroin sample |
|-----------------------------|----------|----------|---------------|
| Morphine                    | 16.9     | 14.5     | (+)           |
| Codeine                     | 36.9     | 28.9     | (+)           |
| Caffeine                    | 40.9     | 72.6     | (+)           |
| O6- monoacetylmorphine      | 66.15    | 27.6     | (+)           |
| Papaverine                  | 68.7     | 78.9     | (+)           |
| Heroin                      | 69.2     | 63.16    | (+)           |
| Acetylmorphine              | 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).
Figure 4: GC of the silylated heroin sample (MSTAF) on (HP-5) column.

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

TMS-mor: Trimethylsilylmorphine; TMS-O6-MAM: Trimethylsilyl-O6 monoacetyl-morphine.
| Component                           | tf min. | Relative % | [M]^+ | Base Peak | Fragment ions m/z (relative intensity) | Reference |
|-------------------------------------|---------|------------|-------|-----------|----------------------------------------|-----------|
|                                    | Before  | After      | Before| After     | HP-S  | DB-1 | HP-S | DB-1 |
| Niacinamide                         | 4.75    | 1.30       | 122   | 122       | 78 (63), 106 (57), 51 (19), 123 (7)     | 34        |
| Isomeconine                         | 0.08    | 2.83       | 194   | 194       | 165 (77), 147 (56), 53 (30), 175 (29), 77 (27) | 32        |
| Meconine                            | 9.82    | 8.42       | 194   | 195       | 147 (77), 176 (47), 118 (30), 119 (30), 77 (27) | 32        |
| Hydrocortisone                      | 10.96   | 1.94       | 221   | 220       | 178 (74), 255 (21), 193 (21), 177 (19)     | 35, 36    |
| Caffeine                            | 11.43   | 7.13       | 194   | 194       | 169 (64), 65 (25), 67 (20), 82 (17), 193 (14), 165 (7), 137 (6) | 34        |
| Methyl palmitate                    | –       | 10.2       | –     | 3.44      | 270 (17)                                 | 37        |
| Methyl phenobarbital (Meprobamal)   | 12.57   | 1.03       | –     | –         | 246 (10)                                 | 34        |
| Phenobarbital                       | 14.03   | 10.6       | 5.02  | 9.65      | 232 (21)                                 | 34        |
| Methyl oleate                       | 15.33   | 12.2       | 0.08  | 4.9       | 250 (10)                                 | 34, 36    |
| Methyl stearate                     | 15.73   | 12.4       | 0.09  | 1.55      | 288 (21)                                 | 34       |
| DEP                                 | 18.82   | 14.6       | 0.69  | 0.98      | 252 (55)                                 | 34        |
| Codeine                             | 20.10   | 15.5       | 4.41  | 2.03      | 259 (20)                                 | 34        |
| TMS-Morphine (Morphinone)           | 20.23   | 18.1       | 0.36  | 1.96      | 422 (52)                                 | 30        |
| Morphine                            | 21.09   | 12.79      | –     | –         | 235 (25), 146 (16), 194 (11), 414 (16)    | 30        |
| Acetylcodeine                       | 21.68   | 16.6       | 7.72  | 5.38      | 341 (31), 282 (15), 229 (23), 204 (23), 124 (14), 128 (8), 59 (10) | 30        |
| O6-MAM                              | 22.06   | 17.1       | 27.29 | 30.7      | 327 (31)                                 | 44, 32    |
| TMS-6-MAM                           | 22.21   | 17.3       | 41.4  | 4.0       | 399 (36), 266 (14), 215 (27), 146 (28), 229 (19), 192 (13), 204 (14), 124 (13), 81 (12) | 41        |
| Heroin                              | 23.22   | 18.0       | 0.54  | 0.72      | 369 (56)                                 | 2         |
| Papaverine                          | 23.51   | 19.6       | 2.18  | 0.99      | 338 (37), 304 (25), 310 (25), 304 (23), 235 (15), 154 (14) | 30        |
| Cryptopine                          | 23.33   | 0.07       | –     | –         | 369 (4)                                  | 30        |

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.

