The Differentiation of Positional Isomers Utilizing GC-IRD

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Abstract

With the emergence of novel synthetic drugs available on the illicit market with markedly similar chemical structures, identification of these substances has become a challenge to the forensic chemist. Many of the substances are positional isomers. The analysis and identification is further complicated by the fact that many times the substance will be found in combination with other compounds, such as diluents, adulterants, cutting agents and other controlled substances. Though GC-MS is a valuable technique used in forensic chemistry for the identification of controlled substances, benchtop instruments may not be capable of differentiating the positional isomers of many drugs of abuse. IR is better suited to the differentiation of isomers, and when coupled with a GC, it can allow for the separation and characterization of these compounds. Data is presented demonstrating the differentiation of the 2-, 3-, and 4-isomers of fluoroamphetamine, as well as the meta-, ortho-, and para- isomers of fluorofentanyl using GC-IR analysis.

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.

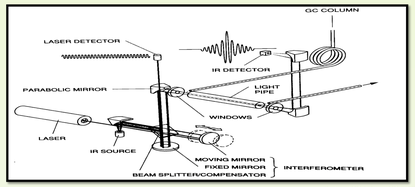
Materials and Equipment

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)



Basics of GC/IRD and GC/IRD/MS. Hewlett Packard, 1993.

Figure 1. Schematic Diagram of IRD

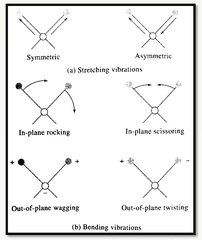
Figure 1 shows the journey of a sample from GC injection to IRD detection

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-4000cm-1
4) Interferometer: consists of the moving mirror, fixed mirror and beam splitter
   - 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 net change in dipole moment. The electric field of radiation is able to interact with the molecule due to it's vibrational and rotational states.
Figure 2 shows the types of molecular 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.



Principles of Instrumental Analysis [4]

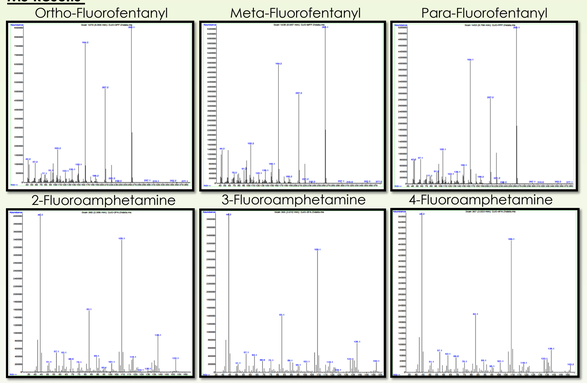
Figure 2. Vibrational Modes

Methods

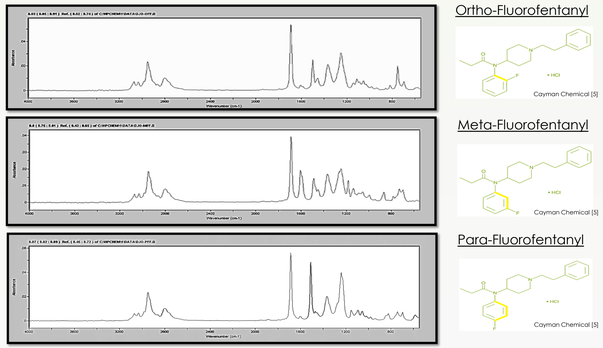
GC-MS Parameters:
COLUMN: 20M DB-17MS, 0.18mm ID, 0.18um FILM THICKNESS
INJECTION: 1.0 uL INJECTOR TEMP: 250C DETECTOR TEMP: 280C
INITIAL TEMP: 100C INITIAL TIME 2.0 MSCAN FROM 40-500m/z
RAMP: 35C/MIN FINAL TEMP: 320C FINAL TIME: 3.5 MIN
GC-IRD Parameters:
COLUMN: 30M DB-5MS, 0.32mm ID, 0.25um FILM THICKNESS
INJECTION: 5.0 uL 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

Results

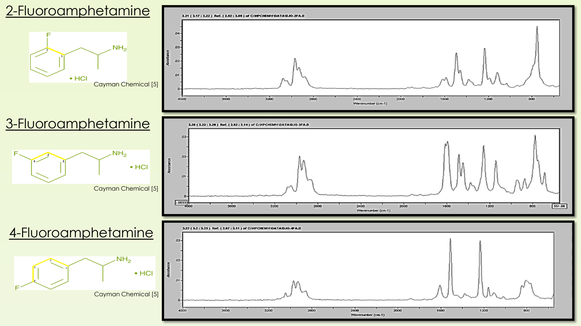
MS Results



IRD Results



Results Cont.



Discussion

Each positional isomer was analyzed by GC-MS and GC-IR, and the resulting spectra compared. The mass spectra of the isomers did not demonstrate any significant differences that would allow for the unique identification of the isomer. Because the fluorine atom is positioned on a stable benzene ring, each isomer produced the same ion fragments in a visually similar pattern. In the IR spectra of the various isomers, however, resulted in are clearly distinguishable data. This is most notable in the area of wavenumbers 1500cm-1 to 800cm-1, aka "the fingerprint region" of the spectra.

Conclusion

Gas chromatography-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 been demonstrated to be a valuable tool for the forensic chemist due to the efficiency of the auto-sampler, the GCs ability to separate compounds in a mixture, and the IRDs ability to produce a spectra that is unique to the molecular configuration. Combining GC-MS and 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

[1] ASTM E2329-17, Standard Practice for Identification of Seized Drugs, ASTM International, West Conshohocken, PA, 2017, www.astm.org
[2] "ASCLD Opioid Task Force :*Opioid Derivatives: Analysis and Instrumentation March 10, 2018*
[3] Basics of GC/IRD and GC/IRD/MS. Hewlett Packard, 1993.
[4] Skoog, Douglas A., et al. *Principles of Instrumental Analysis* 6th ed., Thomson Brooks/Cole, 2007.
[5] Cayman Chemical, http://caymanchem.com, 24 Apr 2018.