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Dr. Nazmul Karim Khan
SSP Chemical Lab, CID
Bangladesh Police, Bangladesh

Md. Nazrul Islam
Chemical Examiner,
Bangladesh Police, CID
Rajshahi, Bangladesh

Vikas Kumar Tomar
AVV Technologies

Harshita Tiwari
AVV Technologies

Vanika Tomar
AVV Technologies

Md. Abdus Salam
Technolab Instruments,
Bob Green SepSolve
Analytical

Dr. Laura McGregor
SepSolve Analytical

Corresponding Author:
Dr. Nazmul Karim Khan
SSP Chemical Lab, CID
Bangladesh Police, Bangladesh

Comprehensive profiling of controlled substances using gas chromatography coupled with time-of-flight mass spectrometry

Dr. Nazmul Karim Khan, Md. Nazrul Islam, Vikas Kumar Tomar, Harshita Tiwari, Vanika Tomar, Md. Abdus Salam and Dr. Lara McGregor

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Abstract
Background: Drug profiling is performed to prove the presence of any controlled substances and to compare sample purity, to determine whether two samples are from the same origin, as well as to assess the expected monetary value of the drug samples. However, conventional analyses by gas chromatography – mass spectrometry (GC-MS) often lacks the sensitivity required to provide a full chemical fingerprint of the trace contaminants and cutting agents. Here, we apply gas chromatography coupled with time-of-flight mass spectrometry (GC-TOF MS) to identify trace differences between seized drug samples.
Material and Methods: Six seized samples of suspected drugs of abuse, were prepared by dissolving of each drug powder in methanol, prior to analysis by GC–TOF MS. Resulting chromatograms were integrated, identified and compared using novel comparative analysis tools to determine trends and differences between the seized samples.
Result: All six seized samples were found to contain diacetylmorphine (heroin), however the trace cutting agents and contaminants present varied significantly. For example, the presence of the common cutting agents, caffeine and acetaminophen (or paracetamol) was only noted for some of the samples, while others contained trace impurities, such as meconin and 6-acetylcodeine, which can be useful in linking samples to the same origin.
Conclusion: Comprehensive profiling of seized drug samples was obtained using GC–TOF MS to identify not only the controlled substances, but also the various cutting agents and trace impurities present.

Keywords: Drugs, controlled substances, heroin, GC-MS, mass spectrometry

1. Introduction
This study describes the use of GC–TOF MS for comprehensive profiling of seized drug samples, for confident identification of controlled substances, as well as adulterants and cutting agents.
Introduction the analysis of controlled substances, such as heroin and cocaine, is commonly performed using gas chromatography coupled with mass spectrometry (GC–MS). However, the quadrupole MS systems traditionally used for GC are restricted in terms of sensitivity when used for screening (i.e. in scan mode). Increased sensitivity may be obtained with selected ion monitoring (SIM) mode, but then whole-sample screening is not viable, resulting in compounds of interest being overlooked. The spectra obtained are also affected by the phenomenon of spectral skew, which could increase the potential for false-positive or false negative results, as well as cause difficulties with spectral deconvolution. These are important issues because forensic laboratories require unequivocal identification of any controlled substances present in a sample – a challenging prospect considering the ever-increasing list of target compounds and the novel 'designer' drugs that are now prevalent (e.g. synthetic cannabinoids).
Additionally, drug profiling involves the comparison of sample purity to determine whether two samples are from the same origin, as well as to assess the expected monetary value of

the drug samples. This involves screening the entire sample for not only the target compounds (i.e. controlled substances), but also any adulterants or cutting agents that are present. BenchTOF2™ time-of-flight mass spectrometers (TOF MS) can address these challenges by providing high-sensitivity screening, with excellent spectral fidelity, for confident identification of targets and non-targets in complex samples. In this study, we will demonstrate these advantages by the analysis of a selection of seized drug samples using GC–TOF MS. Furthermore, smart software tools will be demonstrated, which save time during data analysis for fast and simple comparisons of complex chromatograms.

2. Material and Methods

2.1 Materials and Reagents: All materials and reagents were obtained from authentic suppliers. All reagents used were of analytical grade.

2.2 Sample Preparation

SIX seized samples of suspected drugs of abuse, herein referred to as Samples 1-6.Samples were prepared by dissolving of each drug powder in methanol, prior to analysis by GC–TOF MS.

