CHAPTER 6

CONCLUSION

6.1 Summary of the Findings

From each heroin case, a rich amount of useful forensic information can be extracted for physical and chemical characterization. For the profiling of heroin, six tasks have been performed on a total of 311 heroin cases collected from January to August 2010 at the Department of Chemistry Malaysia. The intelligence data obtained were able to provide new knowledge about the heroin samples at the source/origin, production/manufacturing and distribution/street levels.

Task 1: Preliminary analysis of the heroin seizures was started off with visual examination and physical characterization in order to obtain quantitative measurements and descriptive data about the physical appearance of the seizure. The new photographic technique introduced in this study was found to be economic and practical for routine documentation. The use of the simple color codification system also proved user-friendly. Among the recorded substance colors, off white-brown was found to be the most prevalent color (41.16%) in the local heroin seizures. Besides, the texture of the drug substance varied and this may be useful to indicate the period of distribution of the packaged heroin in the street sale. In particular, 86% of the total heroin cases were found to be in coarse structures. The heroin packer also preferred to adopt the Type 3a wrapping technique to pack the drug substance. The seal patterns were usually clear and complete, with the majority bearing rectangular patterns. The commonly encountered substance weight per package, weight per packet, plastic receptacle width and film thickness were $1.25 - 1.45$ g, $0.46 - 0.54$ g, $6.20 - 6.40$ cm and $42 - 52$ µm respectively. Additionally, validation was done on these four physical measurements in

order to evaluate the reliability of the data and the film thickness was found to be the least reliable parameter as it showed relatively high intra-batch variation. Using these four parameters for statistical clustering, principal component analysis (PCA) suggested a major cluster, in which the cases shared these measurements in close agreement. Documenting visual and physical characteristics could be time-consuming. However, it is eventually worthwhile because a large body of data can be obtained.

Task 2: The polymeric composition of the plastic film is another useful source of intelligence information. At the macro level, five major types of plastic packets were detected with the aid of attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR). In this study, it was found that the quantitative data of the IR spectra could be useful for sample classification. However, data normalization must be carried out before the data are used for distribution/street level sample-to-sample comparison. With the normalized data, the instrument was first checked using film specimens and it was found that the instrument showed repeatable (RSD < 2%) and reproducible (RSD < 3%) readings. When the most frequently encountered Type 1-polypropylene-based plastic packets were further distinguished by PCA after data normalization, 197 case films involved were found to be widely similar at the street level. The IR technique by itself can be used as the preliminary step in forensic profiling. Further analysis on other aspects associated with these packages is required for more precise intelligence work.

Task 3: A method using gas chromatography-flame ionization detection (GC-FID) for the quantification of eight commonly encountered major components in the illicit heroin was optimized and validated. The validation results demonstrated high selectivity with the use of an HP Ultra-2 capillary column. Different solvents were studied and methanol:chloroform (1:9) proved best for sample dissolution. The overall method is rapid (< 12 min), repeatable (RSD < 1.5%), reproducible (RSD < 5% except

for morphine), sensitive (LOD from 0.4 – 6.1 µg/mL), accurate (recoveries from 99 – 102%) and has sufficient linearity ($r^2 \geq 0.9997$) for the target compounds. The capability of the validated method was again evaluated with ten case samples and it was concluded that the GC-FID method has sufficient capability for the determination of the eight target analytes.

The robustness of the optimized GC-FID method was assessed statistically by pattern analysis using 43 heroin samples of unknown origins. Based on the data distribution pattern given by the PCA, the method proved robust for the GC data irrespective of whether the data were calibrated with chemical standards or not. It was shown that assessing the method robustness using pattern analysis is a more reliable technique for the analytical method intended for profiling as it involves the obtained data in the statistical validation process through pattern recognition/sample classification.

Eight simulated links containing 72 samples were prepared to simulate the major component compositions commonly encountered in the real case samples. The simulated dataset was used to validate the best pretreatment method, to find the ideal alkaloid quotients and to verify the best combination of linkage method and distance measure for hierarchical cluster analysis (HCA). All GC data were obtained by analyzing the samples using the optimized method. Through a step-by-step chemometric procedure, Ward-Manhattan was found to be the most suitable statistical technique for HCA by using the Normalization$_{selected}$ + Standardization ($N_{selected}$ + S) pretreatment on eleven chosen quotients. This technique was able to cluster all the linked samples under their respective groups with distinct separation between the unlinked samples despite the wide range of natural compositional variations observed in the dataset.

