DiscovIR

Solid-phase Deposition

FTIR Technology

and Applications

- Potential market and Spectra positioning (industrial sectors, conference presentations, scientific activities, R%D with Professor Luigi Mondello from the University of Messina).

- What makes DiscovIR solid-deposition FTIR technology a unique identification tool?

- Which benefits are to be gained by the online hyphenation GC(LC)-MS-FTIR (parallel detection)?

- Presentation of the FFNSC GC-MS-FTIR Library (flavour and fragrance natural and synthetic compounds) based on GC-EI MS with Linear Retention Indices (LRI) and solid state FTIR spectra.

- The “quantitative” issue.

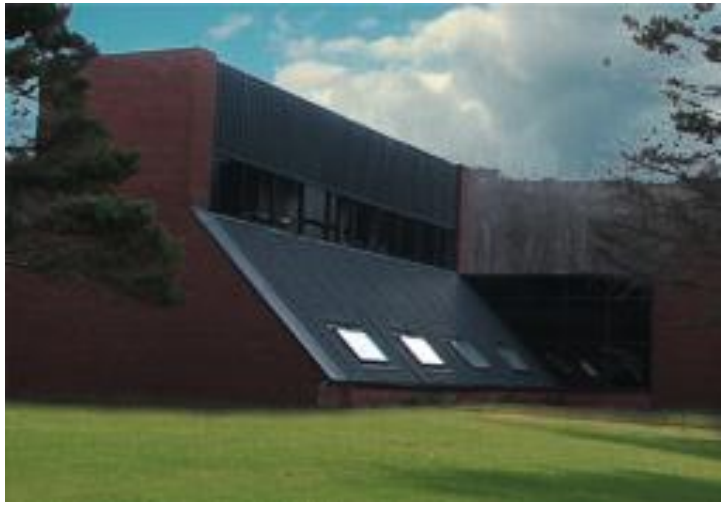
- DiscovIR: how does it work?

- GC-FTIR and LC-FTIR Application fields.

Spectra-Analysis

MARLBOROUGH, MASSACHUSETTS (US)

R&D AND APPLICATIONS
SALES & MARKETING
PRODUCTION & SERVICE
FINANCE & ADMIN



https://spectra-analysis.com/

- The Company develops, manufactures and sells worldwide innovative detectors for GC and LC based on solid phase FTIR.
- The first market approached has been Toxicological/Forensics.
- The company is moving its attention towards Flavour&Fragrance, Food, Petrochemicals and Pharmaceuticals.

BeSep

Research & Development

BeSep S.r.l. was founded in 2019 as an Academic Spin-off of the University of Messina (Italy). It is located at the Department of Chemical, Biological, Pharmaceutical and Environmental Sciences.

http://www.sepsci.unime.it

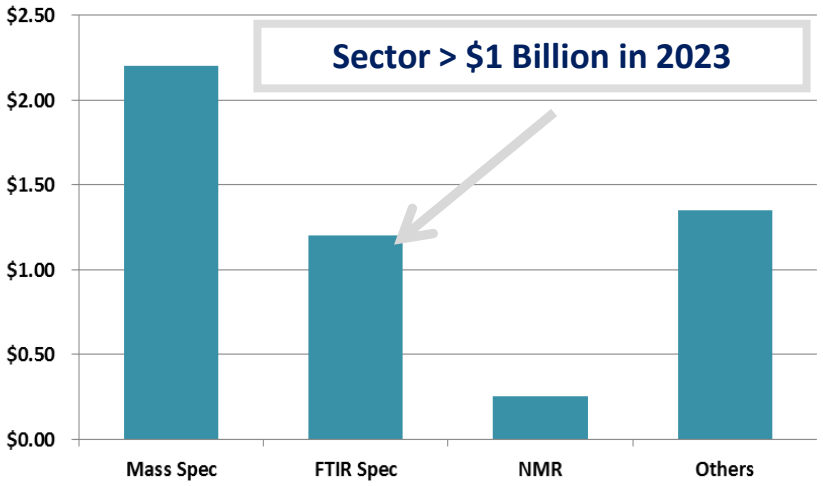
- Research and development of new analytical methodologies using innovative analytical instrumentation.
- Development of dedicated software and libraries, scientific assistance in the area of separation science.
- Established research collaborations with leading analytical companies (Shimadzu, Waters, Merck…).

FTIR – Market

More than $900 million in 2018
Expected to grow 6.5% over the next five years*

- Differentiates 'chemical isomers', analogs of the same compound
- Provides added and complementary information with MS
- Uses same process flow as MS
- Integrates well with chromatography: SEC, HPLC, GC
- DiscovIR is the only 'fully automated solid phase' FTIR

Global Spectroscopy Market



SPECTRA-ANALYSIS'S Share
Hyphenated FTIR: 6% (55 million)

HPLC:

- Polymer Analysis
- Pharmaceuticals
- Chemicals
- Agricultural/Food

GC:

- Forensic Drug Chemistry
- Agricultural/Food
- Oil&Gas

***CAGR data (Compound Annual Growth Rate)**

DiscovIR-LC® Installed Customer Base

PHARMACEUTICAL (Excipients & Process Contaminants)

BASF, Germany	Excipients
MERCK Research Labs, PA	Excipients
ALCON (Novartis), GA	Process Contaminants
CORDIS (J&J), PA	Implant Coatings
SHIRE Pharmaceutical, MA	Process Contaminants

POLYMER ANALYSIS

DOW Chemical (5 systems)	Additives
DuPONT (2 systems)	Fiber Coatings
KIMBERLY CLARK, GA	Healthcare Products
AFTON CHEMICAL, VA	Fuel Additives
WR GRACE, MA	Additives
SABIC Innovative Plastics, NY	Plastic Products
NISSAN Arc, Ltd, Japan	Lubricant Additives
PUBLIC SAFETY, Canada	General Analytics
AXALTA Coating Systems, DE	Coating Additives
KONISHI CHEMICAL, Japan	Additives

DiscovIR-GC® Installed Customer Base

FORENSIC DRUG ANALYSIS, International Labs

Canada Border Services Agency
Forensic Sciences of South Australia
Victoria Forensic Science lab, Australia
Forensic Science Institute of Zurich
Health Canada DAS – (3 systems)
Canada Health Services – (2 systems)
Singapore Health Services
Japan National Police Agency (9 systems)
Japan Customs
ST. Gallen Police Forensic, Switzerland
Forensic Institute – Ljubljana, Slovenia
Landeskriminalamt, Schleswig-Holstein, Germany
Landeskriminalamt, Berlin, Germany
Landeskriminalamt, Munich, Germany
Landeskriminalamt, Dresden, Germany
Finnish Customs, Finland
Malaysia Customs, Malaysia

CHEMICAL WEAPONS ANALYSIS

Oak Ridge National Laboratory, TN
Lawrence Livermore National Lab, CA
Bundeswehr Institute, Germany
US ARMY, ECBC, Aberdeen
US Naval Research Laboratory
ARMSCOR, Dept. Defense, South Africa
OPCW, The Hague
Porton Down research lab, UK

CROP SCIENCE/PHEROMONES

Universidade do Parana, Sao Paulo, Brazil
University of Tokyo, Japan
DuPont, Newark, DE
El Colegio De La Frontera, Mexico
Technical University Braunschweig, Germany

FRAGRANCE

MANE ET FILS, Grasse, France

Current Research Fields – Applications and Scientific Activities

1.Forensic Chemistry - Drugs of abuse -

-Authorization requested to the Italian Minister of Health for purchase and use of all the prohibited substances listed by the Regulation.

-Construction of a solid-deposition GC-FTIR library of all the standard compounds.

-Analysis of drugs of abuse and their metabolites into biological samples (blood, urine).

-Publication of the results into specialized scientific journals: FORENSIC, TOXICOLOGICAL

-Special Issue for Frontiers in Chemistry: "New Approaches in Forensic Analytical Chemistry". Invited paper in preparation: "New psychoactive substances (NPS) analysis in seized material" (publication by March 2020).

-Paper in prep for Analytical and Bioanalytical Chemistry (Springer) on "Determination of Synthetic Cannabinoids and their metabolites by GC/MS/FTIR". Case-study: 3MeO-PCP in seized powder and urine sample (a case report).

- Paper in prep for Drug Testing and Analysis journal on "Integrated analytical platform for reliable drug identification" (GC-EI MS, LC-Orbitrap MS, GC-FTIR).
Case study: 4-CEC.

- Paper in prep for Analytical and Bioanalytical Chemistry on "Integrated analytical platform for reliable drug identification" (GC-EI MS, LC-Orbitrap MS, GC-FTIR).
Case study: Mephedrone and Isomephedrone.

Current Research Fields – Applications and Scientific Activities

2. Food analysis – Nutritional, Safety, Regulation

- Analysis and characterization of food nutrients and bioactive constituents
- Determination of the cis/trans fatty acid content
- Analysis of pesticides/unknown contaminants

3. Explosives

- Document by EPA8330, sample analysis (collaboration with Merck)

4. Petrochemicals

- Lubricant analysis (degradation/contamination), heteroatoms, jet fuels

5. Chiral compounds (2 chiral centres)

- Pesticides, Pharmaceuticals

Explore the feasibility of MDGC for increased selectivity/sensitivity

- Potential market and Spectra positioning (industrial sectors, conference presentations, scientific activities, R%D with Professor Luigi Mondello from the University of Messina).

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- DiscovIR: how does it work?

- GC-FTIR and LC-FTIR Application fields.

FTIR

- The addition of FTIR to an analytical scheme can yield more robust identification of closely related compounds.

- Mass spectrometry techniques aim to identify the chemical composition of a compound. FTIR detects the isomers, that is the ultimate chemical structure.

- MS may not identify different compounds with the same exact mass, IR can.

- Coupling MS to FTIR and specific Libraries provides a unique tool for identification of unknown molecules.

FTIR vs MS

Component Separation

Chromatography

↓

Solvent Removal

Detection	**Mass Spectrometry**	**FTIR**
Measure	Fragments	Bonds
Information content	Elemental composition	Structural & isomers
Sensitivity	High, but suppression issues	Moderate, universal for organics
Synthetic polymers	Interpretation challenges	Ensemble averaging
Pharma Libraries	Confirm composition Find ionizable contaminants	Isomer confirmation Find all contaminants

- Solid-phase FTIR provides excellent resolution (4 cm^{-1})
- Each compound possesses a unique spectral fingerprint

Can yield more robust identification of closely related compounds

UNTARGETED APPROACH

In a peak chromatogram, the maximum absorbance detected anywhere in the mid-IR spectrum is plotted for every point in time.

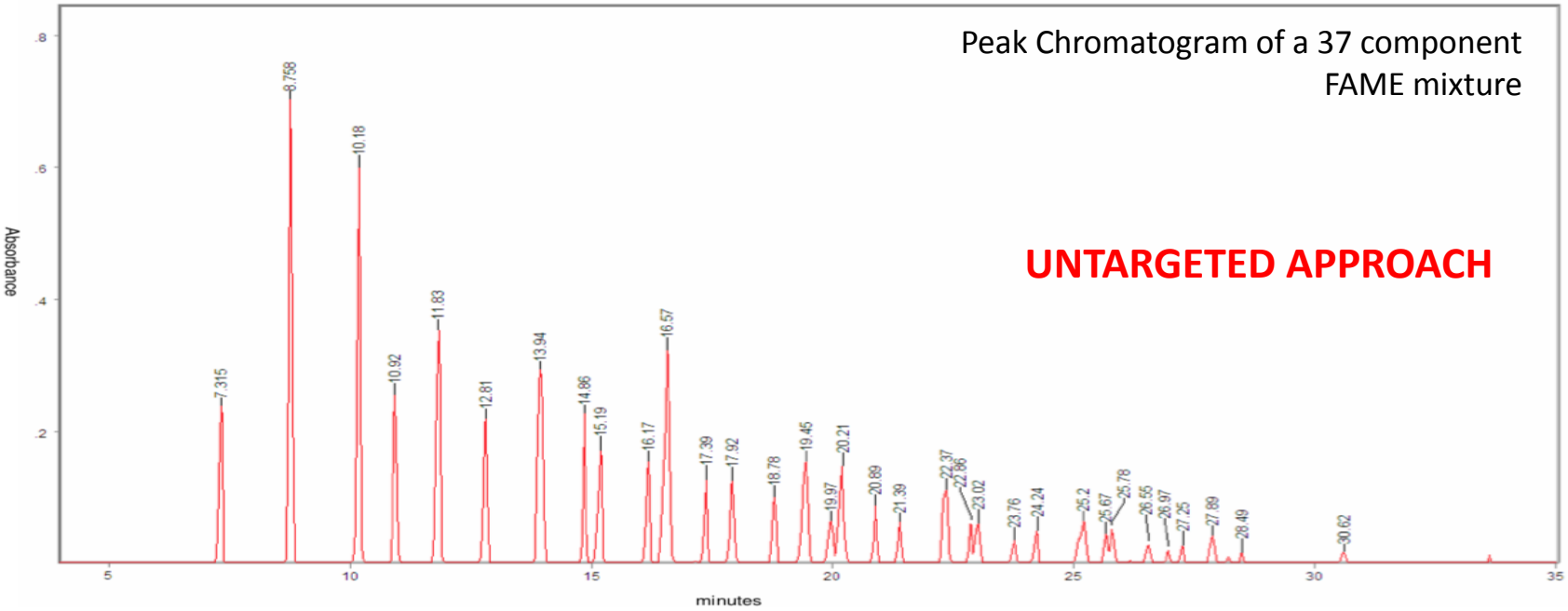
Each exhibits a distinctive pattern as a Spectral Peak Chromatogram (SPC) that plots peaks of highest IR absorbance across all wavelengths

TARGETED APPROACH

In this plot, only the absorbance found in a defined range of the spectrum is plotted. This can be extremely useful when trying to discriminate between compounds on the basis of a particular structural feature.

DiscovIR combines the power of both methods in an automated approach that yields important structural information for each compound in addition to retention time comparison to standards.

Infrared analysis of FAMEs is also an important tool used commonly to identify trans- and cisisomers, among other features.



Sample: Supelco #47885 37 component Fatty Acid Methyl Ester Mixture

Injection: 0.3 µL

Column: Supelco SP-2340-24022, 30m x 0.25-µm [100% poly(bis-cyanopropyl siloxane)].

Helium carrier: 1 µL/min

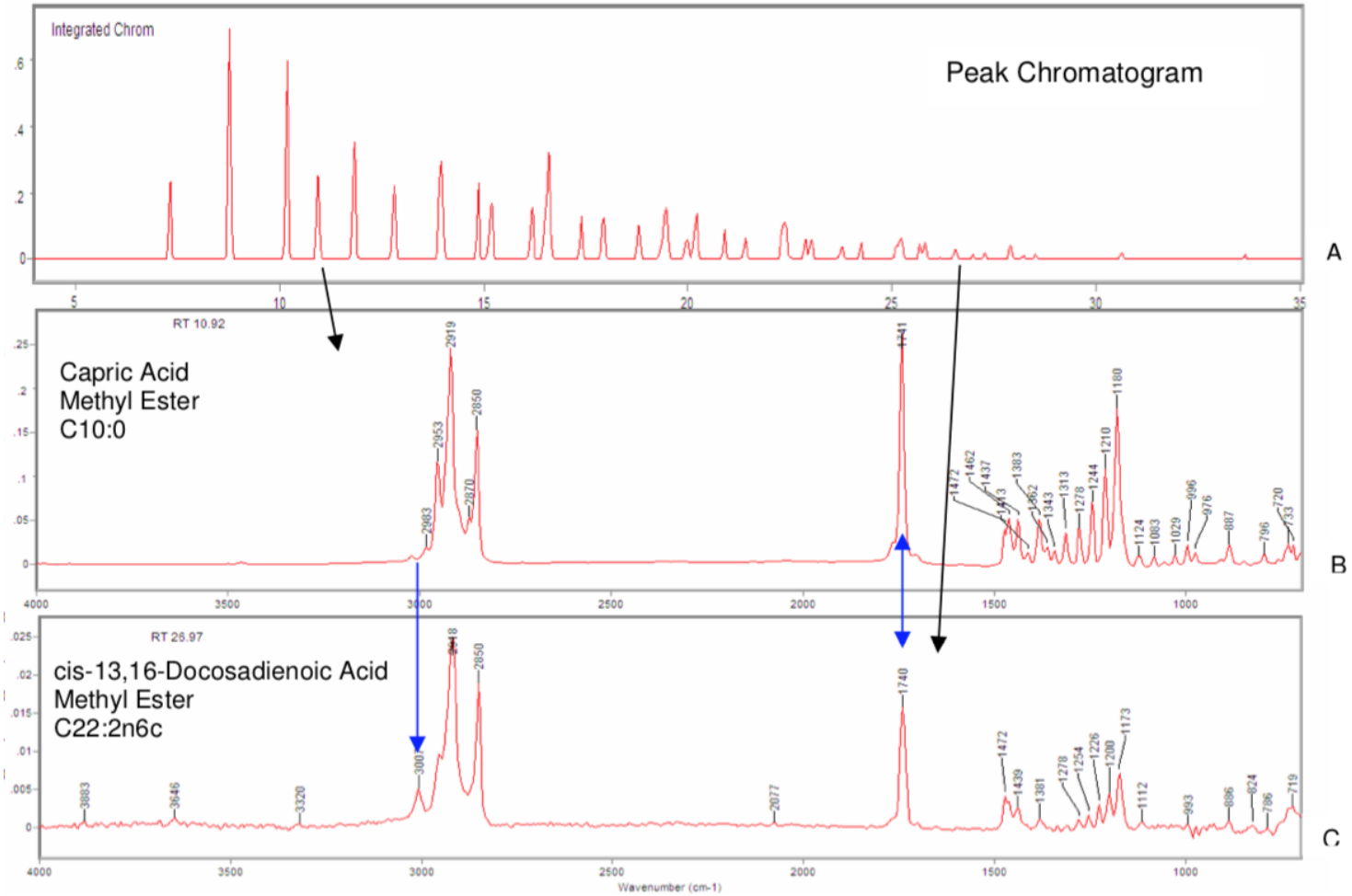
Temp. program: 40 °C for 1 min, 5 °C/min up to 140 °C (hold for 1 min), 4 °C/min up to 230 °C.

Injector, transfer line, restrictor tip: 240 °C.

To prepare samples for analysis, fats are extracted, saponified and methylated to produce the methyl esters. for analysis and quantitation by Gas Chromatography.

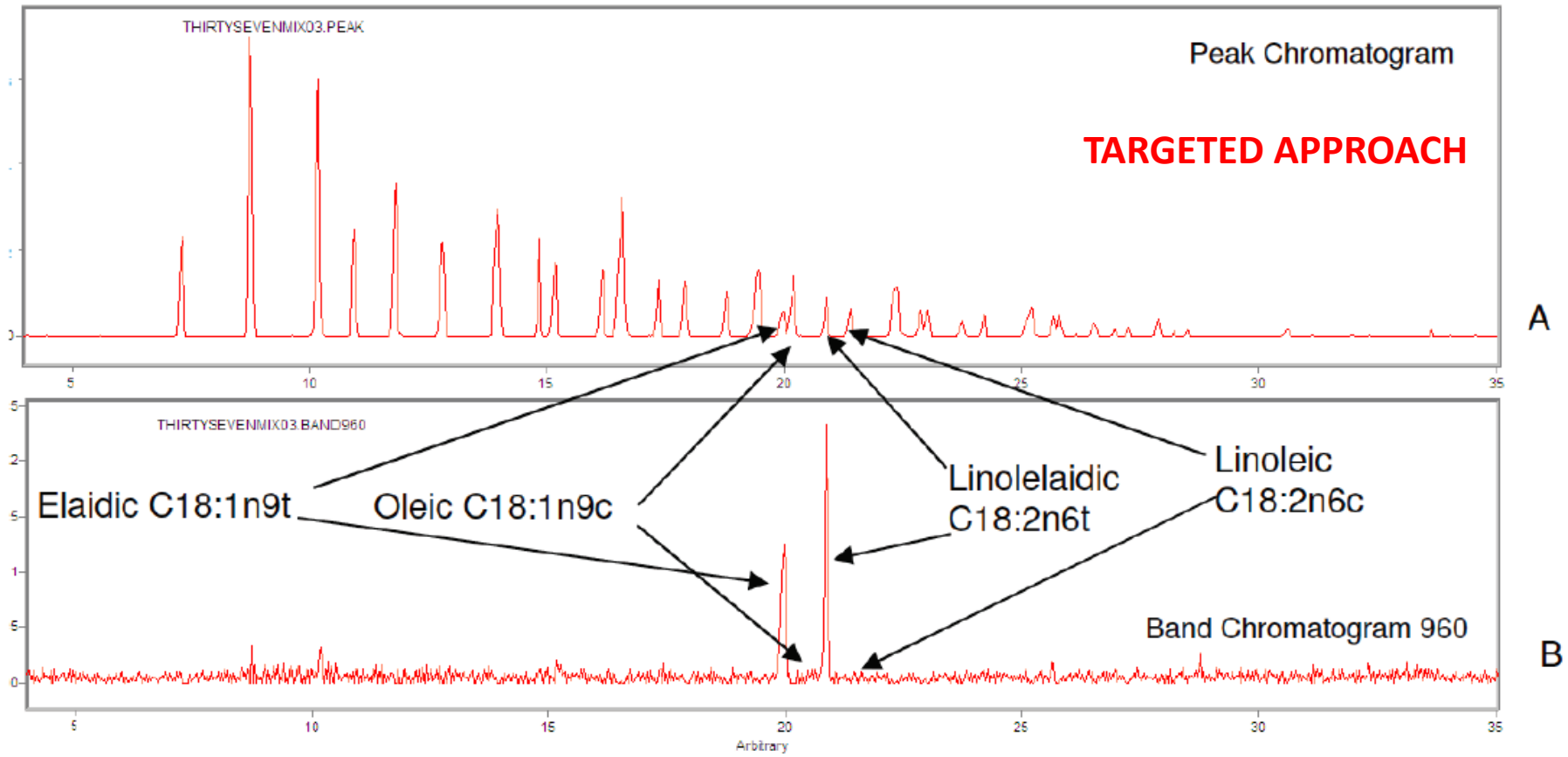
Spectral data about chain length and saturation level, even without retention time are useful for fatty acids whose standards are not commercially available.

UNTARGETED APPROACH



The appearance of the olefinic peak just above 3000 and the downward shift of the carbonyl near 1740 are hallmarks of higher saturation level

Comparison of Peak Chromatogram to Band Chromatogram centered on 960 cm^{-1} of FAME mixture



Elaidic and Linolelaidic Acids, with one and two trans double bonds respectively, show strong relative absorbance in this region compared to the neighboring peaks with only cis configuration.

This alerts the analyst to examine the full spectra of these peaks to confirm that they are consistent with a trans configuration.

Forensic Drug Chemistry – DRUGS OF ABUSE

The **SWGDRUG*** (Scientific Working Group for the Analysis of Seized Drugs) recommend minimum standards for forensic identification of commonly seized drugs. Drugs identification requires the use of multiple uncorrelated techniques. Currently used methods have been divided into three categories, where category A techniques provide the best discriminating power.

Category A	Category B	Category C
Infrared Spectroscopy	Capillary Electrophoresis	Color Tests
Mass Spectrometry	Gas Chromatography	Fluorescence Spectroscopy
Nuclear Magnetic Resonance Spectroscopy	Ion Mobility Spectrometry	Immunoassay
Raman Spectroscopy	Liquid Chromatography	Melting Point
X-ray Diffractometry	Mycrocristalline Tests	Ultraviolet Spectroscopy
	Pharmaceutical Identifiers	
	Thin Layer Chromatography	

**PART III B - Methods of Analysis/Drug Identification Recommendations Version 7.1 SWGDRUG 2016-June-9 -Page 14*

In cases where hyphenated techniques are used they will be considered as separate techniques provided that the results from each are used

Forensic Drug Chemistry – CHEMICAL WEAPONS

The Organization for the Prohibition of Chemical Weapons (OPCW) test mix is a 16-component mixture which is routinely used to calibrate GC and GC-MS systems and QC tests in the OPCW lab in The Hague, Netherlands.

It also is used as GC-based instrument performance check mixture by the CWC Member States' laboratories in their routine analysis of CWA sample (ex. OPCW Proficiency Tests).

The OPCW demands that the identified compounds must be confirmed by at least two different analytical techniques, one of which must be spectrometric.

The coupled technology of solid phase GC-FTIR has received increased attention as its detection limits (ng level) are approaching those routinely reported during GC-MS analysis.

This is a significant improvement of IR detector sensitivity when compared with a gas phase light pipe IR detector which only has ~250 ng detection limits.

The Coupling of GC and LC with FTIR

Over the past years, the coupling of liquid chromatography (LC) and gas chromatography (GC) to Fourier-transform infrared spectrometry (FT-IR) has been pursued by means of different types of interfaces

→ achieve specific detection/identification of sample constituents

INTERFACES

Flow cell (on-line approach)	Solvent removal (semi on-line approach)
The column effluent pass directly through a flow cell and the IR absorption is continuously recorded.	Eluent is eliminated by the interface; compounds are deposited on a substrate prior to the collection of IR spectral data.
Features	Features
➢ Solvents suited for LC have many absorption bands in the IR region ➢ The path length of the flow cell has to be limited ➢ Low LC flow rates are allowed	➢ IR spectra can be recorded independently from the LC conditions ➢ Concentrated deposits may be obtained ➢ Analyte spots can be analyzed repeatedly

Solid phase vs. Vapor phase FTIR

Gas phase GC-IR limitations

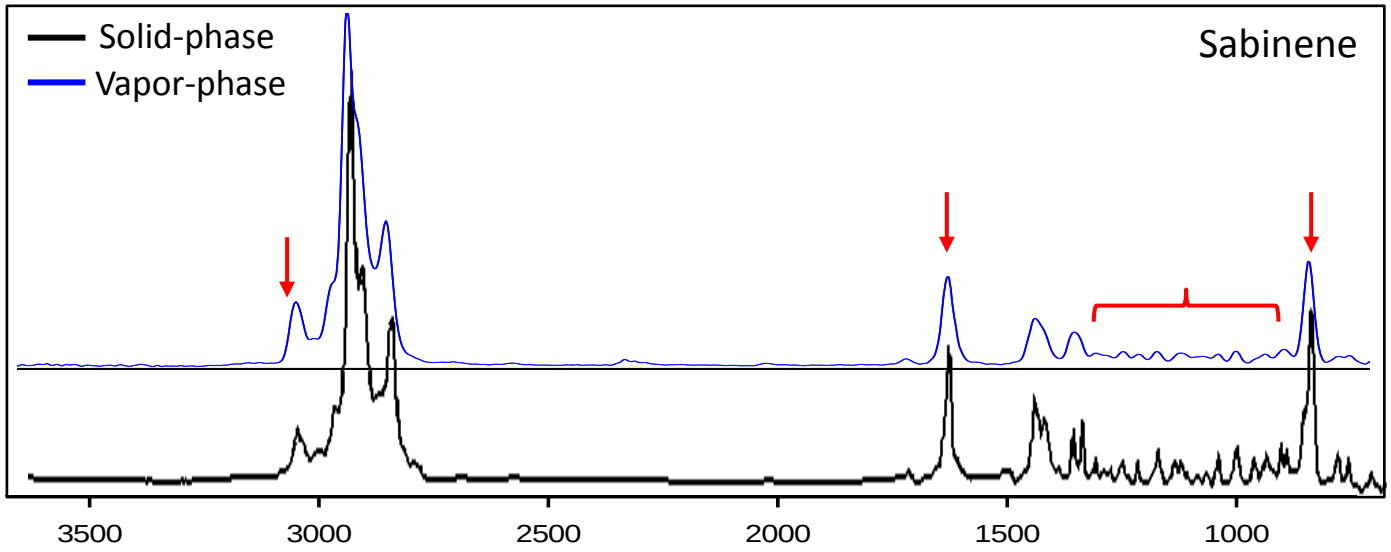
- Gas molecules are free to rotate
- Centrifugal distortion causes diffusion of IR bands in gas phase
- Insufficient spectral resolution

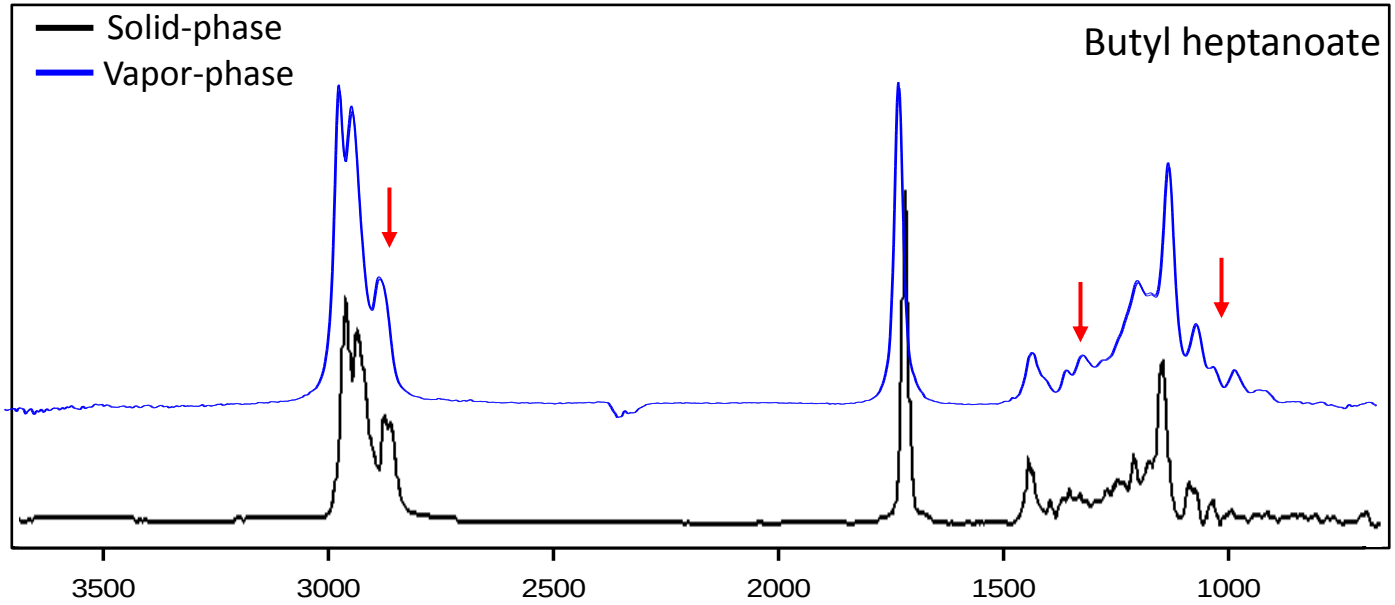
Benefits of condensed phase GC-IR

- Sample is concentrated in small spot
- Distortion of spectra is eliminated in solid phase
- Excellent spectral resolution provides unique IR spectra

