

APPLICATION NOTE 030

Forensic Analysis of Drugs

Drug abuse is a destructive force in our society. There are over 300 substances in use as recreational drugs. This includes the illicit use of various pharmaceutical products, such as opioid analgesics, psychotropics, stimulants, and hallucinogens. It also includes various substances produced in thousands of clandestine labs, ranging from one-person operations to highly sophisticated chemical processing facilities operated by large crime syndicates.

In the US there exists a network of several hundred city, county, state, and national forensic labs; tasked with analysis of samples obtained by law enforcement personnel, and with presentation of evidential materials to US courts.

The analytical laboratory tasks include identification of active ingredients, and of other species (adulterants, excipients, reaction byproducts). With the passing of time, the courts have imposed increasingly stringent standards for forensic scientists testifying as expert witnesses in trials involving illegal manufacture and use of drugs. Rigorous methods are required to provide unequivocal evidence in criminal proceedings.

As of 2008 the Scientific Working Group for the Analysis of Seized Drugs (www.SWGDRUG.org/approved.htm) has emphasized including uncertainty principle in the selection of laboratory methods. The specificity of Infrared Spectroscopy is well suited to reduce uncertainty in the identification of isomers. Spectra Analysis provides an instrument designed to meet the work flow needs of forensic laboratories. The DiscovIR GC is a “walk-up” instrument, capable of high sample volume automated processing. It provides requisite sensitivity for the analysis of drugs and illicit substances. Identification based on infrared spectra is highly discriminating, and fills a gap in identification by mass spectrometry. The DiscovIR is configured for the high workload demands of forensics, provides archived, retrievable data, and makes use of a spectral database search engine to facilitate rapid unequivocal identification of sample unknowns.

The examples provided illustrate the use of this technology in the forensics environment, and highlight the ability of DiscovIR to identify isomeric forms of materials that cannot be readily identified by GC-MS methods.

Methods of Analysis and identification

Categories of Analytical Techniques

The SWGDRUG requires the use of multiple independent identification techniques. They have divided currently used methods into three categories, with category A techniques providing the best discriminating power.

Table I SWGDRUG methods		
Category A	Category B	Category C
Infrared Spectroscopy	Capillary Electrophoresis	Color Tests Fluorescence Spectroscopy
Mass Spectrometry	Gas Chromatography	Immunoassay
Nuclear Magnetic Resonance Spectroscopy	Ion Mobility Spectrometry	Melting Point
Raman Spectroscopy	Liquid Chromatography	Ultraviolet Spectroscopy
	Microcrystalline Tests	
	Pharmaceutical Identifiers	
	Thin Layer Chromatography	
	Cannabis only: Macroscopic Examination Microscopic Examination	

The following are some of the guidelines put forth by SWGDRUG.

Analytical identifications that include a Category A method require the use of at least one other independent method from category A,B, or C. When a Category A technique is not used, then at least three different validated methods must be employed.

The combination shall identify the specific drug present and shall preclude a false positive identification.

All Category A techniques shall have data that are reviewable.

In cases where hyphenated techniques are used (e.g. gas chromatography-mass spectrometry, Liquid chromatogram-diode array ultraviolet spectroscopy), they will be considered as separate techniques provided that the results from each are used.

Workloads are typically high in forensics laboratories, and the analysts must be skilled in the use of all of the methodologies cited in Table I. This favors automated instrumentation with “walk-up” ease of use. Interpretation of results must similarly be quick and straightforward. The use of computer assisted interpretation and materials databases is a requirement for rapid processing and turnaround of results. Data must be stored in a retrievable form. Conventional Infrared spectroscopy of forensic samples carries a burden of sample preparation. Generally the analytes must be purified for identification. Methods of sample preparation, such as KBr pellets, typically require milligram quantities of material. Classical purification methods impose sensitivity limits, a tremendous time burden, and limited output of a lab besieged by many samples per day.

Use of hyphenated methods

Samples of clandestine lab materials and ethical drugs typically contain multiple ingredients. Examples include residual starting materials, off-reaction products, excipients, and isomeric forms of constituents. In general, the highly discriminating analytical techniques require an isolation and purification of an analyte to achieve unambiguous identification. Classical methods of sample preparation would be hugely time consuming step in these analyses, and would influence the speed, discrimination, and accuracy of analytical results. The direct linkage of high resolution chromatography and spectroscopy provides an automated single procedure to fill these requirements.

Chromatography-Mass Spectrometry

In the Category A methods cited above, Mass Spectrometry: provides identification based on molecular weight determination of parent ion and fragmentation products. Chromatography-Mass Spectrometry has been widely adopted as a sensitive and rapid automated method for analyte components identification.

