TECHNICAL NOTE

Comparison of the Novel Direct Analysis in Real Time Time-of-Flight Mass Spectrometry (AccuTOF-DART™) and Signature Analysis for the Identification of Constituents of Refined Illicit Cocaine

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ABSTRACT: The characterization of 25 illicit cocaine samples by a novel application of direct analysis in real time (DART) sample introduction coupled with time-of-flight mass spectrometry (TOF-MS) and cocaine signature analyses is provided. The AccuTOF-DART™ analysis of the cocaine samples resulted in the detection of most analytes, although some compounds were not detected. This new technique is easy, rapid, requires very little sample, and can be used to screen even complex mixtures. Potential applications, including use for signature analyses of controlled substances, are discussed.

KEYWORDS: Cocaine Signature Analyses, DART, TOF-MS, Screening Test, Forensic Chemistry

Introduction

Time-of-flight mass spectrometry (TOF-MS) using exact mass determination has the potential to greatly improve drug screening in forensic laboratories [1-4]. A TOF-DART instrument, which couples a TOF mass spectrometer with a direct analysis in real time (DART) ion source, has been recently introduced. The instrument easily and rapidly screens samples for a wide range of compounds, and requires only minute amounts of sample and little sample preparation. Both sample preparation and sample screening for multiple drug analytes can be completed in minutes with the TOF-DART, whereas conventional cocaine signature analyses or controlled substances screening may take 8 hours or longer. Figure 1 compares the analysis of controlled substances by traditional GC/MS to the novel screening by TOF-DART. The instrument provides sufficient selectivity and accurate elemental composition assignment through exact mass determination, resulting in analytical identification for a wide variety of small molecules, such as drugs and unknown substances (e.g., adulterants, manufacturing solvents, and byproducts), with minimal sample preparation. TOF-DART detects a variety of controlled substances in solid samples or solution preparations [5-6].

In addition to routine sample analysis, AccuTOF-DART™ may have potential as an adjunct technique for signature analyses. While such analyses have become routine in many forensic laboratories, these programs could still benefit from a rapid screening method to identify controlled substances [7]. A procedure with minimal to no

sample preparation would complement existing methods. Determination of complex mixtures of drugs, adulterants, and diluents can help law enforcement track high-level dealers of illicit substances and identify new local or national illicit manufacturing trends. Herein, we provide a direct comparison of cocaine signature and AccuTOF-DART™ analyses of 25 refined illicit cocaine samples.

Experimental

Materials: Twenty-five DEA confiscated cocaine hydrochloride samples were obtained from the National Institute of Drug Abuse's drug supply repository for research (Bethesda, MD). Polyethylene glycol (used as the calibrating reagent) was of reagent-grade quality, and was obtained from Sigma Aldrich Chemical (St. Louis, MO). Cocaine analyte standards were purchased from Cerilliant (Austin, TX) as hydrochloride salt solutions in methanol (cocaine, anhydroecgonine methyl ester, cocaethylene, norcocaine) or acetonitrile (benzoylecgonine), all at 1 mg/mL.

AccuTOF-DART™ Analyses: Analyses were performed at the RTI International's Center for Forensic Sciences using a JEOL USA, Inc. (Peabody, MA) AccuTOF-DART™. The analyses were conducted using positive modes of the DART ion source. The source was operated with a ring lens voltage of 5 V, an orifice 1 voltage of 20 V, and an orifice 2 voltage of 5 V. Electrodes 1 and 2 of the DART source were set to 150 V and 350 V, respectively, while the DART temperature was set to 300^{0}C. The detector was optimized at 2,200 V. The AccuTOF-DART™ was calibrated with polyethylene glycol prior to each sample run. The samples were introduced into the ion source by dipping a glass probe into the sample and passing this through the stream. When available, the mono-isotopic M+H values of the cocaine analytes were verified using certified drug standard solutions.

Cocaine Signature Analyses by Gas Chromatography/Mass Spectrometry (GC/MS): Cocaine signature analyses were conducted by gas chromatography/mass spectrometry, as reported by Casale *et al.* and briefly described herein [7-11]. Analyses were performed using an Agilent (Palo Alto, CA) Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent Model 6890 gas chromatograph (GC). The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34 - 700 mass units, and at 1.34 scans/second. The GC was fitted with a 30 m x 0.25 mm I.D. fused-silica capillary column coated with 0.25 µm DB-1 (J & W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature, 100^{0}C; no hold, program rate, 6^{0}C/min; final temperature, 300^{0}C; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) and at a temperature of 280^{0}C. The auxiliary transfer line to the MSD was operated at 280^{0}C.

