CHAPTER 4

METHODOLOGY

4.1 Preliminary

A vast volume of heroin profiling methodologies have been discussed in the literature. These analytical procedures when applied to the locally seized samples may not be as effective as it should be since the samples seized from different countries may vary significantly in the sample matrix and purity level. In practice, these methods must be re-validated to ensure they function as predicted with the samples under study. Alternatively, method development and method optimization should be performed to establish a new method that is fit for the profiling purpose. As a result, this study seeks to develop and optimize several methods for the profiling of locally seized heroin samples. The overall methodology undertaken in this study emphasized the following:

- Simple and less training is required
- Suitable for routine analysis
- Economic
- Rapid
- Repeatable and reproducible
- Less sample preparation
- Small sample size (maximum 1 g) for the overall analysis

Time and cost, in particular, are the main concerns of this profiling program. The study emphasized a shorter analysis time and a cost effective method. Sometimes, less time is devoted to time consuming laboratory work because timely decisions must

be made. For example, the sampling procedure adopted in this study must be able to provide an immediate decision on the number of samples to be taken for physical examination. It was achieved by using a statistical sampling approach. In addition, expensive methods are impractical for high throughputs. As a result, little and limited chemicals were involved in this study. The following methodology was also planned in line with the availability of the required facilities so that the profiling of heroin can be performed on a regular basis. Deliberate purchases of new instruments are totally impractical and hence are not recommended in this study.

Six specific tasks were undertaken in this study. In order to obtain maximum information from the seized heroin, the following tasks were performed:

- Task 1: Visual examination and physical characteristics
- Task 2: Analysis of plastic receptacles/films
- Task 3: Profiling of major components as Signature 1
- Task 4: Profiling of manufacturing impurities as Signature 2
- Task 5: Profiling of trace elements as Signature 4
- Task 6: Data management and database build-up

The signature numbers follow the order of analyte importance explained by Besacier and Chaudron-Thozet (1999). Apparently, the determination of occluded solvents and isotopes as Signatures 3 and 5 respectively was not performed because the laboratory did not have the facilities for this task. According to Besacier and Chaudron-Thozet (1999), associating the major components with manufacturing impurities and/or occluded solvents is the most reliable procedure for complete characterization of illicit heroin. Therefore, Signature 3 can be optionally taken out since Signatures 1 and 2 were already emphasized in this study. Despite this omission, extra effort was also given to

physical profiling and elemental characteristics of the heroin samples. Throughout the course of the study, only samples submitted as illicit heroin to the Department of Chemistry Malaysia (Headquarters) during a period of eight months (January until August) in the year 2010 were considered in this profiling program.

4.2 Materials

4.2.1 Chemicals and Reagents

HPLC grade methanol, analytical reagent grade chloroform, toluene, n-hexane and sulfuric acid (96%) supplied by Fisher Scientific were employed. Analytical reagent grade ethyl acetate and ultrapure reagent grade nitric acid (< 70%) were purchased from J. T. Baker. Deionized water was obtained from an Elga Maxima (18.2 MΩ) filter.

4.2.2 Chemical Standards

4.2.2.1 Profiling of Major Components

For the quantification of major components, eight target analytes were selected. Paracetamol and dextromethorphan hydrobromide were supplied by Y.S.P. Industries (Malaysia). Acetylcodeine hydrochloride was prepared by the Department of Chemistry Malaysia. Codeine phosphate, morphine hydrochloride and heroin hydrochloride were purchased from Johnson Matthey Macfarlan Smith. 6-Monoacetylmorphine (6-MAM) hydrochloride was commercially obtained from Lipomed. 2,2,2 Triphenyl acetophenone (internal standard, IS) was supplied by Aldrich Chemical Company. Caffeine was purchased from Merck.

Ten food coloring agents (ammarant, tetrazine, green S, rhodamine BS, fast green, brilliant blue, sunset yellow, carmoisine, red 2G and erythrosine BS) involved in the preparation of simulated heroin samples were obtained from the Department of Chemistry Malaysia.

4.2.2.2 Profiling of Manufacturing Impurities

For semi-quantitative determination of manufacturing impurities, seven target n-alkanes including n-pentadecane (C15), n-eicosane (C20), n-pentacosane (C15), n-triacontane (C30), n-tritriacontane (C33), n-pentatriacontane (C35) and n-octatriacontane (C38) were commercially obtained from Dr. Ehrenstorfer GmbH. n-Tetracontane (C40) was obtained from Supelco Inc and it was chosen as the internal standard (IS).

Paracetamol and caffeine respectively supplied by Y.S.P. Industries (Malaysia) and Merck were used for diluting the heroin samples in the preparation of simulated samples.

4.2.2.3 Profiling of Trace Elements

For trace elemental analysis, Instrument Check Standard I containing thirteen minor elements: silver (Ag), aluminium (Al), arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se) and zinc (Zn) each at 10 ppm was commercially obtained from SPEX CertiPrep. Individual stock standards of sodium (Na), magnesium (Mg), potassium (K), calcium (Ca) and iron (Fe) which are the major elements in this study, and another minor element, boron (B) as well as four ISs including scandium (Sc), rhodium (Rh), indium (In) and bismuth (Bi) with a concentration, each at 1000 ppm were purchased from Fisher Scientific. An individual stock solution of mercury (Hg) with a concentration of 1000 ppm was purchased from AccuStandard.

4.2.3 Glassware and Plastic Ware

Glassware was employed for organic analysis. All glassware was thoroughly cleaned with detergent and rinsed with tap water. After the glassware was dried at room

temperature, it was further rinsed with methanol to remove any remaining organic residues.

New plastic ware was used for trace elemental analysis. It was rinsed with 10% nitric acid before use.

4.2.4 Apparatus

The following items were employed throughout the course of analysis. A five decimal point balance was used to weigh the chemical standards. Weighing small items less than 200 g was accomplished by using a four decimal point balance. Large items were weighed with a two decimal point balance. Other apparatus were utilized for specific purposes where appropriate.

a) Analytical balances – i. AND FX-2000 (accuracy = 0.01 g)
   ii. Sartorius BT224S (accuracy = 0.0001 g)
   iii. Denver Instrument AA-200 (accuracy = 0.0001 g)
   iv. Sartorius MC210S (accuracy = 0.00001 g)
b) Ruler (accuracy = 0.5 mm)
c) Electronic micrometer caliper – Chicago (accuracy = 0.001 mm)
d) Sonicator – 5510 BRANSON
e) Centrifuge – Labofuge 200 Heraeus
f) Deionized water filter – Elga Maxima (18.2 MΩ)
g) Steam evaporator
h) Digital camera - SONY Cyber-Shot 6 Megapixels
i) GC capillary columns – i. J&W HP Ultra 2 (Part no.: 19091B-102)
   (length 25 m, i.d. 200 µm, film thickness 0.33µm)
   ii. J&W HP Ultra 2 (Part no.: 19091B-002)
   (length 25 m, i.d. 200 µm, film thickness 0.11µm)

iii. J&W HP-5 (Part no.: 19091J-433)

(length 30 m, i.d. 250 µm, film thickness 0.25 µm)

iv. J&W DB-1 (Part no.: 122-1032)

(length 30 m, i.d. 250 µm, film thickness 0.25 µm)

4.2.5 Instrumentation

Five pieces of equipment were used for instrumental analysis. The specific purposes served by these instruments are summarized in Table 4.1.

Table 4.1: Instruments employed for the profiling of heroin

Instrument	Model	Purpose
Portable spectrometer using attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR)	HazMatID System (Part no.: 023-1002)	To analyze plastic films
Gas chromatography – flame ionization detector (GC-FID)	Agilent Technologies HP6890N GC	To quantify major organic components and manufacturing impurities in illicit heroin
Gas chromatography – mass spectrometer (GC-MS)	Agilent Technologies HP6890N GC with a 5975B inert MSD.	To identify major components in illicit heroin
Gas chromatography – mass spectrometer (GC-MS)	GC-2010 Shimadzu with a GCMS-QP2010Plus	To identify manufacturing impurities in illicit heroin
Inductively coupled plasma-mass spectrometer (ICP-MS)	Perkin Elmer SCIEX ELAN DRC-e	To quantify trace elements in illicit heroin

4.3 Sampling

A submission may contain a number of heroin sample units. However, to analyze every sample unit in each submission would be extremely costly and

impractical. Hence, a sampling procedure that aims to select a small portion of a homogeneous sample plays a significant part in all chemical analyses. It helps to minimize the analysis cost without undermining the validity of the results. Thus, the resultant laboratory sample derived from the sampling process will represent the overall heroin sample in the profiling process. In this study, three sampling procedures were adopted to obtain a valid laboratory sample for analysis. These procedures are described as follows:

- **Gross sampling via subgrouping:** The sample units contained in a submission can vary in number and/or appearance. Therefore, subgrouping was performed to minimize sample inhomogeneity in the submission. Each packet was inspected carefully to note for any disagreement in their general appearance before the sample units were mixed and homogenized. These included the general size and packing of the plastic receptacle as well as the color and texture of the heroin substance. Once observable discrepancies were found in the submission, the sample units were separated and subgrouped accordingly. Each group was treated as an individual case.