Methyl palmitate: (hexadecanoic acid methyl ester); Methyl oleate: (9-Octadecanoic acid methyl ester); Methyl stearate (Octadecanoic acid methyl ester); DEP: 3,6 Dimethoxy- 4,5 epoxypenthanthrene; TMS- Morphine: trimethylsilyl morphine; O6-MAM: O6 monoacetylmorphine; TMS- O6 MAM: Trimethylsilyl-O6 monoacetyl morphine

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

Table 6: Results of GC-MS of identified components 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 |
|---------------------------|-------------|------------|-------|-----------|------------------------------------------|-----------|
| Diphenyamine              | 0.02        | 11.1       | 169   | 169       | 168 (60), 167 (34), 83 (23), 170 (15), 51 (10), 77 (10) | 34        |
| Meconine                  | 10.5        | 4.24       | 154 (57) | 165       | 147 (77), 176 (44), 119 (30), 119 (30), 77 (27) | 32        |
| Caffeine                  | 12.9        | 5.84       | 194   | 194       | 159 (46), 55 (25), 67 (20), 82 (17), 159 (14), 103 (13) | 137 (6)   |
| Methoxyphenobarbital (Mephenobarbital) | 11.7        | 2.84       | 246 (10) | 218       | 117 (33), 144 (26), 114 (15), 114 (15) | 34        |
| Palmitic acid (Hexadecanoic acid) | 14.1        | 1.21       | 255 (59) | 73       | 80 (63), 57 (70), 55 (60), 129 (65), 72 (46), 69 (58), 83 (34), 213 (31), 97 (30), 157 (25) | 34, 36    |
| Phenobarbital             | 16.8        | 12.2       | 232 (20) | 204       | 117 (77), 161 (64), 144 (11), 235 (11), 77 (10) | 34        |
| n-Tricosane               | 18.4        | 0.62       | 324   | 57        | 71 (7), 85 (56), 99 (21), 113 (19), 127 (10), 141 (7) | 34        |
| DEP                       | 19.3        | 2.88       | 252 (65) | 237       | 154 (21), 138 (15) | 38        |
| n-Tetracontane            | 10.0        | 0.50       | 338 (1) | 57        | 71 (7), 85 (56), 99 (21), 113 (19), 127 (11), 141 (7) | 155 (5)   |
| Oleamnic acid (Octadecanamide) | 19.9        | 0.61       | 281 (5) | 50        | 72 (67), 126 (19), 83 (15), 97 (12), 112 (11) | 36        |
| Stearamide (Octadecanamide) | 20.3        | 0.19       | 283 (5) | 50        | 72 (64), 88 (15), 128 (12), 88 (10), 124 (7), 66 (6) | 34        |
| Pentacosane               | 21.2        | 1.23       | 352 (5) | 57        | 71 (7), 85 (59), 99 (25), 113 (19), 127 (10), 141 (7) | 34        |
| Hexacosane                | 22.5        | 0.67       | 365 (7) | 57        | 71 (7), 85 (50), 99 (24), 113 (19), 239 (11) | 34        |
| Octacosane                | 24.8        | 0.7        | 384 (5) | 57        | 71 (7), 85 (60), 99 (25), 113 (19), 127 (11), 141 (7) | 34        |
| Squaric acid              | 25.0        | 0.22       | 419 (1) | 60        | 51 (55), 145 (41), 137 (16), 121 (15) | 34        |
| Nonacosane                | 26.1        | 0.74       | 408 (6) | 57        | 71 (60), 85 (63), 99 (25), 113 (17), 328 (15) | 34        |
| Triacontane               | 27.6        | 0.4        | 422 (2) | 57        | 71 (60), 85 (61), 99 (25), 113 (19), 127 (12), 141 (11) | 34        |
| Henricontane              | 29.5        | 0.18       | 436 (5) | 57        | 71 (7), 85 (60), 99 (25), 113 (17), 127 (12), 141 (11) | 34        |
| mixture of: laudanosoline and N-Acetylnorlaudanosine | 29.9        | 0.20       | 437 (7) | 192       | 357 (3), 253 (12), 191 (10), 107 (6) | 42        |
| Laudanosine               | 30.0        | 0.17       | 411 (5) | 234       | 235 (12), 191 (10), 107 (6) | 32        |
| N-Acetylnorlaudanosine    | 38.9        | 0.17       | 411 (5) | 234       | 235 (12), 191 (10), 107 (6) | 32, 36    |
| Norcamphone               | 32.4        | 0.51       | 413    | 220       | 221 (14), 205 (11) | 2         |
| N-Acetylnoroxycornine     | 39.0        | 0.17       | 411 (5) | 234       | 235 (12), 191 (10), 107 (6) | 32, 36    |

DEP: 3,6-Dimethoxy-4,5-epoxyphenanethrene; (;-) Not detected
Figure (7): EI-MS spectrum of the detected components in heroin samples.

