Characterization and Classification of Heroin from Illicit Drug Seizures Using the Agilent 7200 GC/Q-TOF

Application Note

Forensics

Authors

Koluntaev Dmitry
InterLab Inc.
Moscow, Russia

Sergei Syromyatnikov and
Igor Sarychev
Federal Drug Control Service of Russian Federation
Moscow, Russia

Sofia Aronova
Agilent Technologies, Inc.
Santa Clara, CA

Abstract

An untargeted GC/Q-TOF analysis approach has been used to successfully differentiate street heroin samples that may originate from separate criminal enterprises. The statistical analysis tools in Agilent Mass Profiler Professional Software were used to construct a sample prediction model that was more than 90% accurate in determining to which sample group the unknown sample belonged.

Agilent Technologies

Introduction

Countering illicit drug trafficking is one of the main responsibilities of law enforcement agencies. During the investigation of criminal cases related to the sales of narcotic drugs, one of the main tasks is to identify the criminal organization responsible for bringing narcotic substances into the country. Forensic examination to obtain information about the composition and quality of the seized drugs is crucial to identifying and dismantling such criminal enterprises.

Using the chemical composition of an illicit drug sample to determine its origin requires a comparative study of narcotics obtained from different seizures. The most common illicit drug is heroin, or so-called street heroin. It is usually a multicomponent mixture containing not only the heroin, but also various additives such as pharmacologically active compounds and neutral substances. Determining the composition of such crude mixtures requires a method that can accurately identify and quantitate a large number of chemical components in complex matrixes.

The accurate mass capability of the quadrupole time-of-flight (Q-TOF) mass spectrometer is particularly useful for the characterization of such complex street heroin samples. This application note describes a comparative study of street heroin samples using the Agilent 7200 GC/Q-TOF. The characteristic profiles of heroin samples were the basis for further statistical analysis performed in Mass Profiler Professional (MPP). Two major groups of heroin samples were identified, and a sample class prediction model was constructed that accurately determined the group to which a particular sample belonged. Such models can provide valuable forensic tools for determining the origin of a street heroin sample and identifying the criminal organization that distributed it.

Experiment

Instruments

This study was performed using an Agilent 7890B GC system, coupled to an Agilent 7200 Series GC/Q-TOF system. The instrument conditions are listed in Table 1.

Sample preparation

Samples of street heroin were obtained from 22 seizures. The heroin samples were first extracted by chloroform, followed by ethylamine treatment to remove extenders. The lower phase was collected for further analysis.

Table 1. Agilent 7890 GC and Agilent 7200 GC/Q-TOF Mass Spectrometer Conditions

Condition	Major components	Minor components
Column	HP-5MS 30 m × 0.25 mm, 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 minute	
	10 °C/min to 280 °C, for 3 minutes	
	10 °C/min to 300 °C, for 5 minutes	
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 current	12 µA	35 µA
Ionization parameters used 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

Data processing and statistical analysis

The data were processed by chromatographic peak deconvolution using the Unknowns Analysis tool from MassHunter Quantitative Analysis software package (B.07), followed by compound identification by comparison to the NIST11 mass spectral library. The identification of the compounds was confirmed using accurate mass and relative isotope abundance information as well as MassHunter Qualitative Analysis tools such as the Molecular Formula Generator (MFG) and Fragment Formula Annotation (FFA).

Statistical analysis was performed by MPP (12.6), a multivariate statistical analysis package, to find compounds present at distinct levels in different groups of samples. The data were subsequently used by MPP to build a sample class prediction model.

Results and Discussion

Data acquisition and compound identification

Two different acquisition methods were used to acquire data for major and minor components of the samples. The method for major compounds used reduced emission current on the filament to avoid saturation. Data for minor components were acquired using higher emission current but decreased ionization energy during the elution of major components.

Chromatographic deconvolution was performed using the Unknowns Analysis tool, followed by compound identification by comparison to the NIST11 mass spectral library. The most common components of the heroin samples were morphine alkaloids and morphine derivatives. All, but one sample, contained a monoacyl derivative of morphine, including 6-monoacetylmorphine and acetylcodeine. Many other common alkaloids as well as pharmacologically active substances were also detected in a majority of the samples (Table 2).