After the subgrouping, each case containing a different quantity of physically similar sample units was subjected to two other sampling procedures.

- **Random sampling for visual and physical characteristics**: Owing to the sensitive nature of this narcotic drug, the exposure time of the heroin case samples after discarding from the strong room has to be minimized. Therefore, it was impossible to exhaustively record the physical characteristics of all the sample units in a case. After the subgrouping, if the quantity did not exceed 10

units per case, all sample units were then considered for physical profiling. Alternatively, random samples were taken from every case containing more than 10 units of samples based on the Hypergeometric Distribution Chart (UNODC, 2009b) at 95% confidence using k = 0.9. For example, 18 random samples were sampled if a case contained 40 sample units in total. The selected sample units provided a representative description of the physical characteristics for the case.

- **Bulk sampling for chemical analysis**: All heroin substances in each case were withdrawn from their receptacles and homogenized. Approximately 1 g of the homogenized substance was taken for chemical analysis. Each homogenized sample substance was kept in a separate locker in an air-conditioned environment.

As a minimum of 1 g of illicit heroin was required for profiling, this study was eventually aimed at large contents, especially those substances packed in plastic packets because these items could provide sufficient laboratory sample for analysis. However, substances in straw tubes were excluded in this study because their total amounts were insufficient for sampling.

4.4 Task 1: Visual Examination and Physical Characteristics

Task 1 was accomplished upon receiving the submission from the police. All information was documented before the samples were altered or handled. As previously stated, random sampling may have been performed prior to physical profiling depending on the quantity of the sample units present in the case. In relation to this requirement, physical characterization was only performed on the sampled units. When the necessary information was well-recorded, all the substances in a case were mixed

for bulk sampling. The physical data served as a general description for the case to build up a database in Task 6. Quantitative data derived from the physical measurements in this task were also used to establish the relationships between the cases.

4.4.1 Police Information

The profile of every individual heroin case was started off with its case background. This was achieved by extracting the information from the document (Police Form 31) that was sent together with the case submission. Besides, additional information was also obtained from the Linked Information Management System (LIMS). Table 4.2 lists the common items included under this investigation.

Table 4.2: Police information and case background of a single heroin case

Item	Source	Example	Description
Laboratory number	LIMS	(PJ)FOR1234/10-0	The unique case identity at the Department of Chemistry Malaysia
Police report number	Police form 31	1234-35/10	The unique case identity at the police department
Label	Police form 31	'A'	The unique marking for a specific exhibit/envelope
Branch	Police form 31	Pandan Indah	The location of the police operation branch
Location	LIMS	Cheras	The area in which the operation branch is located
Date of seizure	Police form 31	13 January 2010	The date on which the illicit heroin was seized from the perpetrator
Date of submission	LIMS	14 January 2010	The date on which the illicit heroin was submitted to the Department of Chemistry Malaysia
Case category	Police form 31	39A1	The severity of the case based on its initial sample weight

4.4.2 Photography

A written description of the physical appearance of an entire heroin sample unit (the receptacle and its content) may not be able to offer sufficient details for future comparison. Besides, revisiting of the case sample is impossible since opening up the case sample for detailed examination and in-depth analysis will inevitably alter the physical conditions of the sample. It is therefore critical to preserve the original conditions by means of photography. These photographs will serve as a permanent documentation and representation of the case in the database. A number of photographic techniques adopted for building the ecstasy pill database are costly and cumbersome. In this study, the drop-black technique discussed by Freeman (2004) was employed. This is an economic photographic technique specially designed for taking close-ups. In order to enhance its routine usability among untrained personnel, this technique was modified according to the following setup:

Camera:	SONY Cyber-Shot 6 Megapixels
Illumination:	Two study lamps 9W wrapped with tracing papers
Background:	A polystyrene platform wrapped with black cloth and black paper
Accessories:	A ruler with four standard hues (CMYK)
Editing software:	Photoshop® CS3

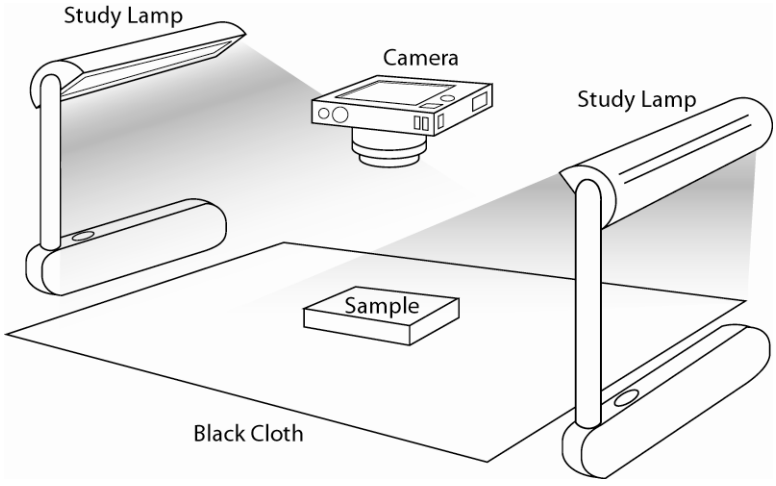
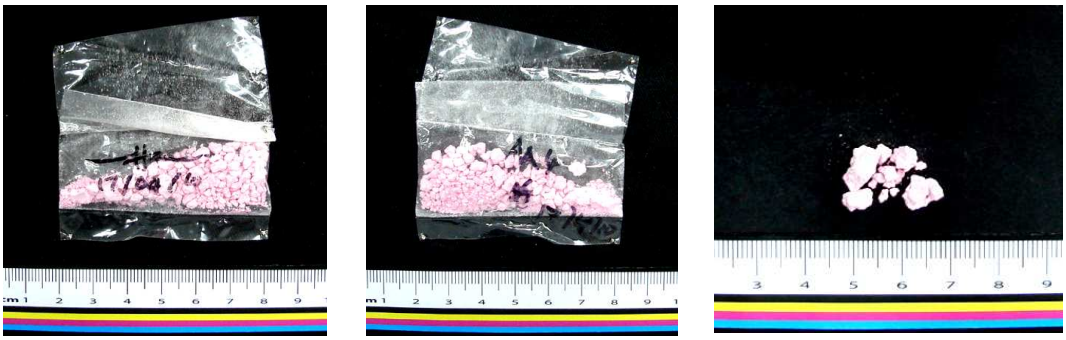


Figure 4.1: General photographic setup

A representative sample was spread out on a polystyrene platform in the centre and a ruler bearing four standard hues (CMYK whereby C = Cyan, M = Magenta, Y = Yellow, K = Black) was placed next to the sample (Figure 4.2). The ruler gave a general measurement for the sample whereas the standard hues provided relative color adjustment for interpreting the photographs especially when different illumination was used for the photographs taken at two different times. Besides, two study lamps wrapped with tracing papers were installed on opposite sides to provide diffuse lighting to the sample. The lights were disregarded if the lighting was sufficient. Direct snaps were taken with a digital camera held perpendicularly above the platform.

(a)



Side A Side B Substance

(b)

Figure 4.2: Elements in photography (A ruler with four standard hues in (a) is used to provide a scale; general photographs in (b) represent the visual documentation for a single case)

For each case, two snapshots (front and back views) were taken from a sample unit in its original condition. The side bearing more features such as folds and significant marks, was labeled as ‘Side A’ while the other side as ‘Side B’. Another

snapshot of the heroin substance that was placed on a piece of black paper was also included in this study. The photographs were edited with the Photoshop® software to achieve a desired contrast, if necessary. All the three photographs were uploaded on the database as a single data profile.

4.4.3 Color of the Heroin Substance

The color of the heroin substance is one of the most prominent characteristics to unaided eyes. Justification suffers when the color description is too detailed, owing to the personal subjectivity in color interpretation. A better description of color was achieved by documenting the color in a general term, without having to describe the color value (e.g. light or dark). For monochrome substances, their colors were chosen from the list (Table 4.3) provided in Color 1. For substances in dual colors, there was a need to provide two observable colors as a general description for the samples. In this case, for a dual colored substance, the lighter color is expressed using the list of Color 1, while the darker color is obtained from the list of Color 2. For multicolored substances, the colors are indicated in the checkbox in the database and only two major colors were recorded for Color 1 and Color 2. To avoid obliteration of the minor color, the colors of the substance were first recorded before the sample was ground/ powdered.