Results and Discussion

Table 1 contains the theoretical M+H values of the target analytes that were detected in the cocaine exhibits by cocaine signature analyses. All values are reported to 0.001 mmu with the exception of petroleum ether (which has a very low mass and thus a larger expected mass error).

The results of the AccuTOF-DART™ analysis of the 25 cocaine samples in comparison to the multi-technique signature analyses are in Table 2. Anhydroecgonine methyl ester (AEME) and cinnamoylcocaine were easily detected in 23 out of the 25 samples, as shown in the AccuTOF-DART™ spectra depicted in Figures 1A - B. In all samples, there was an ion present at *m/z* = 290.139, which is the M+H value of $C_{16}H_{19}NO_4$. This is the molecular formula of the isomeric pair benzoylecgonine (BE) and norcocaine, which have identical (and therefore indistinguishable) masses. Figure 2 shows the presence of the ion at 290.169 in an analyzed sample. The theoretical value of BE and norcocaine is 290.139. Although the difference of 0.030 mmu is not optimal, it may be due to an interferent present at a similar mass, resulting in a skewed *m/z* value. This is a problem that is frequently encountered during TOF-DART analysis. For example, known analytes may be analyzed sequentially and subjected to the same calibration, but while one peak will generate an M+H value 1 or 2 mmu from its

theoretical value, the other will have a difference of more than 10 mmu. Cocaine has a theoretical M+H value of 304.154 and the actual value, as seen in the analysis of a sample in Figure 2, is only 0.002 mmu higher than expected, while this is not the case with the BE/norcocaine isomer. In a recent study, the isomeric pair was analyzed by increasing the orifice 1 voltage to 90, which generated distinguishable ion fragmentation patterns [5]. However, this was done with methanolic standards at a high concentration, and was unsuccessful when analyzing the illicit cocaine samples used in this study.

Tropacocaine and truxillines were present in 5 and 7, respectively, of the cocaine samples (Figures 3A - B), while 3',4',5'-trimethoxycocaine and cocaethylene were undetected. Of the solvents and adulterants/diluents detected by cocaine signature analyses, methyl ethyl ketone (MEK), methyl isobutyl ketone (MIBK) (Figures 4A and B), and dimethylterephthalate (Figure 1A) were all identified by AccuTOF-DART™.

The AccuTOF-DART™ allowed for the rapid introduction and analysis of 25 illicit cocaine samples without the need for sample preparation. However, although this direct analysis resulted in rapid production of data, it also gave inconsistent results. In addition, because the introductions of the powdered samples were done manually, the outcome was analyst dependent (not ideal for signature analyses, where consistency of analysis is critically important). Many samples required multiple analyses to verify the presence or absence of the target analytes. Although analytes such as AEME and cinnamoylcocaine were easily detected in most of the samples, AEME is likely present as an artifact generated from truxillines during analysis. Other analytes such as tropacocaine and 3',4',5'-trimethoxycocaine were minimally detected, if at all.

Conclusions

The AccuTOF-DART™ is a novel approach to forensic analysis; however, its use in the analysis of refined illicit cocaine in this study proved ineffective for detecting the presence of the many compounds that are used to trace a cocaine sample to its geographic origin. In an effort to increase laboratory production, forensic laboratories may wish to utilize AccuTOF-DART™ as a rapid screening test for preliminary sample-to-sample comparison work, which could then be confirmed by more thorough analyses.

Acknowledgments

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Table 1. Theoretical Mono-Isotopic Mass+H of Analytes.

Analyte	Theoretical Mono-Isotopic Mass+H
Anydroecgonine methyl ester	182.117
Benzoylecgonine	290.139
Caffeine	58.958
Cinnamoylcocaine	330.169
Cocaethylene	318.169
Dimethylterephthlate	195.064
Ethyl acetate	89.052
(*Iso-/n-*)Propyl acetate	103.068
Lactose	343.116
Mannitol	303.079
Methyl ethyl ketone	73.064
Methyl isobutyl ketone	101.096
Norcocaine	290.138
Petroleum ether	87-90
Sodium chloride	58.985
3',4',5'-Trimethoxycocaine	393.178
Tropacocaine	246.141
Truxillines	658.325

Table 2. The Number of Samples, out of the Total 25 Analyzed, That Tested Positive for the Various Analytes, Using the AccuTOF-DART™ System and Cocaine Signature Analysis.