2.3 Instrumentation

GC–TOF MS analysis

Table 1: GC-TOF MS analysis

Analytical column	DB-5 MS, 30m × 0.25 mm I.D. × 0.25 µm film thickness
Injection Volume	1 µL
Inlet temperature	250 °C
Carrier gas	1 mL/min, Helium
Split ratio	20:1
Oven temperature gradient	80 °C for 1.6 minutes 12.5 °C/min to 300 °C hold for 7.5 minutes.
Total Run Time	26.7 minutes
Transfer line temperature	250 °C
TOF MS Parameters	
Source	Electron Ionisation (EI)
Ionisation energy	70 eV
Ion source temperature	250 °C
Filament voltage	1.8 V
Mass range	m/z 35-500

2.3.1 GC–TOF MS configuration

GC–TOF MS analyses were carried out on an 8890 GC system (equipped with an autosampler) coupled to a Bench TOF™ time-of-flight mass spectrometer (Sep Solve Analytical, Peterborough, UK). Analytes were separated using a DB-5 MS column (30m × 0.25 mm I.D. × 0.25 µm film thickness).The GC oven ramp was set to: 40 °C (hold for 3 min), 4 °C min-1 to 250 °C (hold 15 min). An injection volume of1 uL was used, with split injection using a split ratio of 20:1. The carrier gas (Helium) was at flow rate of 1 mL/min. He gas of grade was used. Full experimental details are provided in Table 1.

Instrument settings, data acquisition and processing was performed in Chrom Space 1D software (Sep Solve Analytical, Peterborough, UK). Putative identifications were based on spectrum matching against the NIST 2020 and Wiley mass spectral libraries.

3. Results and Discussions

The GC–TOF MS total ion current (TIC) chromatograms obtained for the analysis of the six suspected drug samples are shown in Figure 1. Tentative identifications are provided for the major peaks, which clearly indicate the presence of heroin (diacetylmorphine) in each sample. Screening all peaks against commercial mass spectral libraries also revealed the presence of cutting agents and impurities, such as caffeine and acetaminophen (or paracetamol). The software platform used in this study enabled real-time data processing to be employed during analysis – meaning the chromatograms were background-substracted, integrated, deconvolved and library-searched during acquisition.

Table 2: Tentative identification of peaks labelled in Figure 3.

Peak #	Identification	Description
1	Acetaminophen	Cutting agent (analgesic)
2	Meconin	Trace impurity
3	Acetaminophen acetate	Cutting agent
4	Caffeine	Cutting agent (CNS stimulant)
5	6-Acetylcodeine	Trace impurity
6	6-Monoacetylmorphine	Trace impurity
7	Acetyl thebaol	Trace impurity
8	Heroin	Controlled substance
9	Olanzapine	Cutting agent (Antipsychotic)
10	Azonaphtol OA	Synthetic dye
11	Alprazolam	Cutting agent (Benzodiazepine)
12	Phenolphthalein	Cutting agent (Laxative)
13	Phenolphthalein 2AC	Cutting agent