Table 2. Common Compounds Found in Street Heroin

Alkaloids

Compound	No. of samples containing it
Noscapine	50
Papaverine	50
Meconine	43
Morphine	40
Hydrocotarnine	13
Codeine	10

Adulterants

Compound	No. of samples containing it
Caffeine	55
Dextromethorphan	27
Tolycaine	18
Paracetamol	5

In an effort to characterize the opium alkaloid content of the street heroin samples, relative ratios of alkaloids were determined using the following formula:

$$U_n = \frac{S_n}{S_{ac} \times \Sigma n_i}$$

U_n = Relative ratio of the alkaloid n,
S_n = Area of alkaloid n peak,
S_{ac} = Area of the acetylcodeine peak,
Σn_i = area sum of all detected alkaloid peaks

The relative alkaloid ratios for 20 samples are depicted in graphic form in Figure 1, which shows that the samples can be categorized into two major groups, based on alkaloid composition.

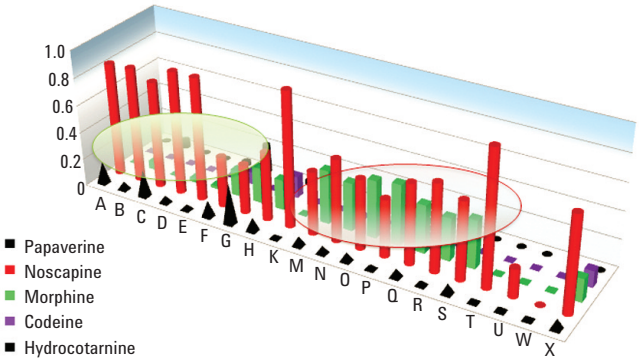

Figure 1. Comparison of the natural alkaloids identified in the street heroin samples, relative to acetylcodeine.

For the few compounds that did not give a high library match score, additional confirmation steps were performed using accurate mass information and MassHunter Qualitative Analysis structure elucidation tools, including Molecular Formula Generator (MFG) with library search and Fragment Formula Annotation (FFA). Figure 2 shows the mass spectrum for one compound tentatively identified as 6-acetyl-crotonosine acetate, which was present in 36 out of 55 samples.

When a library hit is found, the molecular ion is identified by the software. Based on the empirical formula of the hit and accurate mass spectral data, MFG and FFA assign fragment formulas that are a subset of the empirical formula. These annotated ions are colored in green (Figure 2). Whenever a good match cannot be found for a given fragment ion due to interfering ions that do not belong to the compound or poor ion statistics, the ion retains the original red color. The molecular ion and its isotopes have the theoretical isotopic distribution overlaid (pink rectangles). Further confirmation of the identity of this compound could be performed using MS/MS to verify whether ion *m/z* 282.1081 is a product ion of the *m/z* 367.1418 ion.