Chromatography- FTIR Spectroscopy

Infrared Spectroscopy provides identification based on molecular structure, which can generate a unique constellation of absorption bands. Measurements of the band positions and their relative intensities provide the basis for identification. Successful hyphenation of Chromatography and FTIR spectroscopy has resulted in a procedure possessing similar benefits to those of hyphenated MS.

Because infrared spectroscopy provides a different basis for identification than MS, these two spectrometric methods are considered complementary. MS, for example, has very limited ability to discriminate isomeric forms of a molecule, whereas this is readily performed by FTIR.

GC-light pipe based FTIR can be used as a hyphenated method, but suffers from 1) low sensitivity, and 2) broad spectral bands characteristic of vapor phase spectra. These characteristics seriously limit the ability of GC-(light pipe) FTIR in identifying highly similar structures. The advent of the DiscovIR provides an instrument system that overcomes these issues of sensitivity and discrimination.

SWGDRG Part IV C: Quality Assurance/ Uncertainty

In October of 2008 the SWGDRUG made recommendations regarding Quality Assurance/Uncertainty. Uncertainty encompasses limitations of qualitative methods. SWGDRUG considers an understanding of uncertainty to be fundamental to the interpretation and reporting of results and recommends the concept of uncertainty be considered for all analytical results.

SWGDRG Guidelines for qualitative results:

- Relevant limitations of an analytical scheme (e.g. inability to distinguish isomers) should be documented and may need to be included in the report.
- It is expected that an appropriate analytical scheme will result in, effectively, no uncertainty in reported identifications.

The specificity of Infrared spectroscopy reduces uncertainty in the identification of isomers, which reduces uncertainty in the identification of controlled substances.

Summary of needs for controlled substance analysis laboratories:

Conformance with SWGDRUG guidelines
High sample throughput rate
Sub-microgram analysis capability
Minimal post analysis cleanup
Automation
Library capability
Reviewable data
Methods validation capability
Compatibility with other Category A methods
Walk-up capability

DiscovIR™ – GC: a new highly discriminating technology for forensic chemists

System Description

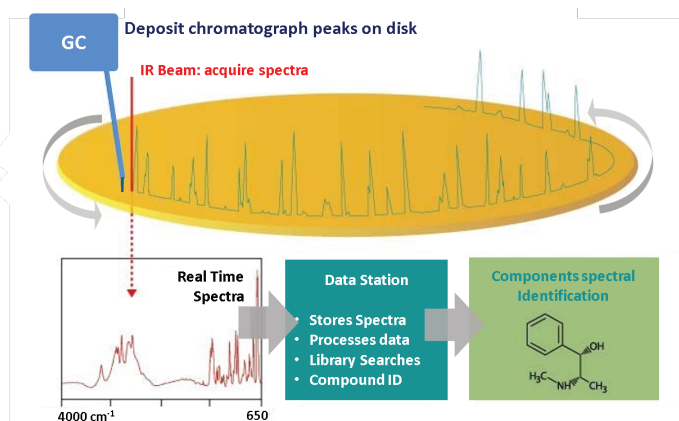
The DiscovIR is an instrument platform that links IR spectrometry to chromatography in a manner that provides real-time data flow, computerized analytical aids, and integrated automation that allows unattended operation for a large batch of samples.

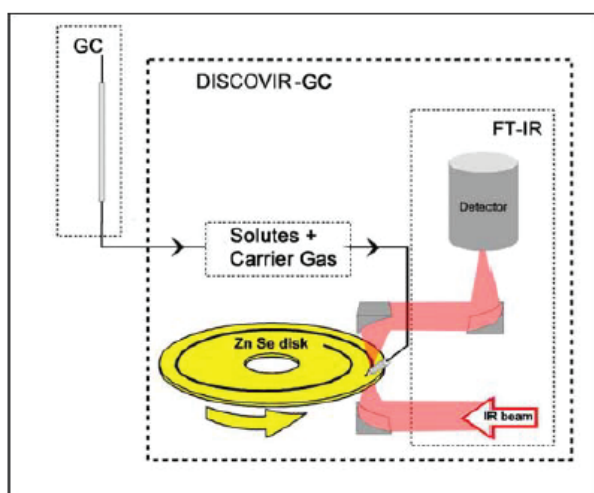


Figure 1 The DiscovIR GC integrated chromatography spectroscopy platform

The photo and diagrams illustrate the DiscovIR GC operating concept. A gas chromatograph performs automatic multi-sample processing, with the column effluent stream passing to the DiscovIR-GC instrument via a heated transfer line.

Carrier gas plus eluants flow through a deposition tip, and eluants are deposited as a collimated jet onto a cryogenically cooled sample collection disk. This disk rotates/translates under the deposit tip to result in a spiral deposit track of samples. As analytes elute from the capillary and onto the cold disk they form a solid phase deposit on the disk.