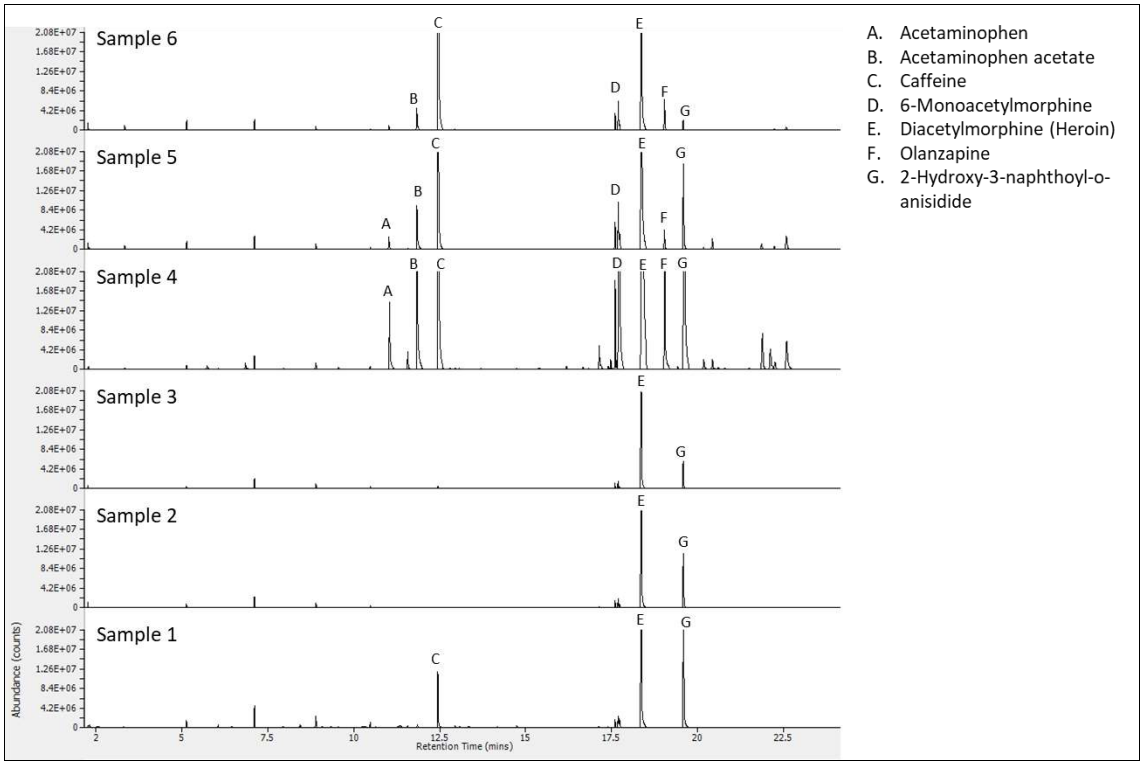

Fig 1: GC–TOF MS (TIC) chromatograms for six suspected drug samples (labelled samples 1-6) with annotations showing tentative identification of the major peaks.

Figure 2 provides an expanded region of Sample 4, whereby two co-eluting components were deconvolved, enabling more comprehensive detail on the sample composition to be obtained. This is an important aspect in profiling of controlled substances,as information on sample purity is used to determine whether two samples are from the same origin, as well as to assessthe expected monetary value of the drug samples.