Analytes	Cocaine Signature Analyses	AccuTOF-DART™
Anhydroecgonine methyl ester	ND	23
Benzoylecgonine	21	25
Cocaethylene	NA	ND
Cinnamoylcocaine	25	23
Norcocaine	21	25
3',4',5'-Trimethoxycocaine	25	ND
Tropacocaine	25	5
Truxillines	25	7

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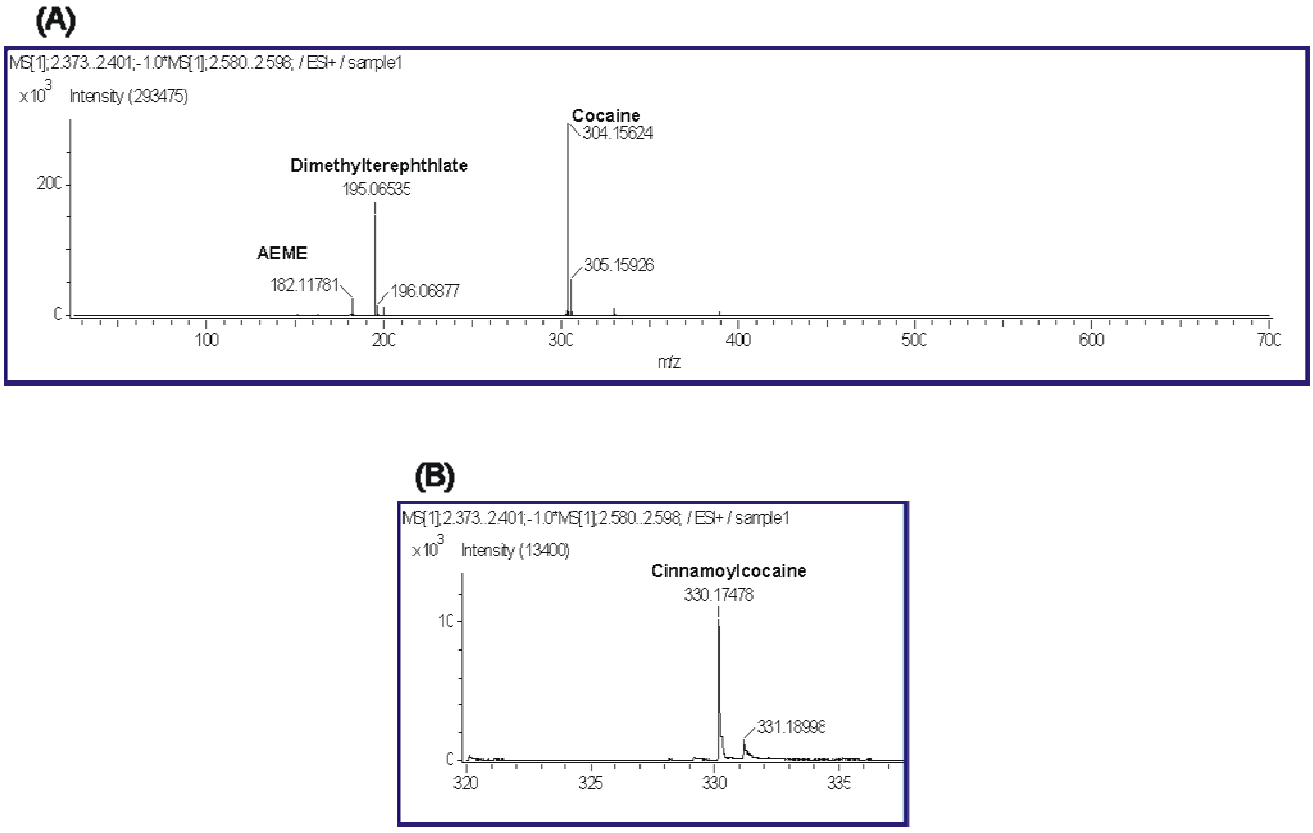

Figure 1. AccuTOF-DART™ Spectra of an Illicit Cocaine Sample Showing the Presence of: (A) Cocaine, Anhydroecgonine Methyl Ester (AEME), and Dimethylphthalate; and (B) Cinnamoylcocaines.