The collection disk is infrared transparent. A beam of infrared energy from an interferometer passes through the disk along the deposition track, and infrared spectra are collected at 0.3 second intervals.

The width of the deposit track and the infrared beam focus point are matched at ca. 100µm. As a result one can obtain good quality spectra on nanogram amounts of analytes.

An infrared spectral data set is thus generated for each sample processed by the chromatograph. All spectra are solid-phase spectra, providing greater resolution and sensitivity than obtainable with traditional light pipe instruments. The spiral deposition track of the collection disk has capacity to accommodate several hundred samples to be analyzed over a run time as long as two days.

All operations and data handling are automated, enabling unattended operation and a high daily sample capacity. DiscovIR GC is compatible with autosamplers that provide for new sample insertions into the sample queue after a run has commenced.

DiscovIR GC Features

- Resolve and identify mixed analytes in a single instrument and procedure.
- GC-FTIR hyphenated method meets SWGDRUG Category A requirements for structural analysis.
- GC-FTIR provides two analytical methods in one operation.
- High resolution solid phase spectra
- Water vapor not an issue
- Self-cleaning IR disk. No need to manually clean off old samples.
- Built-in Reference standard (polystyrene film) and calibration software.
- Unattended automatic operation up to 50 hours: Autosampler-compatible.
- Sample capacity: hundreds. One can batch 50 hours of chromatography.
- Spectral identification library software.
- Integrated Sample collection and FTIR in one instrument.
- Sub-nanogram sensitivity.
- Flow splitting: can interface with existing GC-MS instrumentation.
- Real-time or post-run data collection. Allows re-analysis of deposited samples.
- Higher sensitivity and greater spectral resolution than can be obtained with the old technology of GC-“lightpipe” systems.

Performance

Example1. Clan Lab SX

This example† illustrates the use of GC-FTIR in the analysis of materials seized from a clandestine laboratory. It demonstrates the infrared chromatogram generated by the DiscovIR GC and individual spectra selected from various portions of that chromatogram. Components were identified by comparing to a library of DiscovIR spectra which were prepared from known standards.

Spectra Analysis wishes to thank Alabama Department of Forensic Sciences, courtesy of Stephanie M Fisher and J. Gary Wallace, Mobile Lab in providing the analytical data for the Clan Lab samples and the comparative data for Oxymorphone

Experimental Conditions Summary

Figure 2 shows the infrared chromatogram of peaks generated during the sample elution.

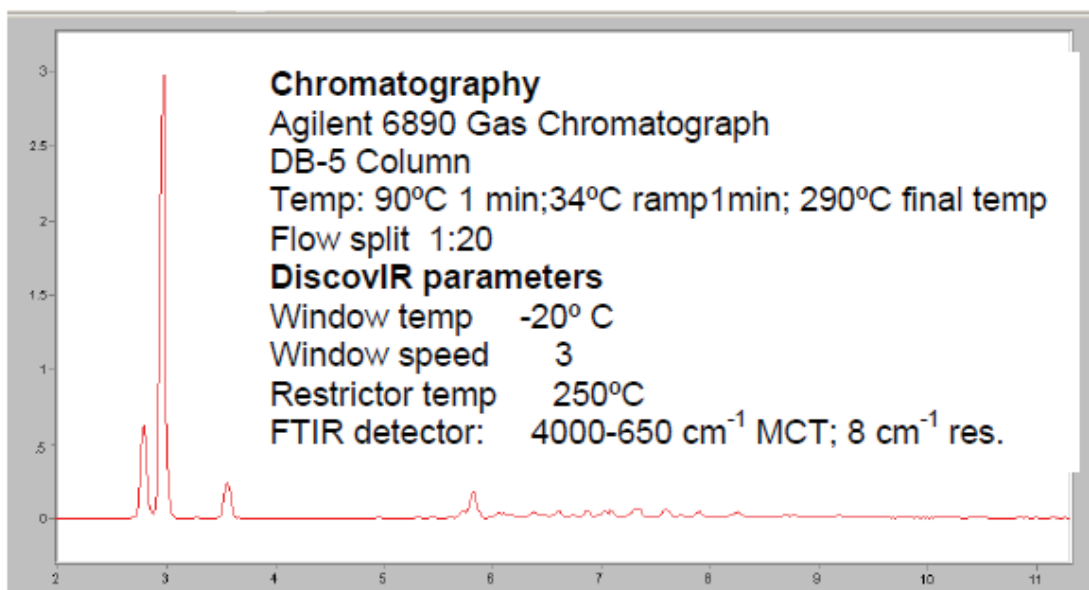


Figure 2 Infrared chromatogram of Clan lab sample

The elution times of the four peaks were 2.79 min, 2.97 min, 3.57 min, and 5.9 min.