	Vibrations	Rotations	Resolution	Spectrum type	Mixture analysis
ATR	Yes	No	≤ 4 cm^{-1}	Solid phase reflectance	De-Convolution
Gas phase GC-IR	Yes	Yes	>16 cm^{-1}	Gas phase absorbance	Limited GC
DiscovIR GC-IR	Yes	No	4 cm^{-1}	Solid phase transmission	Standard GC

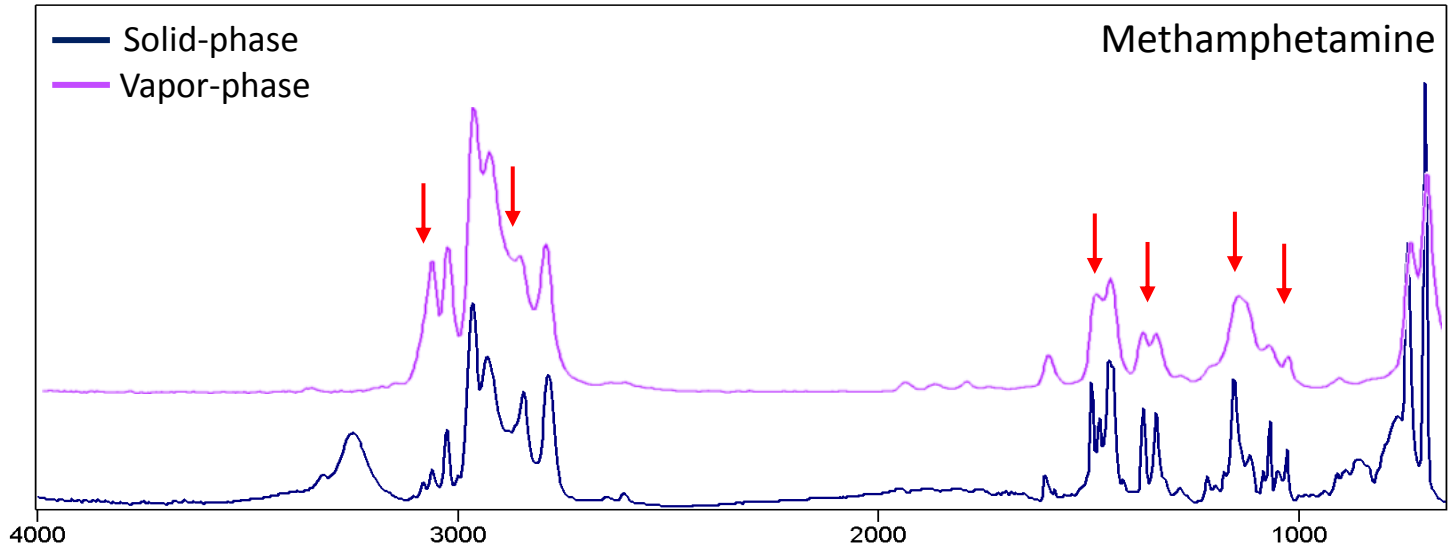
Solid phase vs. Vapor phase FTIR

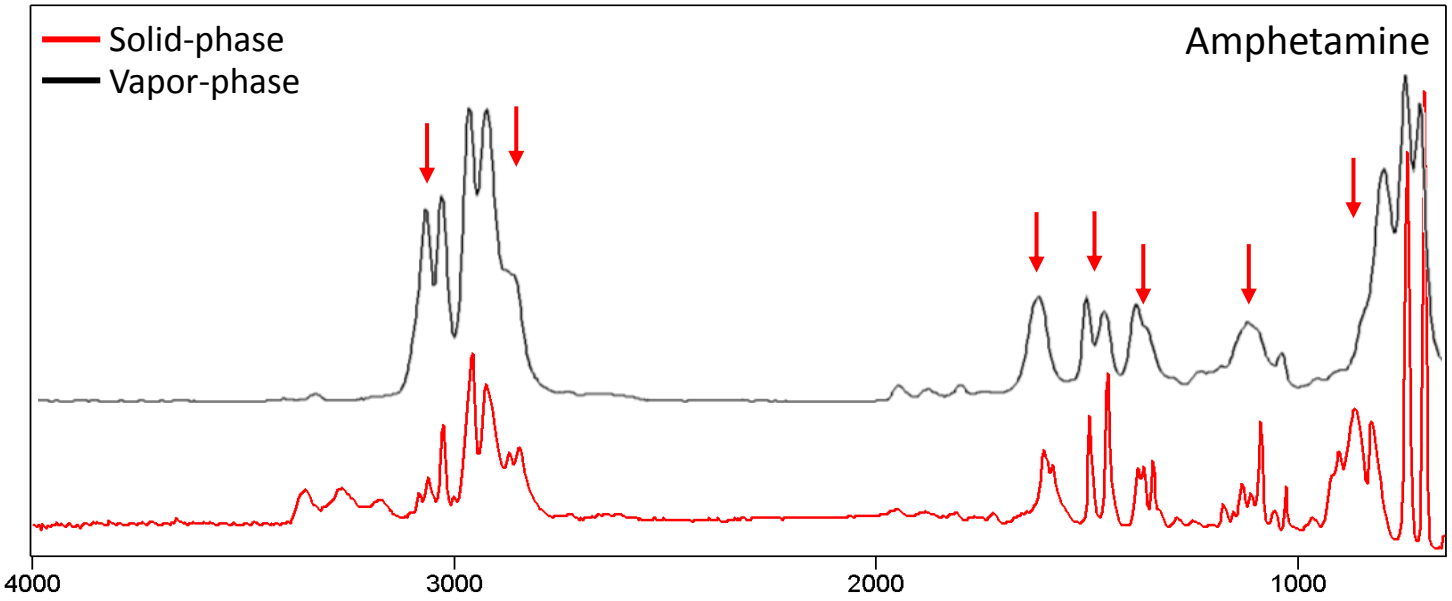




Enhanced resolution

Solid phase vs. Vapor phase FTIR



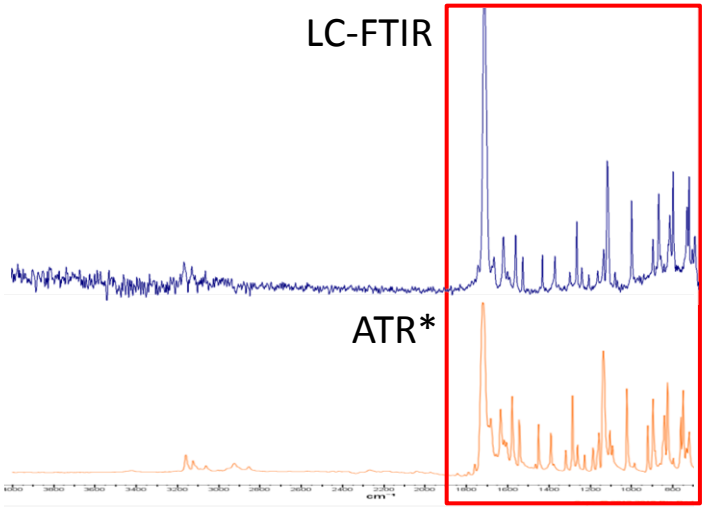


Enhanced resolution

Solid phase FTIR vs. ATR

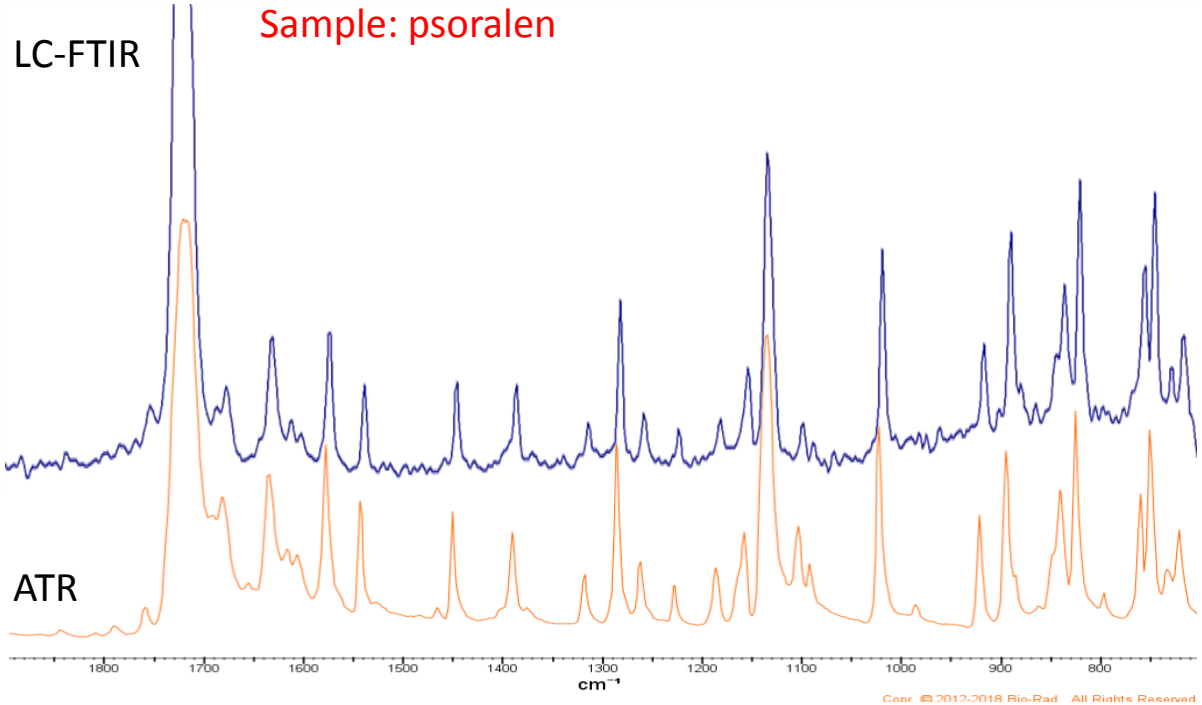
Solid-state FTIR spectra closely resemble ATR ones

Obtain the IR spectra of solid compounds with no need for sample prep

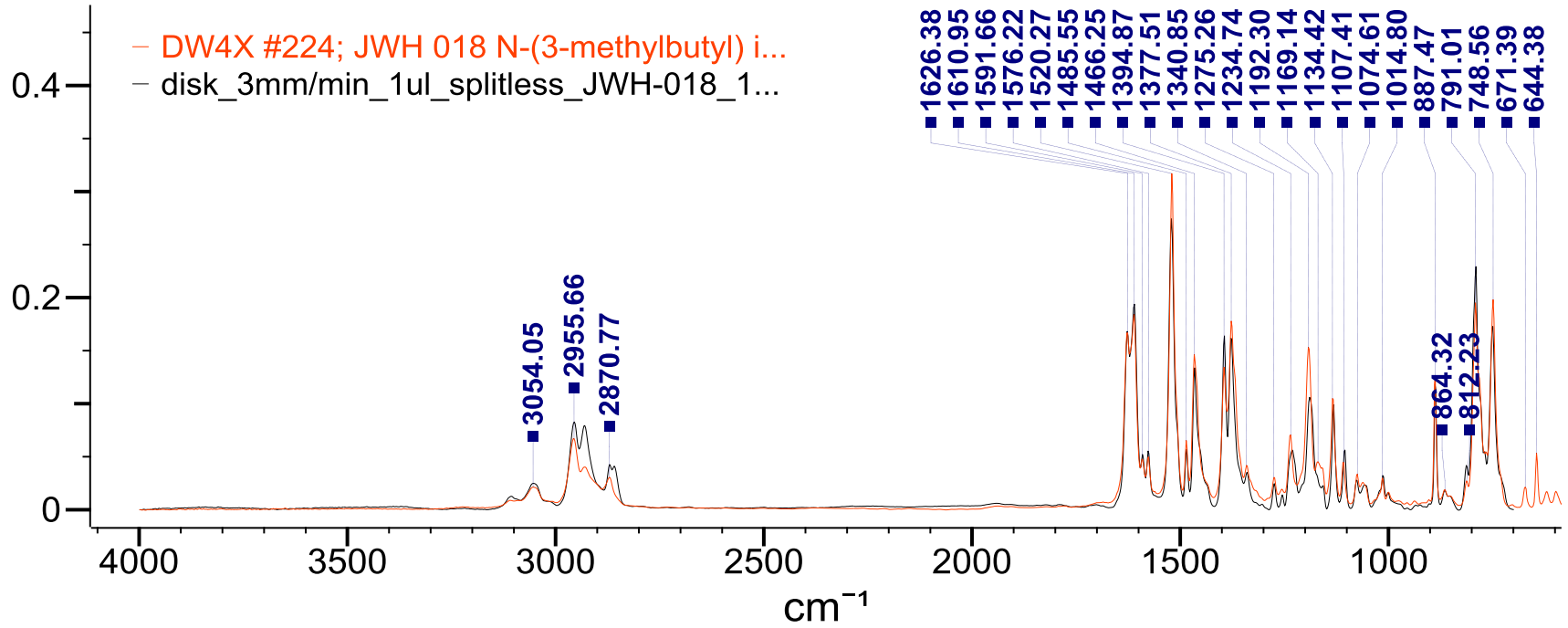


*ATR-Film ($MeCl_2$) from Spectrabase.com

Sample: psoralen



Solid phase FTIR vs. ATR



Source Software: know it all (Biorad)

FTIR spectra obtained by solid-deposition are superimposable with those of ATR Libraries

Comparison of the spectra to the solid-phase IR libraries by the DiscovIR software can uniquely identify the unknowns.

1. Spectral Resolution
2. Sensitivity
3. Compatible with existing solid phase IR libraries
4. Compatible with existing GC-MS columns and methods

When the potent combination of Mass and IR library matching is used, structure assignments can be made with higher confidence.

A key feature of Infrared analysis is that absorption at a certain wavelength is in direct proportional to the amount at hand. Therefore, relative quantities can be determined with confidence, avoiding errors due to disparity in ionization or presence of chromophores.

- Potential market and Spectra positioning (industrial sectors, conference presentations, scientific activities, R%D with Professor Luigi Mondello from the University of Messina).

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- DiscovIR: how does it work?

- GC-FTIR and LC-FTIR Application fields.

Identification in Chromatography: the Key Role of MS

- Structural characterization of chemical compounds
- Identification through search into spectral libraries
- Shotgun approaches and LC-MS or GC-MS techniques are both successfully applied for the analysis of complex samples

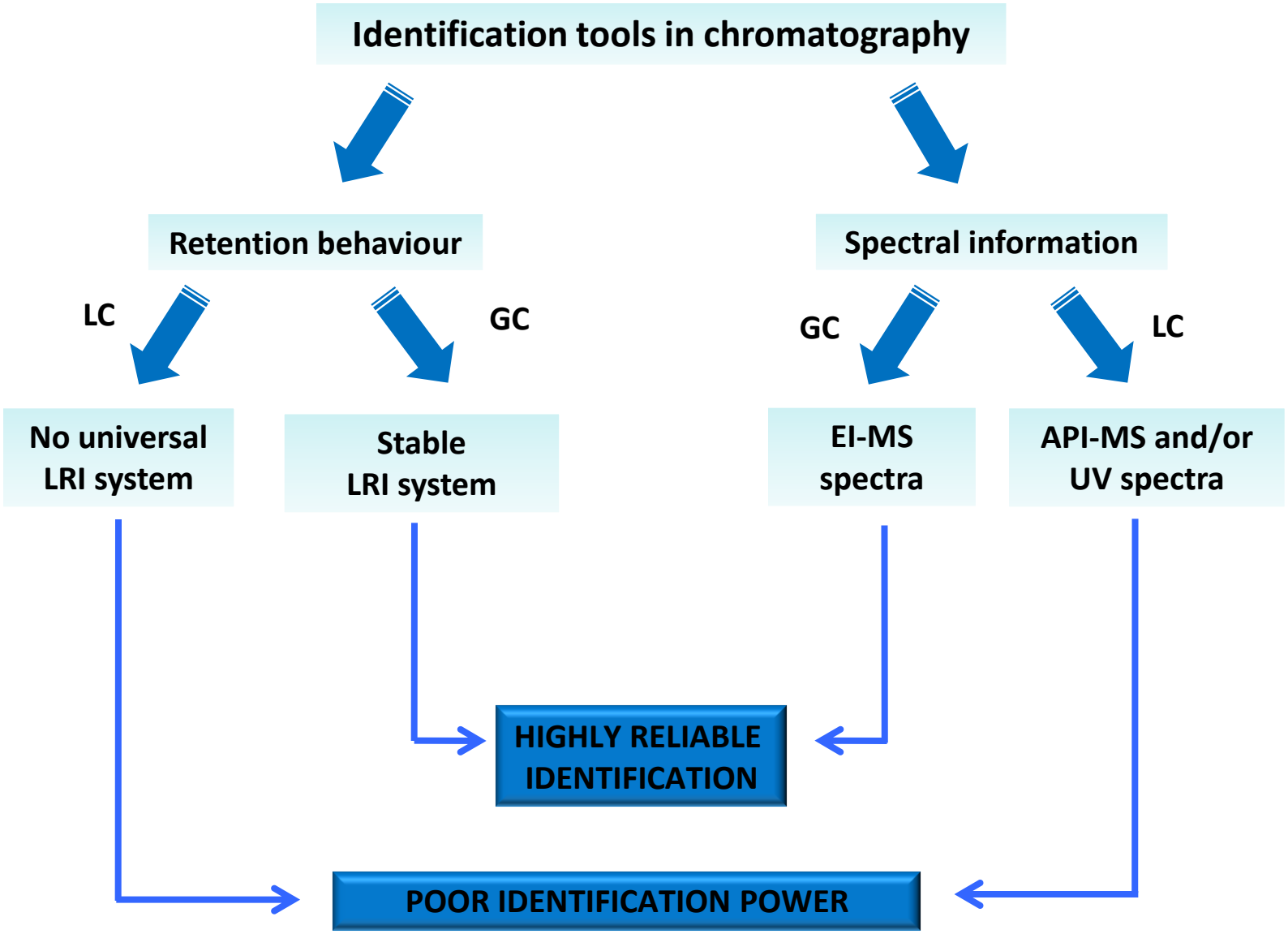
GC-(EI)-MS

- Gas phase ionization
- High vacuum chamber
- No matrix effect
- Strong fragmentation
- Not suitable for thermolabile/non volatile components

LC or SFC-(API)-MS

- Atmospheric pressure ionization chamber
- Great matrix effects
- Low fragmentation
- Particularly suitable for large molecules, but MS/MS needed

Identification in Chromatography

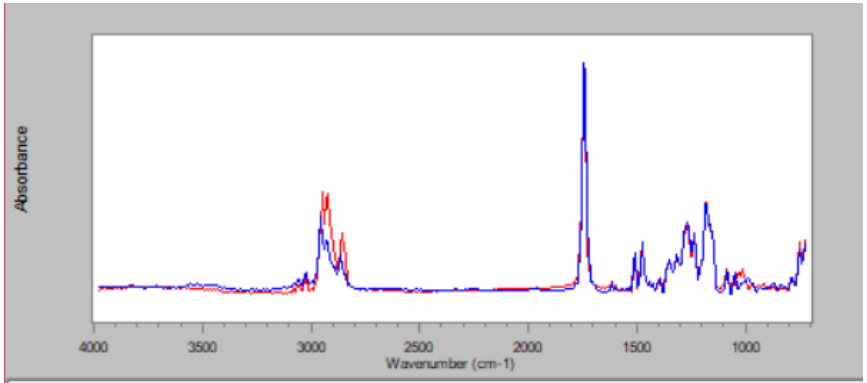


POTENTIAL OF GC-FTIR

The combination of **GC** with **FTIR** is useful because it uses a gas chromatograph to **separate** the components of sample mixtures, and an FTIR spectrometer to provide **structural elucidation** and **compound identification**

Direct complement to MS

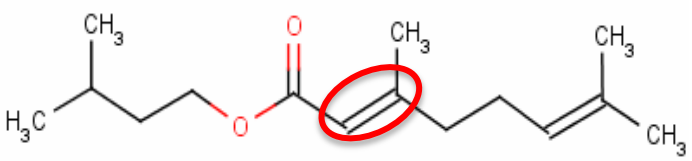
Structural difference between molecules with the same retention behaviour



	Hit	Quality	Memo
▶	1	.125367	Phenylacetate <butyl->
	2	.143293	Phenylacetate <isopentyl->
	3	.26106	Phenylacetate <(2E)-hexenyl->
	4	.281727	Phenylacetate <allyl->
	5	.285918	Phenylacetate <methyl->
	6	.321858	Phenylacetate <isobutyl->

Discrimination of stereoisomers

through the differences in the fingerprint region around 1100 cm^{-1}

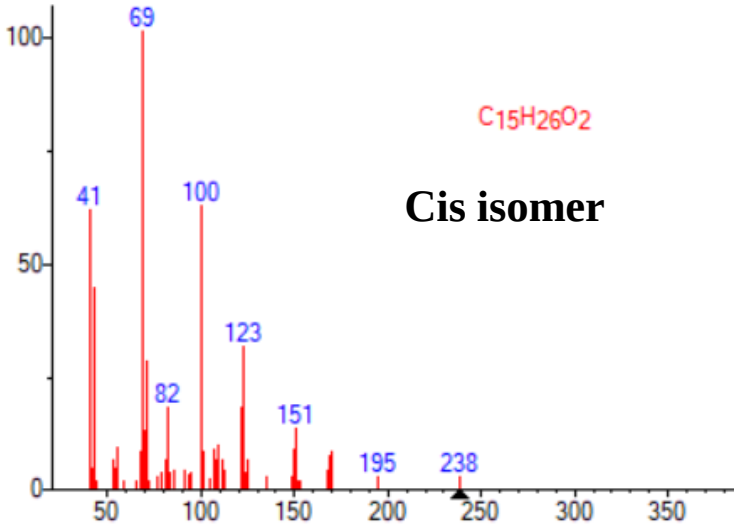


2,6-Octadienoic acid, 3,7-dimethyl-, 3-methylbutyl ester

Cis isomer

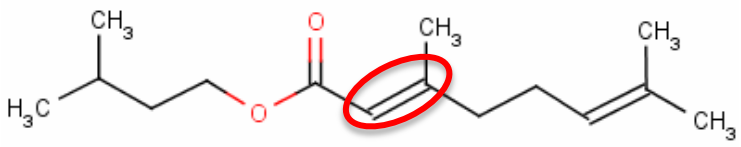
Trans isomer

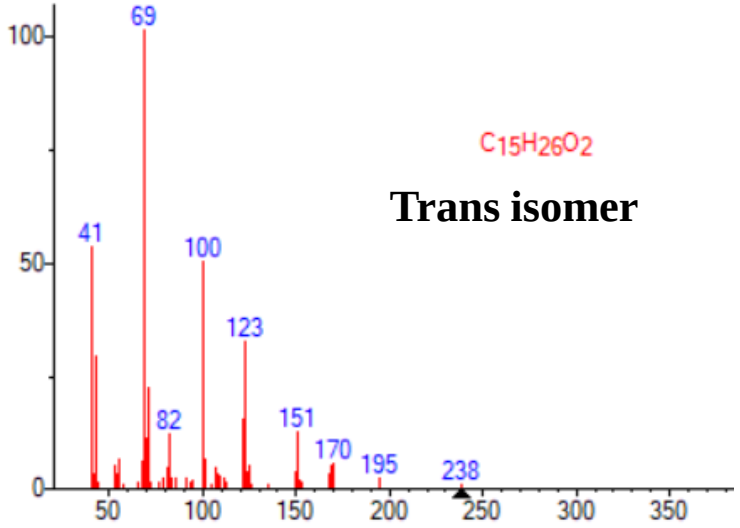
ENHANCHED DISCRIMINATION POWER OF FTIR SPECTRUM



$C_{15}H_{26}O_2$

Cis isomer

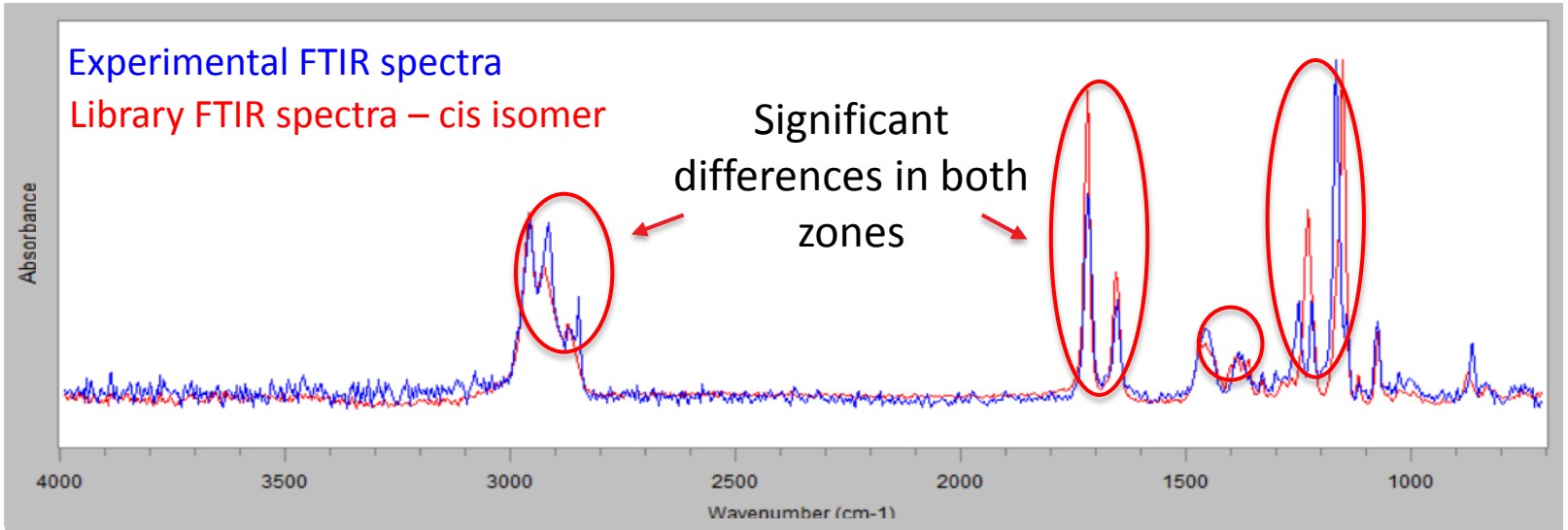


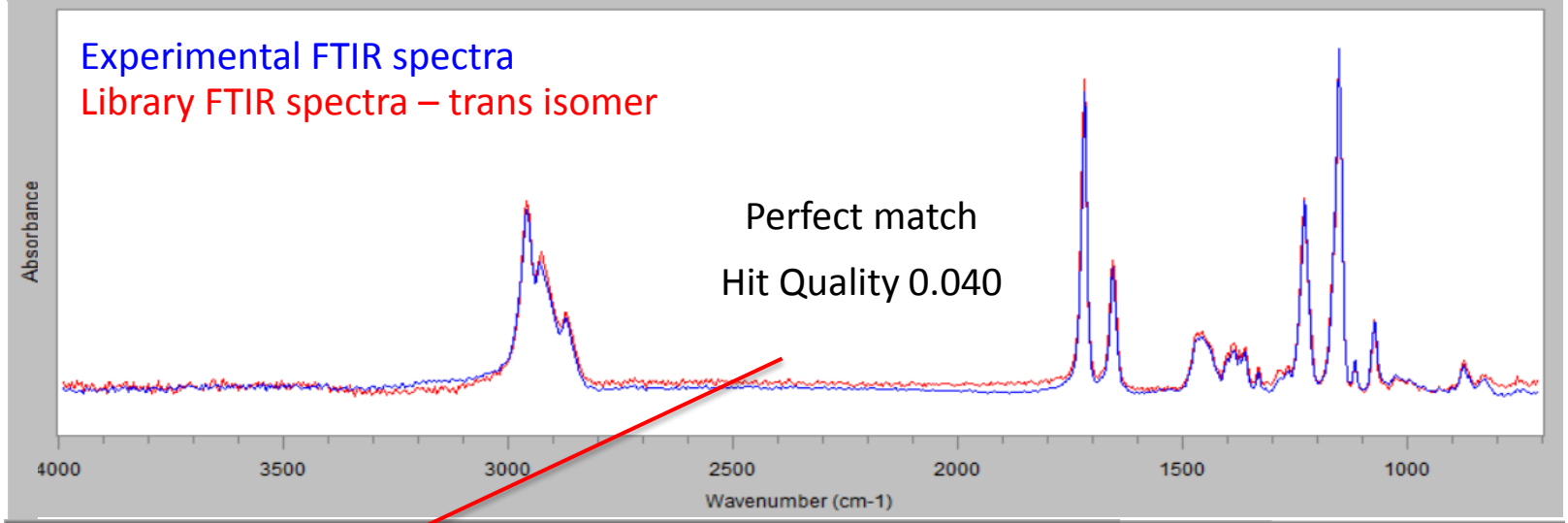


$C_{15}H_{26}O_2$

Trans isomer

ENHANCED DISCRIMINATION POWER OF FTIR SPECTRUM



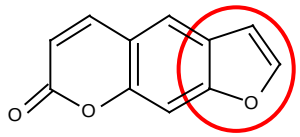


Hit	Quality	Memo	LibName	TextInfo
1	0401344	Octa-(2E),6-dienoic acid <3,7-dimethyl-,	FFNSC_GC-FTIR.lib	Octa-(2E),6-dienoic acid <3,7-dimethyl-,
2	,945067	Octa-(2Z),6-dienoic acid <3,7-dimethyl-,	FFNSC_GC-FTIR.lib	Octa-(2Z),6-dienoic acid <3,7-dimethyl-,
3	,971441	Cinnamyl isovalerate	FFNSC_GC-FTIR.lib	Cinnamyl isovalerate
4	,997495	Ionone <dimethyl->	FFNSC_GC-FTIR.lib	Ionone <dimethyl->

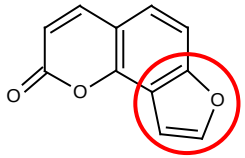
Furocoumarins

- Organic chemical compounds produced by plants
- Structure consists of a furan ring fused with a coumarin
- The furan ring may be fused in various ways producing several isomers

Psoralen
(linear furocoumarins)



Angelicin (isopsoralen)
(angular furocoumarins)