Table 4.3: Color categories for the description of heroin substance

Color 1	Color 2
White	Not applicable
Off white	Pink
Pink	Brown
Brown	Yellow
Yellow	Green
Green	Grey
Orange	Orange

4.4.4 Texture of the Heroin Substance

Texture refers to the general particle size of the substance. The texture of the substance is another useful attribute for distinguishing between samples during subgrouping. Practically, a range of particle sizes in the form of continuous data can be obtained with a set of sieves in highly variable mesh sizes (Holt, 1996). If the sieves are fixed with definite measurements, the particles can be recorded in micrometer (Pye, Blott, Croft & Witton, 2007). However, the sieving procedure is laborious and time-consuming. As only a limited sample size was available for analysis, losses due to handling must be avoided and hence it was decided to collect such data according to their categories. Three categories were selected to offer textural descriptions, and this was based solely on visual inspection with the following criteria (Table 4.4):

Table 4.4: Texture categories for the description of heroin substance

Category	Criteria
Coarse	• Apparently large and visually well-defined bodies of substance • A small amount or less than half of the sample is in the form of a fine powder
Coarse and Fine	• Both visually defined bodies of substance and fine powder are present in approximately equal portions
Fine	• Powdery appearance • A small amount or less than half of the sample is in the form of a coarse substance

4.4.5 Wrapping Style of the Plastic Package

Many different types of wrappers could be used to contain the heroin substance. As previously stated, only those samples contained in plastic packages were considered in this study. In Vietnam, the package was clearly stamped with distinctive logos for direct comparison (Hung *et al.*, 2005). In Malaysia, most of the seized samples were

packed in clear plastic packages/receptacles, rendering the packages less informative. From the forensic intelligence perspective, the differences in the appearance of the receptacles infer that different countries may have different wrapping styles. For example, the 'square like' and 'amorphic' types experimented by Zamir *et al.* (2007) in Ireland are not the popular wrapping styles in Malaysia. In the local context, drugs of different nature such as ketamine and heroin always show a considerable disagreement in their wrappers. The wrapping style could thus be a potential signature for the illicit heroin at the retail distribution level as Zamir *et al.* (2007) noted that different packers would deliberate some action on the package.

In most cases, some of the illicit heroin samples contained in clear packages were submitted together with additional wrappers such as newspapers and commercial plastic bags. To minimize irrelevant information, only the package/receptacle having direct contact with the substance was considered. To document the packages devoid of illicit manufacturer's markings, the plastic wrapping style can be generally defined using seal (S), margin (M) and portion (P):

Table 4.5: General morphology of plastic wrapping styles defined by seal, margin and portion

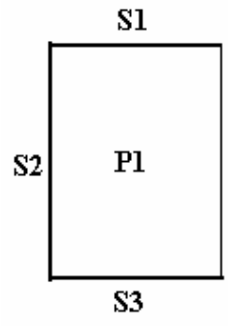
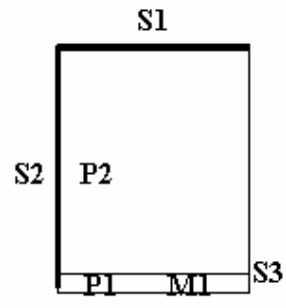
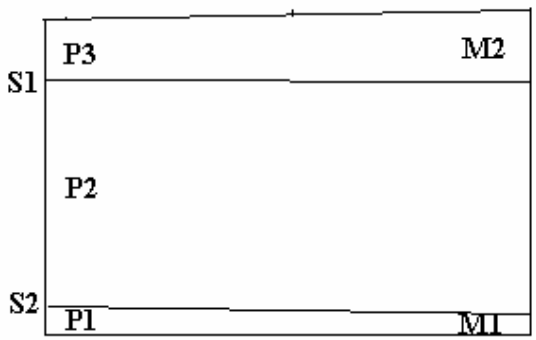
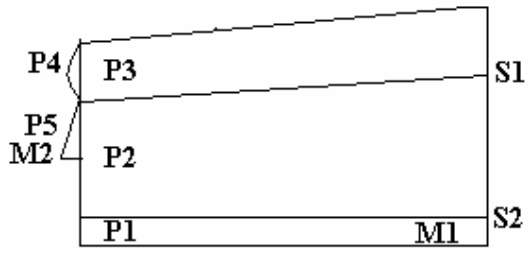
General morphology	Description
	Seals on the brims without any margin. The packet has only 1 portion.

Table 4.5: Continued

General morphology	Description
	2 seals on the brims and 1 seal at the bottom with 1 margin. The packet has 2 portions.
	2 seals on top and bottom with two margins. The packet has three portions.
	2 seals on top and bottom with two margins. The packet has five portions.

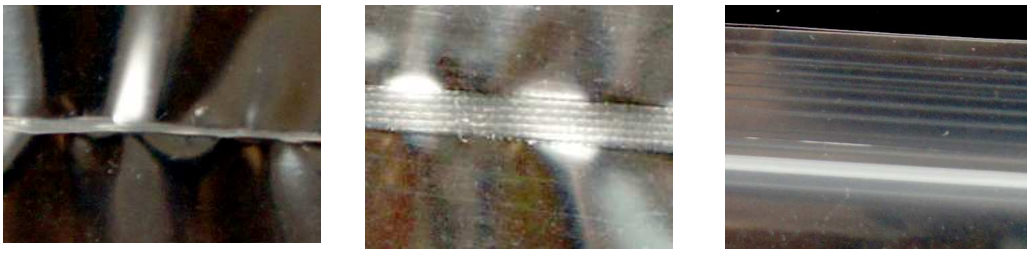
With the predefined morphology, the wrapping of the plastic package was observed and recorded based on the following categories:

Type 1a – seals on brims, no margin, 1 portion
Type 1b – 2 seals, 1 margin, 2 portions
Type 1c – 3 seals, 1 margin, 2 portions
Type 2a – 2 seals, 2 margins, 3 portions
Type 2b – 3 seals, 2 margins, 4 portions
Type 2c – 3 seals, 3 margins, 6 portions
Type 3a – 2 seals, 2 margins, 5 portions
Type 3b – 3 seals, 2 margins, 7 portions
Type 4a – tube with seal(s)
Tube 4b – undefined

4.4.6 Sealing of the Plastic Package

All plastic receptacles (e.g. plastic packets or plastic bags) are usually sealed to firmly secure the heroin substances so that the distributor can handle their stocks with ease. Most of the plastic packets are usually accompanied by at least two seals. One seal at the bottom is usually made by the legitimate plastic manufacturer. With some exceptions, this bottom-seal may also be introduced by the heroin packer if a roll of plastic tube instead of individual plastic packets is used for packing the drug substance. However, it is with great difficulty to determine if the bottom-seal is made by either of the parties with confidence. Hence, this bottom-seal was not included in this study. Other seal(s) are most likely to have been introduced by the drug packer after the drug substances are placed into the plastic packets during packing. The number of such seals together with the seal pattern and seal quality observed in all individual plastic packages were recorded. The following observations summarize the particulars associated with the seal of the drug packer. The number of packer's seals refers to the total number of seals made by the drug packer (not including the manufacturer's seal). Seal patterns are defined according to Figure 4.3. Clarity of seal refers to the quality of the seal observed on the package.

Number of packer's seals:	Not applicable
	Single
	Double
	Triple
Seal pattern/type:	No seal
	Undefined seal pattern
	Rectangle
	Clip seal
Seal quality (clarity):	Not applicable
	Clear and complete
	Clear but incomplete
	Vague but complete
	Vague and incomplete



(a) (b) (c)

Figure 4.3: Appearance of different seal patterns (Three major patterns are (a) undefined seal pattern; (b) rectangle; (c) clip seal)

4.4.7 Weight of the Heroin Substance and Plastic Receptacle

The weight of each plastic receptacle containing the heroin substance was determined using an analytical balance (Sartorius brand for small items or AND brand for large items) and the reading was documented as gross weight, W_g. All the substance was withdrawn from its plastic receptacle, after which the empty plastic receptacle was cleaned thoroughly with a tissue paper. Subsequently, the empty plastic package was weighed on the same analytical balance and the weight of the empty receptacle, W_r was recorded. Finally, the net weight of the substance, W_s withdrawn from each different receptacle was computed using the following formula:

$$W_s = W_g - W_r$$

The procedure was repeated for all the units sampled for physical characterization. The mean values representing the substance weight per package and the weight of an empty plastic receptacle for each case were obtained and expressed as the mean ± standard deviation.