The DiscovIR collects spectra at 0.3 sec intervals during the run. Figure 4 displays spectra taken from the data set at the peak maxima of the first three elution peaks.

The spectra are strong, and possess the fine structural detail typical of solid phase spectra.

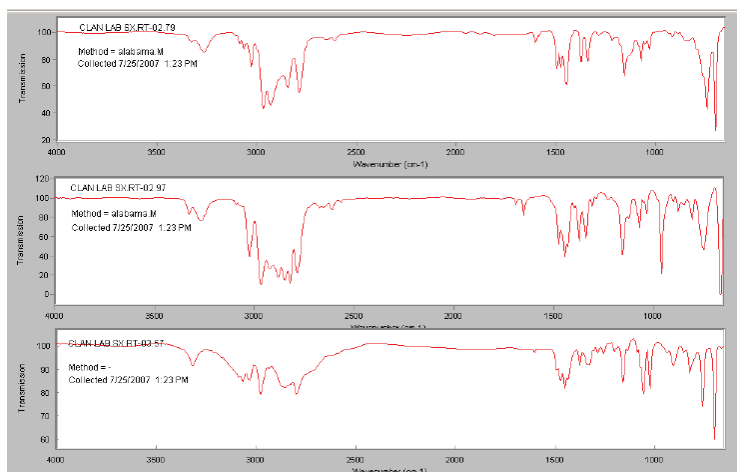


Figure 3 IR spectra from three of the Clan lab elution peaks

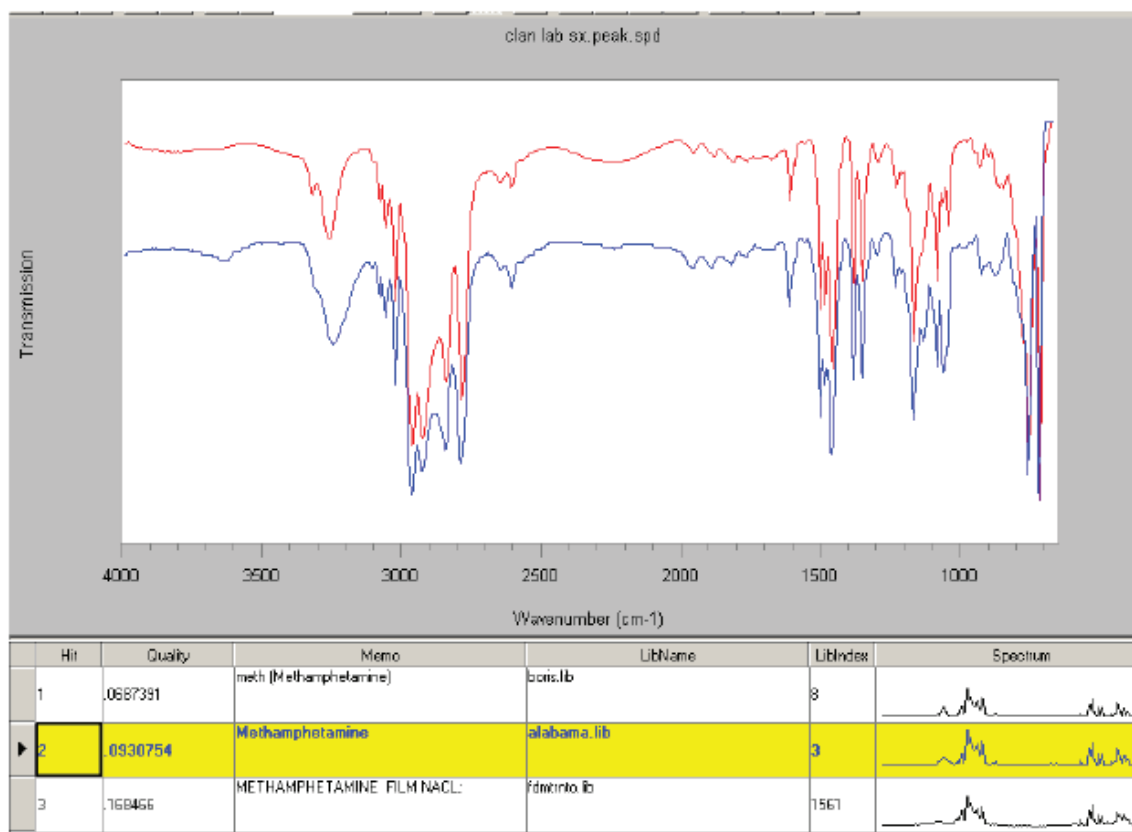


Figure 4 Identification of the 2.79 min peak as methamphetamine

A library of drug spectra was prepared, using the DiscovIR and known standards. The spectra shown in Figure 3 were matched against the prepared library. The peak eluting at 2.79 minutes was identified as methamphetamine (Figure 4). In a similar fashion, the other three peaks were identified by matching their spectra against the drug library.