Some act as strong photosensitizers when applied topically to the skin

Phototoxicity & Harmful effects on humans

Regulation EC n.1223/2009

European parliament and Council on Cosmetic Products
(Annex II: list of substances prohibited in cosmetic products)

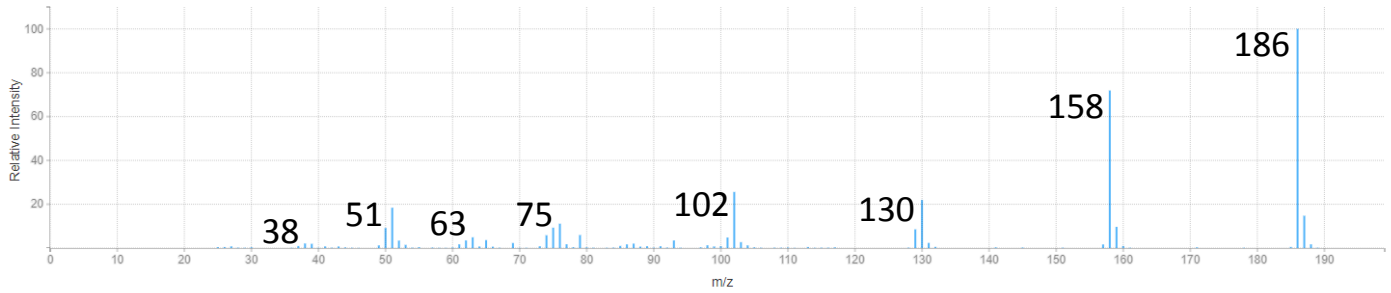
Furocoumarin Positional Isomers

Spectrum Details

HMDB ID: HMDB0034272
Compound name: Psoralen
Spectrum type: Mass Spectrum (Electron Ionization)
Splash Key: splash10-052r-3900000000-eff7b2146482de5c7740 View in MoNA

PSORALEN

Spectrum View

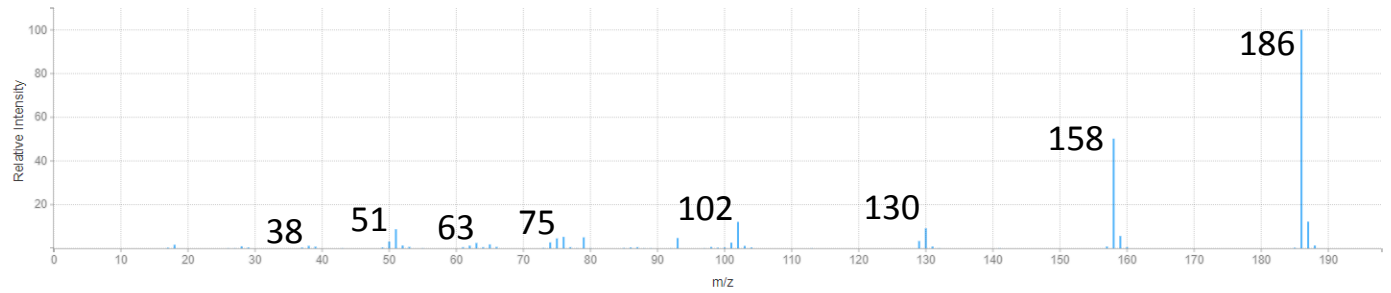


Spectrum Details

HMDB ID: HMDB0033930
Compound name: Angelicin
Spectrum type: Mass Spectrum (Electron Ionization)
Splash Key: splash10-000i-2900000000-b75bda2977a6c5f4a825 View in MoNA

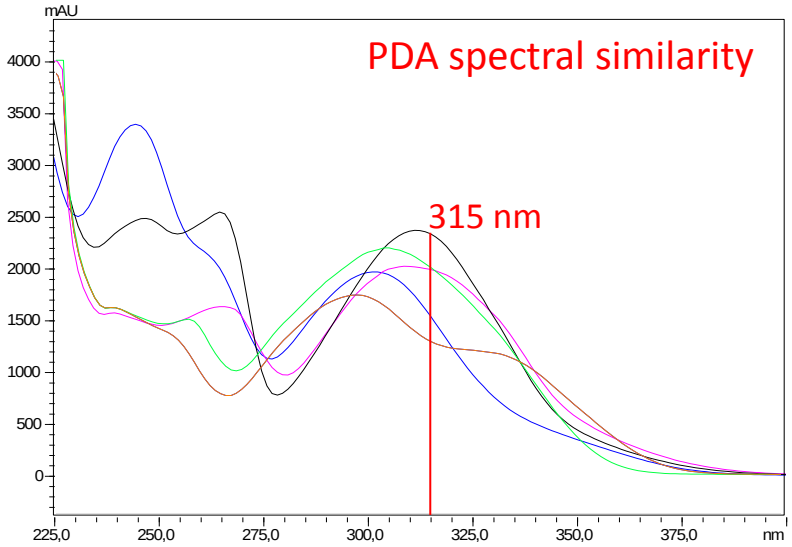
ANGELICIN

Spectrum View

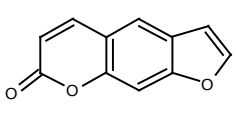


EI-MS spectra do not afford discriminant information

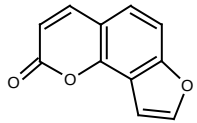
Furocoumarin Positional Isomers



$C_{11}H_6O_3$
MW: 186.166 g/mol

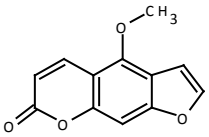


Psoralen

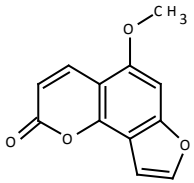


Angelicin

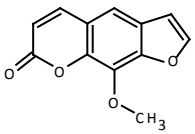
$C_{12}H_8O_4$
MW: 216.19 g/mol



bergapten



isobergapten



8-methoxypsoralen

Identical MRM

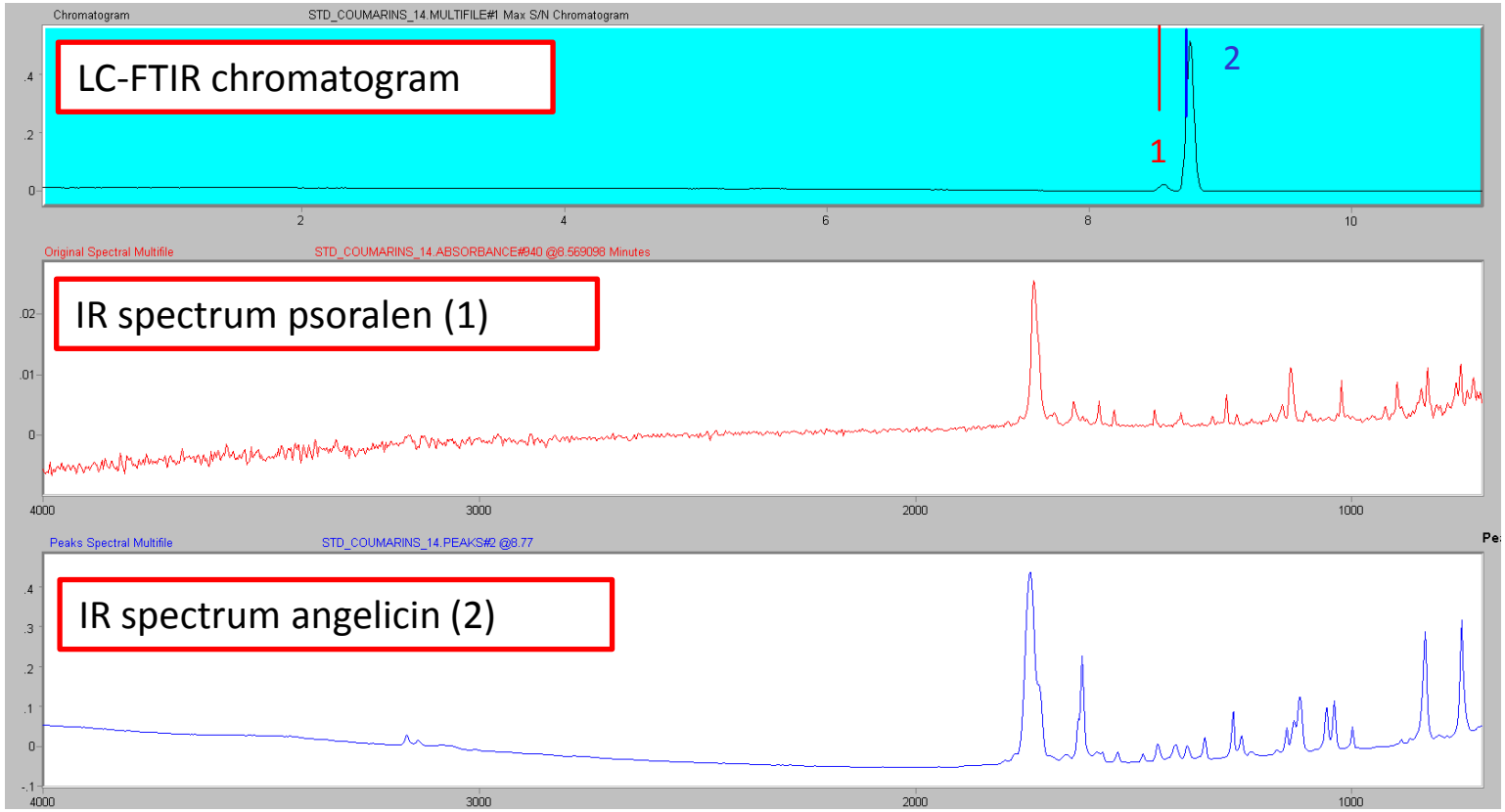
Type	Event#	+/-	Compound Name m/z	Time (0,500 min - 10,200 min)
MRM	1	+	Coumarin 146,80>75,25, 146,80>73,15	
MRM	2	+	Meranzin hydrate 278,90>189,15, 278,90>261,20	
MRM	3	+	Hemiarin 177,00>121,00, 177,00>77,10	
MRM	4	+	Byacangelicin 317,20>231,05, 317,20>233,05, 334,70>1	
MRM	5	+	8-methoxypsoralen 216,90>202,00, 216,90>173,80	
MRM	6	+	Psoralen 186,90>131,10, 186,90>77,20	
MRM	7	+	Angelicin 186,90>131,10, 186,90>77,30	
MRM	8	+	Oxypeucedanine hydrate 304,90>202,90, 304,90>147,15	
MRM	9	+	Citropten 207,00>192,05, 207,00>162,90	
MRM	10	+	Isopimpinellin 246,90>216,95, 246,90>232,10	
MRM	11	+	Meranzin 260,90>188,95, 260,90>131,10	
MRM	12	+	Isomeranzin 260,90>189,25, 260,90>131,15	
MRM	13	+	Heraclenin 286,90>202,90, 286,90>147,05	
MRM	14	+	Bergapten 217,20>202,00, 217,20>174,10	
MRM	15	+	Sinensetin 373,00>343,00, 373,00>312,15	
MRM	16	+	Isobergapten 217,00>201,90, 217,00>174,20	

Identification/quantification approaches rely on the combined use of PDA and LRI or QqQ and LRI*

* A. Arigò, F. Rigano, G. Micalizzi, P. Dugo, L. Mondello, *Flavour Frag. J.*, 2019

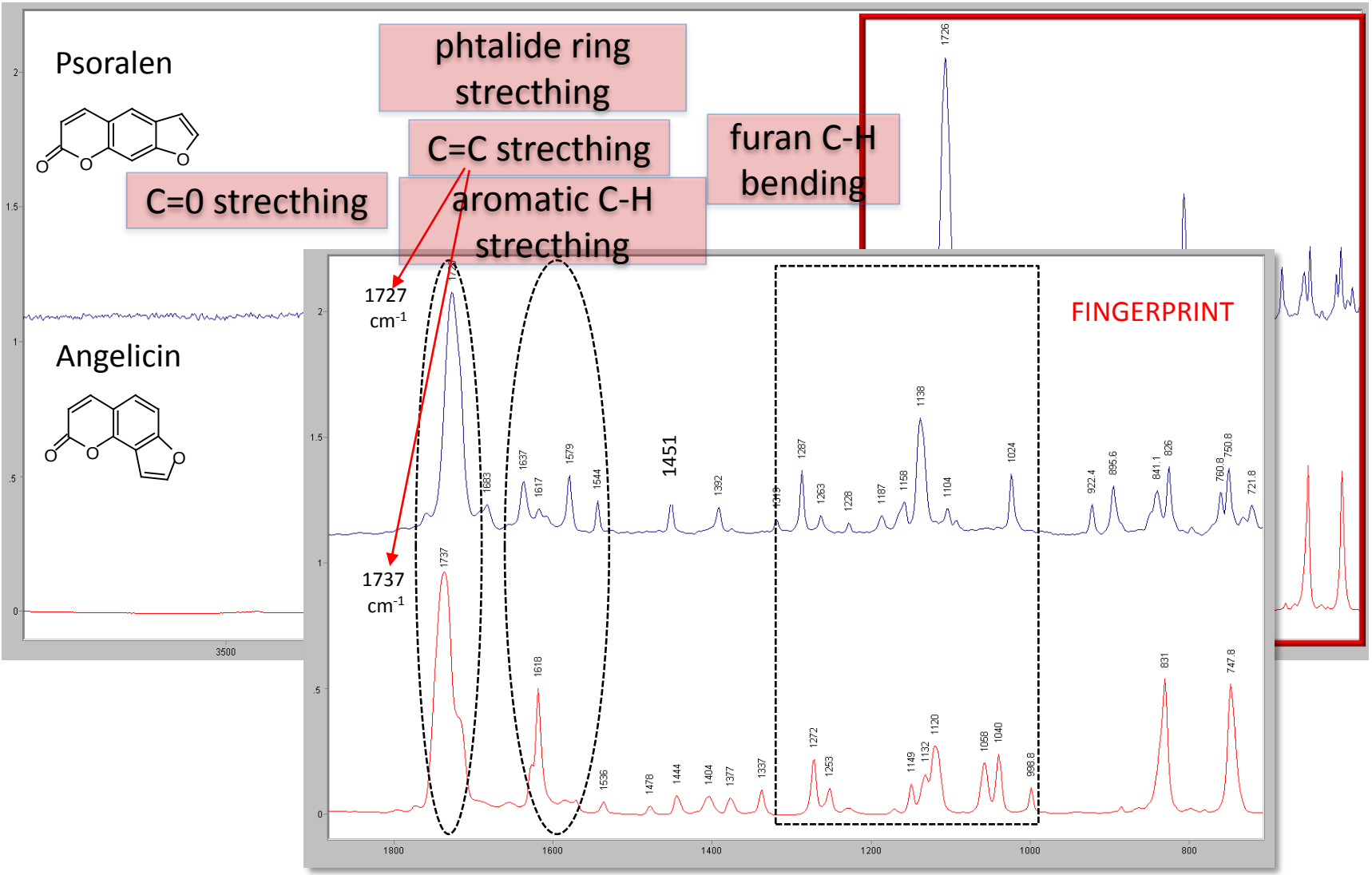
LC-FTIR Analysis

Sample: (1) Psoralen, (2) Angelicin standard mixture in EtOH (5 µg on column)
Column: Ascentis Express C18, 150 mm L × 4.6 mm i.d. × 2.7 µm d.p.
Solvents: A(H_2O), B (MeOH); **Flow rate:** 1 mL/min (gradient mode); **PDA** 315 nm;
Nebulizer voltage: 14 W; **Cyclone temp.:** 180 ° C; **Condenser temp.:** 3-7 ° C; **IR** 4000-700 cm^{-1}

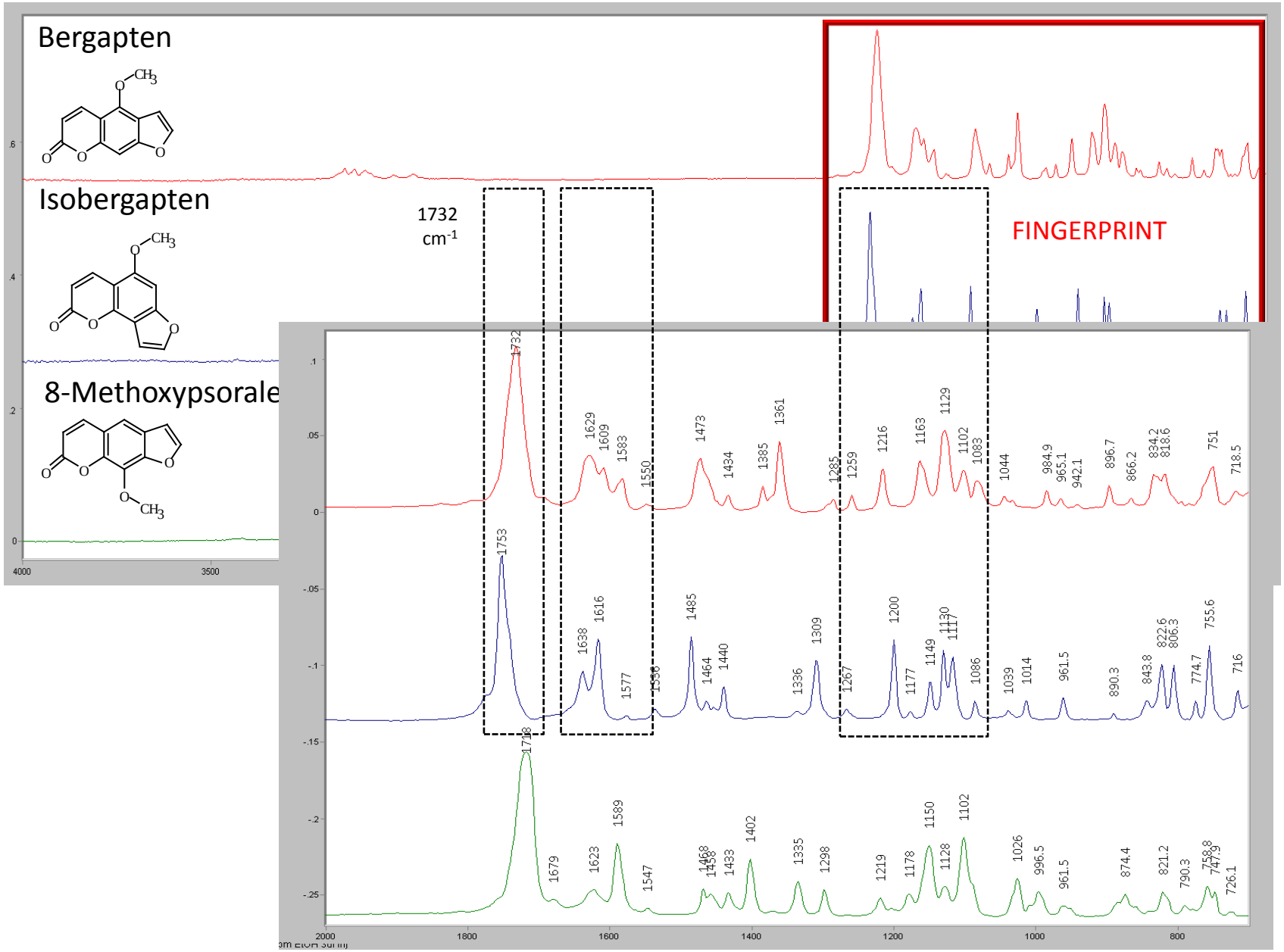


- Each compound in the mixture exits at a different time and possesses a unique spectral fingerprint
- In a peak chromatogram the maximum absorbance detected anywhere in the mid-IR spectrum is plotted for every point in time

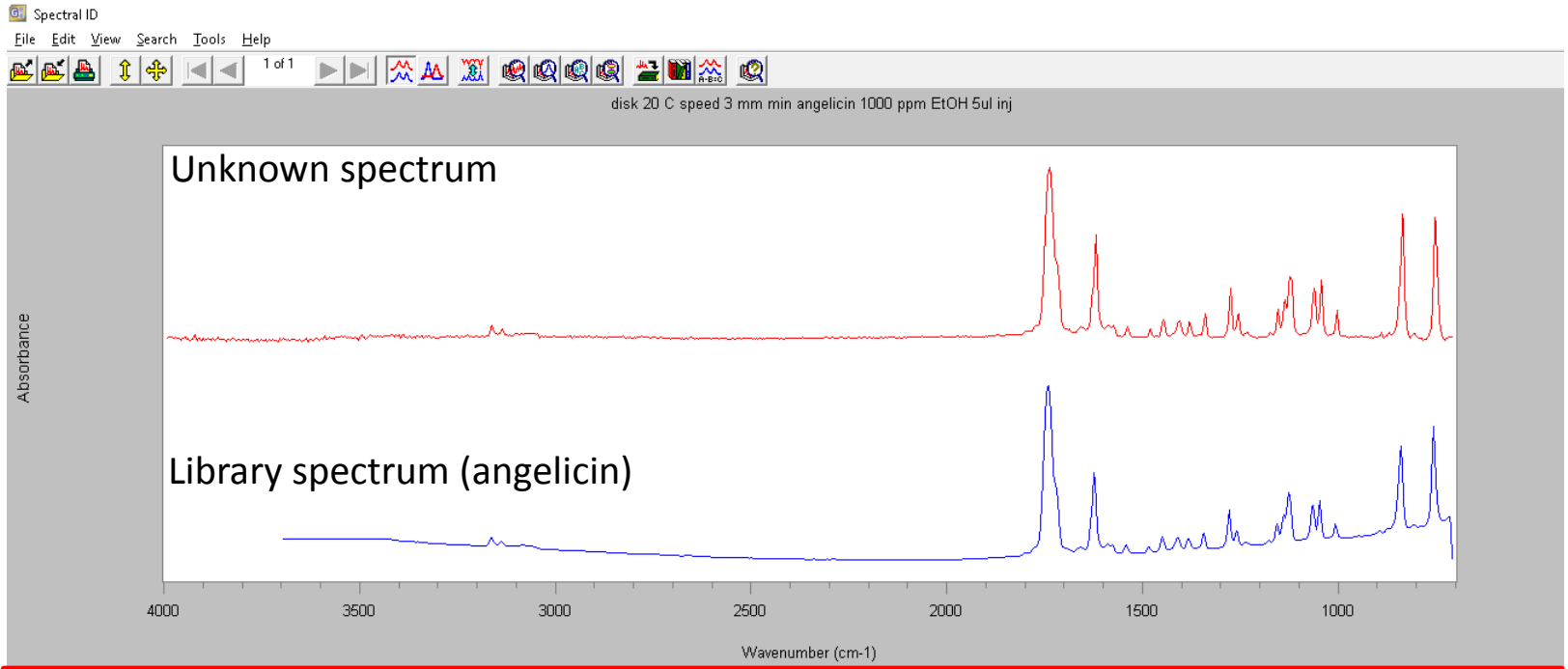
LC-FTIR Spectra of Isomers



LC-FTIR Spectra of Isomers



LC-FTIR Library Search



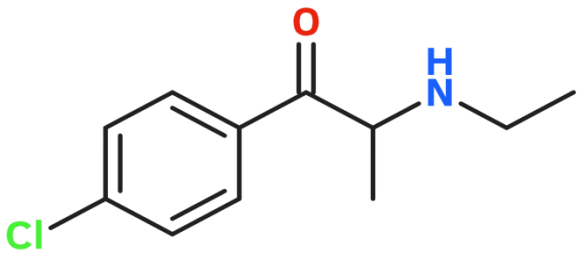
Hit	Quality	Memo	LibName	LibIndex	Spectrum	TextInfo
1	.0315762	Angelicin	Furopolypsor.lib	0		Angelicin
2	.0991837	Angelicin	190403 junk.lib	6		Angelicin
3	.825589	o-Chlorotoluene	Technofleet.lib	651		o-Chlorotoluene
4	.830071	p-Terphenyl (1,4-Diphenylbenzene)	Technofleet.lib	698		p-Terphenyl (1,4-Diphenylbenzene)
5	.864607	Poly(Diethylene Glycol Succinate)	Technofleet.lib	756		Poly(Diethylene Glycol Succinate)
6	.865619	Poly(Diethylene Glycol Adipate)	Technofleet.lib	755		Poly(Diethylene Glycol Adipate)
7	.867179	Adipic Acid Dimethyl Ester	Technofleet.lib	268		Adipic Acid Dimethyl Ester
8	.870296	Adipic Acid Diethyl Ester	Technofleet.lib	265		Adipic Acid Diethyl Ester
9	.873956	Propionic Acid Benzyl Ester (Benzyl Propionate)	Technofleet.lib	774		Propionic Acid Benzyl Ester (Benzyl Propionate)
10	.874192	m-Terphenyl (1,3-Diphenylbenzene)	Technofleet.lib	557		m-Terphenyl (1,3-Diphenylbenzene)
11	.880451	beta-BHC(benzenehexachloride)	Technofleet.lib	310		beta-BHC(benzenehexachloride)
12	.88134	Adipic Acid Di(2-methoxyethyl)Ester	Technofleet.lib	262		Adipic Acid Di(2-methoxyethyl)Ester
13	.882131	Adipic Acid Di-n-propyl Ester	Technofleet.lib	263		Adipic Acid Di-n-propyl Ester
14	.88359	Triethylene Glycol Diacetate	Technofleet.lib	912		Triethylene Glycol Diacetate
15	.887557	Succinic acid di-n-butyl ester (Di-n-butyl succinate)	Technofleet.lib	836		Succinic acid di-n-butyl ester (Di-n-butyl succinate)
16	.887949	Succinic acid diethyl ester (Diethyl succinate)	Technofleet.lib	837		Succinic acid diethyl ester (Diethyl succinate)
17	.889507	Diallyl Phthalate	Technofleet.lib	350		Diallyl Phthalate
18	.890349	Adipic Acid Diallyl Ester	Technofleet.lib	264		Adipic Acid Diallyl Ester
19	.891038	Diethylene Glycol Monoethyl Ether Acetate	Technofleet.lib	371		Diethylene Glycol Monoethyl Ether Acetate
20	.893334	Dimethyl succinate (Succinic acid dimethyl ester)	Technofleet.lib	373		Dimethyl succinate (Succinic acid dimethyl ester)

Hit quality varies from 0 (higher) to 1 (lowest)

CHARACTERIZATION OF 4-CHLOROETHCATHINONE BY GC-MS AFTER 2,2,2-TRICHLOROETHYL CHLOROFORMATE DERIVATIZATION, LC-HRAM ORBITRAP™ MS, AND SOLID DEPOSITION GC-FTIR

An impressive number of new psychoactive substances (NPS), appear on the recreational drug market. Clinical and forensic toxicology laboratories are challenged every day to identify NPS, very quickly and often without the availability of reference standards or analytical data from scientific literature.

Fig. 1



4-CHLOROETHCATHINONE

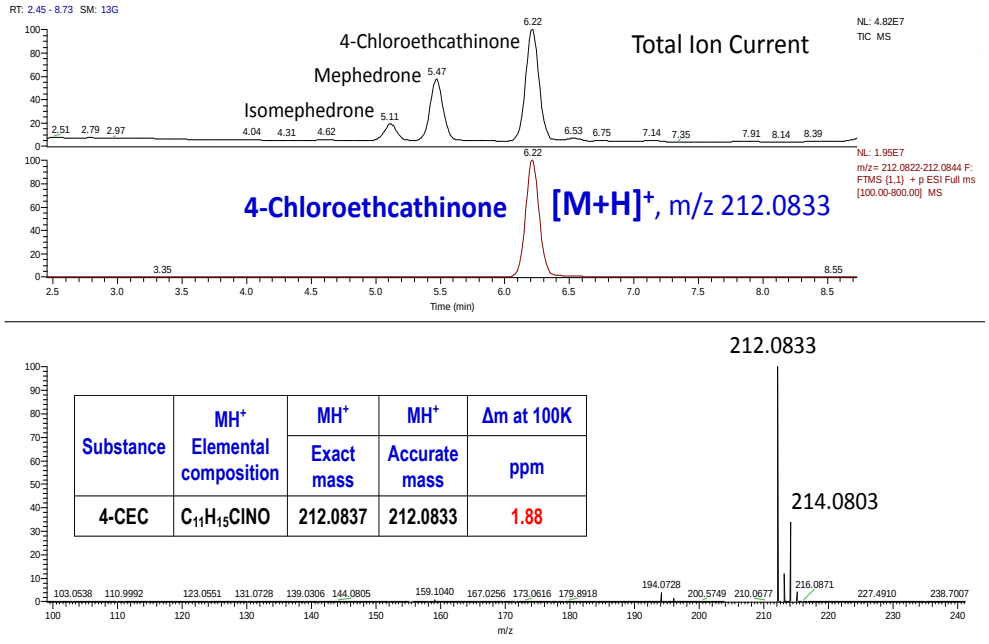
(4-CEC) $C_{11}H_{14}ClNO$, MW 211

57th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Birmingham, UK, 2-6 September 2019

GC-MS (Agilent 7890 series II/ 5975 system), EI full-scan (*m/z* 40-600) conditions with a HP-5MS UI (30 m x 0.25 mm, 0.25 µm film thickness) capillary column

LC – high-resolution accurate-mass Orbitrap™ mass spectrometry (LC-HRAM Orbitrap™ MS, Thermo Scientific Exactive HCD MS system), using a Hypersil Gold PFP analytical column (2.1 x 50 mm, 1.9 µm particle size), a scan range from *m/z* 50 to 800 and mass resolution of 100.000 (HCD off) or 25.000 (HCD on, 25 eV)

solid deposition GC-FTIR, Shimadzu Nexis GC-2030 - Spectra Analysis DiscovIR), utilizing a SLB-5MS analytical column (30 m × 0.25 mm, 0.25 µm film thickness), a 700-4000 cm^{-1} spectrum range, with a 4 cm^{-1} resolution.



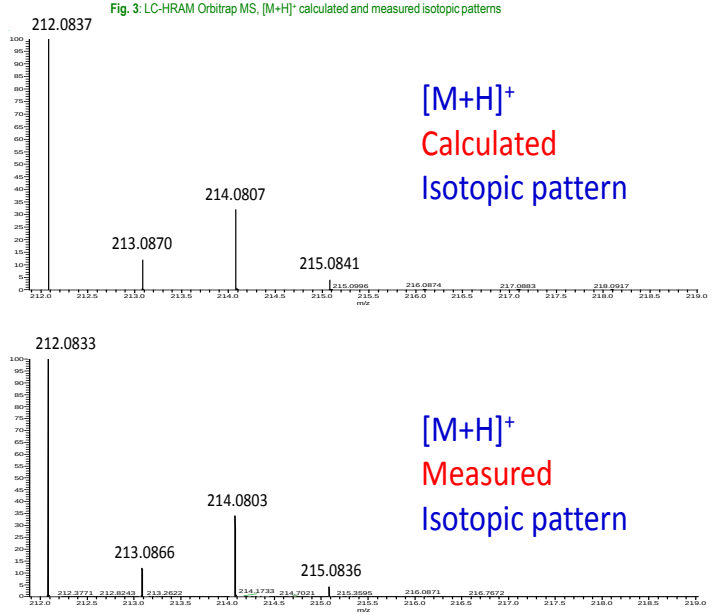
Substance	MH+ Elemental composition	MH+ Exact mass	MH+ Accurate mass	Δm at 100K ppm
4-CEC	$C_{11}H_{15}ClNO$	212.0837	212.0833	1.88

Fig. 2: LC-HRAM Orbitrap MS, full-scan acquisition at 100K RP

Accurate mass measurement of the chloroethcathinone MH^+ ions had a mass accuracy of 1.88 ppm.

