Characterization and Classification of Heroin from Illicit Heroin Seizures by GC/Q-TOF

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Overview

To counter illicit drug trafficking is one of the main responsibilities of law enforcement institutions. Integral and important part in addressing this issue is the forensic examination that helps to obtain maximum information about the composition and quality of the seized drugs.

We have developed a GC/Q-TOF method for comparison of heroin samples and determination of possible distribution linkages between samples from different seizures by impurity profiling. Impurity profiling of "street heroin" performed using high resolution accurate mass GC/Q-TOF system that is highly sensitive in full acquisition mode and therefore ensures high confidence in the identification of the large number of trace components in heroin sample.

Introduction

During investigation of criminal cases related to the sales of narcotic drugs, one of the main tasks is to identify a full group of criminals who mediated the trafficking of narcotic substances within a country. Thus, to address this problem it is necessary to perform a comparative study of heroin samples as their characterization and classification would help establishing common sources of origin.

The present work is focused on the most common illicit drug – heroin, or so-called "street heroin", *i.e.* heroin confiscated from the illicit trafficking. Such heroin is usually a multi-component mixture comprising, apart from the heroin, of various additives such as pharmacologically active compounds as well as neutral substances.

The study benefited from the accurate mass high resolution capability of the Agilent GC/Q-TOF system particularly useful in the identification of the composition of the heroin samples. The characteristic profiles of heroin samples were the basis for further statistical analysis performed in Mass Profiler Professional (MPP).

Methods

The heroin samples were first extracted by chloroform followed by ethylamine treatment to remove extenders. The lower phase was collected for further analysis. The samples were analyzed by GC/Q-TOF in EI mode. Two different acquisition methods were utilized to separately acquire data for major and minor components of the samples. This was done in order to compensate for differences in ion intensities between corresponding to major and minor components. The method for major compounds utilized reduced emission current on a filament in order to avoid saturation. The method used to acquire data for minor compounds, had higher emission current but decreased ionization energy during elution major components (see Table 1 for further details).

This study was performed using an Agilent 7890 GC coupled to an Agilent 7200 Series Quadrupole-Time-of-Flight (Figure 1). GC and MS conditions are described in Table 1.

Figure 1. 7200 series GC/Q-TOF system.

GC and MS Conditions:	Major Components	Minor Components
Column	HP-5MS, 30 m, 0.25 mm ID, 0.25 µm film	
Injection volume	0.3 µL	0.5 µL
Split ratio	400:1	30:1
Split/Splitless inlet temperature	280 °C	
Oven temperature program	100 °C for 1 min 10 °C/min to 280 °C, for 3 min 10 °C/min to 300 °C, for 5 min	
Carrier gas	Helium at 1.2 mL/min constant flow	
Transfer line temperature	290 °C	
Ionization mode	EI	
Source temperature	230°C	
Quadrupole temperature	150°C	
Scan range	50 to 500 m/z	
Spectral acquisition rate	5 Hz, both centroid and profile	
Emission	12µA	35 µA
Ionization parameters used in the method for minor compounds		
Time segments	Ionization Energy, eV	Time range, min
1	12	11,9 – 12,4
2	12	15,0 – 15,8
3	12	19,3 – 19,8

Table 1. GC-MS conditions used in the study.

The chromatographic deconvolution was performed using MassHunter Unknown Analysis software. Impurities of interest were identified by comparison with the NIST11 mass spectral library. The multivariate statistical software package Mass Profiler Professional (MPP) was used to find compounds present at distinct levels in different groups of samples. The data were subsequently used to build a classification model.

Results and Discussion

One of the main goals of this study was to detect and classify opium alkaloids that include natural alkaloids as well as pharmacologically active synthetic or semisynthetic additives.

To identify components in the samples we have first performed chromatographic peak deconvolution using Unknowns Analysis tool of MassHunter Quantitative Analysis software package followed by NIST11 MS library search. The samples from 22 seizures have been processed in MassHunter Quantitative Analysis, and relative ratios of alkaloids were determined according to the formula:

$$Un = \frac{Sn}{S(ac) \times \sum ni}$$

Un – relative ratio of the alkaloid n,
Sn – Area of alkaloid n,
S(ac) – Area of the acetylcodeine,
Σn_i – Area sum of all detected alkaloids

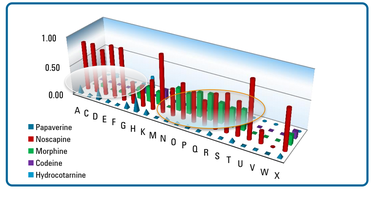

Figure 2. Comparison of the natural alkaloids identified in the samples, relative to acetylcodeine.

Next, Mass Profiler Professional (MPP) software package was used for further statistical analysis. The Filter by Flag tool in MPP was utilized to find the most common compounds across all 22 samples.

Most commonly found components of the heroine samples are morphine alkaloids and morphine derivatives. For example, 54 out of 55 samples contained monoacetyl derivative of morphine: 6-monoacetylmorphine and acetylcodeine. Many other common alkaloids as well as pharmacologically active substances were also detected in the majority of the samples (Table 2).