Gas chromatography-mass spectrometry (GC-MS) was also employed to confirm the identities of the major components that were present in the heroin samples. Prior to use, the instrument preinstalled with an HP-5 capillary column was validated using a standard mixture and a group of ten genuine heroin samples. The instrument showed 100% accuracy in identifying the target analytes. The compounds also showed repeatable relative retention times (RSD < 0.2%). Sufficiently low LODs ranging from 2.5 – 7.9 µg/mL were also obtained. This method is rapid and each sample requires < 9 min to complete the analysis.

As the GC-MS and GC-FID showed high reliability for the profiling of the eight major components, the 311 case samples were subjected to qualitative and quantitative analyses using both the methods. It was found that other excipients such as aminophylline and acetamide (N-phenyl) were also detected in the samples. Among all detected diluents, caffeine was frequently employed for cutting/diluting high purity heroin samples. Two samples were found to be fake heroin. By employing opium-based alkaloids to form eleven quotients pretreated via $N_{selected} + S$ for source/origin level sample classification, PCA grossly suggested that at least two independent sources were responsible for the samples. On the other hand, HCA (Ward-Manhattan) indicated that the samples were closely related in several small clusters at high similarity levels. Several large clusters were also obtained at very dissimilar levels.

With the aid of the opium-based alkaloids, a novel tetrahedron model for sample classification has been developed to simplify the chemometric procedure. This model does not aim to replace the well-established statistical techniques such as PCA and HCA. It serves as an alternative for gross estimation of sample similarity or dissimilarity. The model was found to be suitable for chemists with limited statistical knowledge as this approach employs simple mathematical algorithms. A pilot test

utilizing 9 case samples was performed with this model. The technique was found to show similar results with those obtained with the PCA.

Task 4: A total of 12 impurities have been identified from the case samples. These impurities present in the three selected validation samples were used for optimizing a GC-FID method for impurity profiling. In addition, a series of n-alkanes were also prepared as a control mixture to check the instrument performance. The validation results demonstrated good peak separation with the use of an HP Ultra-2 (0.11 µm) capillary column at 3 µL injection volume and 320 °C injector temperature. Liquid-liquid extraction using different acid strengths and different solvents was investigated and the results showed that 2 N sulfuric acid and toluene gave the best extraction. The overall method is reasonably rapid (< 35 min), repeatable (RSD < 5%), reproducible (RSD < 10% except outliers) and has sufficient linearity (r^2 > 0.968) for the target impurities. It was also found that the related samples can be successfully classified with high correlation despite the poor extraction reproducibility. The limitation of this method is that it requires a large sample amount and a large solvent volume for analysis.

Two separate sets of simulated links respectively containing 55 and 25 samples derived from five unrelated case seizures were prepared. The former set was analyzed using the conventional sample weight equivalent to 15 mg heroin base approach while the latter employed the 650 mg constant weight approach. These datasets were subjected to statistical validation. In HCA, Ward-Squared Euclidean and Ward-Squared Pearson proved superior for both sample weight approaches after the impurity data were pretreated via Normalization + Standardization (N + S). Besides, either of the combinations was also able to cluster all the related samples under their respective links when the two datasets were analyzed simultaneously by HCA.

A total of 298 heroin samples were subjected to impurity profiling using the optimized GC-FID method. The remaining 11 case samples were not analyzed because they contained reactant compounds with the extracting acid. Semi-quantification was performed based on peak areas. Peak identification was confirmed by the relative retention times. Among the 12 impurity peaks, peaks 1, 4 and 5 were detected in all the samples. They were also present in high concentrations. Other peaks were only present in trace amounts. Using the harmonized statistical model to cluster these samples (in N + S impurity data) through HCA (Ward-Squared Euclidean or Ward-Squared Pearson) in order to obtain the final two groups, the results illustrated a major group and a minor group on the dendogram. The major group contained samples exhibiting close relationships at high similarity levels.