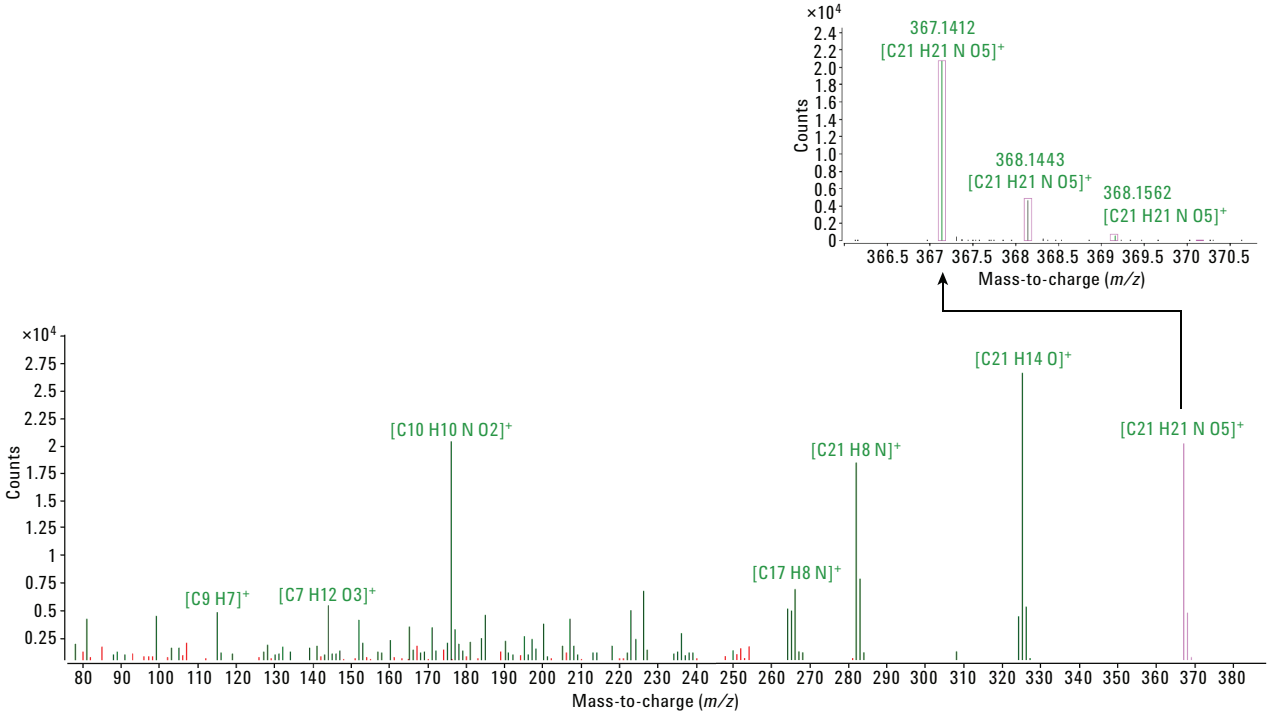

Figure 2. Annotated mass spectrum of a compound tentatively identified as 6-acetyl-crotonosine acetate using MFG with FFA and library search. The molecular ion and its isotopes (expanded in the upper right corner) have the theoretical isotopic distribution overlaid (pink rectangles), illustrating the accuracy of the observed ratios.

Principal component analysis

Principal Component Analysis (PCA) was used to observe clustering of the data for both the major and minor components. The PCA plot in Figure 3 illustrates no significant separation between the analyzed sample groups when the GC/Q-TOF method for major components was applied (A). The data acquired using the method for minor components displayed significant separation into at least two major groups (B).

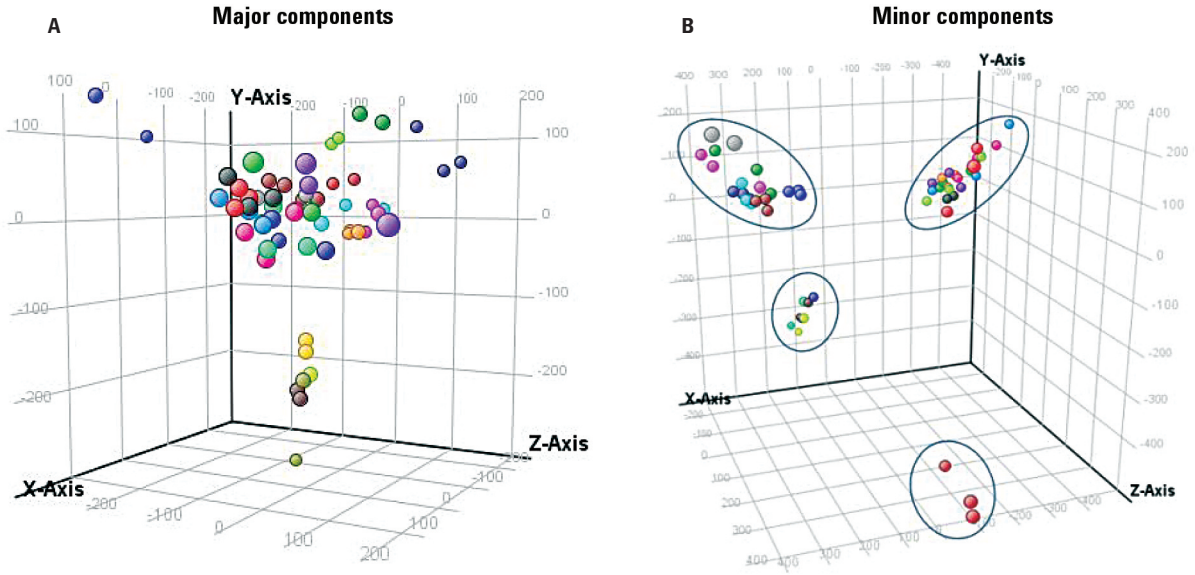

Figure 3. The PCA plot of the components identified by GC/Q-TOF analysis reveals no significant separation between the analyzed sample groups when the GC/Q-TOF method for major components was applied (A). The data acquired using the method for minor components displayed significant separation into at least two major groups (B).

Hierarchical clustering analysis

The minor components were further visualized using Hierarchical Clustering Analysis (HCA) (Figure 4), which confirmed the presence of two major sample groups. Multiple heroin alkaloids were present in both groups as well as other pharmacologically active agents and organic compounds, such as tolycaine, dextromethorphan, and *p*-isopropoxyaniline.