Water and solvent were not used for cleaning the plastic films as they may obliterate or remove the markings put by the police personnel. The plastic receptacle, as a result, was not totally free of the pre-contained substance (especially in the folded areas). A study was performed using small plastic packets to determine the extent to which such un-removed substance and particles would affect the readings. In addition,

the precision of the readings was examined using two different analytical balances (Sartorius BT224S and Denver Instrument AA-200).

4.4.8 Width of the Plastic Receptacle

The width of the plastic receptacle is one of the important dimensions associated with plastic packet and plastic bag. However, certain receptacles have longer widths compared to their lengths. As a result, it was resolved that the width be taken as the side where the seal from manufacture is most likely to be located since almost all types of plastic receptacles have a basic bottom-seal for them to contain the substance before other intended seals were added. The opposite edge was also considered as the width. Two width edges of a plastic receptacle were measured by straightening the edge of the plastic film over a ruler. The procedure was repeated for all the units sampled for physical characterization. The mean value representing the width of the plastic packet for each case was obtained and expressed as the mean ± standard deviation.

Figure 4.4: Plastic packages showing their widths

4.4.9 Thickness of the Plastic Receptacle

The thickness of an object is commonly used as evidence in samples such as hair, paint flakes and pills/tablets. This dimension provides a relatively rapid comparative parameter to distinguish one from another. In ecstasy profiling, the vernier calipers are frequently employed to measure the thickness of the pill since the dimension usually falls in the range of centimeters. To determine the thickness of thin objects such as plastic films, Causin *et al.* (2006) used a Digico 1 (Tesa) micrometer to measure the thickness of plastic bags in the range of micrometers. Similarly in this profiling program, the micrometer calipers (Chicago brand) were employed to determine the thickness of the plastic packet (single film) after it was cleaned thoroughly with a tissue paper. Measurements were made on the less disturbed areas to avoid errors introduced by rough handling. The disturbed areas refer to observable shrinkage, lodgment of particles, folds, etc. Two measurements were recorded from each side of the plastic packet, totaling four readings for both sides of a plastic packet. The procedure was repeated for all the units sampled for physical characterization. The mean value representing the plastic package thickness per packet for each case was obtained and expressed as the mean ± standard deviation.

In addition, the precision of the readings was examined using two different micrometers (both of Chicago brands) and two types of plastic films.

4.5 Task 2: Analysis of Plastic Films by ATR-FTIR

Task 2 was accomplished immediately after Task 1 was completed. In the analysis, various polymeric types of plastic films were first distinguished qualitatively by infrared spectra at the macro level. Then, the plastic types were triangulated with the police information to get an overview of the heroin trafficking activity. At the micro level, the spectral variation of the most frequently encountered plastic type was further

examined quantitatively. Instrumental and statistical validation was performed using specimen plastic films before the quantitative data were used to assess the relationships between the case plastic films.

4.5.1 ATR-FTIR Procedure for Plastic Films

4.5.1.1 Settings for ATR-FTIR

The plastic receptacle used to contain illicit heroin was examined with a HazMatID System portable infrared (IR) spectrometer. The examination was accomplished by the use of a diamond sensor (attenuated total reflectance) with integrated video monitoring. The number of scans was set at 64, with a resolution of 4 and in absorbance mode between 4000 cm^{-1} and 650 cm^{-1}. The instrument was checked on a daily basis with a standard polystyrene film to ensure its optimum condition before use. A background spectrum was run before each sample measurement. The spectra obtained from the instrument were compared manually without the aid of a library. All quantitative IR data were processed by the HazMatID (version 3.1) software.

4.5.1.2 Analysis of Case Plastic Films

For each case, the substance was transferred from the plastic receptacle into a sampling bag and then the inner and outer surfaces of the receptacle were repeatedly and thoroughly cleaned with a tissue paper. The scanned areas were first examined to ensure that they were free of any observable contaminants. Due to limited access to the case samples, only two representative spectra were obtained for each case. The cleaned outer surface of the plastic receptacle was scanned with the HazMatID System portable IR spectrometer with the aid of a machine press.

4.5.2 Instrumental and Statistical Validation for Spectral Variation Using Specimen Plastic Films

Spectral variation was revealed quantitatively by the selected diagnostic peaks. Before the quantitative data were used for statistical interpretation, the reliability of those data was first validated using specimen plastic films. Two sets of specimens A and B (both of Type 1-polypropylene-based identified by IR) were prepared after the polymeric types were identified from the case plastic films. To ensure that specimens A and B were spectrally different quantitatively in the polymer composition of the film, both were purchased commercially from different brands and in different widths. Specimen A simulated sample plastic films that were subjected to uncontrolled variables such as environmental and instrumental factors during IR analysis while specimen B acted as a control for system check-up.

1. **Environmental and instrumental influences:** A specimen plastic film marked 'A' measuring 5.00 cm in width was used to evaluate the effects of optical contact between the film and the diamond surface. Two spots (labeled 'Spot 1' and 'Spot 2') were marked at different positions on the same film to ensure polymeric makeup consistency in each spot during analysis. Each spot was subjected to IR measurement under a set of five different conditions. Four degrees of force (1X, 2X, 3X and machine press) were applied on each spotted area of the film placed on the diamond stage. Another measurement was obtained with the diamond surface covered with a very thin layer of street heroin substance and under machine press. Examination was accomplished by using the HazMatID System portable IR spectrometer. The two spots were used to assess the extent to which uncontrolled factors affected the peak intensities.

2. **Precision:** To assess the intra-day (repeatability) and inter-day (reproducibility) precision of the IR spectral response and intra-batch variability, a batch of seven different specimen plastic packets B measuring 7.40 cm in width were examined and treated like case plastic films to obtain 8 spectra at random on the same day and a total of 20 spectra on different days.

4.6 Task 3: Profiling of Major Components by GC-FID and GC-MS

Task 3 was aimed at quantifying the absolute contents of eight major components (five opium-based alkaloids and three cutting agents) present in the illicit heroin samples. Prior to analysis, optimization and validation of the GC methods were performed. Statistical validation was achieved by employing eight simulated heroin links. Subsequently, these validated analytical and statistical procedures were applied to the case samples. The ultimate use of the GC data was to determine the number of possible origins responsible for the seized heroin.

4.6.1 GC-FID Procedures for the Profiling of Major Components

4.6.1.1 GC-FID Method

Quantitative analysis of eight major components was achieved by using an HP6890N GC-FID preinstalled with an HP7683 series autosampler and a J&W HP Ultra 2 (25 m x 200 µm x 0.33 um) capillary column. Chromatographic separation was accomplished by holding the oven temperature at 240 ^{o}C for 1 min and heating up to 270 ^{o}C at the rate of 12 ^{o}C/min. The oven was then held for 8 min at this temperature. The injector and detector temperatures were set at 290 ^{o}C and an injection volume of 1 µl with a split ratio of 40:1 was employed. The helium carrier gas flow rate was maintained at ~1.0 mL/min. Hydrogen flow, air flow and helium makeup flow were respectively set at 30 mL/min, 300 mL/min and 25 mL/min. The total run time was

approximately 12 min. All GC data were processed by the ChemStation (Rev.A.10.01[1635]) software.

4.6.1.2 Analysis of Heroin Case Samples

i) Internal Standard Solution

An appropriate weight of 2,2,2 triphenyl acetophenone (IS) was weighed into a 2000 mL volumetric flask to which methanol:chloroform in the ratio of 1:9 was added to the mark to obtain a final concentration of 0.18 mg/mL IS solution. The solution was sonicated for 10 min and it was kept for not more than 3 months.

ii) Calibration Standards and Control Sample

Appropriate weights of the eight target analytes (paracetamol, PC; caffeine, CF; dextromethorphan, DM; codeine, CD; morphine, MP; acetylcodeine, AC; 6-monoacetylmorphine, MM; diamorphine (heroin), HR) were weighed in a 25 mL volumetric flask, to which 0.18 mg/mL IS solution was added to the mark. The final concentrations of the analytes were obtained within the preferred ranges that were suitable for the profiling purpose based on the nature of the local samples. Three mixtures of standards were prepared throughout the course of the analysis:

1. **Calibration standard I for method validation and case sample analysis:** 0.16 mg/mL PC, 7.97 mg/mL CF, 0.08 mg/mL DM, 0.05 mg/mL CD, 0.08 mg/mL MP, 0.10 mg/mL AC, 0.26 mg/mL MM and 0.31 mg/mL HR in the presence of 0.18 mg/mL IS.
2. **Calibration standard II for simulated sample analysis:** 0.17 mg/mL PC, 7.64 mg/mL CF, 0.08 mg/mL DM, 0.05 mg/mL CD, 0.09 mg/mL MP, 0.11 mg/mL AC, 0.26 mg/mL MM and 0.30 mg/mL HR in the presence of 0.18 mg/mL IS.