The 2.97 peak was determined to be CMP [1-(1',4'-cyclohexadienyl)-2-methylaminopropane], a byproduct formed in the synthesis of methamphetamine. Peak 3 was identified as pseudoephedrine, and the fourth peak is a hydrocarbon residue. Components are thus identified by two independent methods; their infrared spectra and their chromatographic elution times.

ISOMER Identification: GC-IR and GC-MS

While Mass spectroscopy identification is made based on the molecular weight of the parent ion and its fragmentation pattern, Infrared spectroscopic identification is based on the unique constellation of adsorption bands that the analyte molecule generates. Because of intramolecular resonances within the molecule, seemingly minor chirality differences can affect profound differences in the resultant spectra. The following examples compare the analytical power of infrared spectrometry when dealing with similar compounds which possess the same molecular weight.

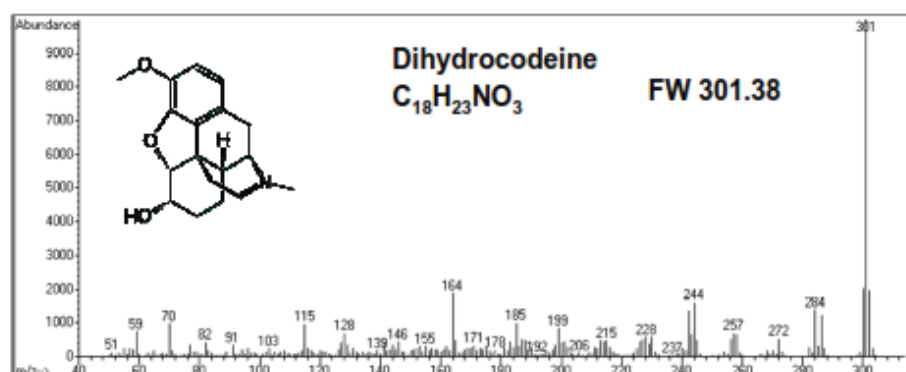
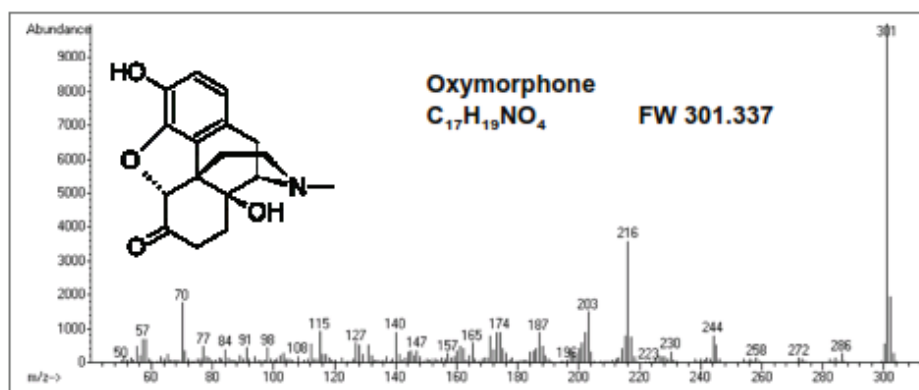
The following examples show three cases of similar molecular structures commonly encountered in the forensic laboratory. Samples of such drugs are readily separated into discrete components. Each component can be subjected to spectroscopic examination. Identification of components is made on the basis of chromatographic elution time and spectra of the components). In the three cases shown, pairs of differing analytes have similar mass. GC-mass spectrometry is compared to GC-FTIR infrared spectroscopy. As in example 1, identification was based on comparison to condensed-phase library spectra prepared on the DiscovIR using known standards.

The following examples illustrate the ability of GC-IR to readily differentiate between compounds possessing similar or identical molecular weights. The examples are presented in order of increasing molecular similarity.

Example 2. Analysis of Oxymorphone and Dihydrocodeine

These compounds are opioid analgesics of similar structure. Although they have differing molecular composition, they possess almost identical molecular weights.

These ethical drugs are produced in various administration forms, and both are subject to illicit usage.



With respect to Mass Spectrometry, Oxymorphone and Dihydrocodeine both generate large base ions and small secondary ions. Identification based on the base ions is not possible, and the limited secondary ion fragmentation patterns make identification quite uncertain.

The infrared spectra of these two compounds, however, show distinctive differences, and permit ready identification. The carbonyl function present in the oxymorphone presents a strong absorbance at 1728 cm⁻¹, and the broad hydroxyl peaks in the 3500 -3000 cm⁻¹ region are much stronger for the oxymorphone. Strong functional group bands for shared molecular structure such as the aromatic group are evident. In the 2000 -800 cm⁻¹ region, the intramolecular vibrational differences of the two molecules result in different sets of bands. These two structures are readily differentiated and identified by IR spectra..