Figure 7: EI-MS spectrum of the detected components in heroin samples.
Figure (7): Cont.
Figure (7): Cont.
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_{23}H_{29}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_{16}H_{34}O_2 | 270 | 3.4 |                                            |
| **Palmitic acid (Hexadecanoic acid)** | C_{16}H_{34}O_2 | 256 | Trace |                                            |
| **Methyl oleate (9-Octadecenoic acid methyl ester)** | C_{18}H_{36}O_2 | 296 | 2.5 |                                            |
| **Methyl stearate (Octadecanoic acid methyl ester)** | C_{18}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_{33}NO      | 283              | Trace           |                                            |
| **Pentacosane**                   | C_{22}H_{52}         | 352              | Trace           |                                            |
| **Hexacosane**                    | C_{26}H_{54}         | 366              | Trace           |                                            |
| **Octacosane**                    | C_{26}H_{58}         | 394              | Trace           |                                            |
| **Squalene**                      | C_{30}H_{50}         | 410              | Trace           |                                            |
| **Nonacosane**                    | C_{24}H_{50}         | 408              | Trace           |                                            |
| **Triaccontane**                  | C_{30}H_{62}         | 422              | Trace           |                                            |
| **Hentriacontane**                | C_{30}H_{64}         | 436              | Trace           |                                            |

Impurities from manufacturing process:
| Component                                      | Formula       | MW  | Purity  |
|-----------------------------------------------|---------------|-----|---------|
| O6 - Monoacetylmorphine                       | C_{19}H_{21}NO_{4} | 327 | Present in high concentration due to hydrolysis of heroin (Small quantities from morphine + Ac_{2}O) |
| Acetylcodine                                   | C_{20}H_{22}NO_{4} | 341 | 7.9     |
| N- Acetylnorlaudanosine                       | C_{22}H_{24}NO_{5} | 234 | 0.06    |
| N- Acetylnornarcotine                          | C_{23}H_{24}NO_{8} | 441 | Trace   |
| N-Acetylanhydroonorcarcine                    | C_{24}H_{26}NO_{8} | 455 | Traces  |
| **Adulterants:**                              |               |     |         |
| Nicotinamide                                  | C_{6}H_{6}N_{2}O | 122 | 1.39    |
| Diphenylamine                                 | C_{12}H_{11}N   | 169 | Trace   |

Table 7: Identified components of the illicit heroin sample.

**Heroin**

![Heroin structure](Heroin_structure.png)

Natural impurities from opium
Figure 8: Identified components of the illicit heroin sample.

Impurities from manufacture process

- 6-O-Monoacetylmorphine
- Acetylcodine
- N-Acetylnorlaudanosine
- N-Acetylnormorphone
- N-Acetylanhydromorphone
Adulterants

![Chemical structures of Adulterants](image)

**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 [1].
   - The TLC data cited in (Table 4), revealed the presence of diacetylmorphine (heroin), O6-monoacetylmorphine (O6-MAM), Acetylcodetaine (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 (O6-MAM) is the dominant component (34.4%) which could be attributed to the following:
   -  The use of excess H2SO4 during the manufacture process of heroin that result in the hydrolysis of heroin to yield high percentage of O6-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 O6-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 O6-MAM during injection into the GC system and/or during the silylation process [27].
2. O3-monoacetylmorphine (O3-MAM) was not detected in our sample, as it is the product resulting from incomplete acetylation of morphine [2,24]. The absence of O3-MAM and the presence of high percentage O6-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-Acetylnornarcotine 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 acetylation agents and support the use of acetic anhydride as an acetylation 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].
It is important to note that, the fatty acid should be present in the original sample as volatile derivative with undetectable molecular ion peaks.

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

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-MAM and the presence of high percentage O-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-MAM at a concentration of about 37.0%; Noscapine (~ 13.7 %), papaverine (~ 2.2%) and Acetylcodaine (~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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