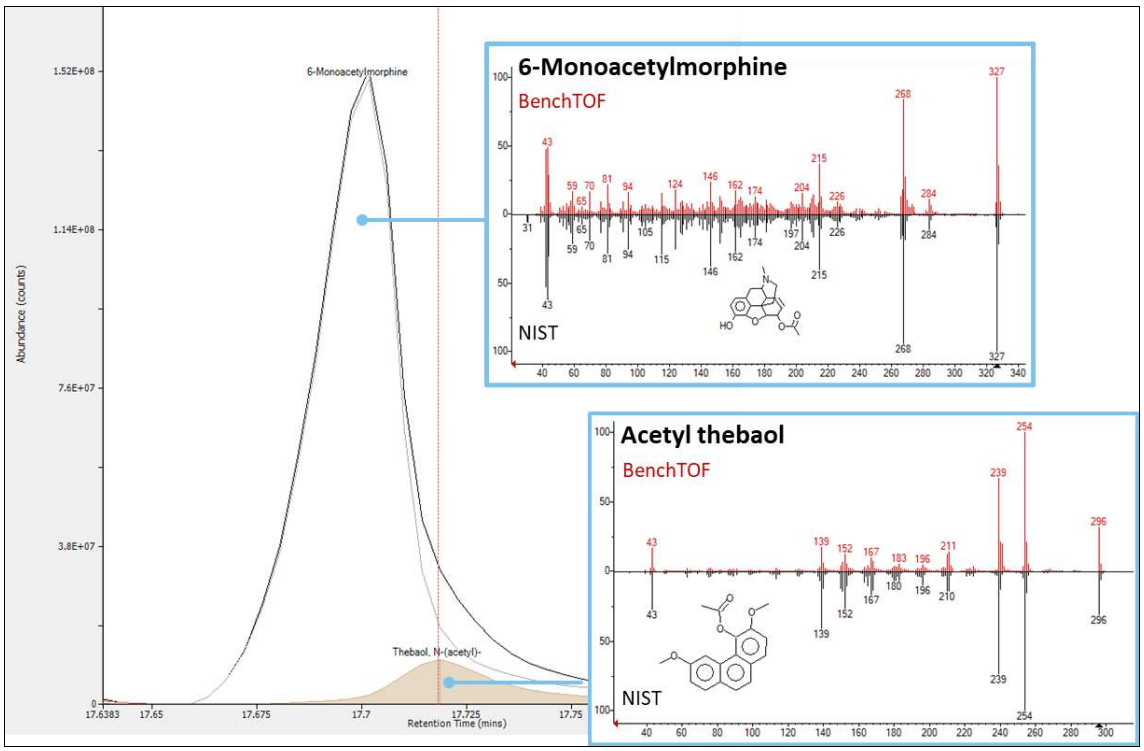

Fig 2: Expanded region of the GC–TOF MS (TIC) chromatogram for Sample 4 showing deconvolution of two co-eluting peaks.

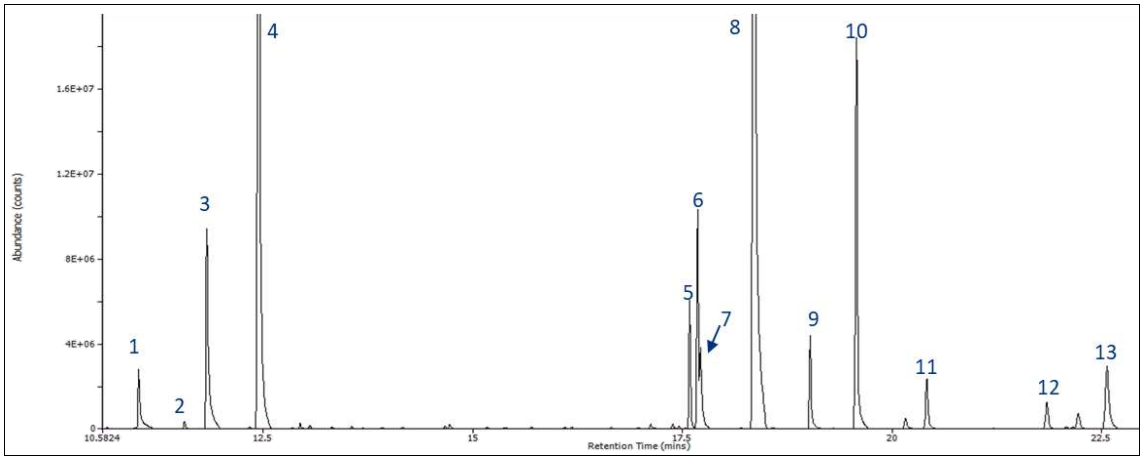

Fig 3: Expanded region of the GC–TOF MS (TIC) chromatogram for Sample 5, with peak identifications provided in Table 2.

Figure 3 shows wide range of trace impurities and cutting agents identified in Sample 5 using this GC–TOF MS approach. The identification of cutting agents, impurities and diluents is an important aspect of drug profiling as it can often provide information on the origin of the drug, as well as distribution networks and manufacturing techniques.

To accelerate the comparison of the drug profiles, novel software tools were utilized to allow the relative EIC peak areas ofthe identified compounds to be displayed as a histogram. The software provides rapid and objective comparisons of multiple data files based on the relative abundances of identified compounds, as displayed in the histograms (Figure 4). A simple matrix of pairwise match factors (between 1 and 1000) is then generated for objective comparisons.

In Figure 4, the comparison of two of seized drug samples is shown, using a suite of identified impurities, adulterants and cutting agents. It can be seen that Samples 4 and 1 have a high match factor of 902, indicating similar profiles. However, the histogram plot also highlights where differences exist, such as the presence of acetaminophen (and its impurities, e.g. acetaminophen acetate) in Sample 4 only.