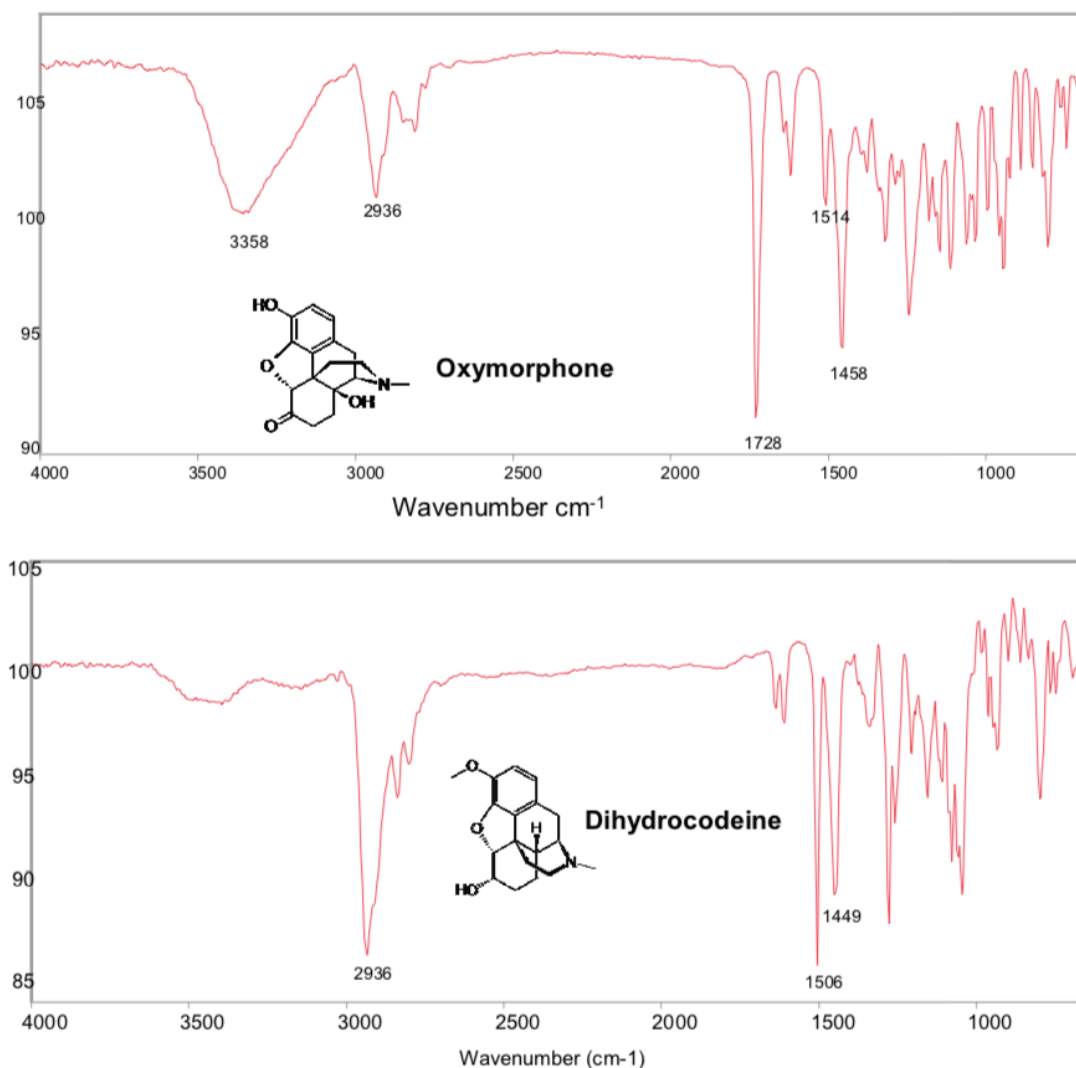


Figure 5 Infrared spectra from chromatograph of sample of oxymorphone and dihydrocodeine

Example 3: Constitutional isomers

Methamphetamine is one of the principal products of clandestine labs in various parts of the world. The positional isomer, phentermine, is produced for use as an appetite suppressant. It is similar to the amphetamines and is classified as a Schedule IV controlled substance under the US controlled substances act. A chromatography separation and deposition on the DiscovIR disc provides the following spectra.