Differences in relative concentrations of the various heroin alkaloids contribute significantly to the separation between the two sample groups. Zooming in on two small vertical sections of the HCA graph, across all of the samples, illustrates the contribution of these alkaloids. For example, higher relative concentrations (shown in red) of 7,8-dihydro-3-desoxymorphinone and hydrocotarnine characterize the right sample group, while lower relative concentrations (shown in blue) of meconine and papaverine characterize the left sample group.

Compounds not derived from heroin can also be used to characterize the two major sample groups. For example, lower relative concentrations of *p*-isopropoxyaniline characterize the left sample group, while higher relative concentrations are characteristic of the right sample group (Figure 4).

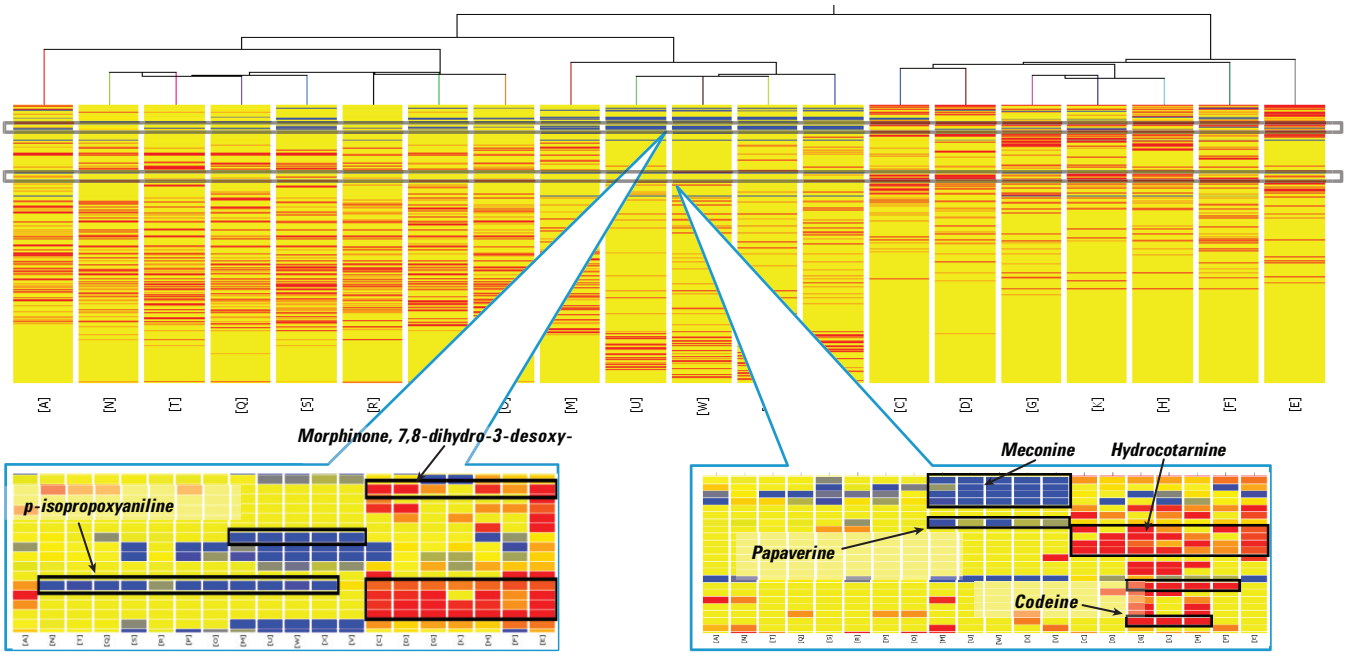

Figure 4. Hierarchical Cluster Analysis (HCA) demonstrated separation of the samples into two major groups. A few characteristic compounds that are likely to contribute to the separation of the two sample groups are shown.

Sample class prediction model

The presence of two major clusters of compounds using both PCA and Hierarchical plots was consistent with the results of semiquantitative analysis of the impurities identified in the samples (Figure 1). These two major clusters were further used to construct a sample class prediction model in MPP. The samples that were earlier separated into the two largest groups were further evaluated using a second PCA plot to confirm clustering of the compounds when only these two major sample groups were included (Figure 5). A volcano plot was also used in Figure 5 to visualize compounds specifically contributing to this separation. The volcano plot displays fold-change differences in abundance between the two sample groups. Using a cutoff of ± 2 fold-change and a p value of 0.05 assured that the differences in the two sample groups were statistically significant.