3. **Quality Control (QC) sample:** 0.12 mg/mL PC, 7.24 mg/mL CF, 0.12 mg/mL DM, 0.08 mg/mL CD, 0.06 mg/mL MP, 0.09 mg/mL AC, 0.27 mg/mL MM and 0.20 mg/mL HR in the presence of 0.18 mg/mL IS.

All the standards were refrigerated for not more than 3 months. The calibration standard mixture was used for daily GC-FID calibration (one-point calibration). The concentration of each compound in the mixture was expressed in base form.

iii) Preparation and Analysis of Case Samples

For each case, approximately 80 – 100 mg of the homogenized sample was weighed and dissolved in 10 mL of 0.18 mg/mL IS solution in a glass volumetric flask, followed by 10 min of sonication. Each aliquot of the sample was injected into the GC-FID pre-calibrated by the standard mixture. For quantitative analysis, each sample was analyzed in duplicate and the results expressed in mg/mL were calculated to arrive at a mean concentration for each target compound. A QC sample was inserted between 20 runs to check for the system stability. Only when the percent errors of the QC sample fell within the acceptance limits, those runs before this QC sample can then be accepted for data analysis.

4.6.1.3 Optimization and Validation of the GC-FID Method

GC-FID parameters were optimized based on the peak resolution and overall peak shape through:

1. **Column choice:** Two sets of GC conditions were achieved by employing two GC capillary columns, namely Option 1: J&W HP Ultra 2 (25 m x 200 µm x 0.33 µm) and Option 2: J&W HP-5 (30 m x 250 µm x 0.25 µm) for separating a

mixture of the eight target compounds and the IS (each at approximately 0.1 mg/mL). Injector and detector temperatures, temperature programming, flow rate and split ratio were optimized based on the best achievement in peak resolution and peak height.

After the optimized GC method, using Option 1 (using J&W HP Ultra 2 capillary column) was decided, the sample preparation procedure was first checked with:

2. **Solvent studies:** Chloroform (100%) and methanol (100%) and the combinations of both in different ratios of methanol: chloroform (1:9; 3:7; 5:5; 7:3; 9:1) were investigated using a mixed standard of the nine compounds (the eight target analytes and the IS, each at 0.1 mg/mL). Each of the seven solvent types was injected six times consecutively.

With the overall optimized method, the GC was validated for the following aspects:

3. **Precision:** A standard mixture containing the eight target analytes at the preferred concentrations (ranging from 0.05 – 7.97 mg/mL depending on the analytes) in the presence of 0.18 mg/mL IS was used to study the repeatability and reproducibility of the instrument. For the repeatability, the standard was injected ten times consecutively on the same day to assess the intra-day precision. The GC was also programmed to inject every 3 hours up to 28 hours for ten injections to assess the inter-hour precision. Then, separate injections performed on ten different days were used to assess the inter-day precision (reproducibility).

4. **Linearity:** Linearity was studied using a series of standards. The initial concentrations (ranging from 0.1 – 10.0 mg/mL depending on the analytes) in a standard mixture were diluted to obtain a series of eight concentration levels with the IS held constant at 0.18 mg/mL. Each level was injected six times consecutively.

5. **Limit of detection (LOD):** The LOD of the instrument was determined from the lowest detectable concentration prepared at 1 μg/mL, 5 μg/mL and 10 μg/mL, for each component based on the signal-to-noise level of 3:1.

6. **Recovery studies:** A known amount of each target analyte was spiked into a sample matrix to yield a high spiked sample from which two dilutions were prepared to obtain medium and low spiked samples. The high, medium and low spiked samples were used for recovery studies.

With the validated method, other factors were also examined:

7. **Dissolving vessels and sample weight studies:** The extent of artifact formation in the polypropylene centrifuge tube (PT) and glass volumetric flask (VF) was investigated with three case samples respectively marked ‘A’, ‘B’ and ‘C’. From each sample, four quantities: 50 mg, 60 mg, 70 mg and 80 mg of the heroin substance were placed in both the PT and VF. The substance was dissolved in 10 mL of 0.18 mg/mL IS solution. Each aliquot was injected six times. The samples were analyzed on the day of preparation and then after four days. The loss of analytes and the presence of artifacts were evaluated. A preferred quantity was also determined.

8. **Sample stability:** Sample stability was examined by a pair of low and high concentration samples injected thrice on the first day and at every seventh day over a 1-month period.

9. **Method capability:** Ten case samples were analyzed using the optimized and validated method under different conditions (quantity, dilution factor and difference in daily calibration) to evaluate the method capability for profiling.

Statistical robustness of the optimized GC-FID method (Option 1) was evaluated against a second method (Option 2) by employing 43 case samples. The performance of the second method was investigated using a standard mixture containing the analytes at approximately the same concentrations used in the method validation of Option 1, but with 0.20 mg/mL IS (the optimized concentration for Option 2). Option 2 was used as a negative control to assess the robustness of the GC data obtained with Option 1. Nine other case samples were used to develop a novel chemometric procedure for sample classification.

4.6.2 Statistical Validation Using Simulated Heroin Links

4.6.2.1 Preparation of Samples for Simulated Links

Four individual homogenized samples (A1, B1, C1 and D1) were taken from separate unrelated seizures which were submitted as trafficking case samples. Prior to the cutting process, separate portions of samples A1, B1 and C1 were respectively mixed with selected cutting agents or/and opium-based alkaloids in small amounts to give three other derivative samples, A2, B2 and C2 (refer to footnote below Table 4.6). A mixed standard containing all the eight target analytes and phenolphthalein was also prepared in the laboratory to give sample E1. Table 4.6 summarizes the initial contents

of the eight initial samples that served as pre-cut samples. The eight components (excluding phenolphthalein) present in the precut samples were chosen because they are the commonly encountered analytes found in the illicit heroin seized in Malaysia.

Table 4.6: Contents (%) of eight target components in eight pre-cut samples

Sample ID	PC	CF	DM	CD	MP	AC	MM	HR
A1	~2.05	14.59	5.85	~0.05	0.19	3.15	3.28	47.27
A2	~2.05	13.87	5.65	~0.05	1.33	3.12	3.48	46.04
B1	0.02	5.08	4.30	0.40	2.68	3.54	13.95	38.03
B2	0.00	4.91	6.10	0.35	4.22	5.84	14.45	35.68
C1	17.46	29.69	3.36	0.39	1.12	2.10	5.34	23.05
C2	30.68	25.59	3.00	0.30	0.99	1.87	4.90	20.50
D1	0.59	11.18	6.36	0.20	0.68	3.84	4.62	49.14
E1	3.60	8.86	2.69	3.19	5.12	8.00	13.38	22.80

~ = Approximate value.
Note: A2 = MP and MM have been added to A1
B2 = DM, MP, AC and MM have been added to B1
C2 = PC has been added to C1

Prior to cutting, the eight pre-cut samples were respectively ground to homogeneous fine powders. On the other hand, two mixtures of cutting agents were prepared according to the ratios of PC: CF: DM (6:70:4) and PC: CF: DM (9:70:1) and marked ‘X’ and ‘Y’. These ratios simulated the frequently detected amounts in the local street samples which contained moderately low amounts of paracetamol and dextromethorphan in the presence of a significantly high amount of caffeine. Each pre-cut sample was mixed with either mixture of the cutting agents in one of four specified proportions in order to obtain four post-cut samples. A small quantity of water was added to transform the powder into a semisolid state to allow for mixing. Stirring the whole post-cut sample with a small quantity of water was to ensure even distribution of the eight components during cutting before the sample was heated to dryness. One half of the sample was kept as an uncolored portion while the other half was mixed with a

prescribed food coloring agent and water before it was heated to dryness. Table 4.7 summarizes the compositions of the post-cut samples prepared from the original eight pre-cut samples. Each of the 32 post-cut samples was divided into two and one half was colored while the other half remained uncolored, generating 64 post-cut samples. Samples derived from the same pre-cut sample are termed 'linked samples'; samples from different pre-cut samples thus 'unlinked samples'.