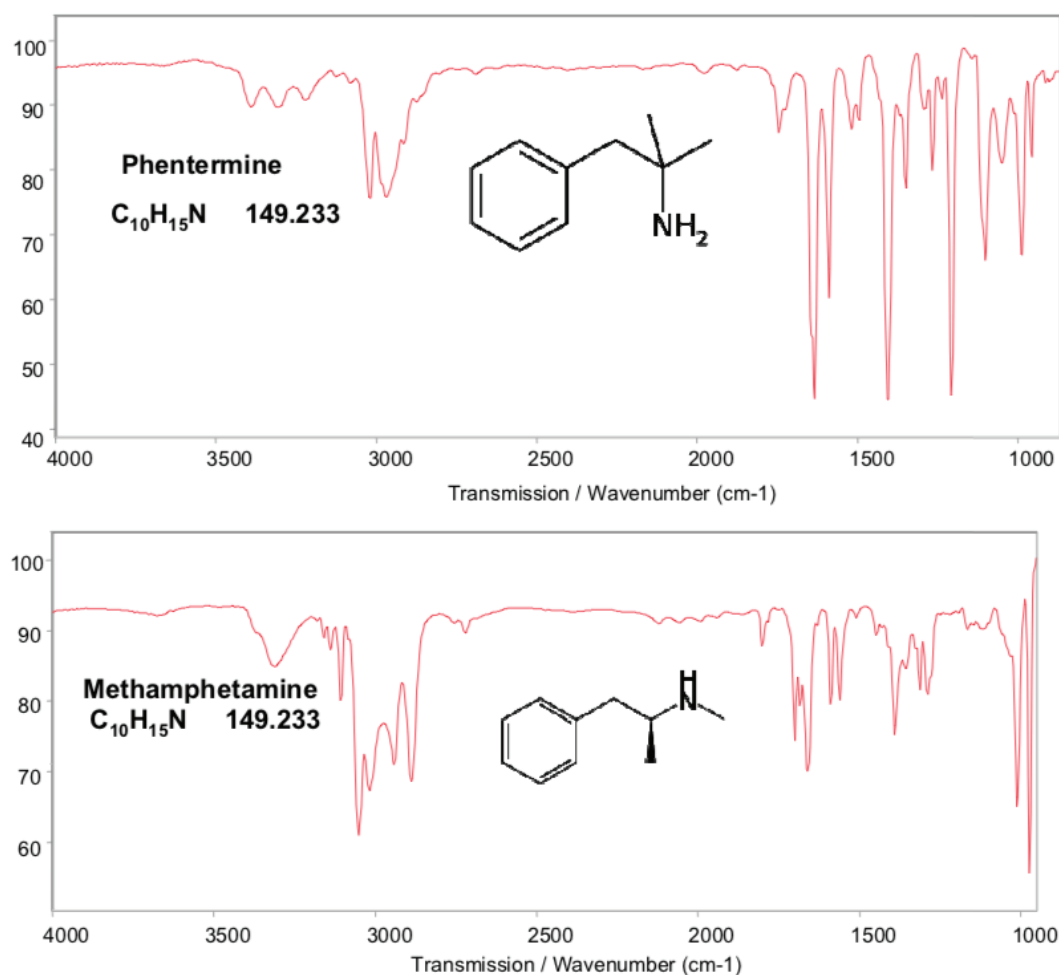


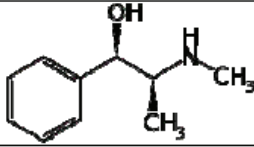
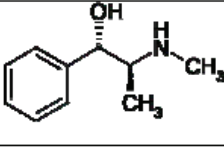
Figure 6 Infrared spectra from chromatogram of a mixture of methamphetamine and phentermine

These two substances have identical molecular composition. Mass Spectroscopy reveals identical parent ions, and very minor differences in the fragmentation ions. The structural differences of the isomers yield distinctly different patterns in the IR fingerprint region, as well as the methyl stretches of the methyl groups adjacent to the primary and secondary amines.

Example 4: Diastereomers Ephedrine and Pseudoephedrine

Samples of diastereomers typically produce similar mass spectra and chromatographic retention times. Ephedrine and pseudoephedrine are both employed in various OTC and prescription therapeutic agents. Either of these materials are also used by clandestine laboratories as precursors to methamphetamine. As such the identification of these substances is useful in forensic identification of residuals in methamphetamine preparations, and in the analysis of processing equipment and materials.

These diastereomers possess two chiral centers. The enantiomers with opposite stereochemistry around the chiral centers (1R,2S), (1S,2R) are designated ephedrine. Pseudoephedrine, by contrast, has same stereochemistry around the chiral carbons (1R,2R), (1S,2S). The molecular diagrams show that these isomers differ only in the R/S configuration at the carbon holding the OH group.