Fully superimposable experimental and calculated MH^+ isotopic patterns were obtained, with relative isotopic abundance (RIA1 and RIA2) error values of 0.59 and 3.09 %, respectively.

Fig. 3: LC-HRAM Orbitrap MS, $[M+H]^+$ calculated and measured isotopic patterns

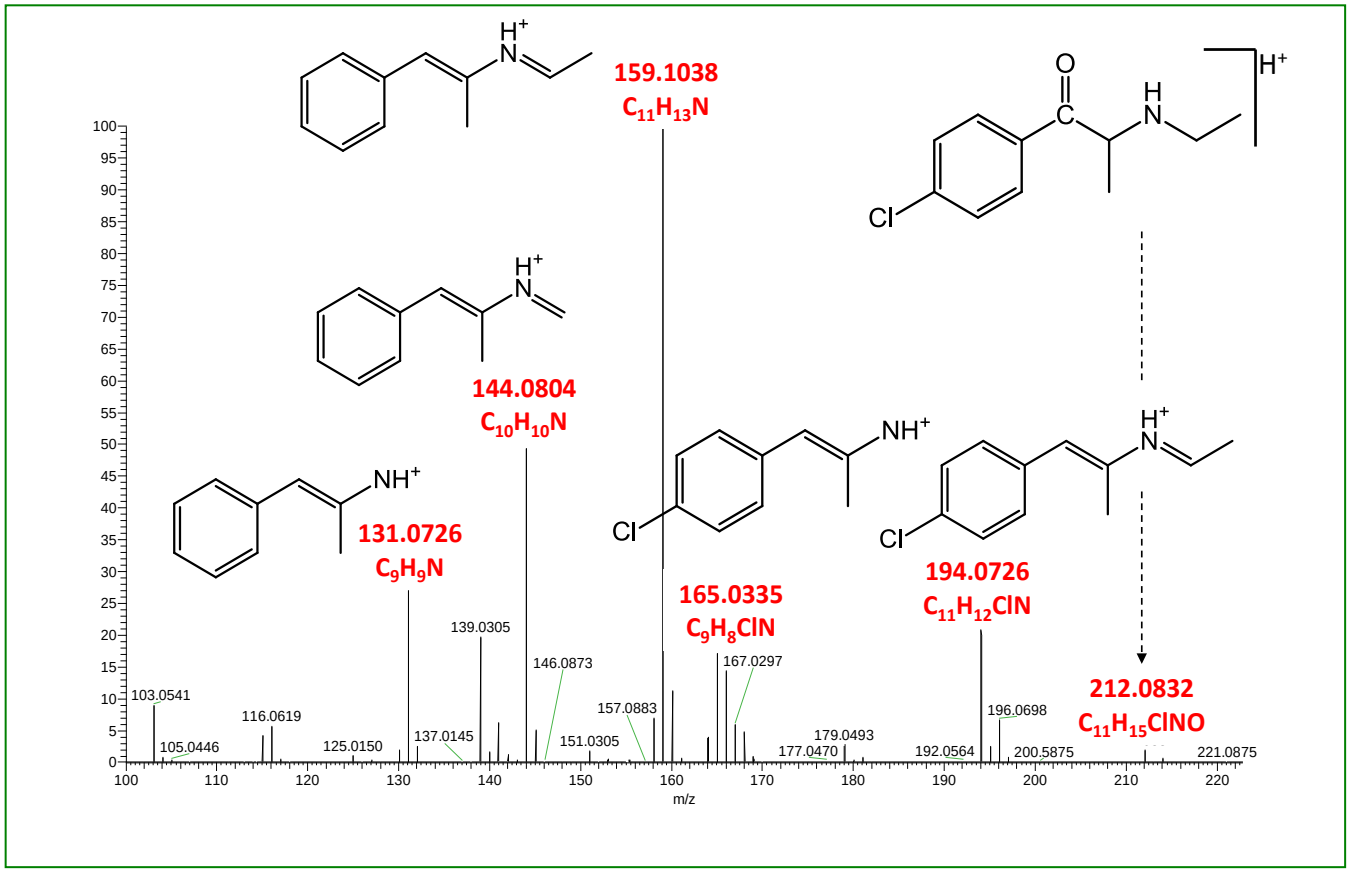


RIA1:
M+1 / M+0 (^{13}C / ^{12}C)

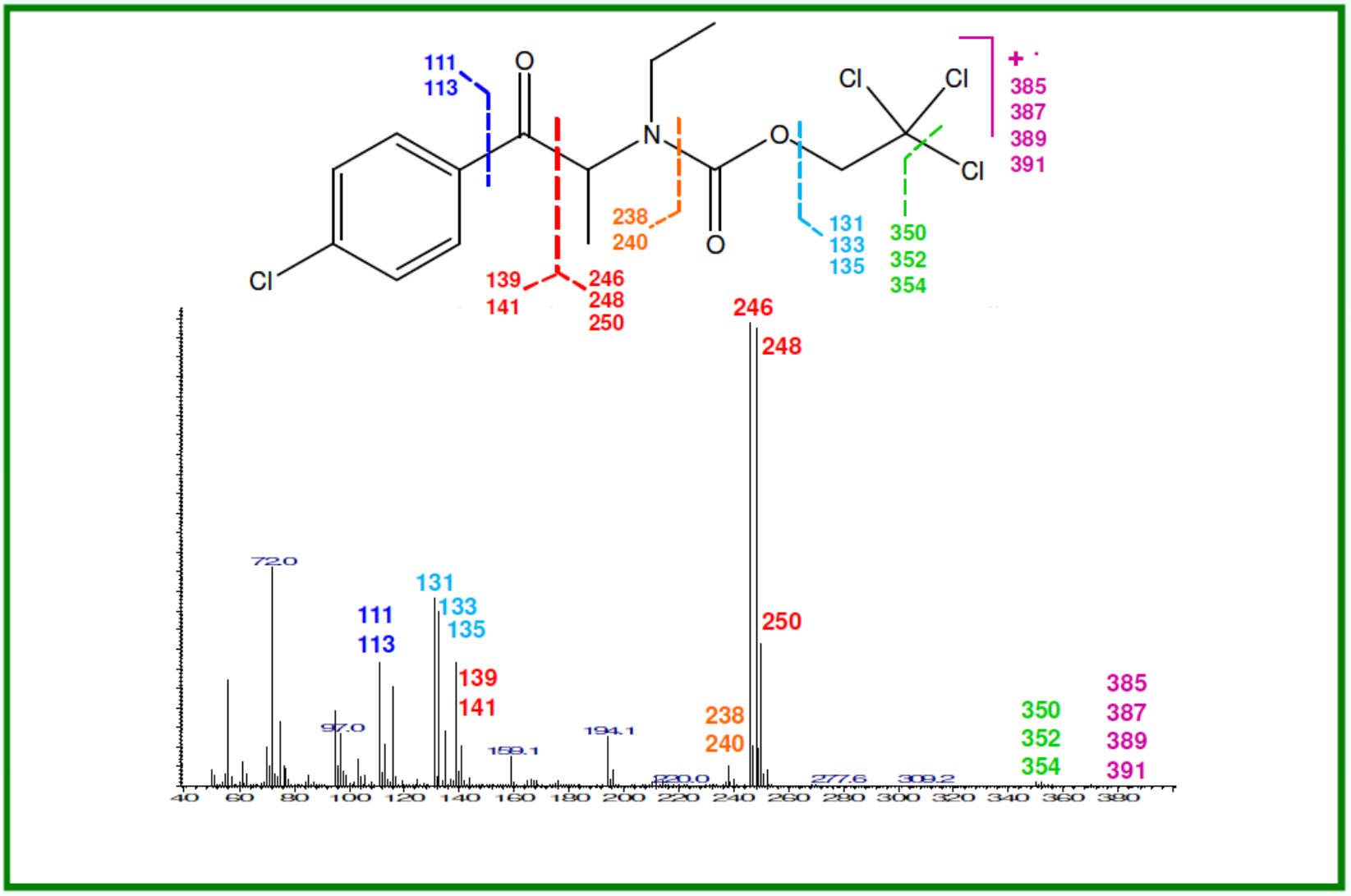
RIA2:
M+2 / M+0 (^{37}Cl / ^{12}C)

RIA error (%):
$RIA_{meas} - RIA_{calc} / RIA_{calc} \times 100$

Substance	RIA1 error (%)	RIA2 error (%)
4-CEC	0.59	3.09

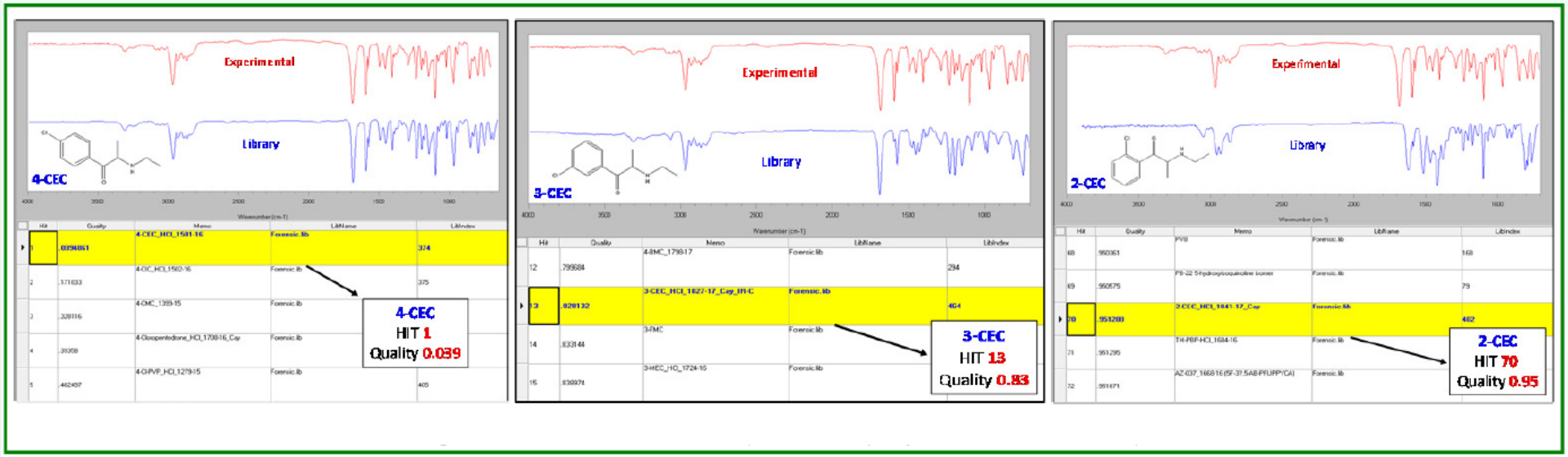


Accurate mass measurement of the characteristic MH^+ collision-induced product ions obtained from LC-HRAM Orbitrap™ MS analyses were in full agreement with the expected structures and consistent with those previously reported using low-resolution MS^3



The application of GC-MS after derivatization with 2,2,2-trichloroethyl chloroformate permitted to obtain highly informative EI mass spectra.

In particular, the presence of a characteristic base peak cluster at *m/z* 246/248/250 confirmed the presence in the molecule of the N-ethyl moiety.



The application of solid deposition GC-FTIR allowed discriminating among chloroethcathinone positional isomers (*i.e.* 2', 3', or 4' positions of the chlorine atom on the benzene ring), confirming the presence of the 4' isomer (4-CEC)

- Potential market and Spectra positioning (industrial sectors, conference presentations, scientific activities, R%D with Professor Luigi Mondello from the University of Messina).

- What makes DiscovIR solid-deposition FTIR technology a unique identification tool?

- Which benefits are to be gained by the online hyphenation GC(LC)-MS-FTIR (parallel detection)?

- Presentation of the FFNSC GC-MS-FTIR Library (flavour and fragrance natural and synthetic compounds) based on GC-EI MS with Linear Retention Indices (LRI) and solid state FTIR spectra.

- The “quantitative” issue.

- DiscovIR: how does it work?

- GC-FTIR and LC-FTIR Application fields.

Unified instrument for F&F identification

LRI information

Retention behavior

SPLIT

MS spectrum

Chemical class, structure

Unified database

FTIR spectrum

Functional group position, stereospecificity

Univocal identification

Limitations of GC-MS with LRIs approach – FTIR information support

- Components with similar MS spectra and/or Linear Retention Index

Flavour and fragrance field

- Hydrocarbons monoterpenes and sesquiterpenes
- Oxygenated monoterpenes and sesquiterpenes
- Pheromone isomers

Lipid-like compounds

- Stereoisomers of fatty acid methyl and ethyl esters with insaturations
- Sterol compounds

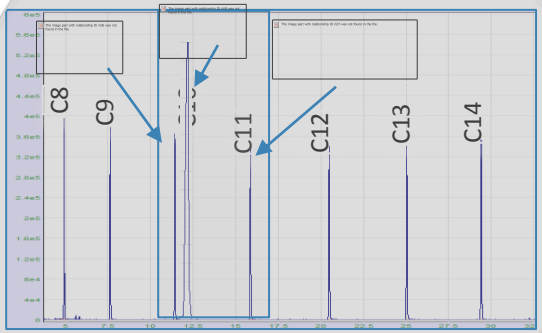
- New designer molecules: slight chemical modifications of known substances
  - Drugs and other prohibited substances
  - Designer doping compounds – FTIR can be a rapid, structurally sensitive method of analysis of suspicious materials

IR spectra may support the MS library search results

Limitations of GC-MS with LRIs approach – FTIR information support

UNIVOCAL IDENTIFICATION FOR ALL TYPES OF COMPOUNDS

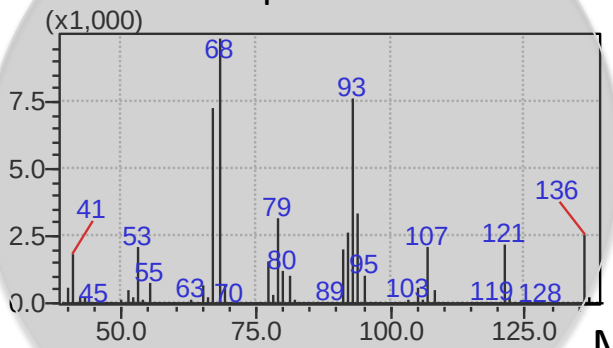
Linear retention index



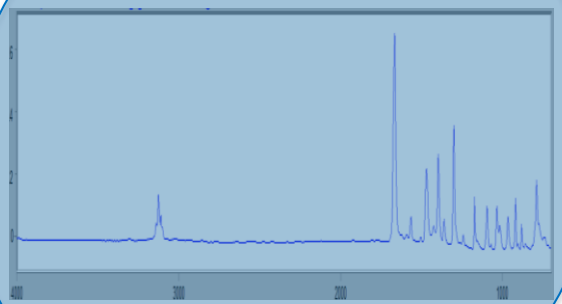
Diastereoisomers
Positional isomers

Saturated and unsaturated members of the same compound family

MS spectrum



FTIR spectrum



Members from the same homologue series

FLAVOUR AND FRAGRANCE NATURAL AND SYNTHETIC COMPOUNDS
FFNSC GC-FTIR Library 1.0

About 1500 pure flavour and fragrance compounds containing:

- ✓ Systematic name
- ✓ Common name
- ✓ CAS number
- ✓ LRI values
- ✓ Molecular weight
- ✓ Molecular formula
- ✓ Standard and column information
- ✓ 8 experimental LRI values

Column	Reference series	No. FFNSC 1.0 LRI
SLB-5ms	ALKANEs	1500
SLB-5ms	FAMEs	1500
SLB-5ms	FAEEs	1500
Supelcowax-10	FAMEs	1500
Supelcowax-10	FAEEs	1500
Equity-1	ALKANEs	1500
Equity-1	FAMEs	1500
Equity-1	FAEEs	1500

FUTURE PERSPECTIVES

- Update of the F&F GC-FTIR/MS Library: Addition of more than 500 pure standard with embedded linear retention indices:

  About 100 new selected flavour and fragrance compounds to be purchased

  Collaborations to obtain pure synthesised standards (oximes, acetals, homologue series)

  Selected real samples (essential oils, foodstuff and other) for isolation by means of preparative LC-GC-GC-GC analysis to identify and append new compounds

  Distillation of particular natural matrices (marine algae, herbs, fruits)

- Identification of unknown components (flavour and fragrance positional isomers, FAMEs stereoisomers, designer doping, ect.) in different matrices prior to separation and purification by means of preparative LC-GC-GC-GC system

FORENSIC DRUG CHEMISTRY

The **SWGDRUG*** (Scientific Working Group for the Analysis of Seized Drugs) recommend minimum standards for forensic identification of commonly seized drugs. Drugs identification requires the use of multiple uncorrelated techniques. Currently used methods have been divided into three categories, where category A techniques provide the best discriminating power.

Category A	Category B	Category C
Infrared Spectroscopy	Capillary Electrophoresis	Color Tests
Mass Spectrometry	Gas Chromatography	Fluorescence Spectroscopy
Nuclear Magnetic Resonance Spectroscopy	Ion Mobility Spectrometry	Immunoassay
Raman Spectroscopy	Liquid Chromatography	Melting Point
X-ray Diffractometry	Mycrocristalline Tests	Ultraviolet Spectroscopy
	Pharmaceutical Identifiers	
	Thin Layer Chromatography	

**PART III B - Methods of Analysis/Drug Identification Recommendations Version 7.1 SWGDRUG 2016-June-9 -Page 14*

In cases where hyphenated techniques are used they will be considered as separate techniques provided that the results from each are used

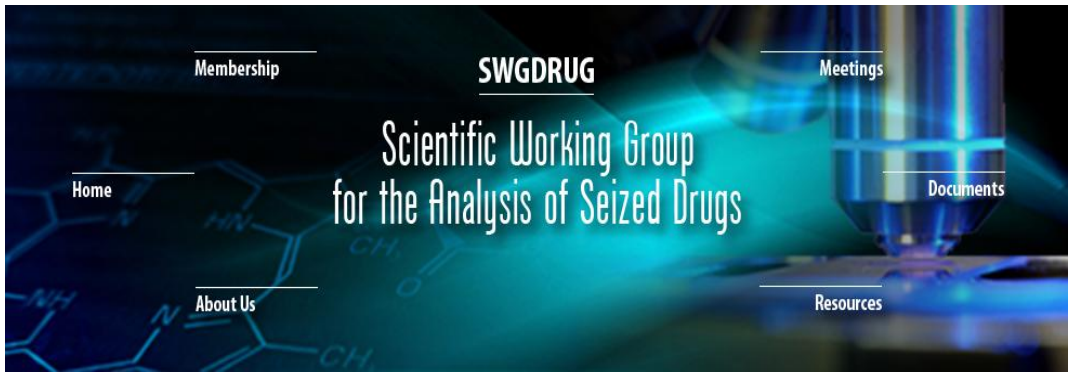
DRUG GC-FTIR/MS LIBRARIES PROJECT

- Creation of GC-FTIR/MS Libraries for Forensic Drug Analysis and Banned compounds in sport:

Combine the GC-MS spectra and LRI with FTIR spectra of Designer Drugs, New Psychoactive Substances (NPS), and doping compounds presented in WADA prohibited list.

State of the art

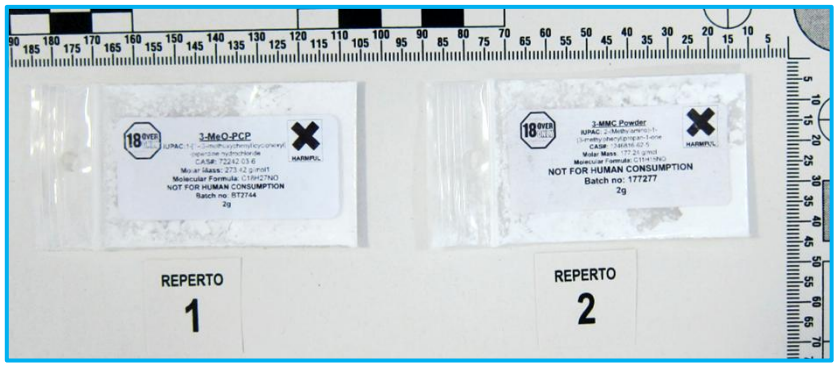
GC-FTIR library with about 500 spectra of compounds belonging to the family of the synthetic cannabinoids, cathinone, NBoMe, steroids



FORENSIC ANALYSIS

Case of study

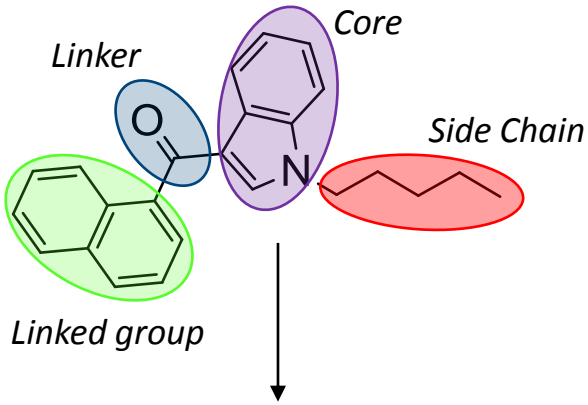
- Sensitivity evaluation using JWH-018 standard
- Identification of 4-CEC in seized sample
- Identification of 4-MMC and 3 MMC in seized sample
- Identification 3-MeO-PCP in seized and urine samples

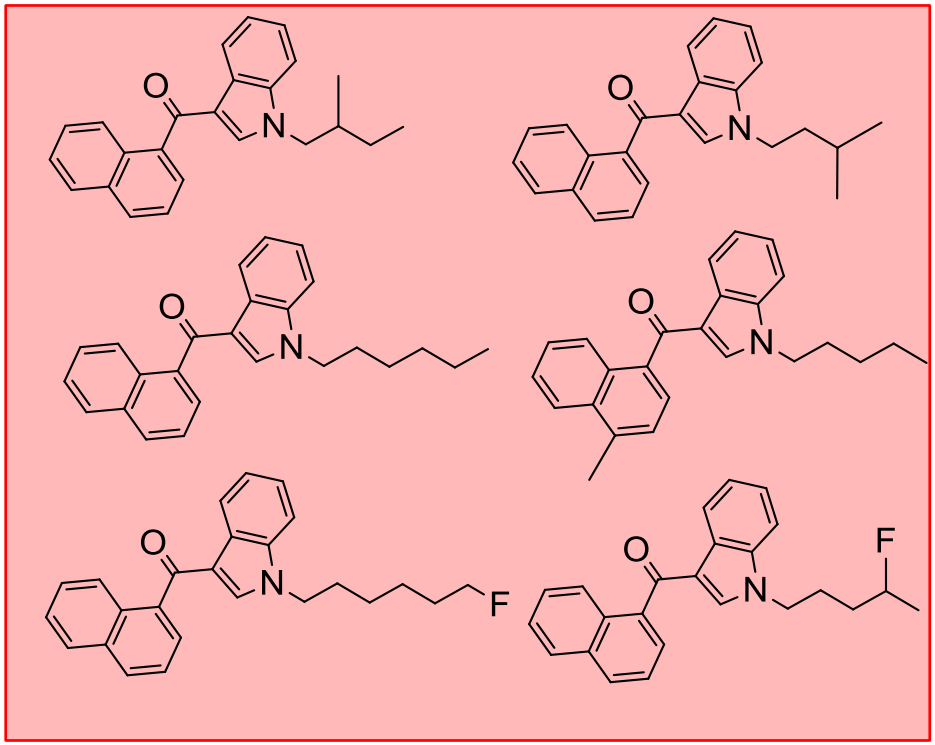


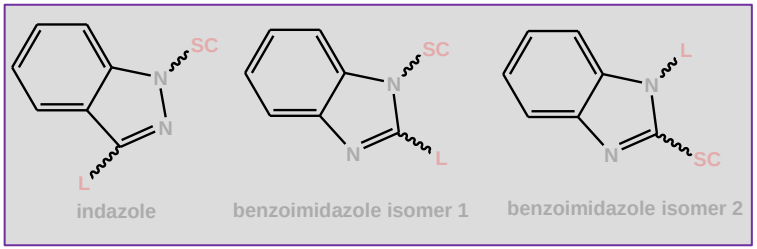
SYNTHETIC CANNABINOIDS: JWH-018

JWH-018 (**1-pentyl-3-(1-naphthoyl)indole**) is a synthetic cannabinoids presenting the core structure of the indole family. JWH-018 and derivatives act as a full agonist at both the CB_1 and CB_2 cannabinoid receptors. These synthetic cannabinoid receptor agonists (SCRAs), present problems for legislatures.

There are more than 120 chemical variants for synthetic cannabinoids and controls struggles to cope with the structural diversity.







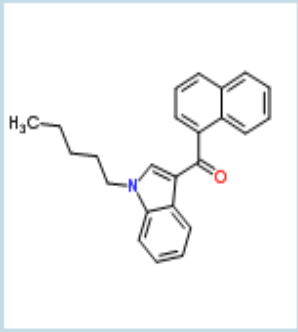
These compounds can appear in a number of regioisomeric and isobaric forms and require unique analytical methods for differentiation.

SYNTHETIC CANNABINOIDS: JWH-018

Sample: JWH-018 standard in EtOAC

JWH-018
CAS # 209414-07-3

Compound Structure and Properties



Molecular Weight	341.4455
Formula	$C_{24}H_{23}NO$

Data above sourced from ChemSpider

Instrument GC-FTIR

Shimazdu Nexis GC-2030 Gas chromatograph
Spectra-Analysis DiscovIR solid phase FTIR

GC parameters

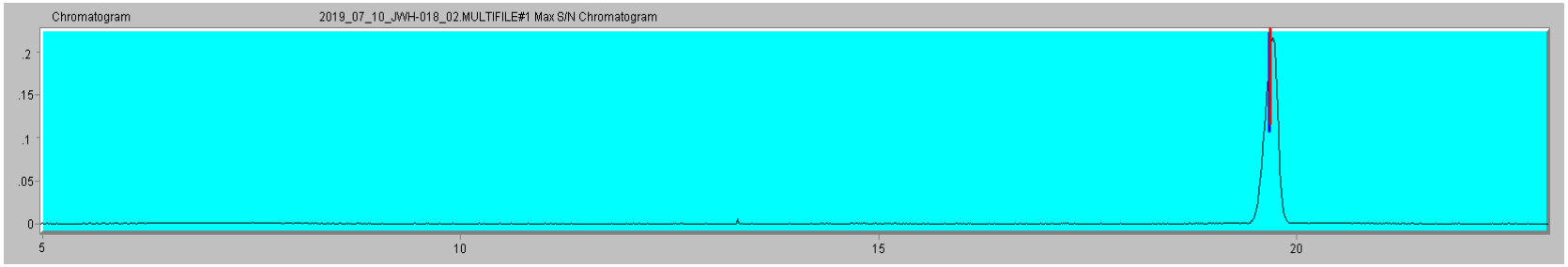
Column: SLB-5ms (Merck KGaA), 30 m L x 0.25 mm i.d. x 0.25 µm $_{d.f.}$
Injector: 280 ° C
Injection volume: 1 µL splitless, 1.50 min (1:20)
Carrier gas: Helium
Linear velocity mode: 30 cm/s
Oven program: 100 ° C initial temp. for 2 min, ramp to 350 ° C at 15 ° C/min
Time analysis: 24 min

FTIR parameters

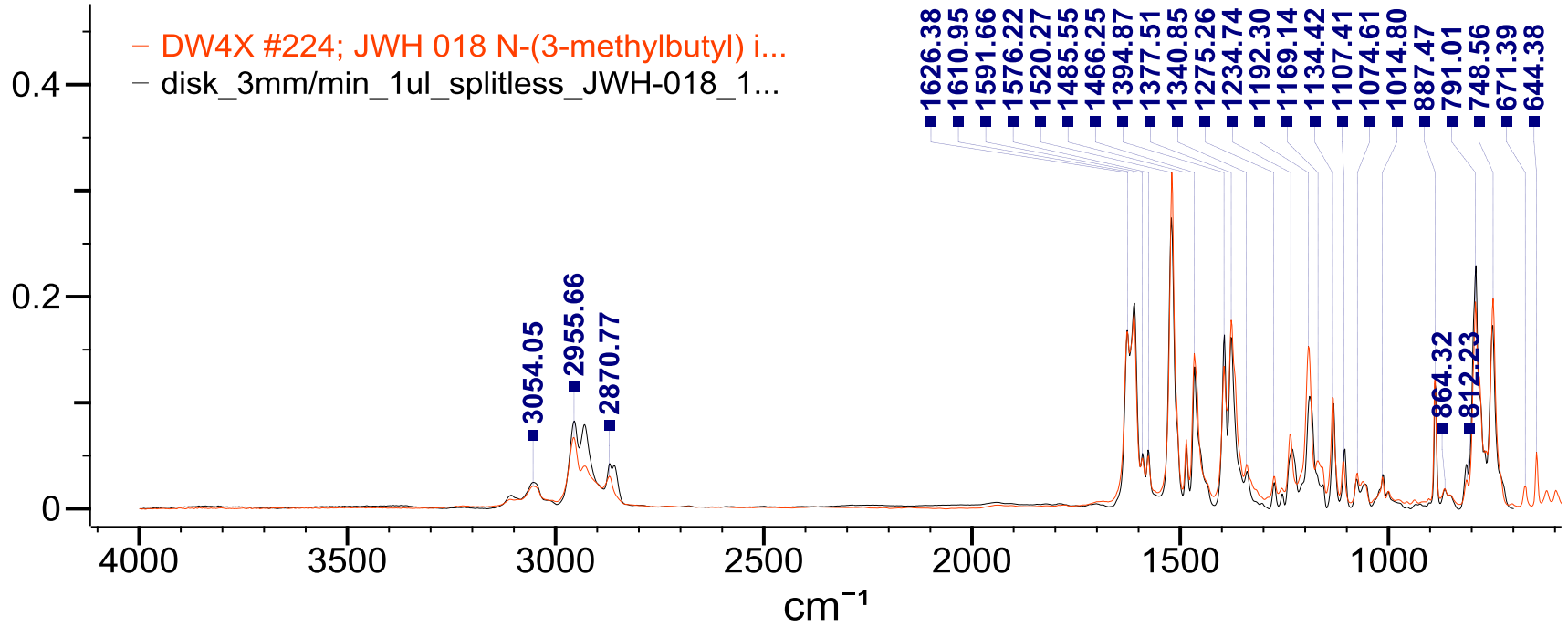
Disk temp.: -50 ° C
Disk speed: 3 mm/min
Detection: MCT IR detector 4000-700 cm^{-1}
Resolution: 4 cm^{-1}
Transfer line temp.: 280 ° C
Restrictor temp.: 280 ° C