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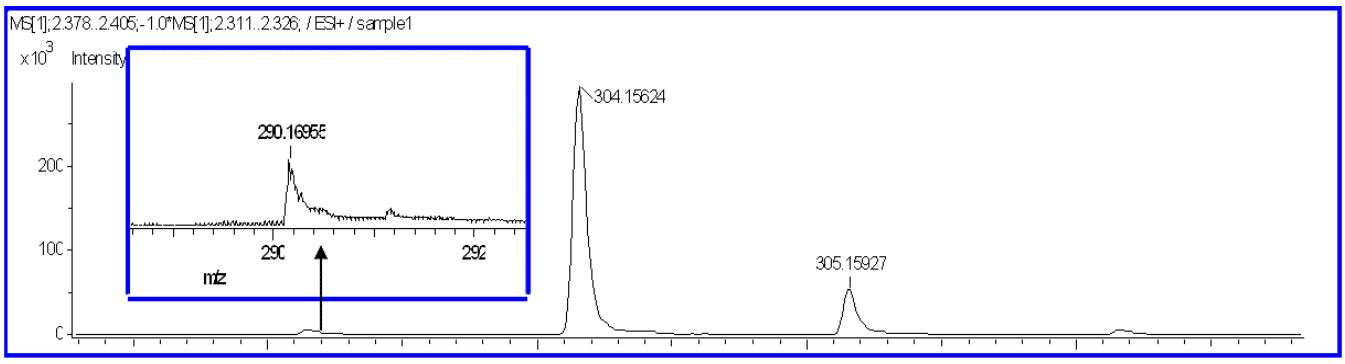

Figure 2. AccuTOF-Dart™ Spectra of an Illicit Cocaine Sample Showing the Presence of Possible Norcocaine and Benzoylecgonine (both at *m/z* = 290.17; see Expansion Window).

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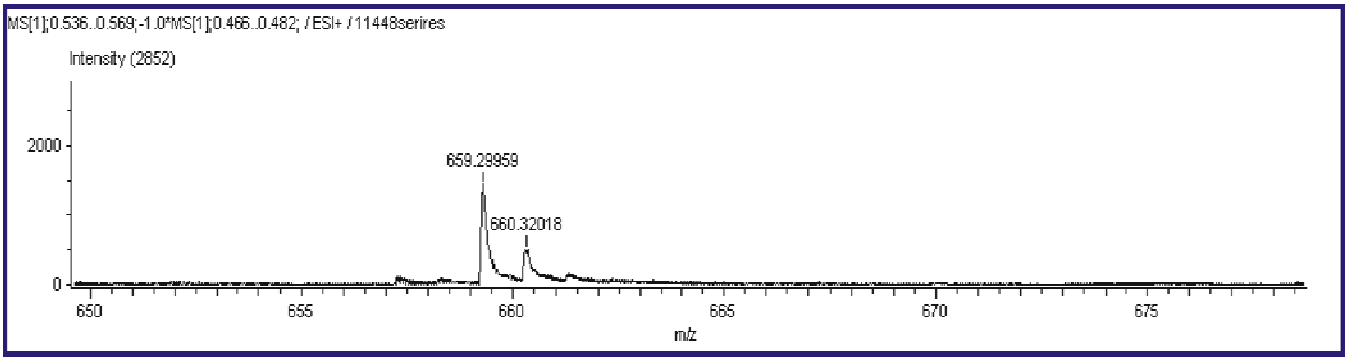

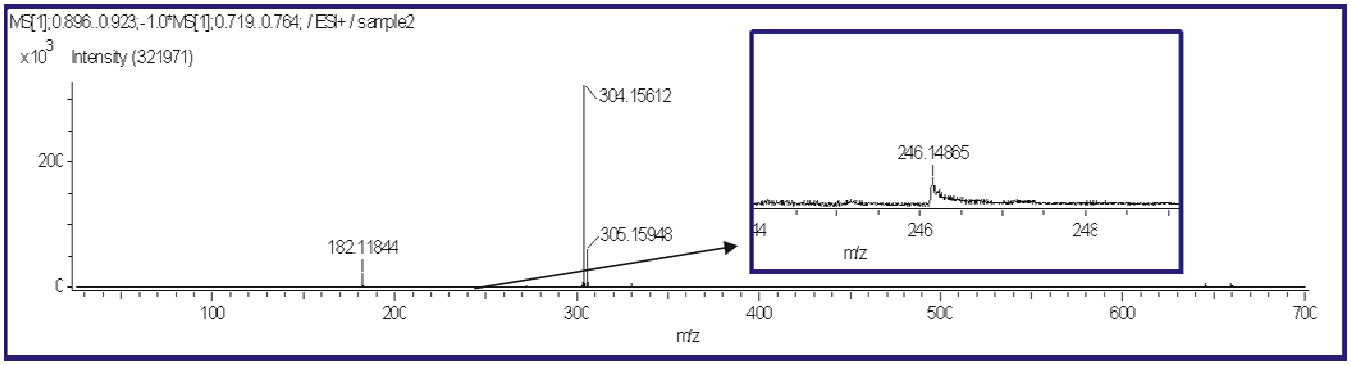

Figure 3. AccuTOF-DART™ Spectra of an Illicit Cocaine Sample Showing the Presence of: (A) Truxillines; and (B) Tropacocaine (in Expansion Window).