Table 4.7: Composition of post-cut samples generated from eight pre-cut samples

Pre-cut sample	Cutting agent[a]	PPS[b], % (Coloring agent)[c]				
		Cutting 1[d]	Cutting 2[d]	Cutting 3[d]	Cutting 4[d]	OR[e]
A1	**X**	5 (AR)	12.5 (TZ)	25 (GS)	50 (RBS)	100
A2	**Y**	5 (FG)	5 (BB)	25 (SY)	50 (CS)	100
B1	**X**	5 (AR)	12.5 (TZ)	25 (GS)	50 (RBS)	100
B2	**Y**	5 (R2G)	12.5 (EBS)	25 (AR)	50 (TZ)	100
C1	**Y**	5 (GS)	12.5 (RBS)	25 (FG)	25 (BB)	100
C2	**Y**	2.5 (R2G)	7.5 (EBS)	12.5 (AR)	25 (SY)	100
D1	**X**	2.5 (FG)	7.5 (BB)	12.5 (SY)	25 (CS)	100
E1	**X**	2.5 (SY)	7.5 (R2G)	12.5 (EBS)	25 (CS)	100

[a]Cutting agent = X contains PC: CF: DM (6:70:4); Y contains PC: CF: DM (9:70:1).
[b]PPS = The proportions of pre-cut sample (PPS) were designed as such in order to yield a wide range of low heroin contents (e.g.: 0.5%, 1%, 2%...) in the post-cut samples based on the pre-cut contents.
[c]Food coloring agents are applicable to the second portions of the post-cut samples. AR = Ammarant, TZ = Tetrazine, GS = Green S, RBS = Rhodamine BS, FG = Fast Green, BB = Brilliant Blue, SY = Sunset Yellow, CS = Carmoisine, R2G = Red 2G, EBS = Erythrosine BS.
[d]Cutting 1-4 = Each pre-cut sample was cut according to four specified proportions based on PPS (e.g.: 5% of A1 was mixed with 95% mixture X in the first cutting. Half of it was left uncolored whereas the other half was colored with AR.)
[e]Original pre-cut samples were not colored.

4.6.2.2 Analysis of Simulated Heroin Samples

To simulate the usual procedure adopted by a dealer who would scoop out the sample for packing, the individual post-cut sample was not homogenized through powdering. From each post-cut sample, three samples weighing 80 – 85 mg each were taken at random (any accessible powders and granules through scooping) and then introduced into three separate 10 mL volumetric flasks. The sample was prepared following the procedure for the case sample except that each solution was analyzed in

triplicate. The eight major components in 8 links X 9 samples X 3 random parts per sample X triplicate per solution = 648 aliquots were analyzed by the GC-FID.

4.6.3 GC-MS Procedures for the Profiling of Major Components

4.6.3.1 GC-MS Method

Qualitative analysis of the eight target analytes was achieved by using an HP6890N GC coupled with a 5975B inert MSD and preinstalled an HP7683 series autosampler. The GC was also preinstalled with a J&W HP5 (30 m x 250 µm x 0.25 µm) capillary column. Chromatographic separation was accomplished by holding the oven temperature at 240 ^{o}C for 1 min and heating up to 270 ^{o}C at the rate of 12 ^{o}C/min. The oven was then held for 8 min at this temperature. The temperatures of both injector and transfer line were set at 290 ^{o}C with a split ratio 10:1 and injection volume 3 µL. The helium carrier gas flow rate was maintained at ~1.0 mL/min. For the MS, a scan mode was employed to examine the mass/charge range of 40 – 450 m/z at 70eV. The total run time was less than 9 min. Each eluted peak in the total ion chromatogram (TIC) was integrated using the Enhanced ChemStation (version E.01.00.237) software. The peaks were identified by searching against the NIST98 library.

4.6.3.2 Analysis of Heroin Case Samples

Each sample was prepared following the procedure as for the GC-FID. The aliquot was injected once into the GC-MS.

4.6.3.3 Validation of GC-MS Method

Three validation aspects were emphasized for the GC-MS in order to ensure that it provides repeatable and reproducible results for qualitative analysis.

1. **Specificity and precision of results:** Ten independent case samples were spiked with a standard mixture containing the eight target analytes each at 0.08 mg/mL in the presence of 0.18 mg/mL IS. Each sample was analyzed individually. The occurrence of false negatives was recorded. The precision of the retention time under the influence of different sample matrices was also investigated.

2. **Precision of retention time:** A single mixture containing the eight target analytes each at 0.1 mg/mL in the presence of 0.18 mg/mL IS was injected ten times consecutively.

3. **LOD:** The LOD of the instrument was determined from the lowest detectable concentration prepared at 0.03 mg/mL, 0.05 mg/mL and 0.1 mg/mL, for each component based on the signal-to-noise level of 3:1.

4.7 Task 4: Profiling of Manufacturing Impurities by GC-FID

Task 4 was carried out when the heroin purity level for each case has been determined by Task 3. Prior to analysis, optimization and validation of the GC method were performed. Statistical validation was achieved by using two sets of five simulated heroin links. The first set utilized the 15 mg heroin base approach whereas the other employed a constant 650 mg substance approach. Subsequently, these validated analytical and statistical procedures were applied to the case samples. The ultimate use of the GC data was to determine the number of possible manufacturing batches responsible for the seized heroin.

4.7.1 GC-FID Procedures for the Profiling of Manufacturing Impurities

4.7.1.1 GC-FID Method

Quantitative analysis was performed on an HP6890N GC-FID with an HP7683 series autosampler. Chromatographic separation was achieved by temperature programming: 145 ^{o}C to 190 ^{o}C for 0.4 min at 8 ^{o}C/min, and to 320 ^{o}C for 5 – 7 min at 6 ^{o}C/min on a J&W HP Ultra 2 (25 m x 200 µm x 0.11 µm) capillary column. The helium carrier gas flow rate was maintained at ~1.2 mL/min. The injector temperature was set at 320 ^{o}C with a split ratio 1:25. The FID detector was maintained at 330 ^{o}C. Hydrogen flow, air flow and helium makeup flow were respectively set at 30 mL/min, 400 mL/min and 30 mL/min. The total run time was approximately 35 min. All GC data were processed by the ChemStation (Rev.A.10.01[1635]) software.

4.7.1.2 Analysis of Heroin Case Samples

i) 2 N Sulfuric Acid

An appropriate amount of sulfuric acid (96%) was pipetted out into a 1000 mL beaker containing 500 mL deionized water. The solution was mixed and stirred well before it was transferred into a 2000 mL volumetric flask. Deionized water was added to the mark to obtain a final concentration of 2 N sulfuric acid. The solution was sonicated for 10 min and it was kept for not more than 3 months.

ii) Extraction Solvent

An appropriate weight of n-tetracosane, C40 (the IS) was weighed in a 1000 mL volumetric flask. Toluene was added to the mark to obtain a final concentration of 0.6 µg/mL IS solution. The solution was sonicated for 10 min and it was kept for not more than 2 weeks.

iii) Sample preparation by liquid-liquid extraction

The method utilized two sample weight approaches during analysis: 1) a weight equivalent to 15 mg heroin base or 2) a constant weight of 650 mg illicit heroin. The weighed heroin substance was placed in a glass centrifuge tube. Liquid-liquid extraction (LLE) was performed by adding 5 mL of 2 N sulfuric acid and 5 mL extraction solvent to the tube. The mixture was vortexed vigorously and sonicated for 10 min. Phase separation was accomplished by centrifuging the mixture at 1800 rpm for 10 min. The upper organic phase was removed and evaporated to dryness. The residues were reconstituted in 100 µL toluene and the extract in a GC insert was injected 3 µL in duplicate in the GC. As the method was designed for semi-quantitative analysis, the duplicate results were obtained in 'peak area' and computed to arrive at a mean concentration for each target analyte.

4.7.1.3 Optimization and Validation of the GC-FID Method

i) Optimization and Validation by a Control Sample

A control mixture was prepared by dissolving seven n-alkanes (C15, C20, C25, C30, C33, C35 and C38) in n-hexane to obtain a concentration of 10 µg/mL for each n-alkane in the presence of 25 µg/mL C40 (IS). This control mixture was used to check the stability of the GC system in the absence of interference from the sample matrix. The optimum chromatographic separation was studied by:

1. **Column choice:** Four GC capillary columns were used to study peak heights. They included J&W HP-5 (30 m x 250 µm x 0.25 µm), J&W DB-1 (30 m x 250 µm x 0.25 µm), J&W HP Ultra 2 (25 m X 200 µm X 0.33µm) and J&W HP Ultra 2 (25 m x 200 µm x 0.11µm).

Upon completing the above task, the ideal column (J&W HP Ultra 2 with film thickness 0.11µm) was determined. With the chosen column, the following were further examined:

2. **Injection volume:** The control sample was injected four times consecutively at each of six injection volumes: 0.2 µL, 1.0 µL, 2.0 µL, 3.0 µL, 4.0 µL and 5.0 µL.