SYNTHETIC CANNABINOIDS: JWH-018

GC-FTIR Chromatogram



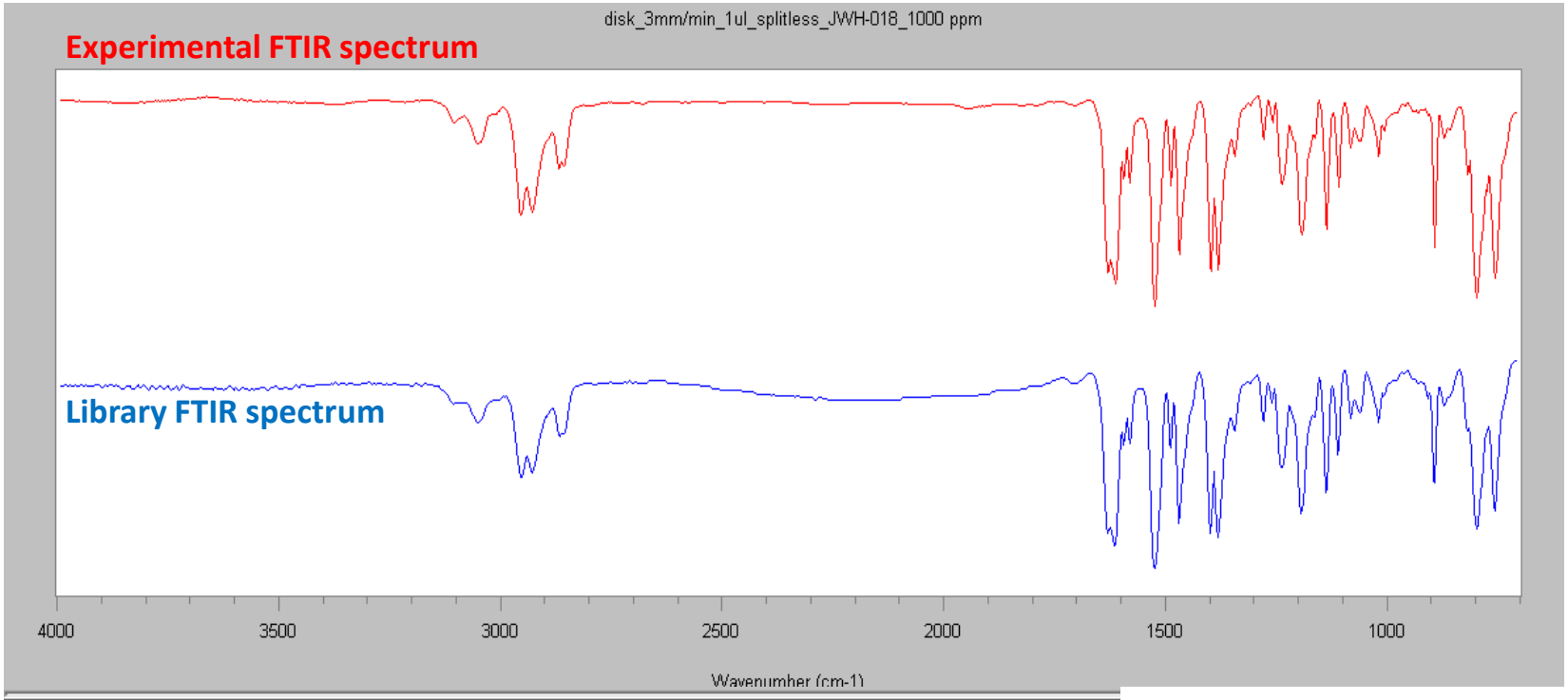
ATR VS SOLID STATE FTIR
JWH-018



Source Software: know it all (Biorad)

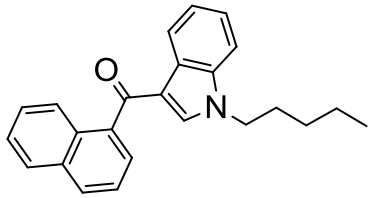
FTIR spectra obtained by solid-deposition are superimposable with those of ATR Libraries

LIBRARY SEARCH



Hit	Quality	Memo	LibName
1	**.0584263**	**JWH-018**	**Forensic.lib**
2	.124419	JWH-019	Forensic.lib
3	.17672	JWH 019 N-(6-fluorohexyl) isomer	Forensic.lib
4	.218861	AM2201 N-(4-fluoropentyl) isomer	Forensic.lib

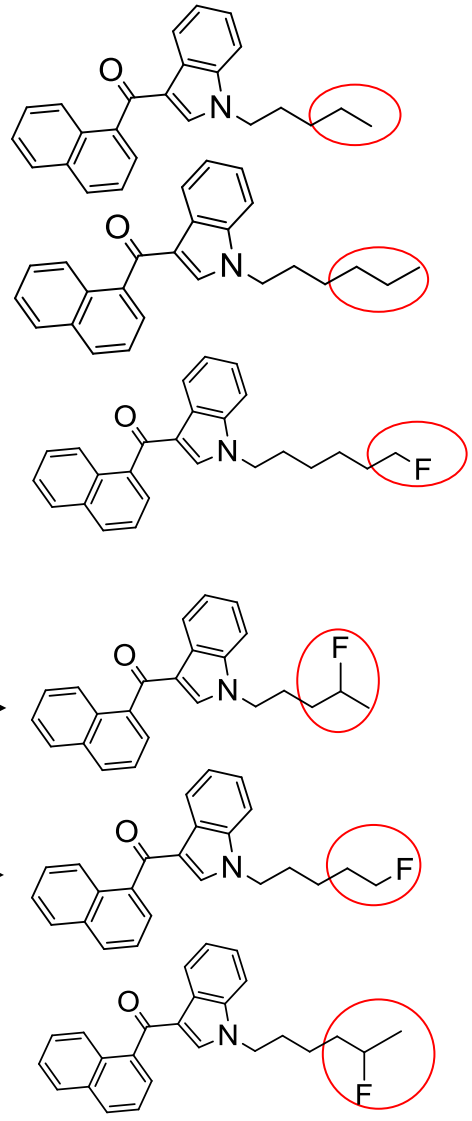
HIT 1 JWH-018
Quality 0.058



HIT Quality vary from 0 (highest) to 1 (lowest)

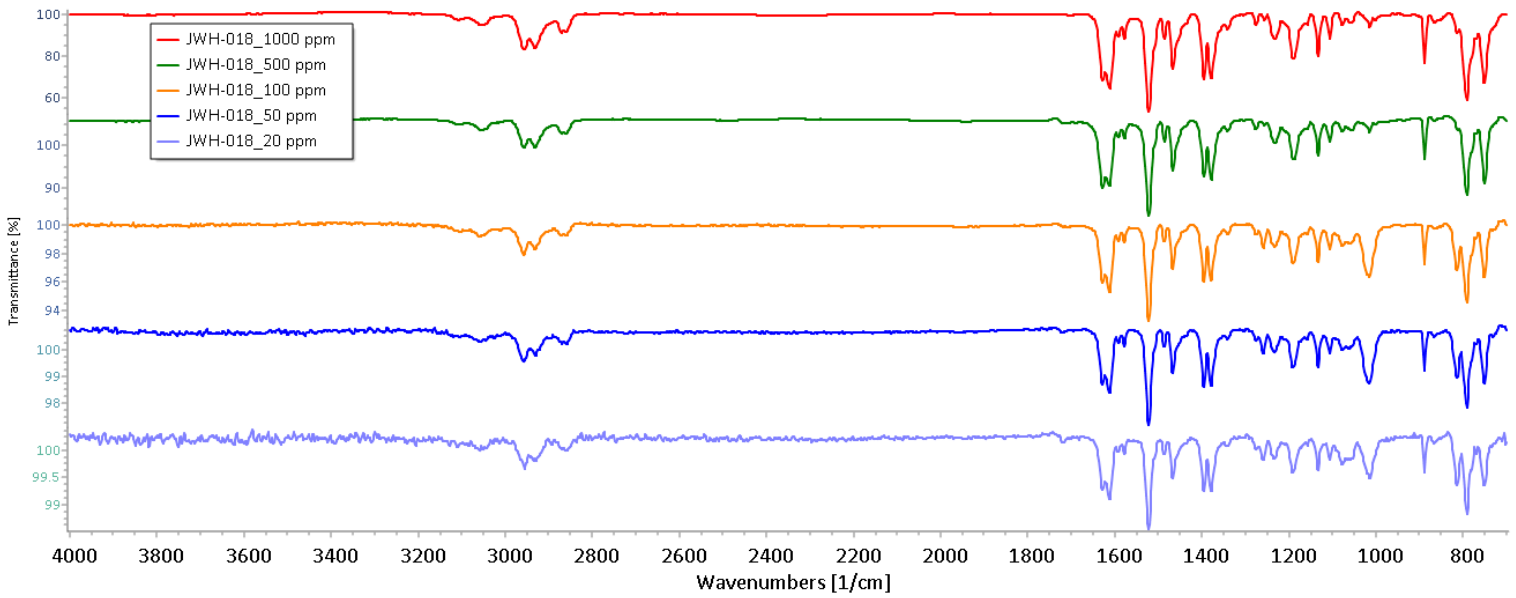
Discriminate Related Compounds Through Library Search

	Hit	Quality	Memo
▶	1	.0584263	JWH-018
	2	.124419	JWH-019
	3	.17672	JWH 019 N-(6-fluorohexyl) isomer
	4	.218861	AM2201 N-(4-fluoropentyl) isomer
	5	.227453	AM2201
	6	.230262	JWH 019 N-(5-fluorohexyl) isomer

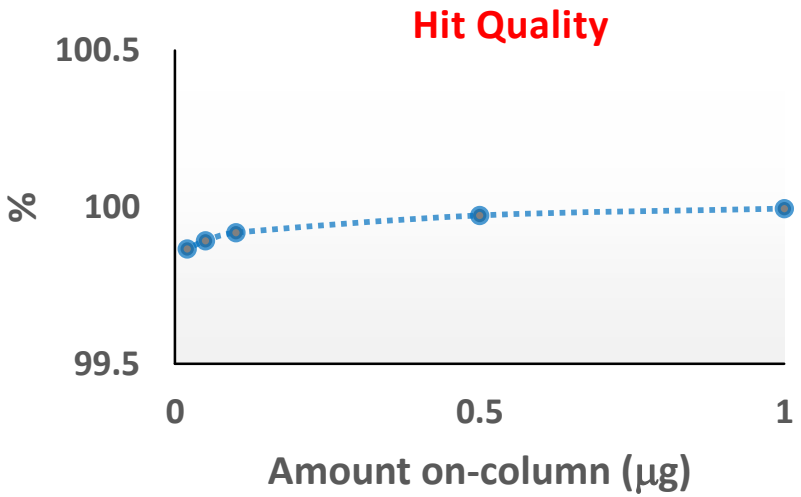


Reliable identification between structural isomers and closely related compounds

IDENTIFICATION QUALITY



Amount on column (ng)	Hit quality	S/N
1000	0.005193	352
500	0.026949	182
100	0.08197	48
50	0.1078	34
20	0.1338	22



LOI: Quality IR spectra obtained from down to 20 ng on column

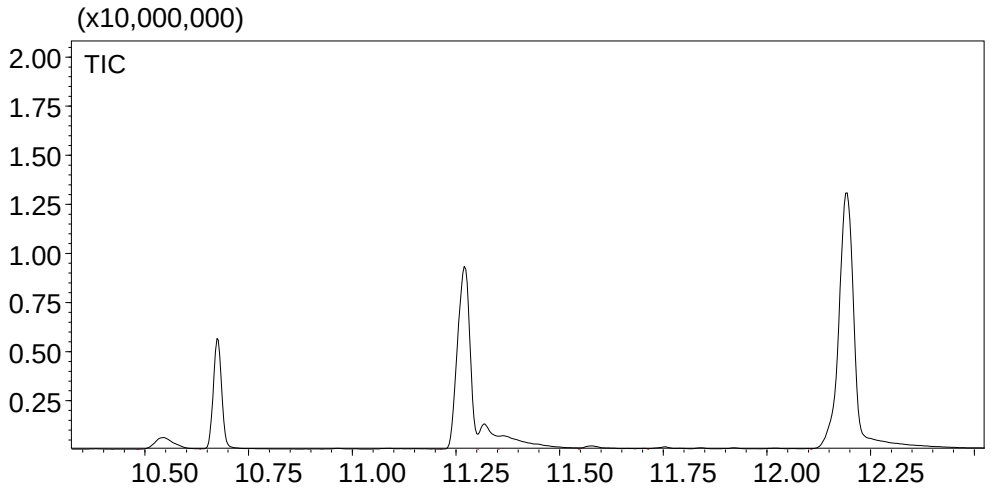
CHARACTERIZATION OF 4-CHLOROETHCATHINONE (4-CEC), ISOMEPHEDRONE AND MEPHEDRONE (4-MMC) IN SEIZED POWDER SOLUTION BY GC-MS AND GC-FTIR

Synthetic cathinones are powerful drugs of abuse, derivatives of the naturally occurring compound cathinone. Alterations in structure produce considerable changes in terms of the perceived effects by the drug user, and are also made to circumvent drug legislation.

GC-MS ANALYSIS CONDITION

Instrument GC-MS:

GC-QP2020 Shimadzu



Column: SLB-5ms (Merck KGaA), 30 m L x 0.25 mm i.d. x 0.25 µm d.f.
Injector: 250 °C
Injection volume: 1 µL split 10, 2.00 min
Carrier gas: Helium
Linear velocity mode: 35 cm/s
Oven program: 60 °C (2 min) to 270 °C at 15 °C/min

GC-FTIR ANALYSIS CONDITION

GC-FTIR Instrument:

Gas chromatograph**: Shimadzu Nexis GC-2030**
Solid phase IR detector: **Spectra-Analysis DiscovIR**

GC parameters

Column: SLB-5ms (Merck KGaA) 30 m L × 0.25 mm i.d. × 0.25 μm *d.f.*
Injector: 250 °C
Injection volume: 1 μL splitless, 2.00 min (1:20)
Carrier gas: Helium
Linear velocity mode: 35cm/s
Oven program: 60 °C (2 min) to 270 °C at 15 °C/min
Time analysis: 16 min

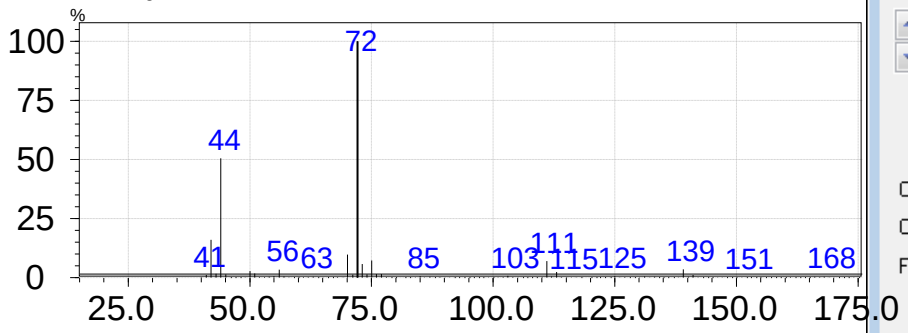
FTIR parameters

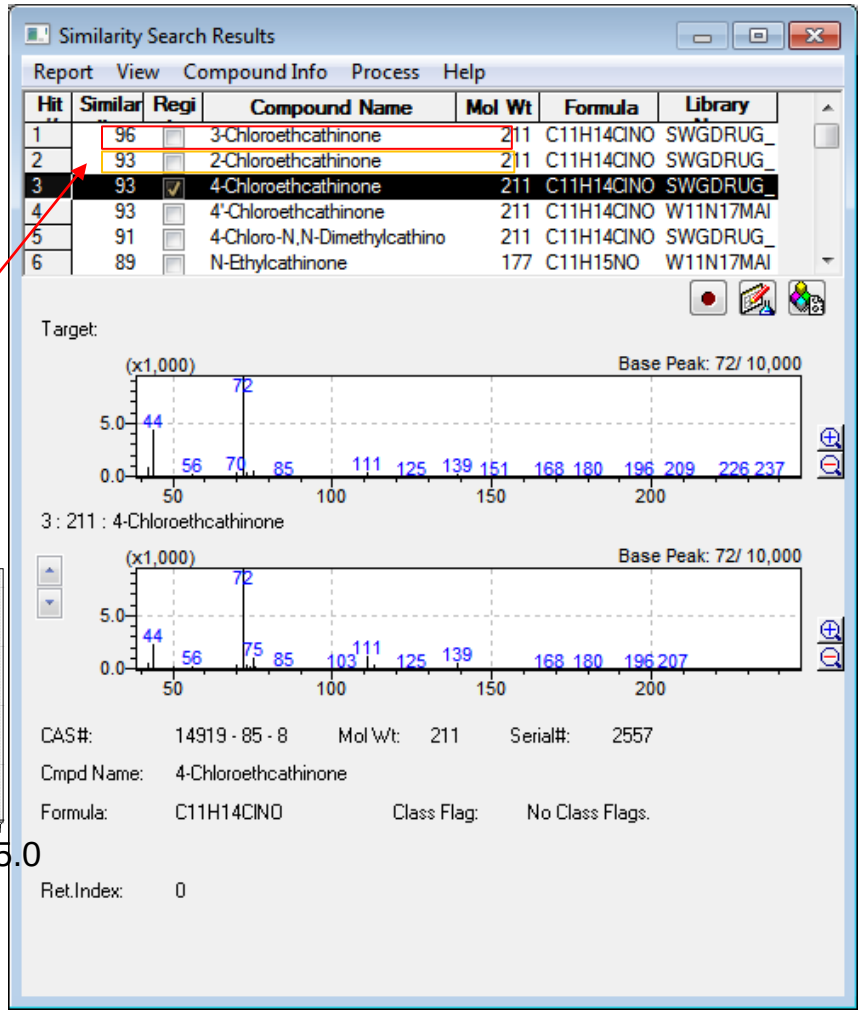
Disk temp.: -50 ° C
Disk speed: 3 mm/min
Detection: MCT IR detector 4000-700 cm^{-1}
Resolution: 4 cm^{-1}
Transfer line: 280 °C

GC-MS IDENTIFICATION OF 4-CHLOROETHCATHINONE IN A SEIZED POWDER SOLUTION ≈1.0% IN WITH LIBRARY SEARCH

Good similarity also with other positional isomers. It's necessary to confirm the identification with FTIR

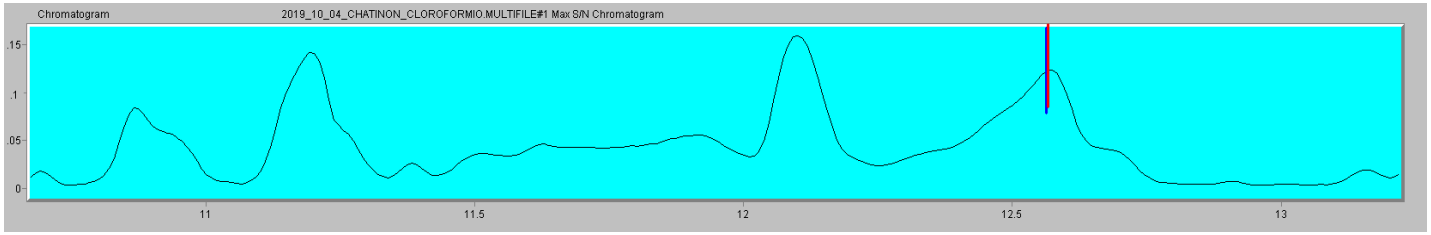
R_t=12.383

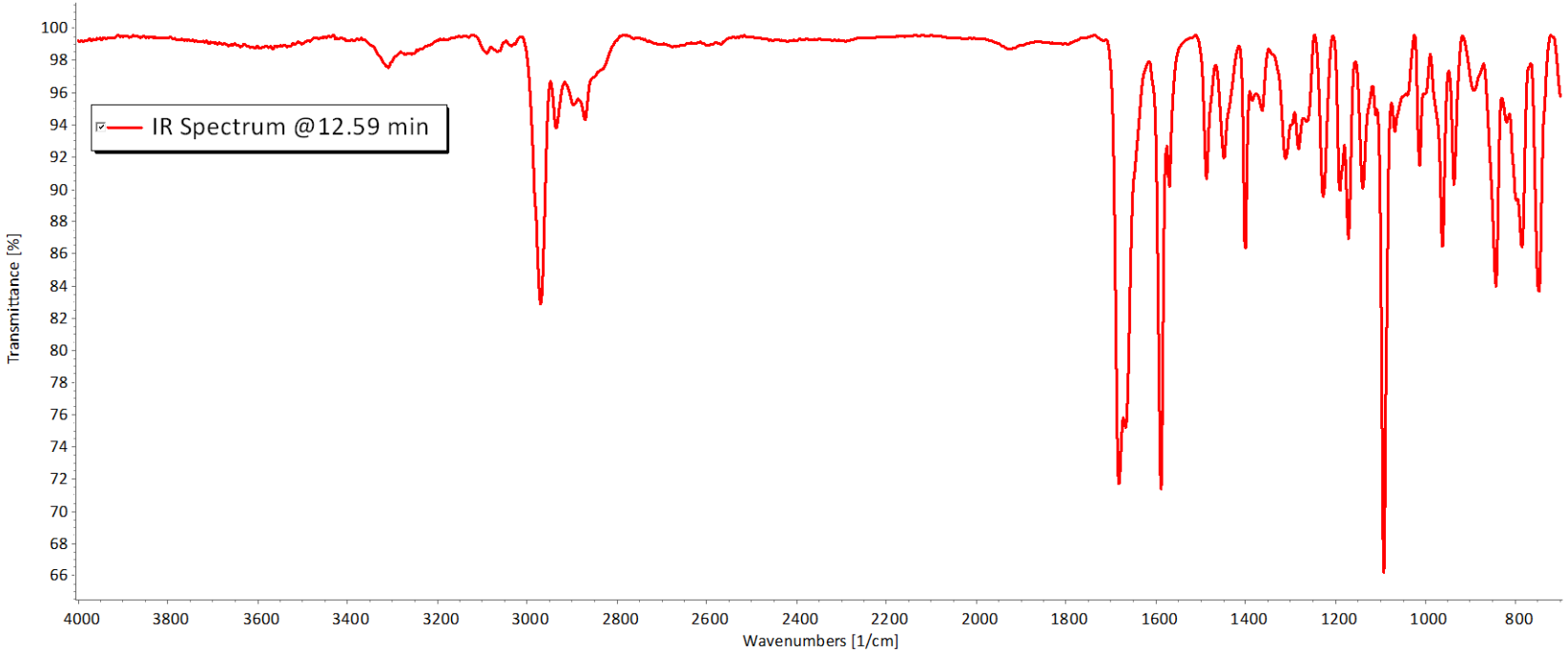




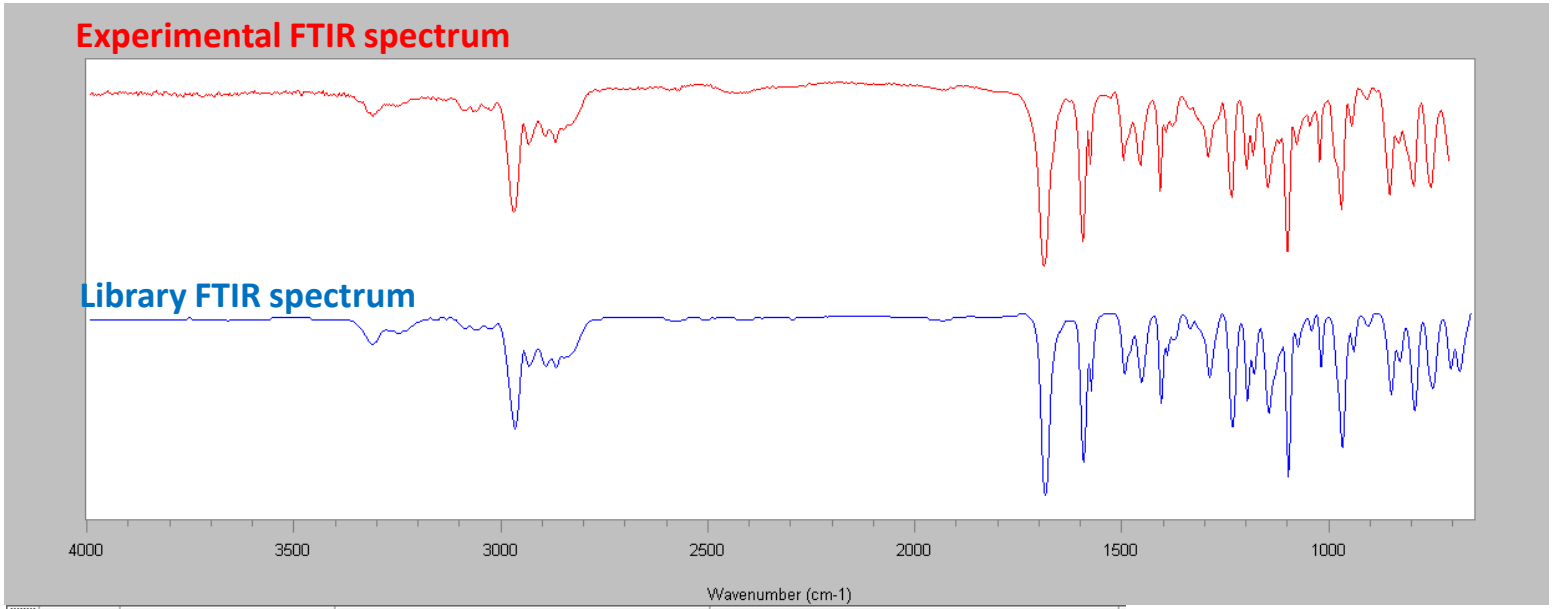
GC-FTIR IDENTIFICATION OF 4-CHLOROETHCATHINONE IN A SEIZED POWDER SOLUTION ≈1.0%

GC-FTIR Chromatogram



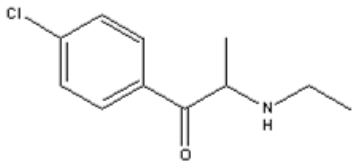


GC-FTIR IDENTIFICATION OF 4-CHLOROETHCATHINONE IN A SEIZED POWDER SOLUTION ≈1.0% WITH LIBRARY SEARCH

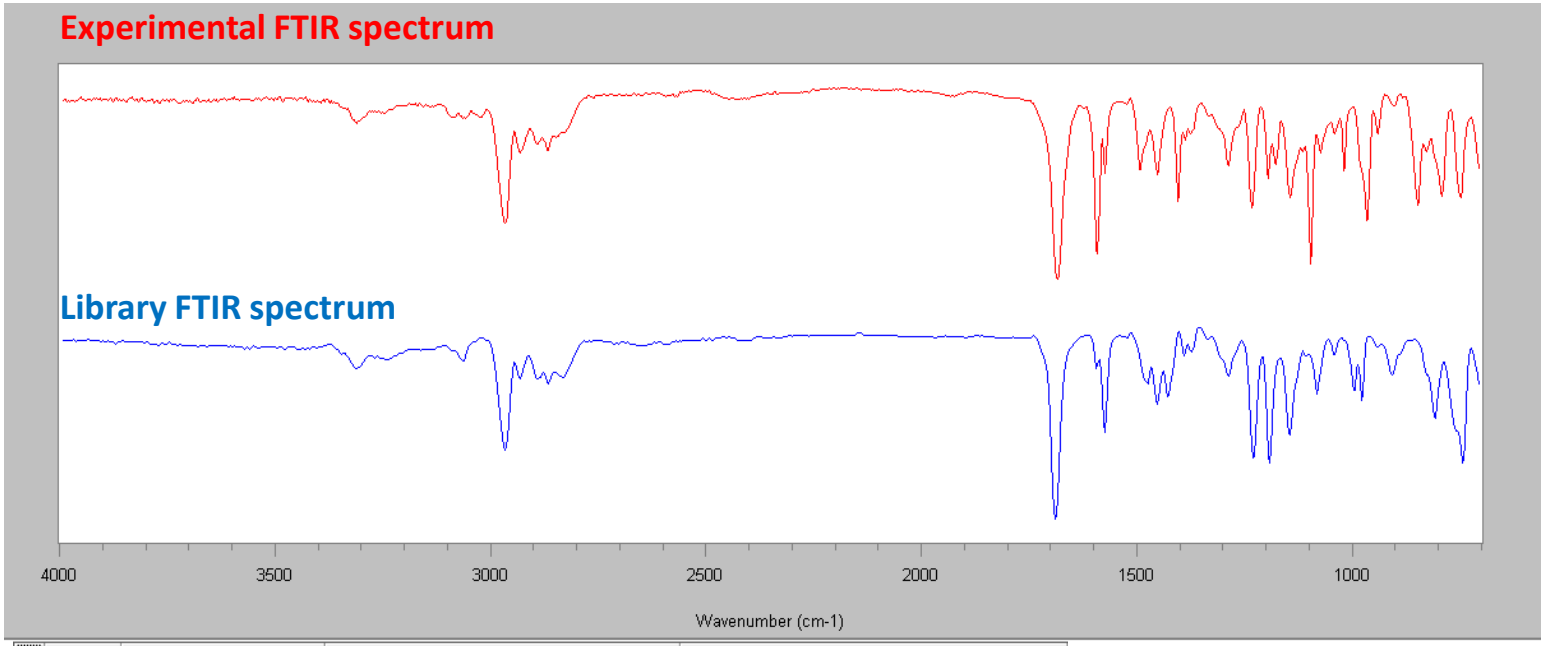


Hit	Quality	Memo	LibName
1	**.0394861**	**4-CEC_HCl_1501-16**	**Forensic.lib**
2	.171833	4-ClC_HCl_1502-16	Forensic.lib
3	.328116	4-CMC_1399-15	Forensic.lib
4	.39358	4-Cloropentedrone_HCl_1708-16_Cay	Forensic.lib