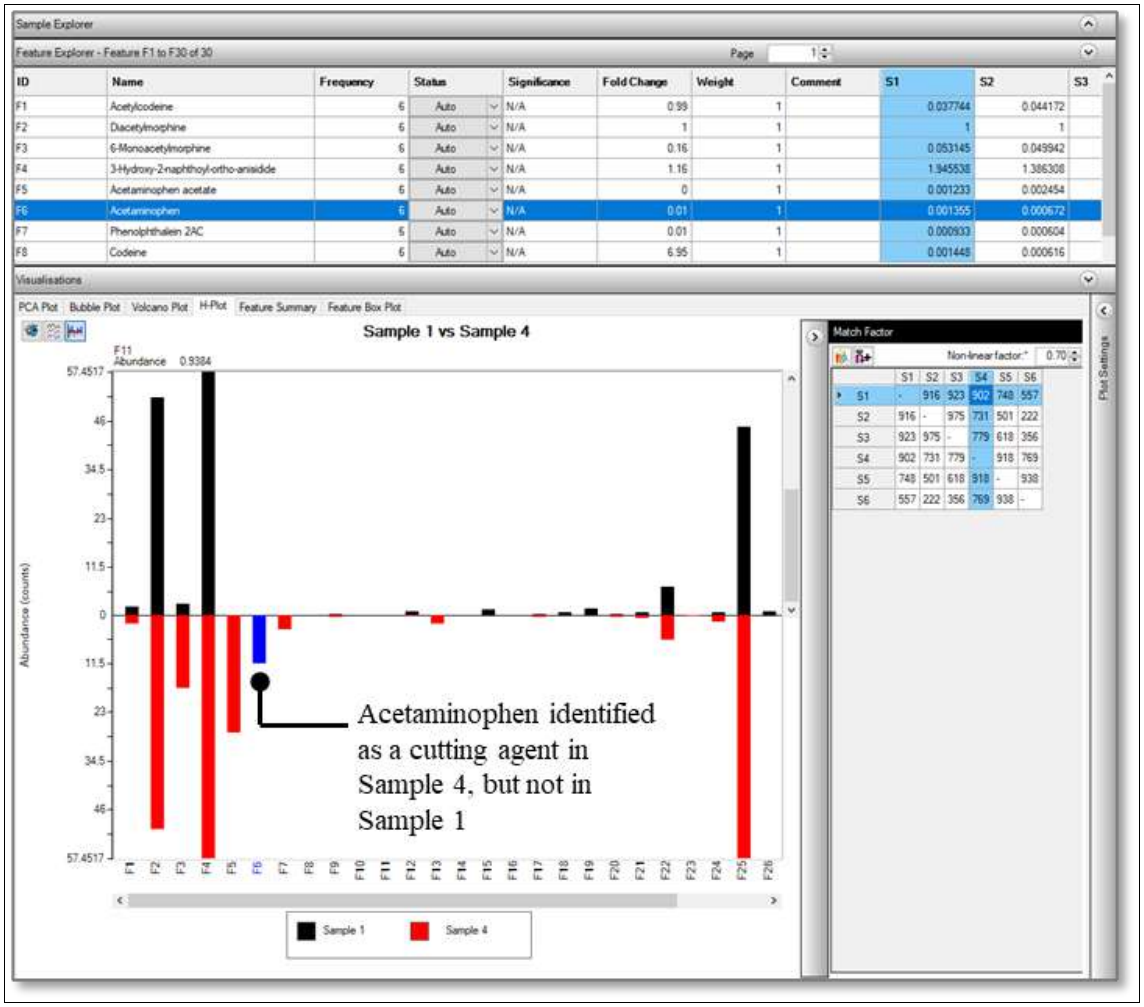

Fig 4: Histogram plot showing the comparison of impurities, diluents and cutting agents in two seized drug samples (Samples 1 and 4).

In a similar way to spectral libraries, databases can be created for these histograms, meaning that subsequent drug samples can be screened quickly and easily. In other words, the match factor will quickly show whether the new sample is similar to an existing entry in the databases, which could help to indicate the same source.

It is important to note that targeted analysis is performed for specific drugs and impurities, using instruments such as single quadrupole MS (in Selected Ion Monitoring (SIM) mode) or triple quadrupole MS, important compounds may be overlooked. On the other hand, TOF MS provides comprehensive screening of the entire sample in a single analysis, meaning that all of information is available for robust profiling and retrospective analysis.

4. Conclusions

This work demonstrates the comprehensive profiling of seized drug samples using GC–TOF MS. The ability to provide fast and objective comparison of cutting agents and trace impurities present in seized drug samples helps to identify samples from the same supplier or distribution network.

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