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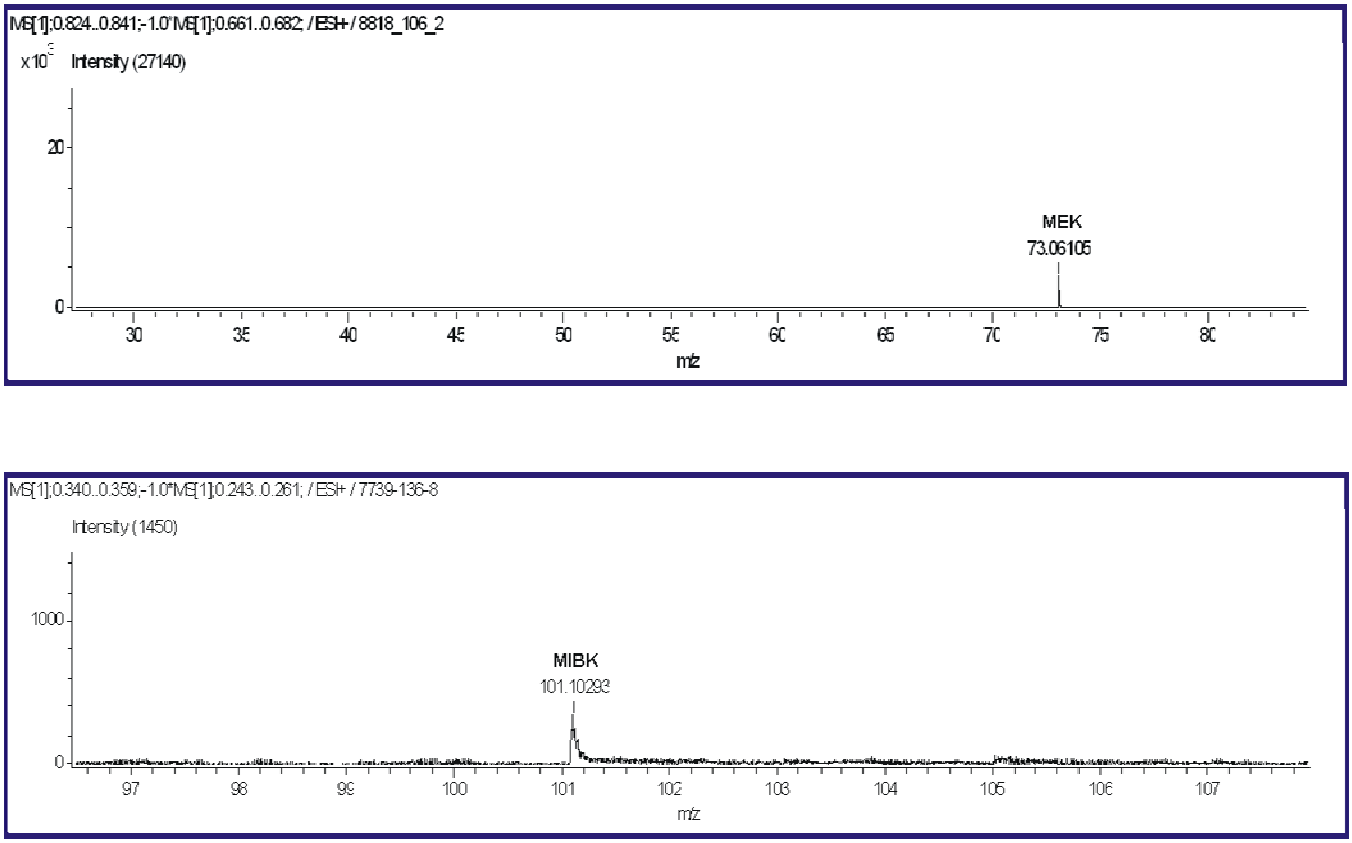

Figure 4. AccuTOF-DART™ Spectra of an Illicit Cocaine Sample Showing the Presence of: (A) Methyl Ethyl Ketone (MEK); and (B) Methyl Isobutyl Ketone (MIBK).

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