HIT 1 4-CEC
Quality 0.039

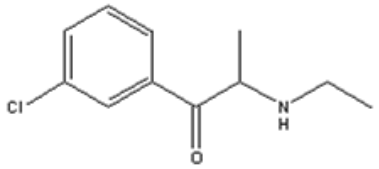


GC-FTIR IDENTIFICATION OF 4-CHLOROETHCATHINONE IN A SEIZED POWDER SOLUTION ≈1.0% WITH LIBRARY SEARCH

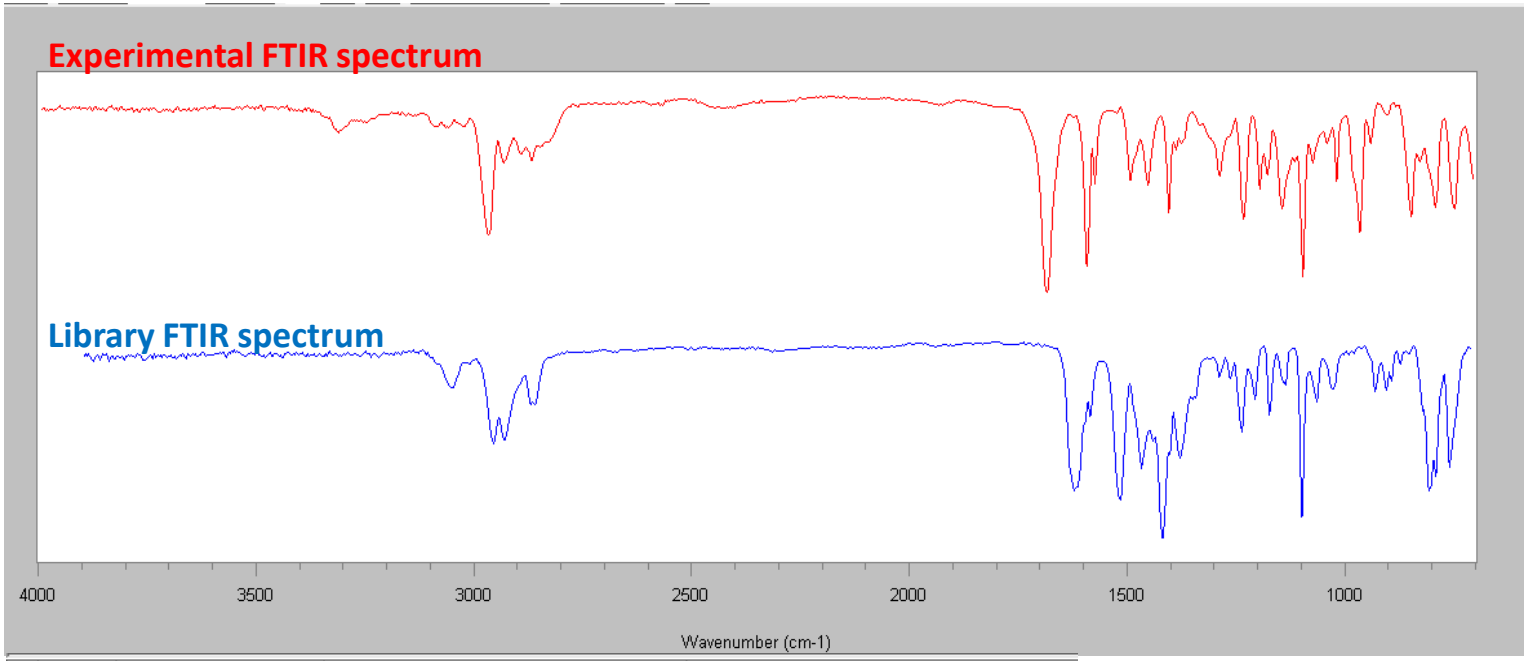


Hit	Quality	Memo	LibName
12	.799684	4-BMC_1798-17	Forensic.lib
13	**.828132**	**3-CEC_HCl_1827-17_Cay_IR-C**	**Forensic.lib**
14	.833144	3-FMC	Forensic.lib
15	.838974	3-MEC_HCl_1724-16	Forensic.lib

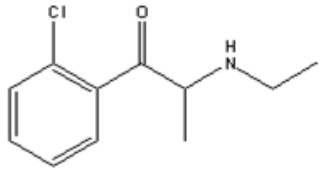
HIT 13 3-CEC
Quality 0.828



GC-FTIR IDENTIFICATION OF 4-CHLOROETHCATHINONE IN A SEIZED POWDER SOLUTION ≈1.0% WITH LIBRARY SEARCH

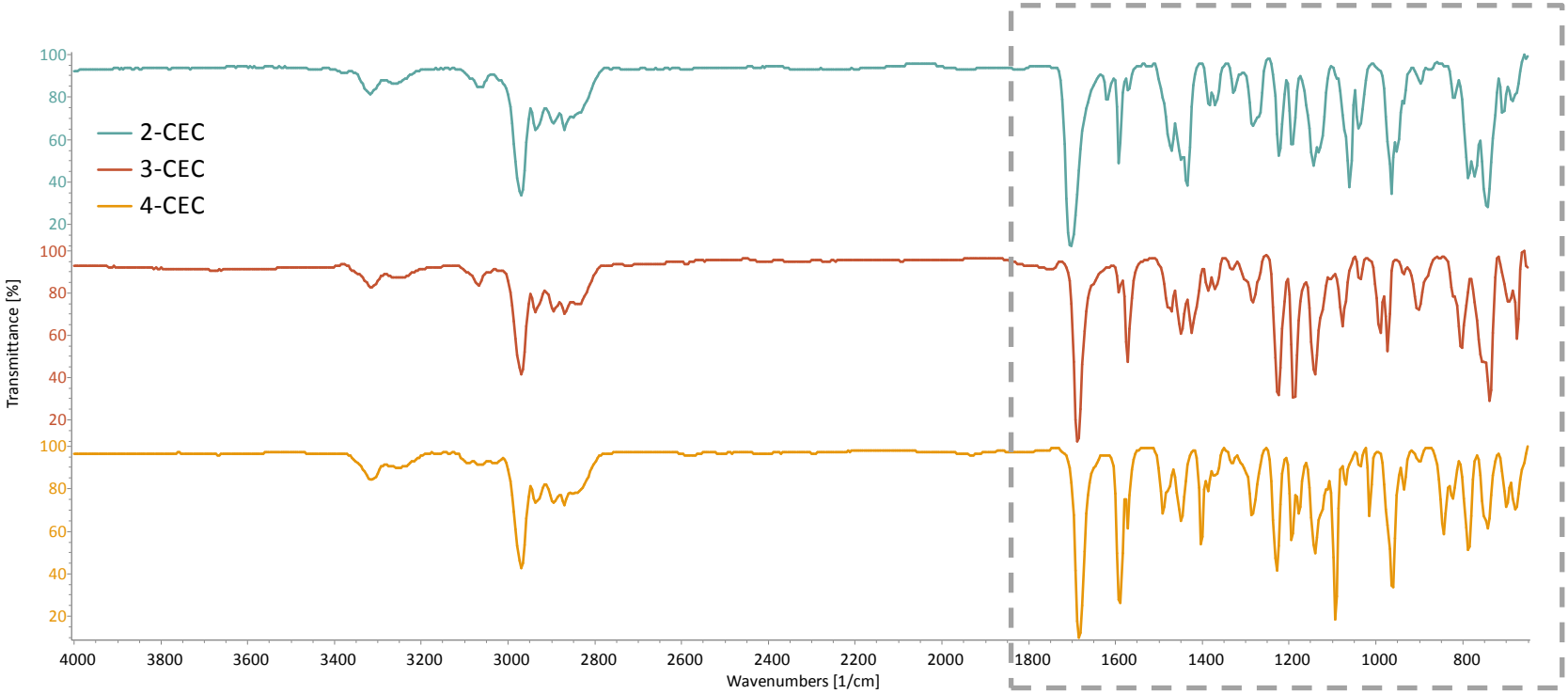


Hit	Quality	Memo	LibName
68	.950361	PV8	Forensic.lib
69	.950575	PB-22 5-hydroxyisoquinoline isomer	Forensic.lib
70	**.951288**	**2-CEC_HCl_1841-17_Cay**	**Forensic.lib**
71	.951295	TH-PBP-HCl_1684-16	Forensic.lib



HIT 70 2-CEC
Quality 0.951

IR SPECTRA COMPARISON BETWEEN 4-CHLOROETHCATHINONE AND OTHER POSITIONAL ISOMERS

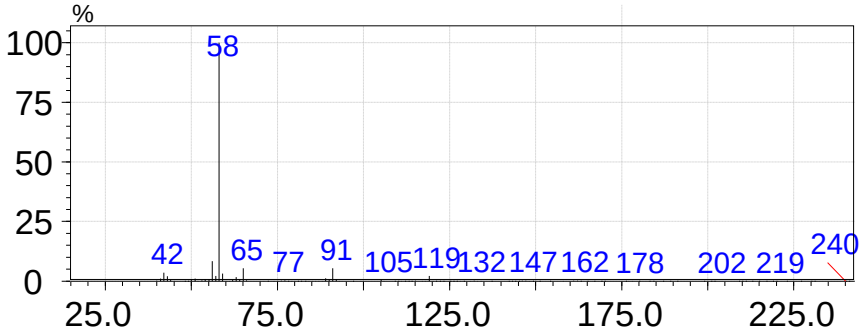


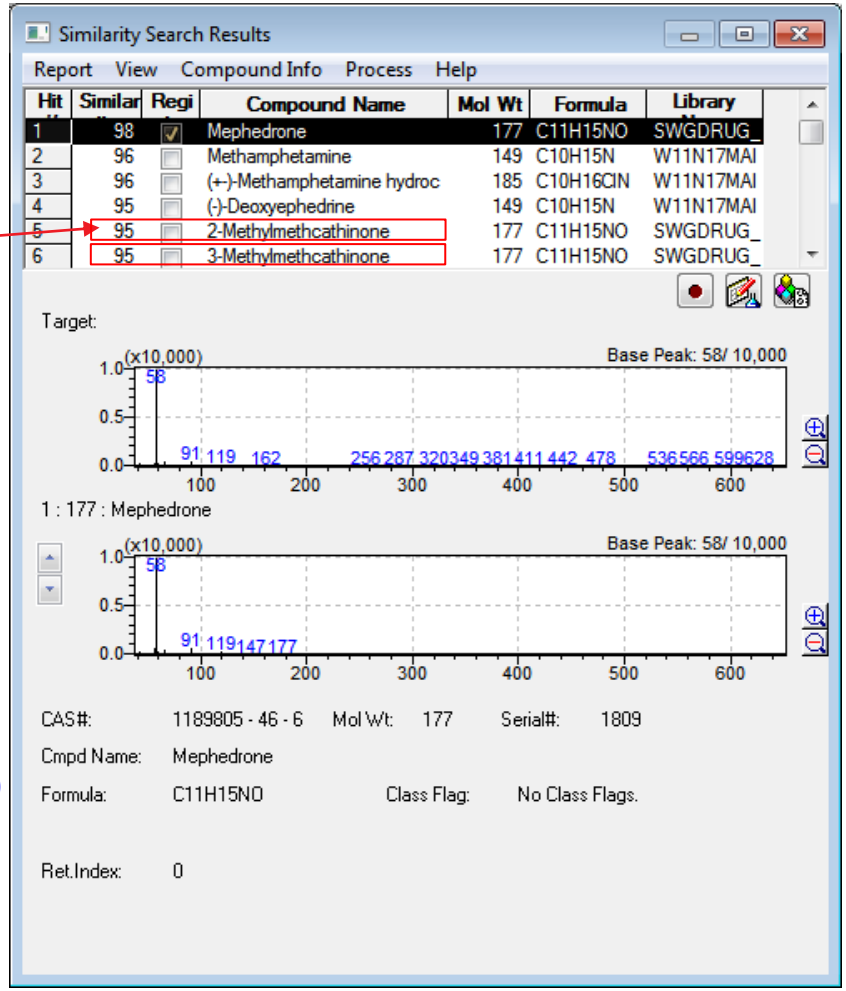
Source Software : Spectragryph

GC-MS IDENTIFICATION OF MEPHEDRONE IN A SEIZED POWDER SOLUTION ≈1.0% WITH LIBRARY SEARCH

Good similarity also with other positional isomer. It's necessary to confirm the identification with FTIR

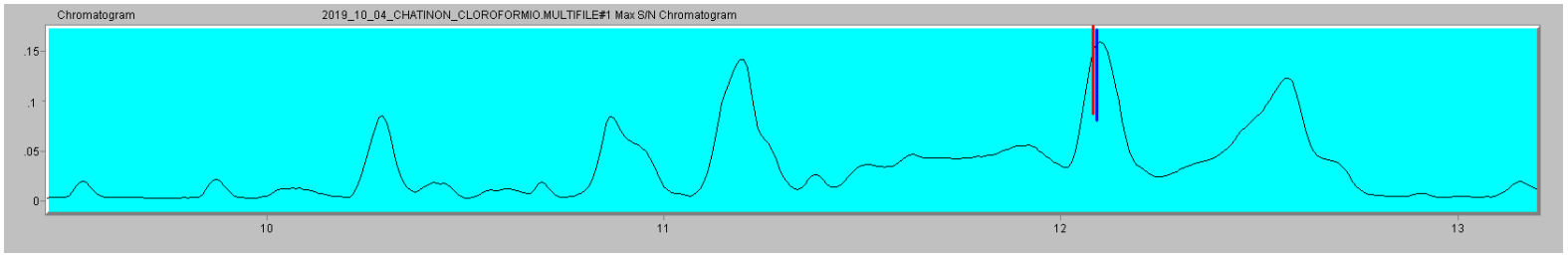
R_t=11.271



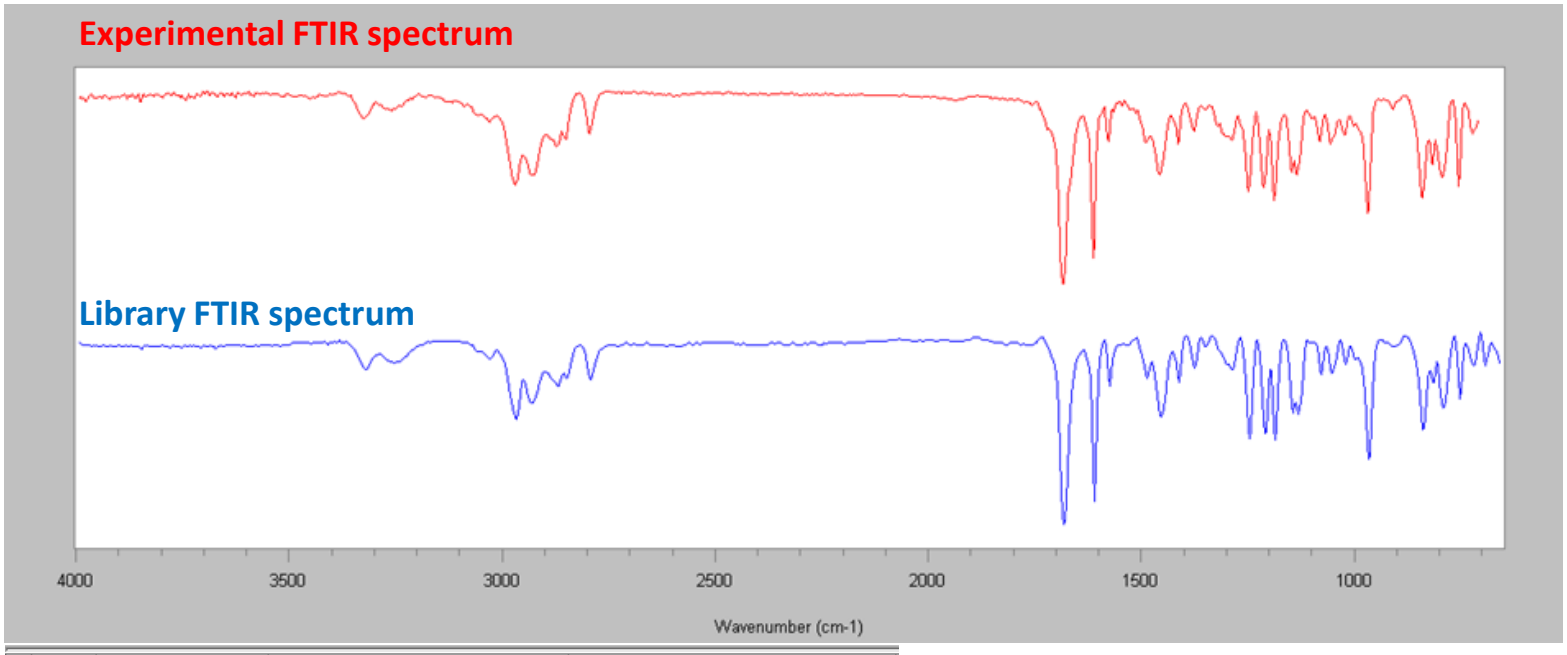


GC-FTIR IDENTIFICATION OF MEPHEDRONE IN A SEIZED POWDER SOLUTION ≈1.0%

GC-FTIR Chromatogram

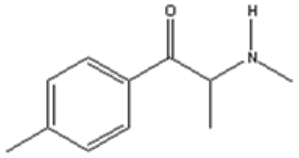


GC-FTIR identification of Mephedrone in a seized powder solution ≈1.0% with Library search

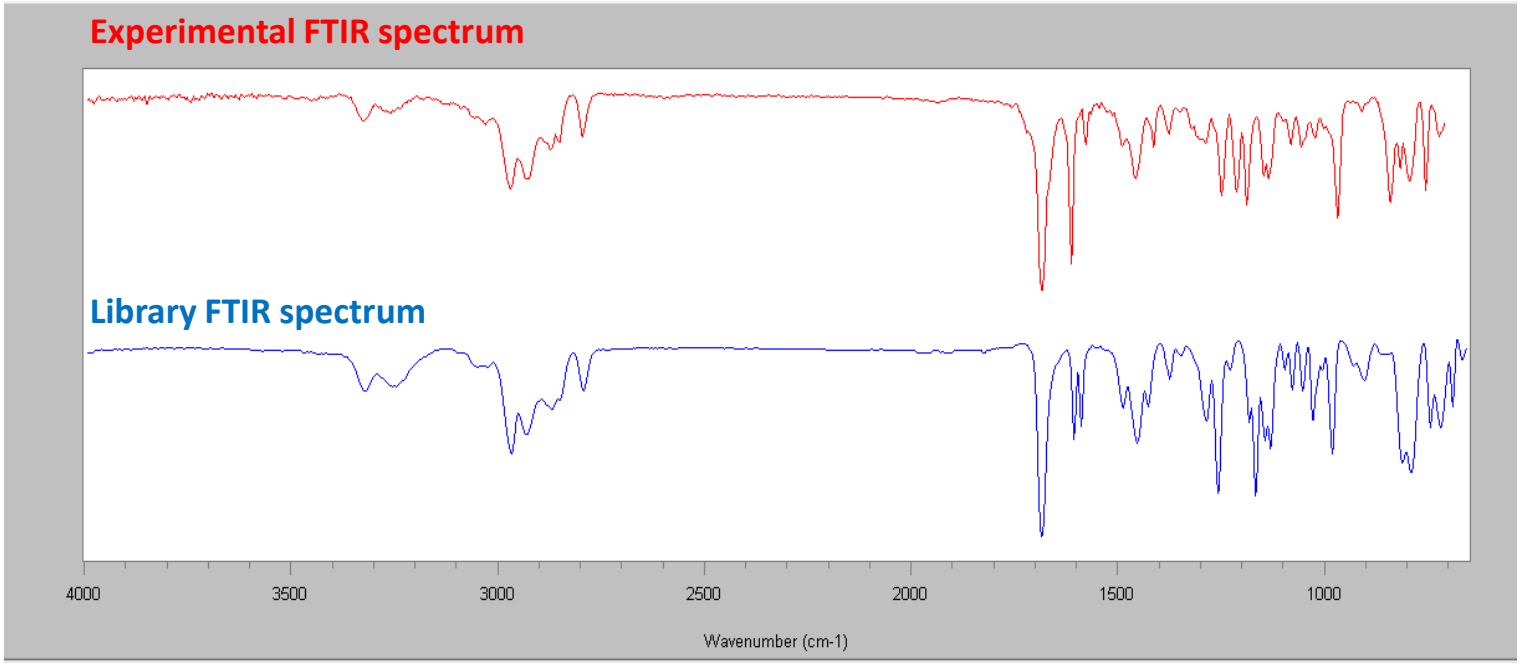


Hit	Quality	Memo	LibName
1	.0440409	4-MMC	Forensic.lib
2	.568783	3,4-DMMC	Forensic.lib
3	.618157	Pyrovalerone	Forensic.lib
4	.665336	4-methyldiethcathinone 4005	Forensic.lib

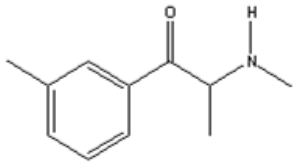
HIT 1 4-MMC
Quality 0.043



GC-FTIR IDENTIFICATION OF MEPHEDRONE IN A SEIZED POWDER SOLUTION ≈1.0% WITH LIBRARY SEARCH

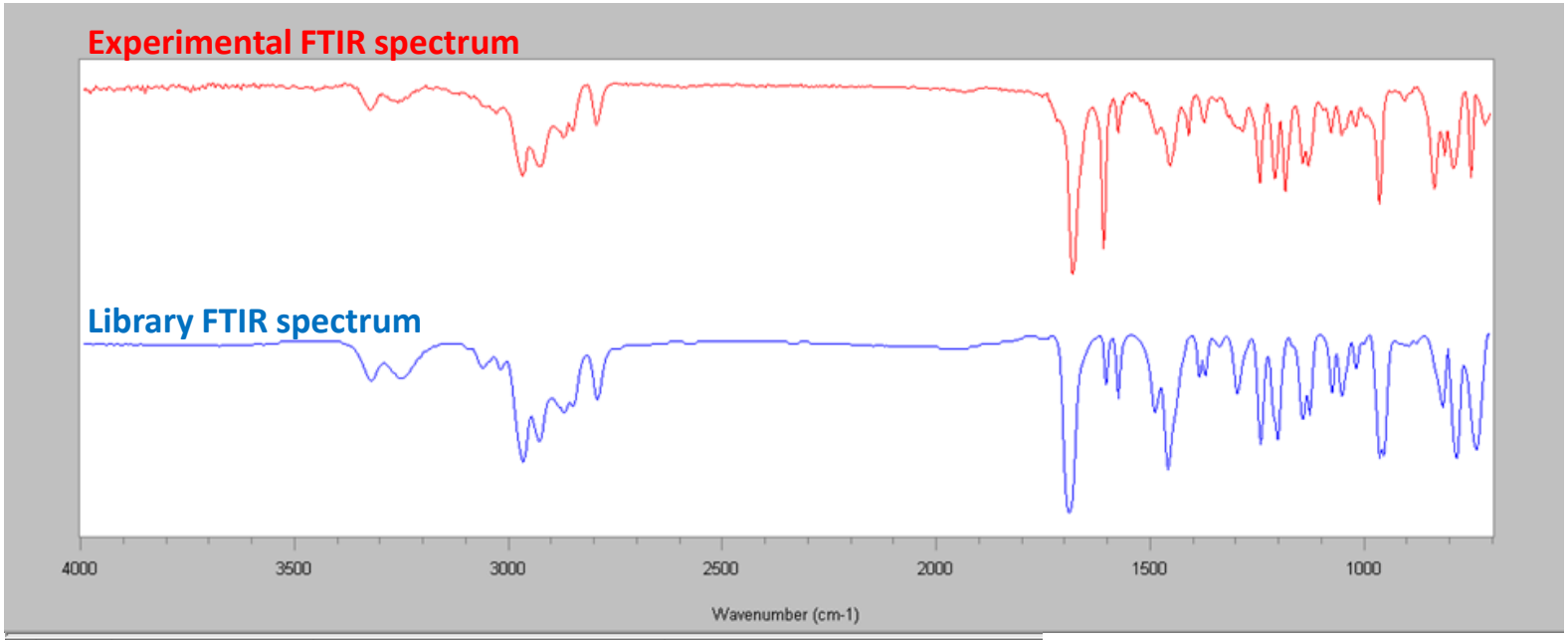


	Hit	Quality	Memo	LibName
	2	.568783	3,4-DMMC	Forensic.lib
	3	.618157	Pyrovalerone	Forensic.lib
	4	.665336	4-methyldiethcathinone 4005	Forensic.lib
▶	**5**	**.828254**	**3-MMC**	**Forensic.lib**

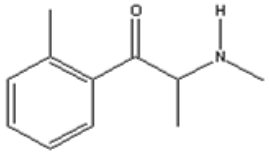


HIT 5 3-MMC
Quality 0.828

GC-FTIR IDENTIFICATION OF MEPHEDRONE IN A SEIZED POWDER SOLUTION ≈1.0% IN ETOAC WITH LIBRARY SEARCH

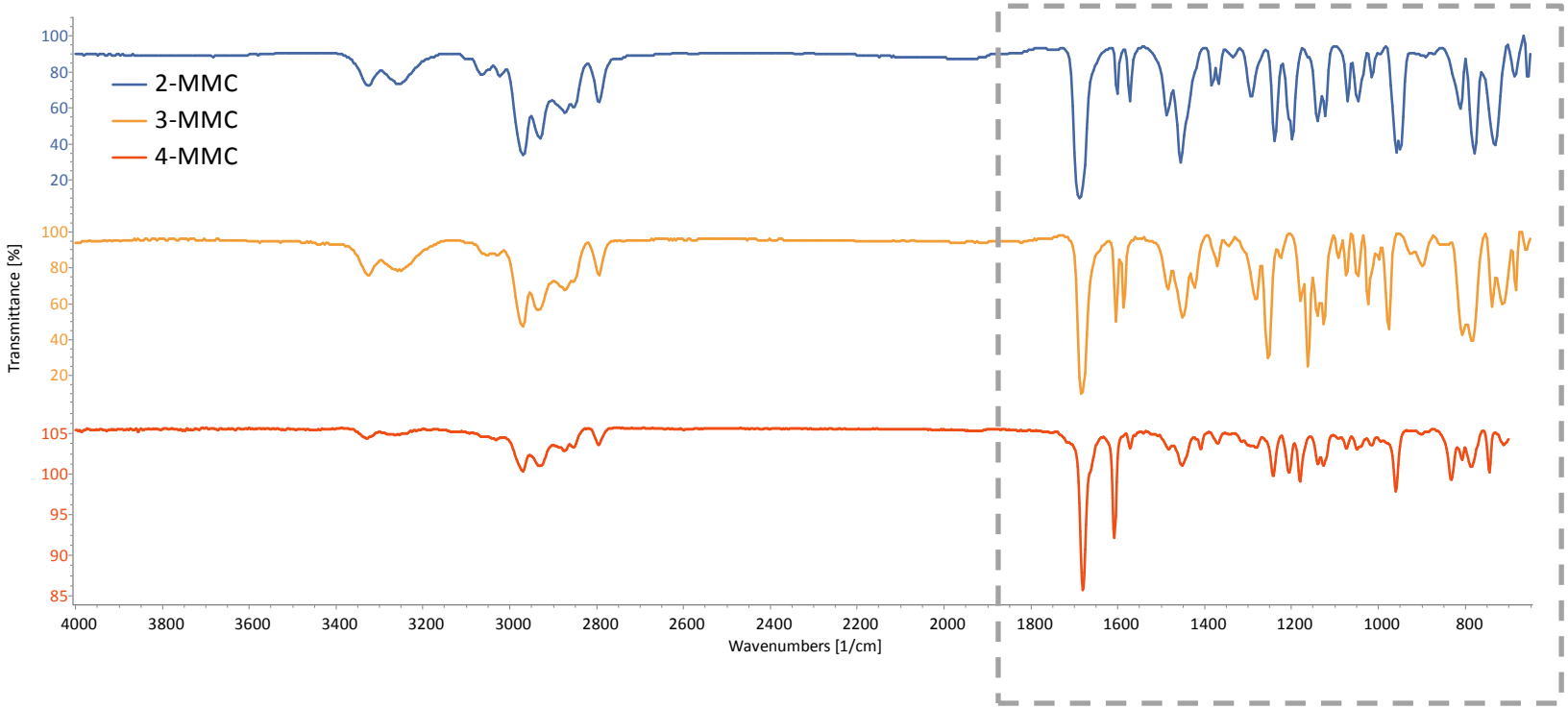


Hit	Quality	Memo	LibName
8	.857553	a-Ethylaminopentiophenone	Forensic.lib
9	.870037	N-Ethylcathinone	Forensic.lib
10	**.890361**	**2-MMC**	**Forensic.lib**
11	.898134	Testosterone Cypionate	Forensic.lib



HIT 10 2-MMC
Quality 0.890

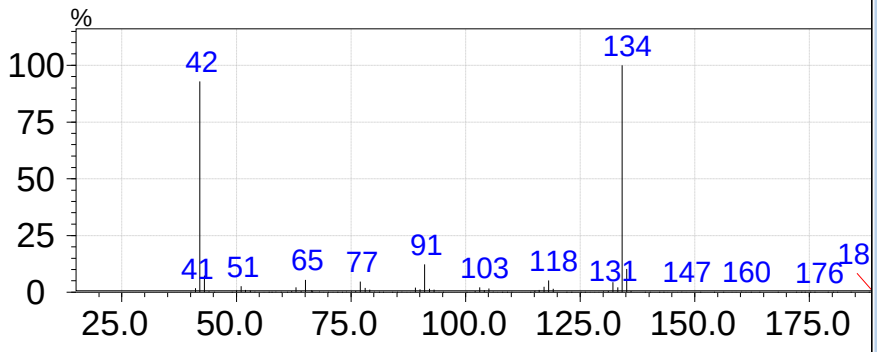
IR SPECTRA COMPARISON BETWEEN MEPHEDRONE AND OTHER POSITIONAL ISOMERS

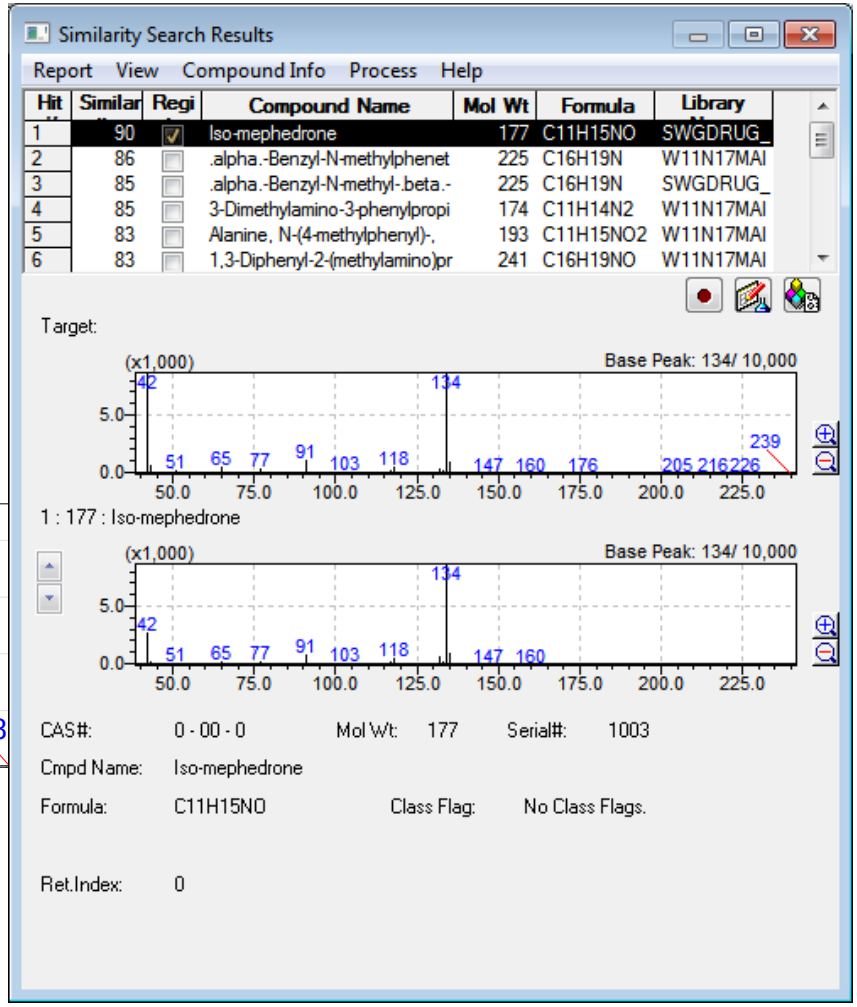


Source Software : Spectragryph

GC-MS IDENTIFICATION OF ISOMEPHEDRONE IN A SEIZED POWDER SOLUTION ≈1.0% WITH LIBRARY SEARCH

R_t=10.677





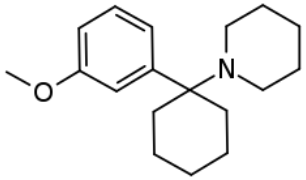
For GC-FTIR there are no standard available for iso-mephedrone

PHENCYCLIDINE ANALOGUES
CASE OF STUDY: OVERDOSE OF 3-MEO-PCP

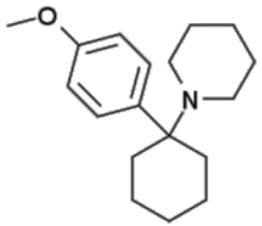
3-Methoxyphencyclidine (**3-MeO-PCP**) is a dissociative hallucinogen of the class related to phencyclidine (PCP) which has been sold online as a designer drug.