3. **Precision:** Intra-day precision (repeatability) was studied by injecting the control sample ten times consecutively on the same day. Inter-day precision (reproducibility) was achieved by investigating ten injections performed on different days.

4. **Linearity, LOD and limit of quantification (LOQ):** Linearity was performed using ten concentration levels covering 0.01 – 100 µg/mL n-alkanes with the IS held constant at 25 µg/mL. LOD and LOQ were theoretically determined based on three times the signal-to-noise ratio (S/N) and ten times the S/N respectively. Each level was injected six times consecutively.

ii) Optimization and Validation Using Validation Samples

Three heroin case samples (marked ‘A’, ‘B’ and ‘C’) were used as validation samples to check the suitability of the method as a whole in the presence of matrix interference for the following eleven aspects. Each validation sample was subjected to LLE using sulfuric acid and toluene containing the IS.

Freshly prepared extracts of the validation sample were combined and kept in individual inserts for optimizing aspects 1 to 6:

1. **Column choice:** The above-mentioned four GC capillary columns were used to study the peak shape and baseline.

2. **Ramping rate:** Six ramping rates: 2 ^{o}C/min, 4 ^{o}C/min, 6 ^{o}C/min, 8 ^{o}C/min, 10 ^{o}C/min and 12 ^{o}C/min were tentatively selected to study the peak resolution and peak symmetry.

With the chosen column (J&W HP Ultra 2 with film thickness 0.11µm) and desired ramping rate, the impurities of interest present in the validation samples were determined by:

3. **Peak identification:** A Shimadzu GC-2010 coupled with a GCMS-QP2010 Plus was employed to investigate the impurities present in the heroin samples with the aid of a CTC Analytics PAL System autosampler. Temperature programming was achieved using the same GC-FID conditions specified in Section 4.7.1a. The transfer line was set at 320 ^{o}C. For the MS, a scan mode was employed to examine the mass/charge range of 40 – 550 m/z at 70eV. Data processing was achieved by employing the Labsolutions (version 2.53 SU1) software. In this respect, 20 other different samples were used to further confirm the peak identities.

With the target impurities, the following were further optimized:

4. **Injection volume:** Each validation sample was injected four times consecutively at each of six injection volumes: 0.2 µL, 1.0 µL, 2.0 µL, 3.0 µL, 4.0 µL and 5.0 µL.

5. **Injector temperature:** Four injector temperatures: 260 ^{o}C, 280 ^{o}C, 300 ^{o}C and 320 ^{o}C were investigated by injecting each validation sample four times consecutively.

With the optimized injection volume and injector temperature, the method was validated by the following:

6. **Precision:** Intra-day precision (repeatability) was examined using ten consecutive injections of each of the three validation samples. Inter-hour precision (reproducibility) was achieved by injecting each validation sample every 3 hours to obtain a total of 17 injections per sample within 48 hours.

7. **Sample linearity**: Six concentration levels were prepared for each validation sample. To do this, 100 mL IS solution was evaporated and reconstituted with 2 mL toluene to give solution X. A concentrated extract (without IS) of the sample was prepared and reconstituted with solution X to give extract Y. Six concentration levels of the impurities were prepared by pipetting out 100 µL, 90 µL, 80 µL, 70 µL, 60 µL and 50 µL of extract Y intoindividual inserts which were then made up to 100 µL with solution X. This was to ensure that the IS was held at a constant concentration in each vial. Each level was injected four times consecutively.

Besides, other parameters were performed to optimize the extraction method:

8. **Extraction solvent:** Four organic solvents: n-hexane, ethyl acetate, chloroform and toluene were investigated to study the abundance of peak impurities.

9. **pH study**: Four equal quantities of each of the validation samples were placed in four individual tubes respectively containing 0.5 N, 1.0 N, 1.5 N and 2.0 N sulfuric acid. The sample was extracted three times consecutively. The performance of the acid was assessed based on the total recovery obtained from three sequential extractions.

10. **Extraction reproducibility**: This was examined by investigating six separate extractions of the validation sample.

11. **Extraction vessels**: Blank extracts obtained from a plastic tube and a glass centrifuge tube were investigated.

4.7.2 Statistical Validation Using Simulated Heroin Links

Five illicit heroin seizures respectively marked 'M', 'P', 'K', 'T' and 'Z' were utilized for statistical evaluation. They were treated as being unrelated ('unlinked samples') based on the histories of the seizures.

4.7.2.1 Preparation of Samples for Simulated Links Using the Weight Equivalent to 15 mg Heroin Base Approach

From each seizure, eleven related samples ('linked samples') were prepared: five samples of the original heroin from the seizure, two samples of the original sample cut with 100% caffeine, two samples with caffeine-paracetamol (2:1) and two samples with caffeine-paracetamol (1:1). From the heroin seizure, an appropriate weight equivalent to 15 mg heroin base was placed in each of eleven centrifuge tubes. Six of them were subjected to cutting. In each of the six tubes, the cutting agent was added according to the weight specified in Table 4.8. Therefore, all samples submitted for

analysis contained 15 mg heroin base despite the varying sample weights doubled by the cutting agents in the tubes. A total of 55 samples were subjected to LLE and treated like the case samples.

Table 4.8: Proportions of pre-cut sample and cutting agents for five simulated links using the sample weight equivalent 15 mg heroin base approach

Pre-cut sample	Weight equivalent to 15 mg heroin base (mg)	Cutting agents (mg)		
		100% CF	CF: PC (2:1)	CF: PC (1:1)
M	33	100	150	150
P	32	100	150	150
K	252	50	100	100
T	148	50	100	100
Z	38	120	60	60

Note: All samples contained 15 mg heroin base in the tubes.
CF = Caffeine; PC = Paracetamol

4.7.2.2 Preparation of Samples for Simulated Links Using the Constant 650 mg Weight Approach

From each of the five seizures, five related samples ('linked samples') were prepared: each heroin sample weighing 15 – 250 mg (depending on the heroin purity level) was placed in a centrifuge tube. Caffeine was added to make up the weight to 650 mg for extraction (Table 4.9). Therefore, each tube contained less than 15 mg heroin base (except for the fifth cutting of M, P, T and Z) but the sample weight in the tube was kept constant at 650 mg. A total of 25 post-cut samples were subjected to LLE and treated like the case samples.

Table 4.9: Proportions of pre-cut sample and caffeine for five simulated links using the constant 650 mg weight approach

Pre-cut sample	Weight equivalent to 15 mg heroin base (mg)	Pre-cut sample (mg) : CF (mg)				
		1	2	3	4	5
M	33	15:625	20:630	25:625	30:620	35:615
P	32	15:625	20:630	25:625	30:620	35:615
K	252	50:600	100:550	150:500	200:450	250:400
T	148	30:620	60:590	90:560	120:530	150:500
Z	38	20:630	25:625	30:620	35:615	40:610

Note 1: After cutting, only samples 5 contained approximately 15 mg heroin base.
Note 2: All samples were at 650 mg irrespective of the amounts of heroin base present in the samples.
CF = Caffeine

4.8 Task 5: Profiling of Trace Elements by ICP-MS

Task 5 was performed to quantify the elemental content in the illicit heroin. The ICP-MS method was validated both by standard mixtures and heroin samples. Statistical validation was performed by using eight replicates of six unrelated samples. Subsequently, the overall analytical and statistical procedures were applied to the case samples. Comparison between the elemental compositions in the heroin samples and other sources (drinking water and contaminated water) was also performed. The ultimate use of the ICP-MS data was to determine the relationships between the samples at the distribution/street level.

4.8.1 ICP-MS Procedures for the Profiling of Trace Elements

4.8.1.1 ICP-MS Method

A Perkin Elmer SCIEX ELAN DRC-e ICP-MS equipped with an ASX-520 autosampler was used in this study. The operating parameters of the instrument are summarized in Table 4.10. The ICP-MS data were processed by the ELAN (version 3.4) software.