Ephedrine	Pseudoephedrine
	
C ₁₀ H ₁₅ NO FW 165.23	C ₁₀ H ₁₅ NO FW 165.23

It would be problematic to use gas phase spectra to distinguish these two compounds. Solid phase infrared spectroscopy, however, produces high resolution spectra that readily distinguishes the two stereoisomers. Shown below are the DiscovIR spectra of the ephedrine and pseudoephedrine. The isomers give rise to intramolecular resonances that yield distinctly different sets of spectral peaks. In the fingerprint region, we see that three major bands in the 1100 - 1200 cm⁻¹ are shifted in frequency from one spectrum to the other, as well as the band near 750 cm⁻¹. These features provide ready differentiation of the stereoisomers.

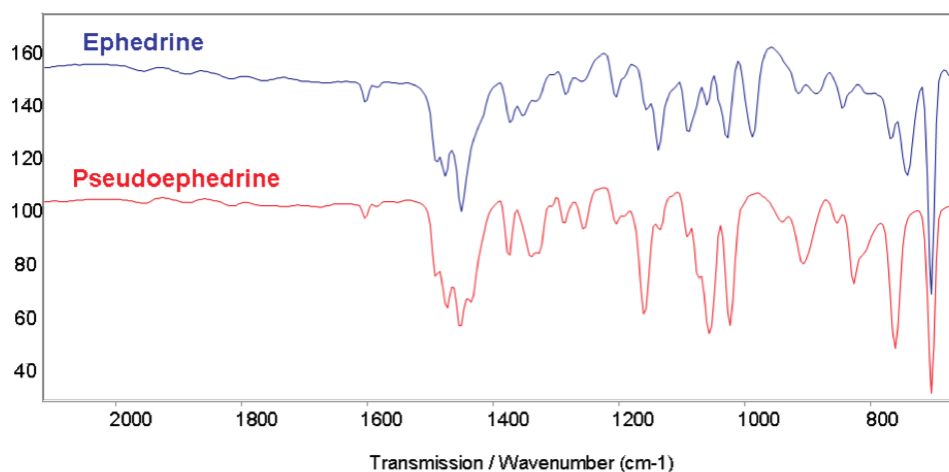
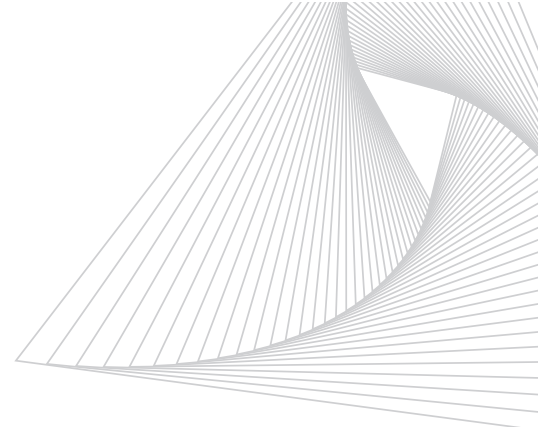


Figure 7 Spectra from a separation of ephedrine and pseudoephedrine



Conclusion

Spectra Analysis provides a “hyphenation” system designed to the needs of modern forensic drug analysis laboratories. It provides the capability of unattended automated operation, data archiving, and software for identification of analytes. The daily processing capacity can be as high as several hundred samples/day.

The technology is particularly useful in dealing with isomers and chiral molecules. Materials of identical molecular weight are difficult to distinguish by mass spectra, but are readily distinguishable by high resolution solid phase IR spectroscopy. The examples presented here illustrate the complementary aspects of GC-IR and GC-MS.

Specifications

System Overview

Operating Principal	<i>Direct deposition of column eluant on cryogenic cooled ZnSe sample disk</i>
Detection Method	<i>Built-in FTIR</i>
IR detector	<i>0.1 X 0.1 MCT</i>
IR Range	<i>4000 – 700 wavenumbers</i>
Resolution	<i>4 or 8 cm⁻¹</i>
Data Collection	<i>Real-Time, plus post-run rescan</i>
Spectrum type	<i>Transmittance through disk and solid phase sample</i>
Disk capacity	<i>~ 20 hours of chromatography</i>
Disk controlled temperature	<i>-100 to -30°; ambient to 100°C</i>
Unattended operation	<i>10 hours; Autosampler compatible</i>

Data Station

Platform	<i>Desktop computer w/Microsoft Windows</i>
Spectroscopy Package	<i>GRAMS/32 AI™ Thermo Scientific</i>
Standard Features	• <i>Real-time and post-run data collect</i> • <i>Chromatographic/Spectral workup</i> • <i>Band chromatograms for chemical classes</i> • <i>Ratio chromatograms for profiling trends</i> • <i>Alignment and tuning tools</i>
Library search	<i>Included Grams add-in search engine</i>

DiscovIR-GC Configuration

GC flow rates accepted	<i>0.1 to 5 ml/min</i>
GC temperatures	<i>Programmed up to 420°C</i>
Sensitivity	<i>Sub-nanogram</i>
Chromatograph	<i>Compatible with all GC systems (user supplied)</i>