Analysis of seized powder and urine

Aim: confirm that the powder contains 3-MeO-PCP (3 methoxy-phencyclidine) and not the 4 positional isomer



3 MeO-PCP
M.F. $C_{18}H_{27}N$
MW:273.420 $g \cdot mol^{-1}$



4 MeO-PCP
M.F $C_{18}H_{27}N$
MW:273.420 $g \cdot mol^{-1}$

GC-FTIR ANALYSIS

Instrument GC-FTIR

Shimazdu Nexis GC-2030 Gas chromatograph
Spectra-Analysis DiscovIR solid phase FTIR

GC parameters

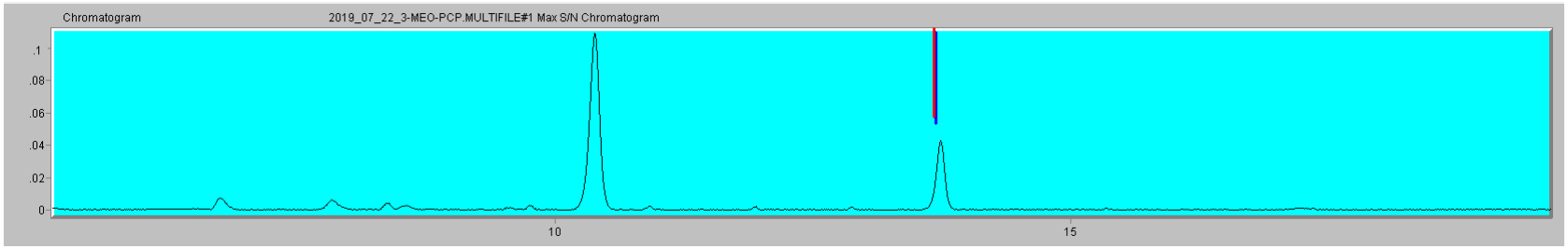
Column: SLB-5ms (Merck KGaA) 30 m L x 0.25 mm i.d. x 0.25 μm *d.f.*
Injector: 280 °C
Injection volume: 1 μL splitless,
Sampling time: 1.50 min (1:20)
Carrier gas: Helium
Linear velocity mode: 30 cm/sec
Oven program: 100 °C (2 min), ramp to 350 °C at 15 °C/min, hold 5 min
Time analysis: 24 min

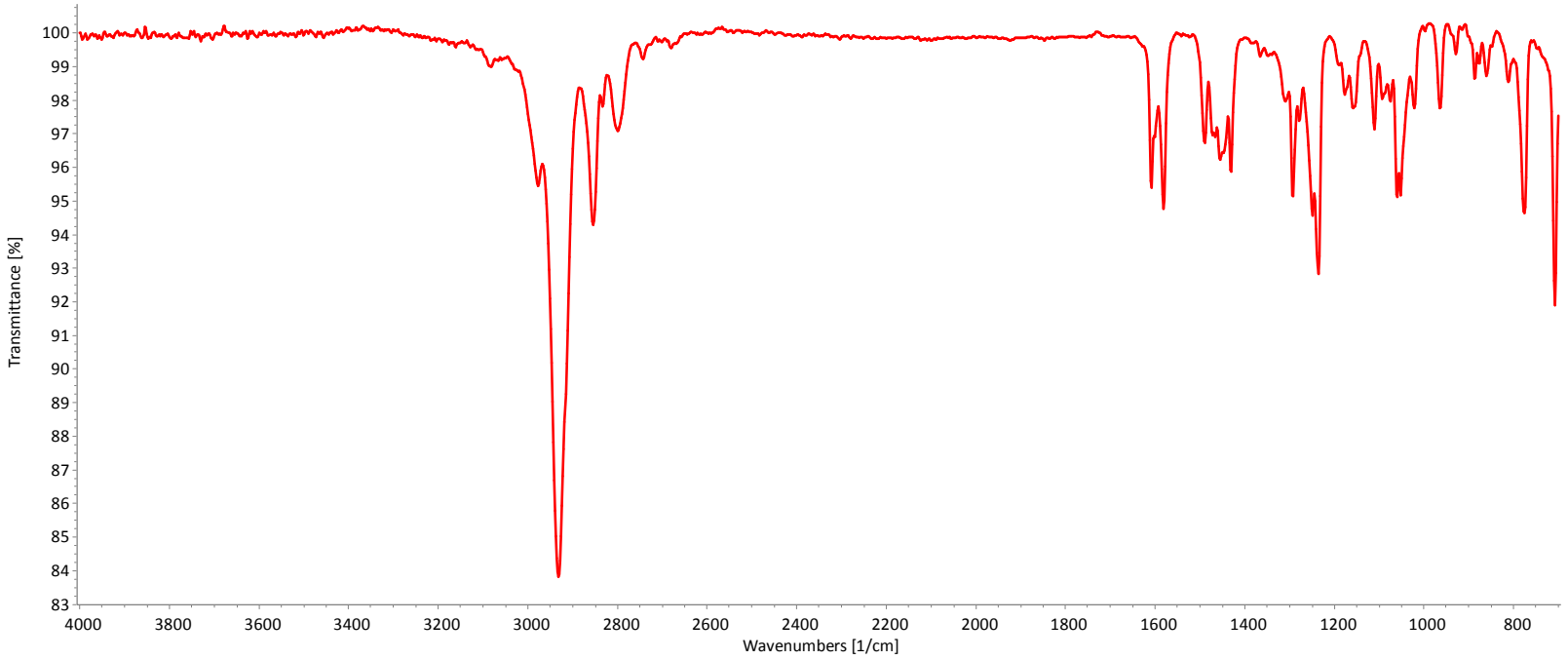
FTIR parameters

Disk temp.: -50 °C
Disk speed: 3 mm/min
Transfer line temp.: 280 °C
Restrictor temp.: 280 °C
Resolution: 4 cm^{-1}
Detection: MCT IR detector 4000-700 cm^{-1}

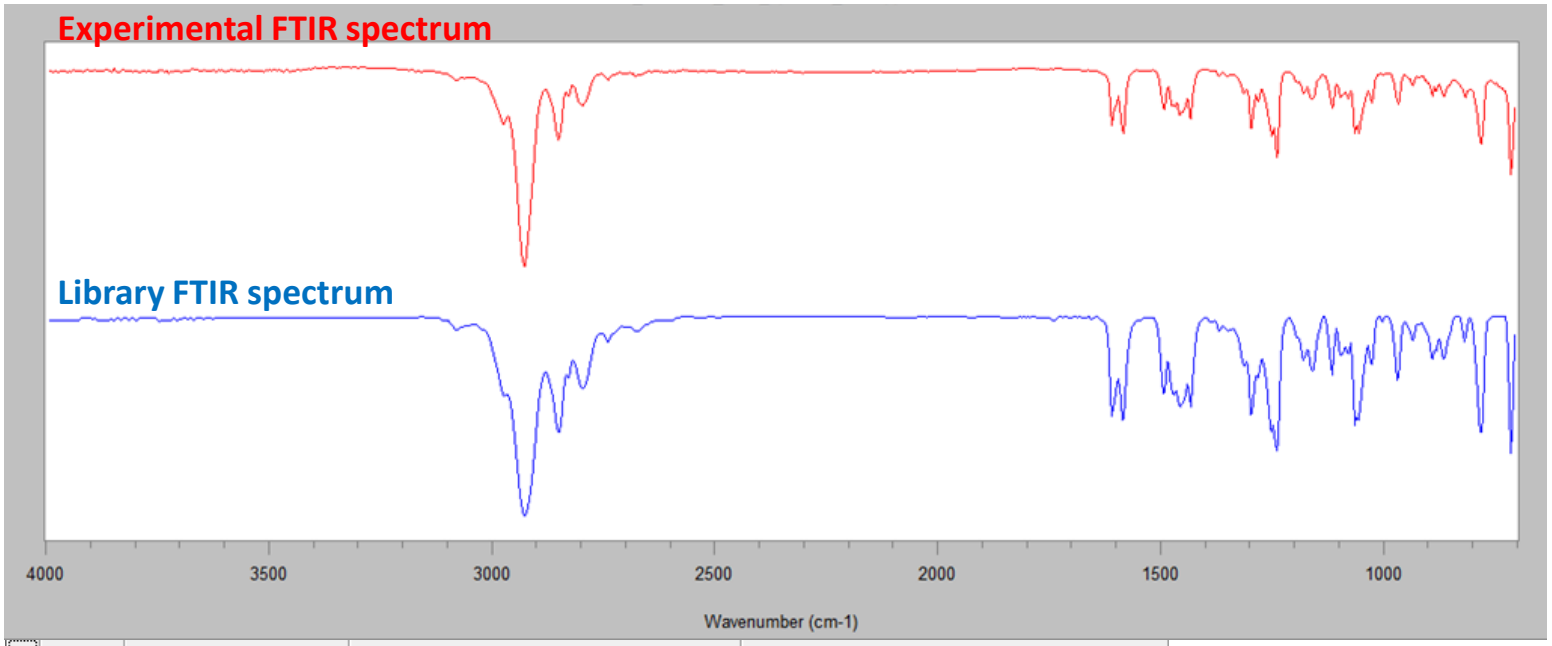
GC-FTIR ANALYSIS

GC-FTIR Chromatogram



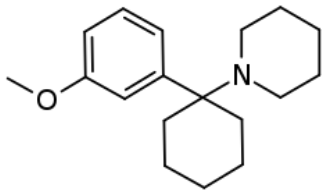


LIBRARY SEARCH

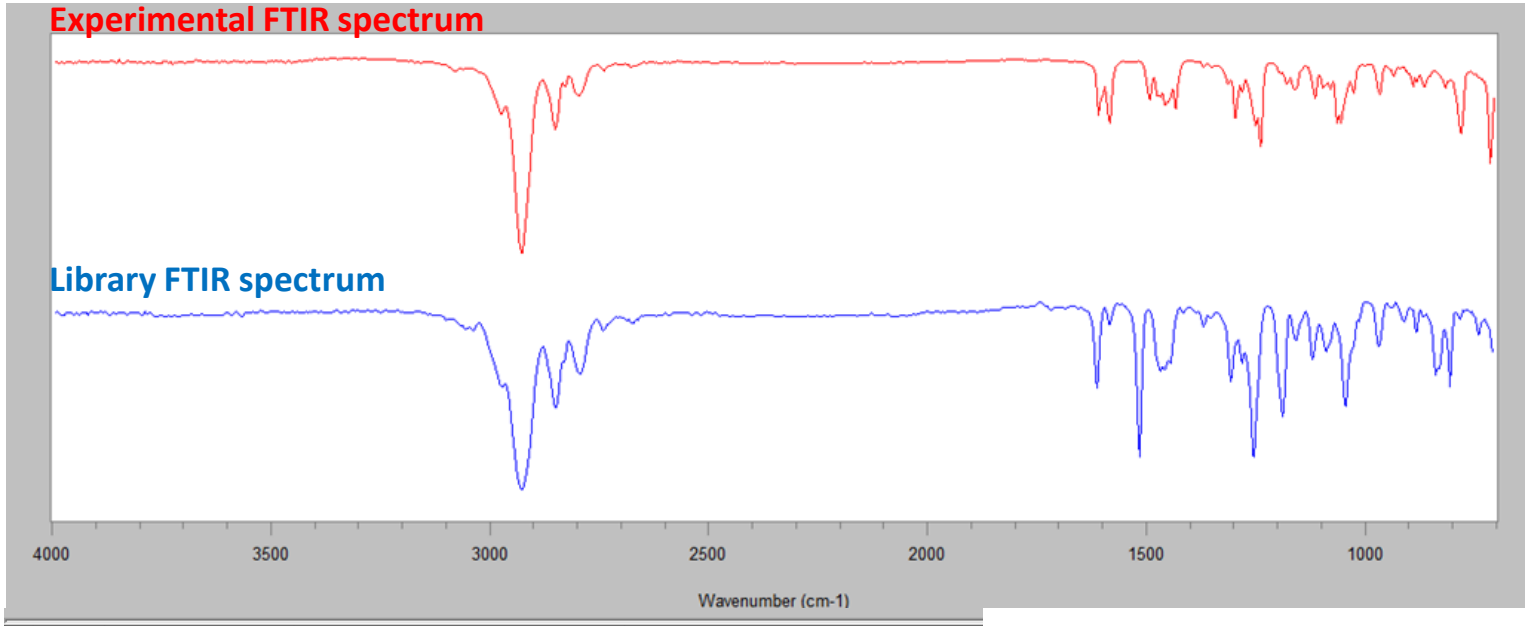


Hit	Quality	Memo	LibName
1	.0358651	3-MEO-PCP_HCl_1262-15_IR-C	Forensic.lib
2	.0454876	3-Methoxy-PCP_HCl_1468-16_Cay	Forensic.lib
3	.599315	3-MeO-PCE_HCl_1732-16	Forensic.lib
4	.799852	3-HO-PCP_HCl-HBr_1904-18_TMS	Forensic.lib

HIT 1 3-MeO-PCP
Quality 0.035

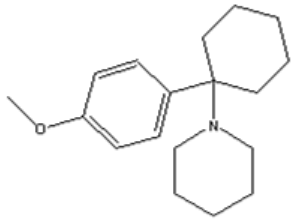


LIBRARY SEARCH



Hit	Quality	Memo	LibName
9	.882031	3-MeO-PCMo-1431-16	Forensic.lib
10	**.901541**	**4-MeO-PCP_1356-15**	**Forensic.lib**
11	.904228	Methoxetamine(MXE) HCl 1237-15	Forensic.lib
12	.914481	PCEEA_HCl_1612-16	Forensic.lib
13	.915608	Cyclopentenyl-fentanyl-HCl_1863-17_Cay	Forensic.lib

HIT 10 4-MeO-PCP
Quality 0.901



The results confirm that the compound is 3-MeO-PCP

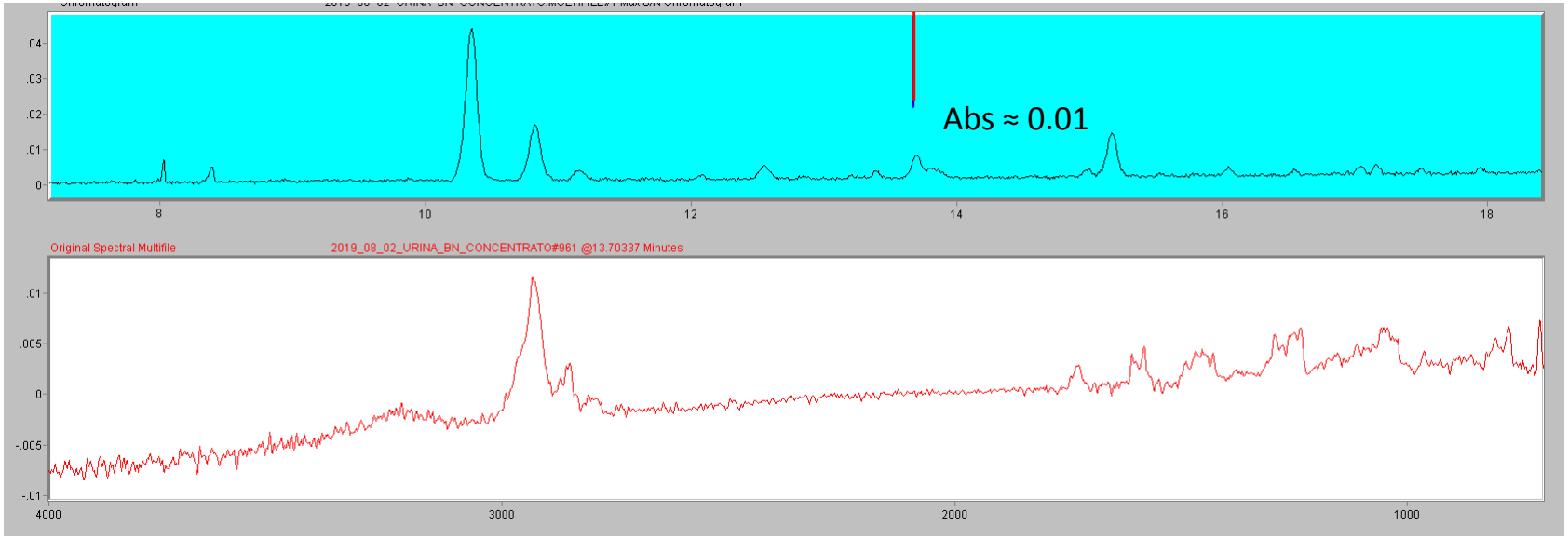
URINE SAMPLE

Urine samples (1 ml), were diluted with 6 mL of 0,1 M phosphate buffer and extracted on Bond Elute Certify 130 mg columns (Agilent Technologies, USA).

The column was first conditioned with CH_3OH, H_2O and buffer, then washed with H_2O, HCl and CH_3OH. The compounds were eluted with CH_3OH and NH_4OH and the organic phase obtained was reduced to dryness. The residues were taken up with 100 µL of ethyl acetate.

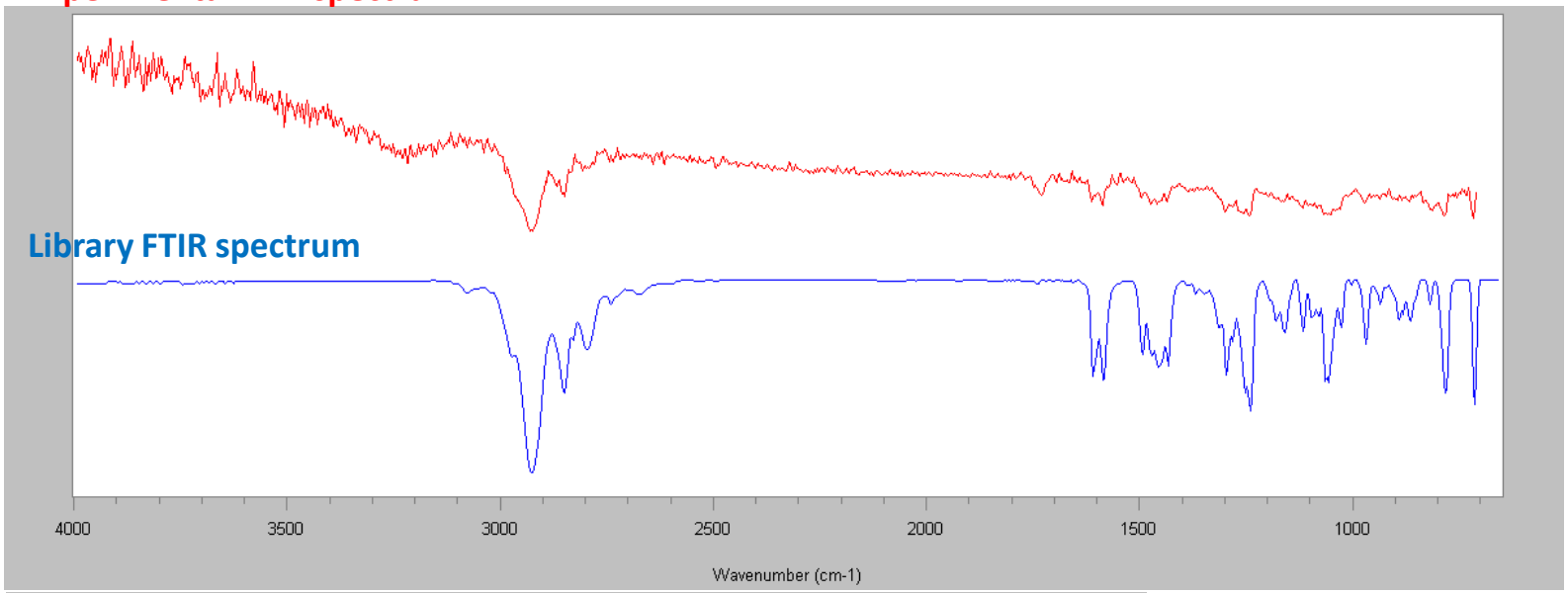
1st deposition

GC-FTIR Chromatogram



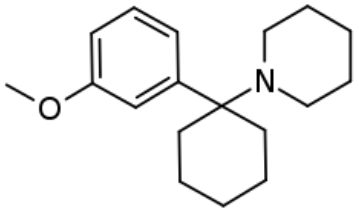
LIBRARY SEARCH: URINE SAMPLE

Experimental FTIR spectrum



Hit	Quality	Memo	LibName
1	**.596014**	**3-MEO-PCP_HCl_1262-15_IR-C**	**Forensic.lib**
2	.610041	3-Methoxy-PCP_HCl_1468-16_Cay	Forensic.lib
3	.812172	3-MeO-PCE_HCl_1732-16	Forensic.lib

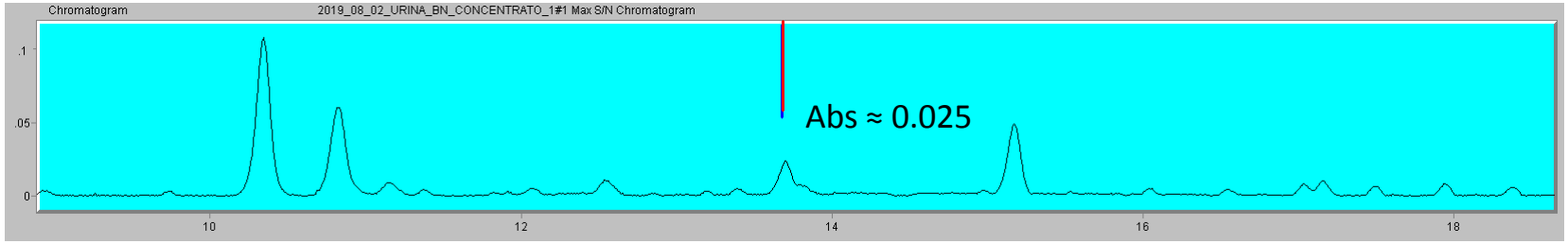
HIT 1
3-MeO-PCP
Quality 0.596

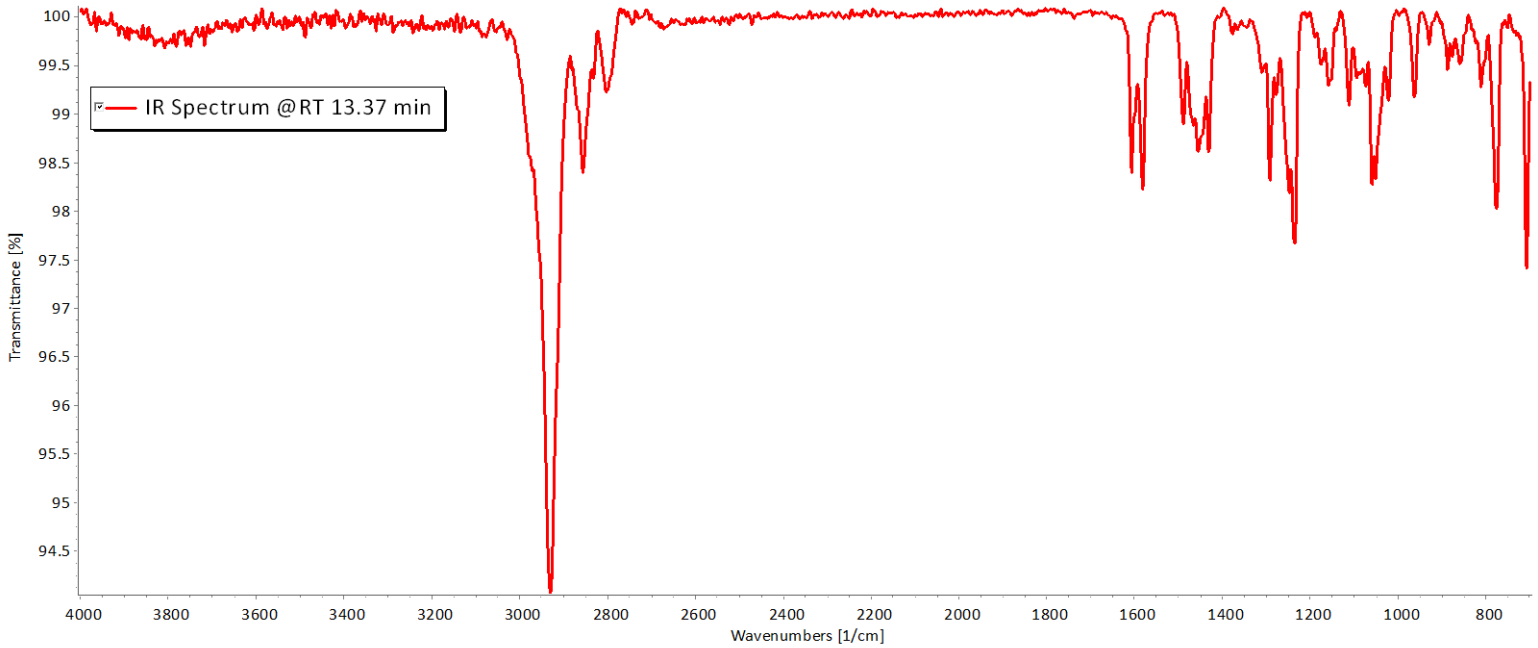


URINE SAMPLE: ANALYSIS IN REDEPOSITION MODE

2nd deposition

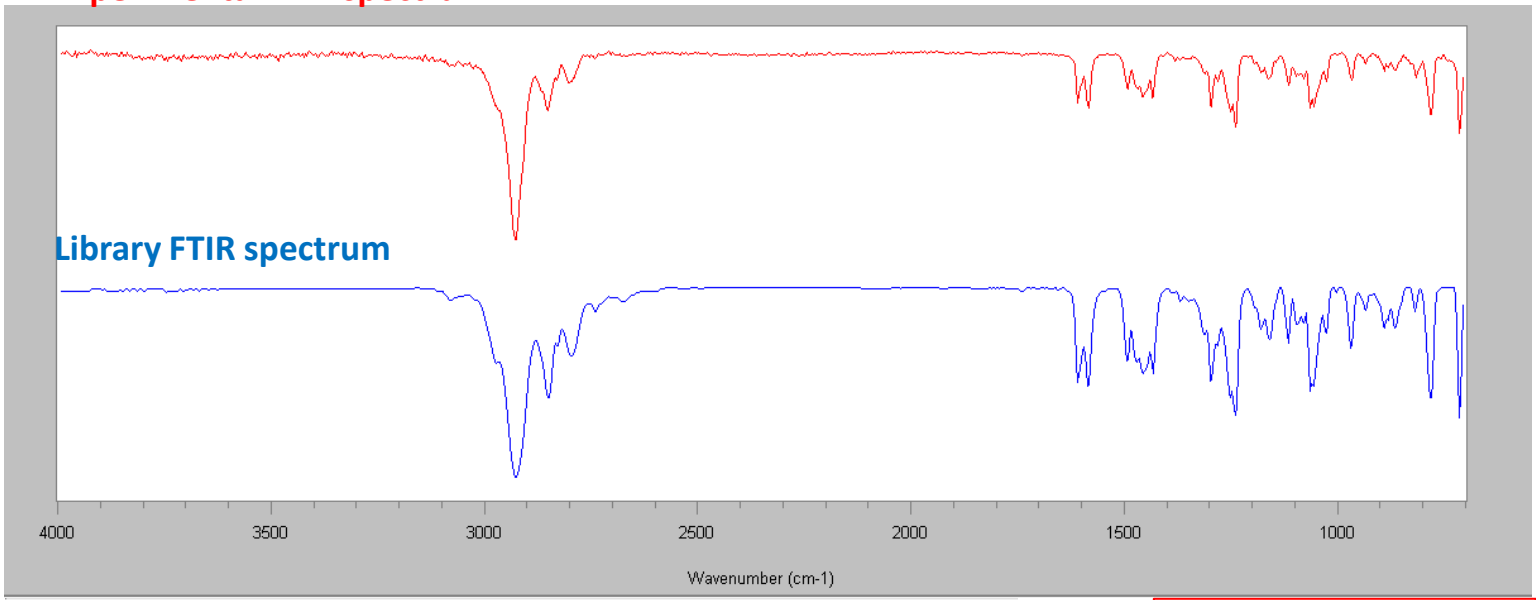
GC-FTIR Chromatogram





URINE SAMPLE AFTER REDEPOSIT
LIBRARY SEARCH

Experimental FTIR spectrum

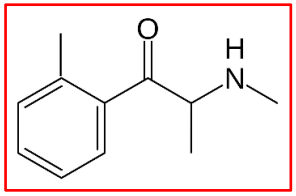


Hit	Quality	Memo	LibName
1	**.0910384**	**3-MEO-PCP_HCl_1262-15_IR-C**	**Forensic.lib**
2	.106525	3-Methoxy-PCP_HCl_1468-16_Cay	Forensic.lib
3	.588626	3-MeO-PCE_HCl_1732-16	Forensic.lib
4	.819258	3-HO-PCP_HCl-HBr_1904-18_TMS	Forensic.lib

