Introduction

Due to the increase in lethality of synthetic substances and legal definitions it can be important to differentiate between isomers of illicit drugs. To comply with accepted standards for identification, this typically requires a combination of instrumental and chemical techniques. Gas chromatography-mass spectrometry (GC-MS) is a powerful tool used in forensic chemistry for the identification of drugs of abuse. It allows the separation and identification of the complex mixtures often encountered by the forensic chemist. In gas chromatography, compounds are separated from a mixture and eluted from a column based on the structure’s affinity for the stationary phase. The elution is measured by retention time, and while the retention time of a given compound in a GC method can provide clues to identity, retention time is not unique to any compound. In mass spectrometry, a molecule is bombarded with high energy electrons, creating charged ions. Typically, these ions are unstable and will undergo fragmentation producing a mass spectrum pattern based on mass to charge ratio that can then be compared to known standards. Many times, this mass spectrum is sufficient to identify the compound of interest in a forensic setting. In the case of positional isomers, however, the ions produced during fragmentation may not be unique, and the resulting mass spectra are indistinguishable in benchtop GC-MS instrumentation. In order to overcome this limitation, another technique is needed for the differentiation of these substances. The American Society of Crime Laboratory acknowledges the need to couple mass spectrometry with other instrumentation in order to properly identify positional isomers [2].

In infrared spectrophotometry, a molecule is exposed to infrared radiation. Based on the arrangement of the substituents in the molecule, it will either absorb or transmit the various wavelengths of radiation. The resulting IR spectrum can then be compared to known standards. Unlike in mass spectrometry, where the movement of a functional group in a positional isomer cannot be differentiated, slight changes in the position of a substituent can be detected in the IR spectrum. When coupled with a gas chromatograph (GC-IR), this technique allows for the characterization of these isomers with the advantage of the separation afforded by the gas chromatograph.

To demonstrate the advantages of GC-IR over GC-MS in the determination of positional isomers, the 2-, 3-, and 4- isomers of fluoroamphetamine and the ortho-, meta-, and para- isomers of fluorofentanyl were analyzed by both GC-MS and GC-IR.

Materials and Equipment

Materials:
1 mg/ml Ortho-Fluorofentanyl (Cayman Chemical)
1 mg/ml Meta-Fluorofentanyl (Cayman Chemical)
1 mg/ml Para-Fluorofentanyl (Cayman Chemical)
1 mg/ml 2-Fluoroamphetamine (Cayman Chemical)
1 mg/ml 3-Fluoroamphetamine (Cayman Chemical)
1 mg/ml 4-Fluoroamphetamine (Cayman Chemical)

Equipment:
Gas Chromatograph-Mass Spectrometer (Agilent 6890N/5973)
Gas Chromatograph-Infrared Detector (AgilentGC6890N/ASAP IR DETECTOR II)
Figure 1 shows the journey of a sample from GC injection to IRD detection

1) GC: houses the injection port, column, and controls
   a) Injection Port: sample injected with a syringe where the sample vaporizes
   b) Column Oven: contains the column and can be temperature controlled to optimize separation
   c) Column: separates the mixture into components
2) Interface: direct line of transfer between GC and IRD
3) IR Source: creates a stable infrared region from 500cm\(^{-1}\)—4000cm\(^{-1}\)
4) Interferometer: consists of the moving mirror, fixed mirror and beam splitter
   A) An interferogram is produced based on the pattern of light from the laser through which the beams traveled and is sent to the flow cell
5) Light Pipe or Flow Cell: sample bounces between two mirrors and absorbs IR light
6) IR Detector: senses the molecules as they elute from the column
7) Readout System: graphical representation of how much the sample is able to absorb or transmit infrared light at a given wavenumber or wavelength

In order for a molecule to absorb infrared radiation, the molecule must undergo a met change in dipole moment. The electric field of radiation is able to interact with the molecule due to its vibrational and rotational states. Figure 2 shows the types of molecule vibrations. Vibrations are divided into two categories: stretching and bending. **Stretching** consist of continual interatomic distance in one plane between two atoms **Bending** involves a change in angle between two molecules such as rocking, scissoring, wagging, and twisting.

**Methods**

**GC-MS Parameters:**
COLUMN: 20M DB-17MS, 0.18mm ID, 0.18um FILM THICKNESS
INJECTION: 1.0 µL
INJECTOR TEMP: 250C
DETECTOR TEMP: 280C
INITIAL TEMP: 100C
INITIAL TIME: 2.0 MIN
SCAN FROM 40-500m/z
RAMP: 35C/MIN
FINAL TEMP: 320C
FINAL TIME: 3.5 MIN

**GC-IRD Parameters:**
COLUMN: 30M DB-5MS, 0.32mm ID, 0.25um FILM THICKNESS
INJECTION: 5.0 µL
INITIAL TIME: 1.0 min
INITIAL TEMP: 80C
RATE: 30C/min
FINAL TEMP: 320C
FINAL TIME: 4.0 min

**Data Analysis Software:**
Chemstation V. B.01.00
Grams V. 414 Level I

Figure 2. Vibration Modes
Results

Ortho-Fluorofentanyl  Meta-Fluorofentanyl  Para-Fluorofentanyl

2-Fluoroamphetamine  3-Fluoroamphetamine  4-Fluoroamphetamine

Ortho-Fluorofentanyl  Meta-Fluorofentanyl  Para-Fluorofentanyl

Ortho-Fluorofentanyl  Meta-Fluorofentanyl  Para-Fluorofentanyl
Conclusion

Gas chromatograph-mass spectrometry is often used for the identification of drugs of abuse; however, it has limitations in the differentiation of positional isomers. When analyzing positional isomers, gas chromatography-infrared spectrophotometry has demonstrated to be a valuable tool for the forensic chemist due to the efficiency of the autosampler, the GC's ability to separate compounds in a mixture, and the IRD's ability to produce a spectra that is unique to the molecular configuration. Furthermore, vapor phase GC-IR spectra are highly reproducible and free of matrix effects such as polymorphism unlike condensed phase GC-IR.

Combining GC-MS and vapor phase GC-IR analysis provides a further layer of quality analysis and identification that exceeds the SWG/DRUG and ASTM recommendations for seized drug identification [2].

References


Acknowledgements

ASAP Analytical would like to thank the following individuals for sharing this information.

Danielle Ostrow, B.S.; Michael Gilbert, B.S.
Pinellas County Forensic Laboratory, 10900 Ulmerton Road, Largo, FL 33778