Task 5: Quantification of trace elements using inductively coupled plasma-mass spectrometry (ICP-MS) is not only simple and rapid; it can also offer a rich amount of data in less than a minute. The overall ICP-MS method was validated and it was found to be repeatable (RSD < 6%), reproducible (RSD < 6%), sensitive, accurate (recoveries from 92 – 108%) and have sufficient linearity for 18 target trace elements. In this study, the use of direct dissolution of a 30 mg sample saves time and sample size. However, solutions that show undissolved particles will still require a filtration step. Unfortunately, the elemental contents may be affected by filtration. Notwithstanding this, the results obtained from direct dissolution and filtered solutions were able to provide forensic values in the street level sample classification when suitable data pretreatment was carried out.

During the analysis, only 16 trace elements were chosen. Boron (B) and antimony (Sb) were excluded as they showed unreliable results in certain samples. A wide range of variations in the original ICP-MS data was observed when six batches of eight related samples were analyzed with the validated method. The Normalization +

Fourth root (N + 4R) pretreatment method was useful to minimize the variation. PCA was tested to have insufficient discriminative power in segregating certain samples using the pretreated ICP-MS data. Similarly, HCA (Ward-Squared Euclidean) showed only 8% mistakes in clustering the related samples under their respective batches using the pretreated data.

To evaluate whether the metal contaminants found in the heroin samples were from other sources such as tap water and contaminated utensils, the elemental compositions of 309 genuine heroin samples were compared against the metal contents of 103 samples of tap water taken from various locations within Malaysia. In addition, the discrepancy in the elemental compositions between the heroin samples and those from known piped water and water from rusty containers is also investigated. It was discovered that the elements in the street heroin products are the sum of contaminants acquired from plant uptake to packing. In particular, sodium (Na) and calcium (Ca) were frequently found in high concentrations in the heroin samples. Minor elements were potassium (K), zinc (Zn), manganese (Mg) and iron (Fe). Data of 16 N + 4R pretreated trace elements detected in the 309 samples were analyzed by PCA and HCA (Ward-Squared Euclidean), and both techniques indicated that a large number of samples could have originated from two major sources. Each source is considered to have a specific contamination history. This in turn infers that most heroin samples have undergone two specific sets of distribution activities. However, exceptional cases were also detected. These samples have undergone contamination histories that are measurably different from those of the two major sources.

Task 6: A database was established to document the physical and chemical data of the 311 cases considered in this study. The database was designed to be user-friendly. A single profile or matched profiles can be retrieved by specifying the categorical data.

Among all the analyses, the major components were found to be the most reliable indicators for the profiling of heroin. First, this task requires less sample preparation and analysis can be accomplished very rapidly. Second, the concentrations of the compounds are sufficiently high to reach the linearity range. Third, a relatively smaller sample weight (< 100 mg) is required. Fourth, the absolute concentrations of the adulterants and opium-based alkaloids can be used to link samples at the street level. Fifth, opium-based alkaloids can be normalized for source/origin level comparison. In contrast, the trace elements are probably the least reliable data as the elements showed highly variable data and are only useful to indicate sample relationships at the distribution/street level.

6.2 Suggestions for Future Work

More thorough analysis can be performed in Task 1. For example, measuring the pH of the seized heroin and collecting the particle sizes of the substance can be included to add more parameters to the physical characterization.

The IR data of the plastic films in Task 2 are useful for street level package-to-package comparison. To maximize the utility of this data, it is proposed that a database be established to store the information of the drug plastic packages as well as the commercial plastic packets/bags. Comparison between the drug plastic packages can reveal the street level relationships. On the other hand, commercial plastic films may be indicative of the locations from which the drug packages were purchased.

In Task 3, chloroquine should be included as the ninth major component for the Malaysian heroin. As the developed GC method is able to separate this compound from other compounds, hence additional validation studies can be easily done on chloroquine for it to be used in profiling.

The GC technique coupled with the liquid-liquid extraction in Task 4 may be less efficient in extracting and separating most of the manufacturing impurities from highly cut samples. More advanced and sensitive techniques should be developed. For example, solid phase microextraction (SPME) could be a potential technique to extract additional impurities.

More trace elements can be incorporated in Task 5. Despite the highly variable data, the qualitative data may still be useful to distinguish between samples.

Finally, the database established in Task 6 should be maintained on a regular basis. At the very least, approximately 30 random samples per month should be updated to the database so that it can keep track of the general development of the trafficking of illicit heroin.