Several techniques have been developed for the purpose of constructing sample class prediction models. Five algorithms are provided by the MPP software for building such models: Partial Least Squares Discriminant Analysis (PLS-DA), Support Vector Machines (SVM), Naive Bayes (NB), Decision Tree (DT), and Neutral Network (NN). The DT algorithm, one the most successful for unsupervised classification learning, was used to construct a Class Prediction model for the two sample groups from data derived from 31 samples (Figure 6). The model proved to be 91.7% accurate for sample Group 1 and 100% accurate for sample Group 2. The sample class prediction model was then tested using these 31 samples and six additional samples that were not used to construct the model. All but two samples were classified correctly, demonstrating that this is a legitimate statistical procedure.

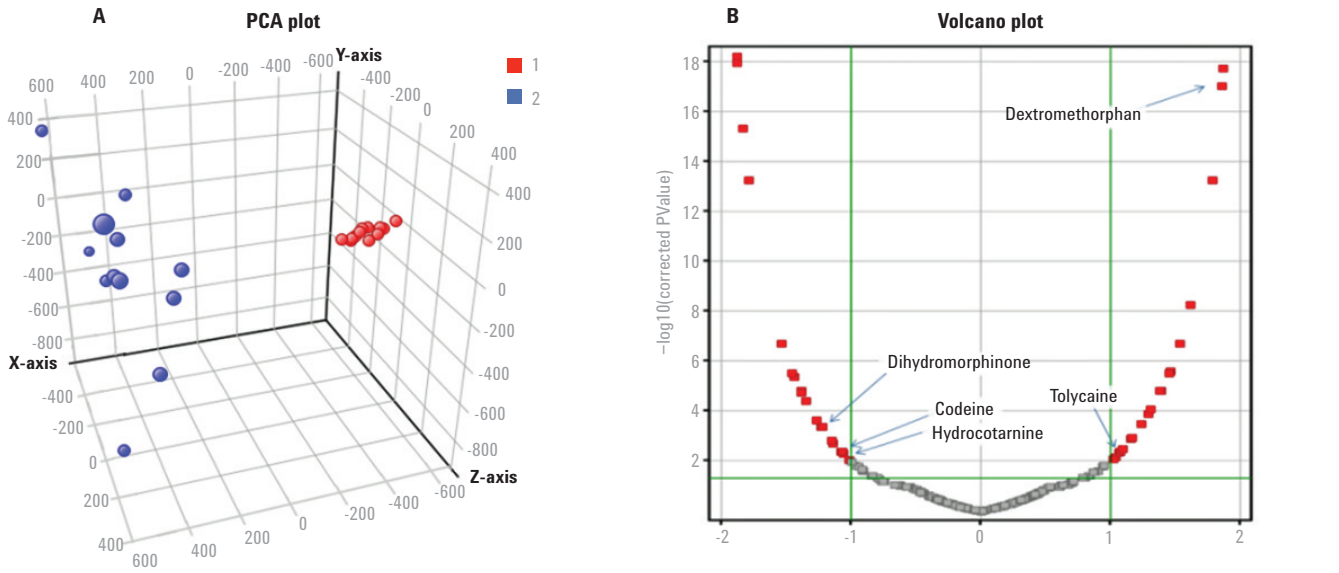

Figure 5. A) PCA plot of the two evaluated subgroups of samples chosen to build the sample classification model, and (B) the volcano plot showing compounds that are present at significantly different levels between the two subgroups of samples.

	[1] (Predicted)	[2] (Predicted)	Accuracy
Sample Group 1	11	1	91.667
Sample Group 2	0	12	100.000
Overall Accuracy			95.833

Figure 6. Validation algorithm outputs for the Decision Tree sample class prediction model constructed with the data from the GC/Q-TOF analysis of the street heroin samples. The model was 91% accurate for prediction of samples belonging to sample Group 1, and 100% accurate for samples belonging to sample Group 2.

Conclusions

An untargeted GC/Q-TOF analysis approach can be used to successfully differentiate street heroin samples that may originate from separate criminal enterprises. Accurate mass information provided by the Agilent 7200 series GC/Q-TOF was essential for identification and quantitation of several opiate alkaloids as well as other nonopiate pharmacologically active agents. Mass Profiler Professional software enabled the automatic mining and processing of the data to find the most characteristic compounds and construct a highly accurate sample class prediction model. This model enables an unknown heroin sample seized by law enforcement to be assigned to one of the two groups of street heroin samples identified by this study.

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Published in the USA
May 2, 2014
5991-4369EN

Agilent Technologies