Table 4.10: Operating parameters for the ICP-MS

Parameter	Magnitude
Nebulizer gas flow/L min^{-1}	0.82
Argon Auxiliary gas flow/L min^{-1}	1.2
Argon plasma gas flow/L min^{-1}	15
Lens voltage/V	6
ICP radiofrequency power/W	1550
Analog stage voltage/V	-1850
Pulse stage voltage/V	950
Sampler cone	Ni
Skimmer cone	Ni
Nebulizer	Ryton, double-pass Scott-type spray chamber and the GemTip Cross-flow
Scanning mode	Peak hopping

4.8.1.2 Analysis of Heroin Case Samples

i) Dissolving Acid Solution (1% HNO_3)

An appropriate amount of nitric acid (< 70%) was respectively added to 1) a plastic bottle containing deionized water generated from an Elga Maxima (18.2 MΩ) filter to obtain a final solution of 1 L of 1% HNO_3, and 2) a plastic bottle containing the same deionized water to obtain 100 mL of 10% HNO_3. The 1% acid solution was prepared freshly on a daily basis.

ii) Standard Preparation

An appropriate amount of each of the standards was pipetted out from the element stock solution and placed in a plastic tube. The mixture of the standards was then diluted with 1% HNO_3. Three standard mixture solutions and a quality control (QC) sample were prepared in the following concentrations:

1. **Standard 1:** minor elements at 0.01 ppm, major elements at 0.1 ppm, Hg at 0.001 ppm
2. **Standard 2:** minor elements at 0.02 ppm, major elements at 1.0 ppm, Hg at 0.002 ppm
3. **Standard 3:** minor elements at 0.1 ppm, major elements at 10.0 ppm, Hg at 0.005 ppm
4. **QC sample:** minor elements at 0.05 ppm, major elements at 5.0 ppm, Hg at 0.0025 ppm

All the standard mixtures were prepared freshly on a daily basis. A mixture of the four ISs was prepared in a large container at 0.01 ppm each. The instrument was supplied with the IS mixture from this external source.

iii) Calibration

Prior to calibration, the instrument was flushed with 1% HNO_3 for 30 min after which a daily internal performance check was performed automatically by the instrument. After the instrument passed the check, the three standard solutions were run on the instrument to obtain calibration curves for the target elements. Subsequently, the QC sample was analyzed and the quantitative results of all the target elements in the QC sample were obtained from the curves. The reliability of the curves were verified when the percent error in the concentration of each element was better than ± 20% (e.g. the maximum acceptable error decided for this task).

iv) Sample Preparation

Approximately 30 mg heroin sample was weighed into a 15 mL plastic tube, to which 10 mL of 1% HNO_3 was added. The solution was shaken vigorously and

sonicated for 10 min. Each solution was checked for the presence of particles. Another 10 min of sonication may be required to completely dissolve the particles. If further sonication did not dissolve the particles, filtration was performed using a filter paper (90 mm ø x 100 circles) that was previously rinsed with deionized water. Each aliquot was analyzed in duplicate by the ICP-MS pre-calibrated by Standards 1, 2 and 3. The QC sample followed by a blank was inserted before the sample sequence and carried out after every fiftieth run. Only when the percent errors of the QC sample fell within the acceptance limits, those runs before this QC sample can then be accepted for data analysis.

4.8.1.3 Partial Method Validation

Six aspects of method validation were performed using a standard mixture, QC sample and illicit heroin samples.

1. **Precision:** The Standard 3 and QC sample respectively containing 20 target elements at the desired concentrations were injected eight times consecutively on the same day to assess the intra-day precision (repeatability). Inter-day precision (reproducibility) was studied by injecting each of the mixture once on eight different days.

2. **Linearity, LOD and LOQ:** A series of ten levels covering 0.01 – 10000 ppb of the major elements, 0.001 – 1000 ppb of the minor elements and 0.0001 – 100 ppb Hg were used to study the linearity of the detector's response. Each level was injected six times consecutively. The LOD was determined from the lowest detectable level, whereas the LOQ was assessed based on the precision of the analyte level.

3. **Recovery studies:** A mixture of standards containing 5 ppb Hg, 100 ppb of the minor elements and 10000 ppb of the major elements was prepared. A sample was spiked with 6 mL, 4 mL and 2 mL of the mixed standard in three tubes before they were made up to 10 mL to yield the high, medium and low levels of spiked samples for recovery studies.

4. **Sample precision:** Three random case samples (marked 'X', 'Y' and 'Z') were chosen for sample precision studies. For the intra-sample precision, each sample was prepared in a tube that was analyzed repeatedly for six times. For the inter-sample precision, the sample was prepared in six different tubes, each was analyzed in duplicate.

5. **Sample weight studies:** Two samples (high and low heroin purity respectively marked 'H' and 'L') were prepared using six different weights in individual tubes: 10 mg, 20 mg, 30 mg, 40 mg, 50 mg and 60 mg to determine the most suitable weight for analysis. Each tube was analyzed six times.

6. **Dissolution vessels:** The contamination levels of two different commercial brands of 15 mL plastic tubes were investigated.

7. **Filtration:** The contamination and loss of elements arising from filtration were estimated using three case samples.

4.8.2 Statistical Validation Using Simulated Heroin Links

Six samples (marked '1' to '6') showing highly different elemental compositions were selected for this study. Each sample was weighed into eight different

plastic tubes, totaling to 48 samples for analysis. The samples were prepared like case samples and colloidal samples were filtered if necessary. Each aliquot was analyzed in duplicate by the ICP-MS.

4.8.3 Additional Studies

Household water could have been used by the dealer to cut/dilute relatively pure heroin samples. Therefore, the street heroin may contain metals coming from various sources including the drinking/tap water. In this regard, metal contents in various water sources were also investigated.

4.8.3.1 Drinking Water

Data of 103 water samples were obtained from the Department of Chemistry Malaysia. These samples were sampled from different sources/states by the local enforcement body in late-July, 2011 within the country and they were submitted as drinking water samples (containing 1% HNO_3) for trace metal analysis. The water samples were analyzed by the same ICP-MS instrument using the same settings. However, the obtained data were only spatially representative of different locations and not temporally representative of the sampling times.

4.8.3.2 Piped Water and Water from a Contaminated Container

The following procedure was carried out in early-August, 2011.

Three different taps directing treated water for immediate household consumption were selected. For each of the three selected taps, three samples were taken. For each sample, 1 mL of 10% HNO_3 was added into a 15 mL pre-rinsed plastic tube containing 9 mL piped water. This was to ensure that the final solution contained 1% HNO_3 to preserve the target elements.

Some of the water was kept in a rusty container from which three samples were taken and prepared and treated like the piped water.

Each of the above 12 samples was analyzed in duplicate by the ICP-MS.

4.9 Task 6: Data Management and Database Build-up

4.9.1 General Procedure

All instrumental findings were collected and collated. Data manipulation and data storage were accomplished by using the following:

1. Microsoft® Excel 2002: Descriptive statistics and data pretreatment were performed prior to multivariate analysis
2. Minitab 15: Inferential statistics (especially t-test and correlation analysis) and multivariate analysis were carried out.
3. SPSS (ver. 18.00): Multivariate analysis was done to supplement information not obtainable with Minitab 15
4. FileMaker Pro 8: All physical and chemical information of the heroin cases were collated and recorded as individual profiles in a database.

4.9.2 Pretreatment Methods

Prior to sample classification, the data were subjected to a number of pretreatment methods such as normalization (N), standardization (S), logarithm (L) and fourth root application (4R). They were performed independently or in combination with two or three pretreatments. Normalization will first organize each sample/row of data in a comparable format, limiting the data to the maximum value of 1 when divided by the sum of the variables of the sample. After the normalization, some variables may show high values (e.g. 0.193) as compared to those with low values (e.g. 0.073). If the

normalized data are not further pretreated appropriately, the clustering outcome will most probably be influenced by the high values. Hence, the second pretreatment step will organize each variable/column in a comparable format, equating the role of all the variables in the dataset. The specific pretreatments chosen for each task are discussed in their respective sections.

4.9.3 Multivariate Analysis

Four major multivariate analyses, namely principal component analysis (PCA), hierarchical cluster analysis (HCA), K-means clustering (K-MC) and discriminant analysis (DA) were chosen. They were used to cluster the samples using data containing at least four variables. Specifically, PCA commonly operates in correlation mode and this is also the default mode used by most chemists. The final results obtained in this mode are usually standardized data and therefore all the variables have the same weightage in the dataset. Preferably, the data are analyzed in this mode if data standardization is not intentionally ruled out.

For statistical validation, the covariance mode was chosen to evaluate pretreatments if standardization is deliberately ruled out. Instead, if standardization is required, it was performed separately prior to PCA. For pretreatment methods ended with standardization as the last step such as N + 4R + S, the use of N + 4R in correlation mode during PCA will reveal the same data distribution as the N + 4R + S in the covariance mode. Depending on the objective, the mode of PCA is also mentioned in the related sections to indicate the application of standardization.

In this study, the correlation coefficient was adopted to mean the coefficient of determination (r^2) obtained from the calibration regression line. Pearson correlation coefficient (r^2) was used to measure the association between two target variables. For the latter, the symbol of r^2 instead of r is used throughout this study because it shows the

relationship of the two variables being multiplied as per r X r = r^2. In this study, the symbol of r^2 is also used to mean the degree of agreement between the two variables or two sets of data without specifically referring to its true meaning in the strictest sense.