Alkaloidal		Adulterants	
Noscapine	50	Caffeine	55
Papaverine	50	Dextromethorphan	27
Meconine	43	Tolycaine	18
Morphine	40	Paracetamol	5
Hydrocotarnine	13		
Codeine	10		

Table 2. Alkaloidal impurities and non-opiate pharmacologically active cutting agents identified in heroin samples.

The clustering of the data acquired for both major and minor components was evaluated using Principal Component Analysis (PCA) plot (Figure 3).

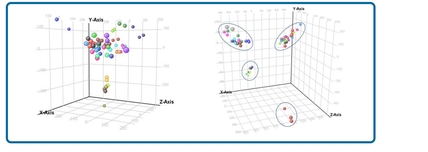

Figure 3. PCA plot illustrates no significant separation between the analyzed sample groups when the GC/Q-TOF method for major components was applied (left). The data acquired using the method for minor components displayed significant separation into at least two major groups (right).

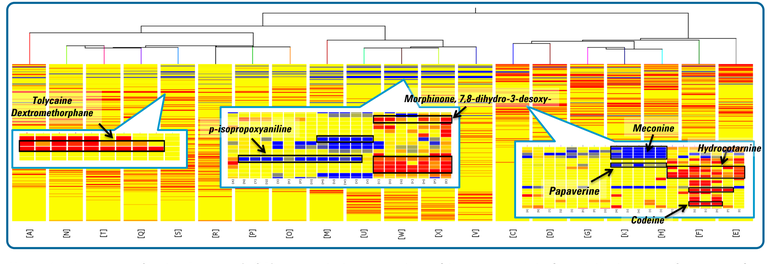

Figure 4. Hierarchical Cluster Analysis (HCA) demonstrated separation of the samples into few distinct groups. Shown are few characteristic compounds that are likely to contribute into separation of the samples.

Results and Discussion

The compounds that contributed to the major difference between isolated sample groups were further visualized using Hierarchical Clustering (Figure 4). Specifically, we were able to observe multiple alkaloids of heroin as well as pharmacologically active agents that contributed into the sample clustering. Hierarchical clustering also confirmed the presence of two major sample groups (Figure 4).

For few compounds that did not give a high library match score we performed additional confirmation steps using accurate mass information as well as MassHunter Qualitative Analysis structure elucidation tools. An example is shown on Figure 5. The compound present in 36 out of 55 samples was tentatively identified as 6-acetyl-crotonosine acetate. The identity was evaluated using Molecular Formula Generator (MFG) with library search and Fragment Formula Annotation (FFA) tools (Figure 5). Further confirmation of the identity of this compounds will be performed using MS/MS to verify whether ion m/z 282.1081 is a product ion of the m/z 367.1418.

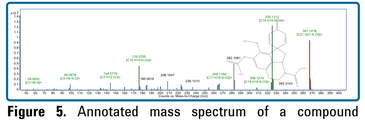

Figure 5. Annotated mass spectrum of a compound tentatively identified as 6-acetyl-crotonosine acetate.

Thus, using GC/Q-TOF acquisition method for minor components in combination with statistical analysis performed in MPP we were able observe separation of the data into distinct clusters. The presence of two major clusters using both PCA and Hierarchical plots was consistent with the results of semiquantitative analysis of the impurities identified in the samples (Figure 2). These two major clusters were further used to construct a class prediction model in MPP. The samples that were earlier separated into the two largest groups were further evaluated using PCA plot to confirm clustering as well as volcano plot to visualize compounds specifically contributing into this separation (Figure 6).

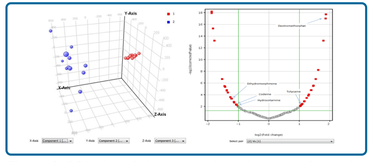

Figure 6. PCA plot of the two evaluated groups of samples chosen to build classification model (left); volcano plot showing compounds that present at significantly different levels between two groups of samples (right).

Results the validation of the class prediction model using Decision Tree algorithm are shown in Figure 7.

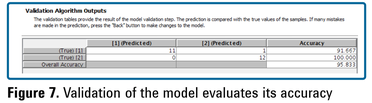

Figure 7. Validation of the model evaluates its accuracy

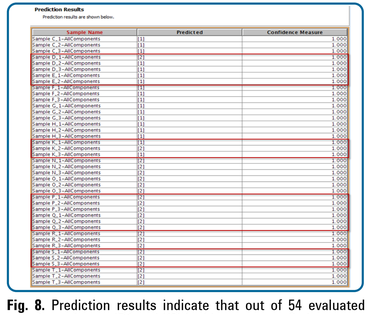

Fig. 8. Prediction results indicate that out of 54 evaluated samples only two (D1 and K2) had incorrect prediction.

Conclusions

- Multiple statistical approaches using MPP were utilized to isolate the heroin samples, analyzed by GC/Q-TOF, into distinct clusters
- Class Prediction model was built in MPP based on two largest groups of heroin samples
- Accurate mass information helped to confirm the identity of alkaloids and other pharmacologically active compounds found in the samples