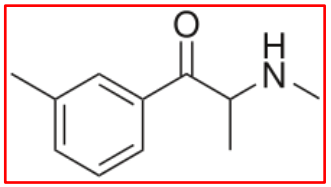
HIT 1
3-MeO-PCP
Quality 0.091

3-MethylmethCathinone

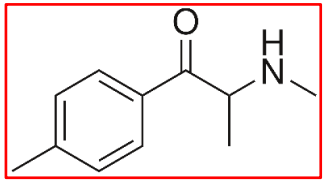
Aim: confirm that the powder contains 3 MMC and not 4 MMC or 2 MMC (positional isomers)



2 MMC
M.F C11H15NO
MW: 177.24 g/mol



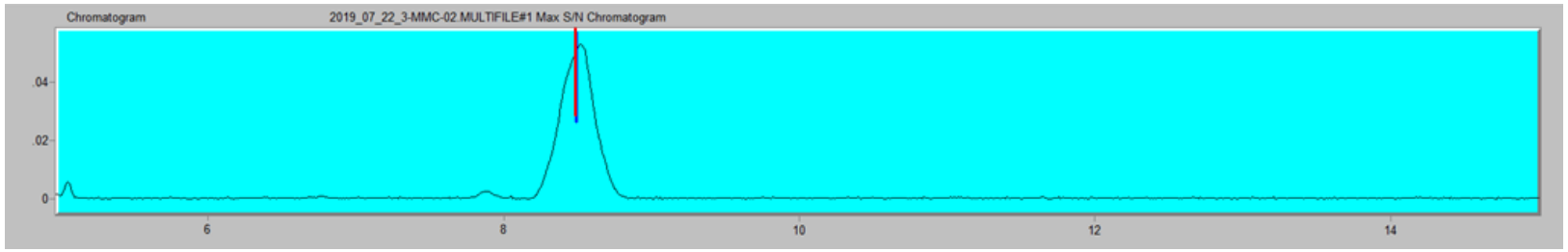
3 MMC
M.F C11H15NO
MW: 177.24 g/mol

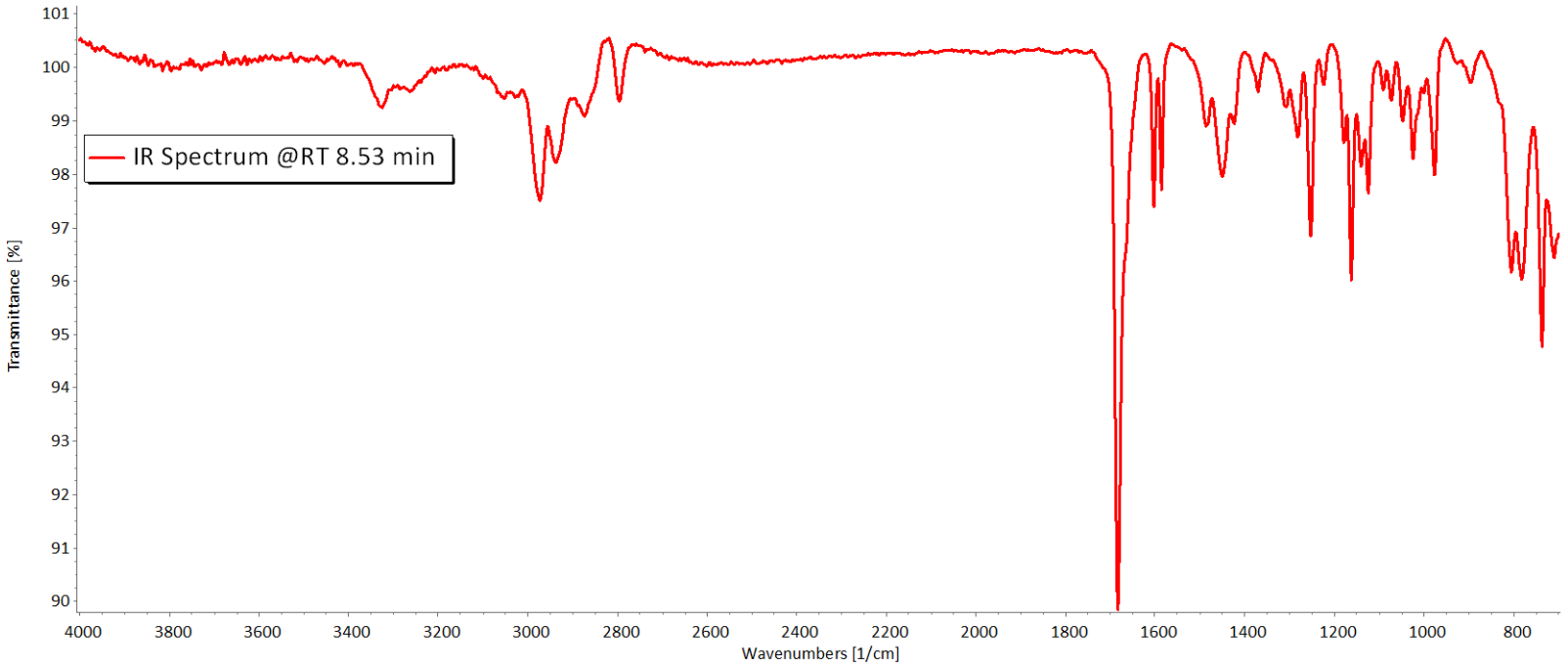


4 MMC
M.F C11H15NO
MW: 177.24 g/mol

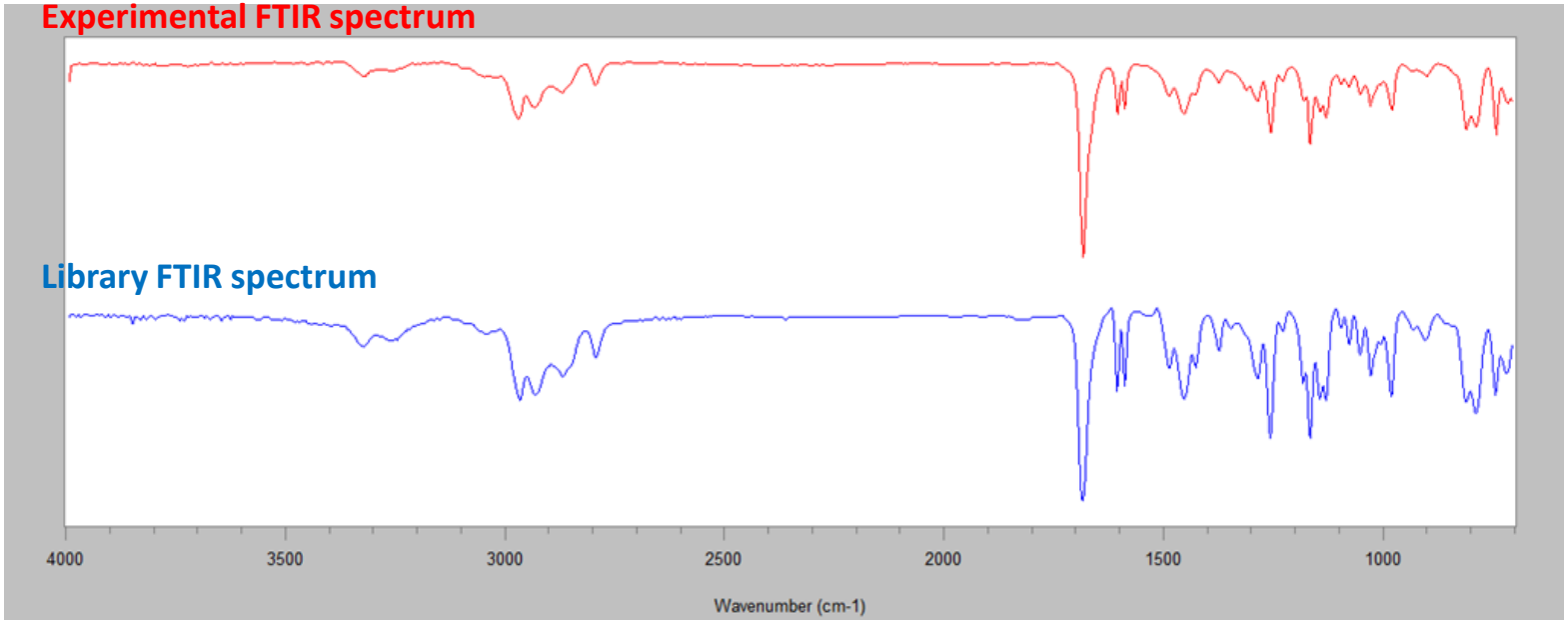
GC-FTIR ANALYSIS

GC-FTIR Chromatogram



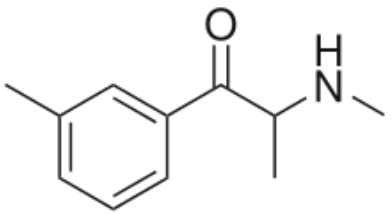


LIBRARY SEARCH

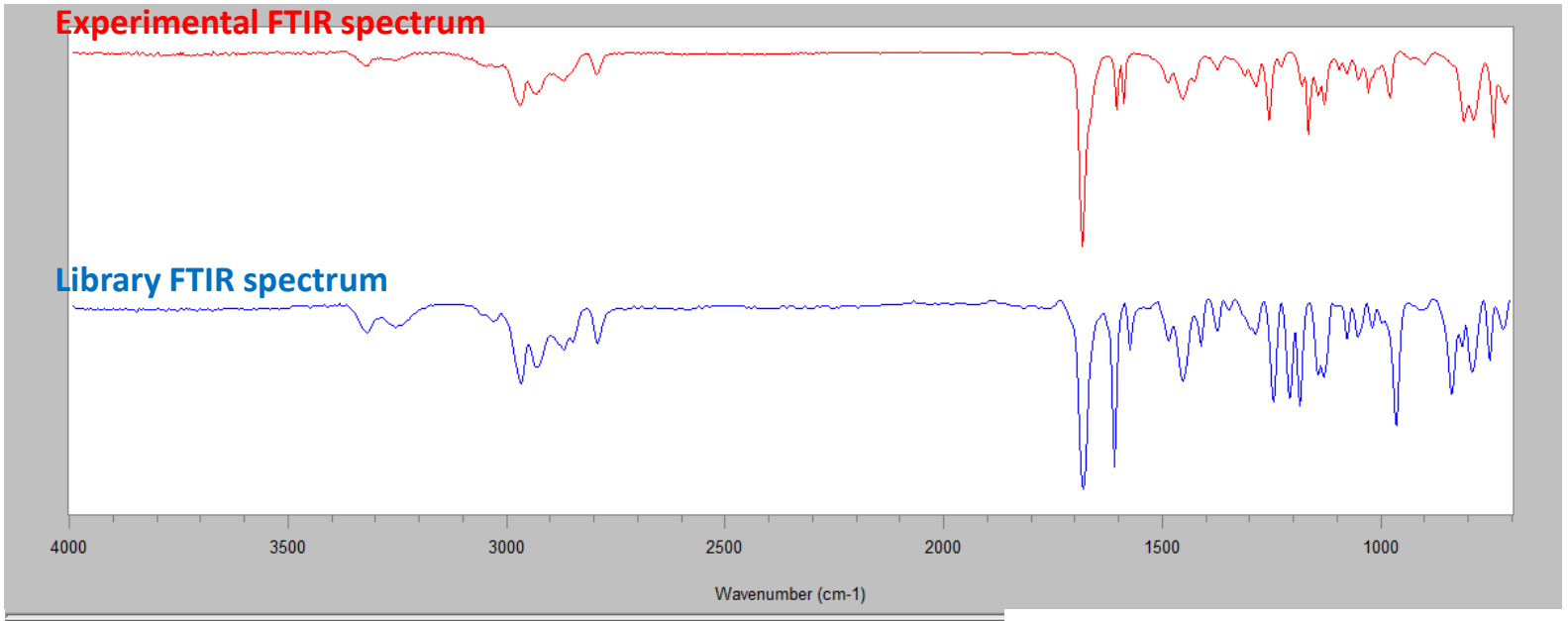


Hit	Quality	Memo	LibName
1	.0840468	3-MMC	Forensic.lib
2	.344382	3-MEC_HCl_1724-16	Forensic.lib
3	.38281	3-Ethylmethcathinone sample 21	Forensic.lib
4	.411988	3-Methylethcathinone sample 49	Forensic.lib
5	.695303	3-Methyl-a-pyr-pro-phen sample 51	Forensic.lib

HIT 1 3-MMC
Quality 0.084

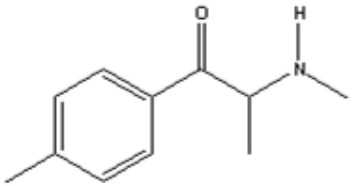


LIBRARY SEARCH

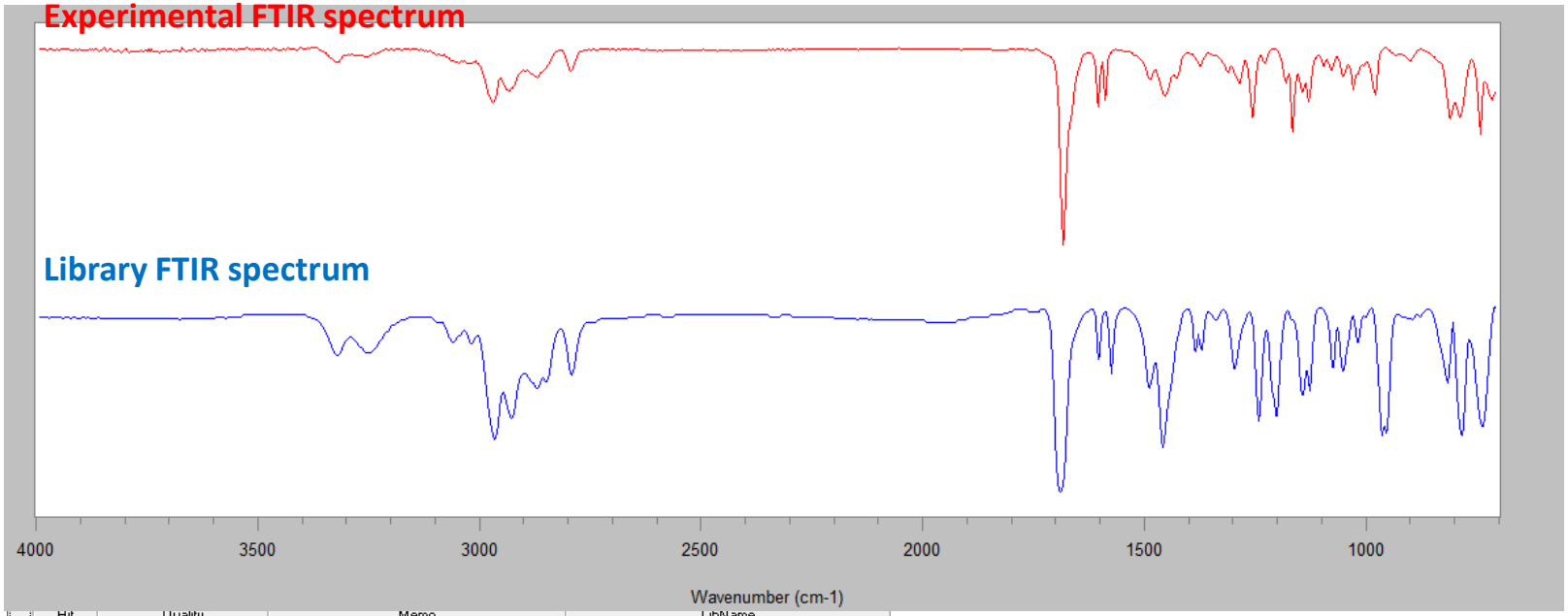


Hit	Quality	Memo	LibName
21	.858147	4-MMC	Forensic.lib
22	.860871	3-Methoxymethcathinone sample 31	Forensic.lib
23	.861033	4-methyldiethcathinone 4005	Forensic.lib
24	.865005	Diethylcathinone	Forensic.lib
25	.865408	3-F-Ethcathinone sample 18	Forensic.lib

HIT 21 4-MMC
Quality 0.858

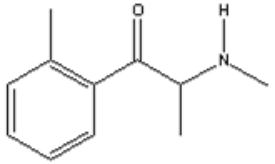


LIBRARY SEARCH



Hit	Quality	Memo	LibName
32	.889261	4-Methylethcathinone sample 5	Forensic.lib
33	**.889867**	**2-MMC**	**Forensic.lib**
34	.891408	2-Methylmethcathinone sample 63	Forensic.lib
35	.891875	a-pyr-pent-phen sample 40	Forensic.lib
36	.892837	a-PVP	Forensic.lib

HIT 33 2-MMC
Quality 0.889



The results confirms that the powder contains 3-MMC

- Potential market and Spectra positioning (industrial sectors, conference presentations, scientific activities, R%D with Professor Luigi Mondello from the University of Messina).

- What makes DiscovIR solid-deposition FTIR technology a unique identification tool?

- Which benefits are to be gained by the online hyphenation GC(LC)-MS-FTIR (parallel detection)?

- Presentation of the FFNSC GC-MS-FTIR Library (flavour and fragrance natural and synthetic compounds) based on GC-EI MS with Linear Retention Indices (LRI) and solid state FTIR spectra.

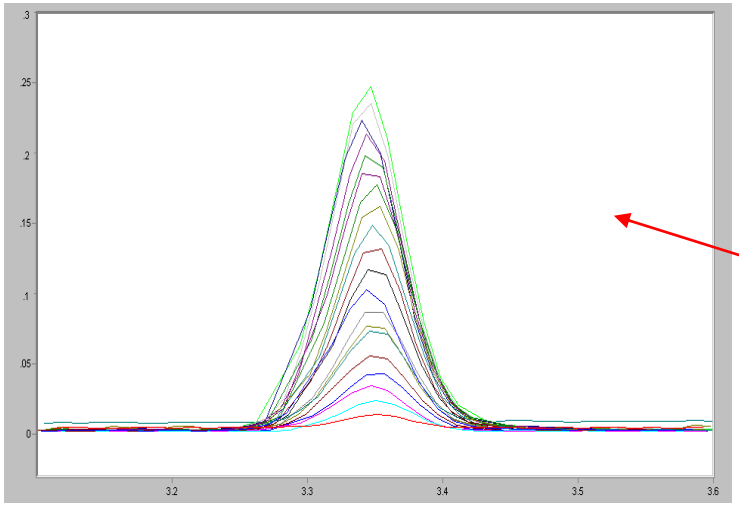
- The “quantitative” issue.

- DiscovIR: how does it work?

- GC-FTIR and LC-FTIR Application fields.

Enhance FTIR Spectrum Quality

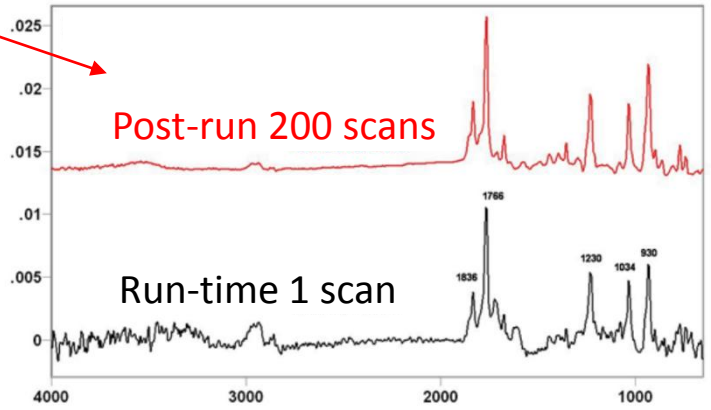
Typically, FTIR has not been regarded as a technique for quantitative analysis, due to the low sensitivity mainly associated with gas-phase spectra.



Concentrated deposits may be obtained through multiple deposition

Twenty consecutive runs overlaid on the disk, yields 5x improvement in S/N

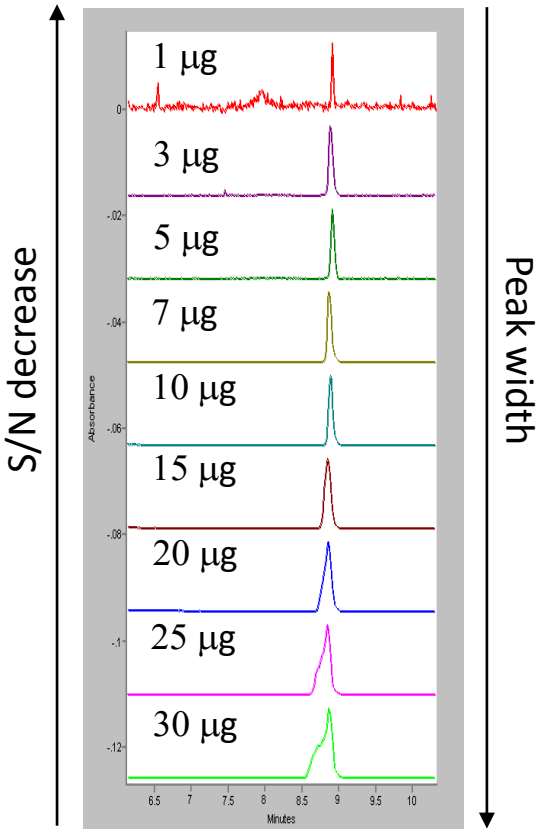
- A table of the peaks of interest may be defined for automatic re-scanning.
- The noise of an FTIR spectrum decreases as the square root of the number of scans.
- By scanning the deposit after the run is complete, it is possible to gain in S/N, which translates to sensitivity.



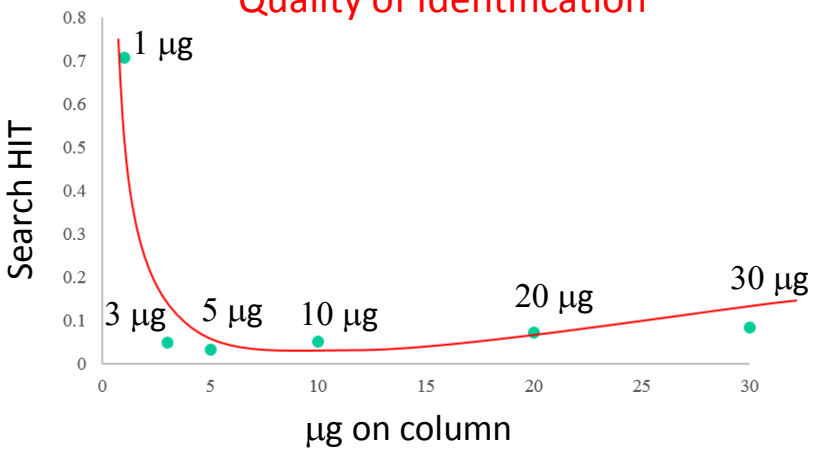
LC-FTIR Library Search

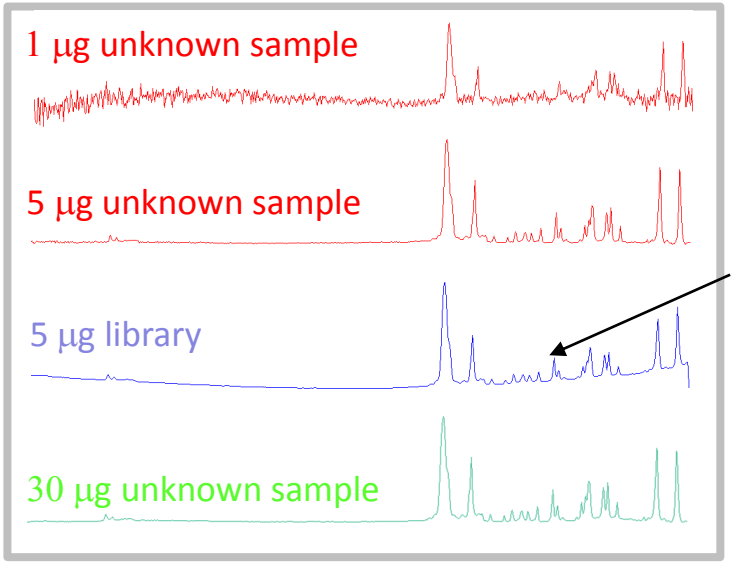
Quantities in real sample may vary to a large extent

LC-FTIR Angelicin



Quality of Identification





Hit .706111
S/N ≈2

Hit .031576
S/N >10

Hit .084026
S/N ≈50

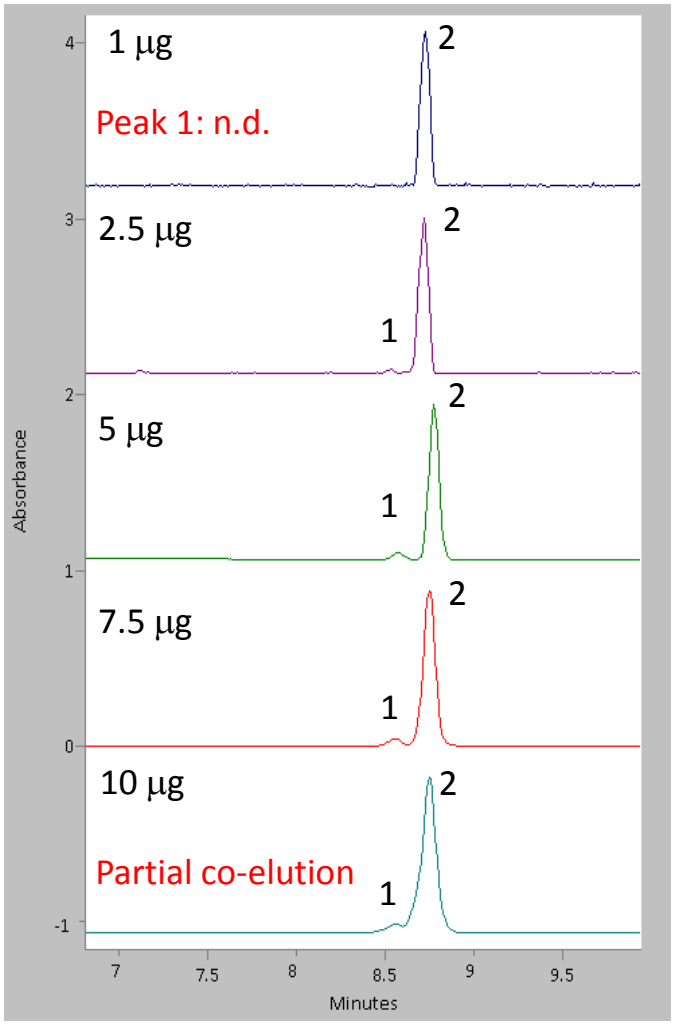
Achieve quality IR spectra for reliable identification

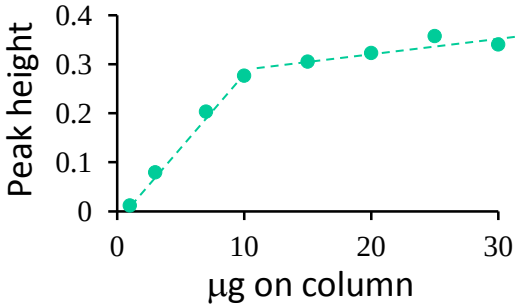
Response vs Amount on Column

Critical pair

Compounds: Psoralen (1), Angelicin (2)

Disc: 3 mm/min, 20 ° C





LOD = 1.09 µg
LOQ = 4.70 µg

1	Peak area	Peak height	*Abs@ 1727 cm^{-1}
slope	0.0005	0.0026	0.0062
R^2	0.9618	0.9905	0.9972
intercept	0.0005	0.0018	0.0106

(n=5)	Peak R_t	Peak area
RSD%	0.11	2.95

LOD = 0.52 µg
LOQ = 0.76 µg

2	Peak area	Peak height	*Abs@ 1737 cm^{-1}
slope	0.0103	0.0295	0.1105
R^2	0.9955	0.9963	0.9776
intercept	0.0082	0.0121	0.0794

(n=5)	Peak R_t	Peak area
RSD%	0.13	2.25

*C=O stretching

Effect of Disc Speed

Critical pair

Compounds: Psoralen (1), Angelicin (2)

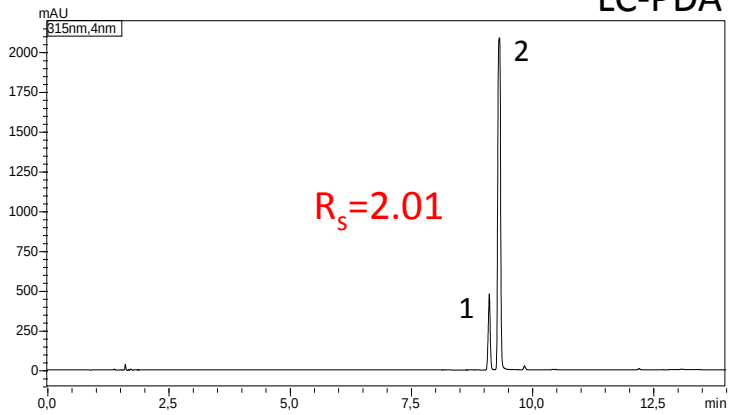
5 µg on column

Disc: 20 ° C

The disc speed influence the shape of the deposit hence the sensitivity of the technique

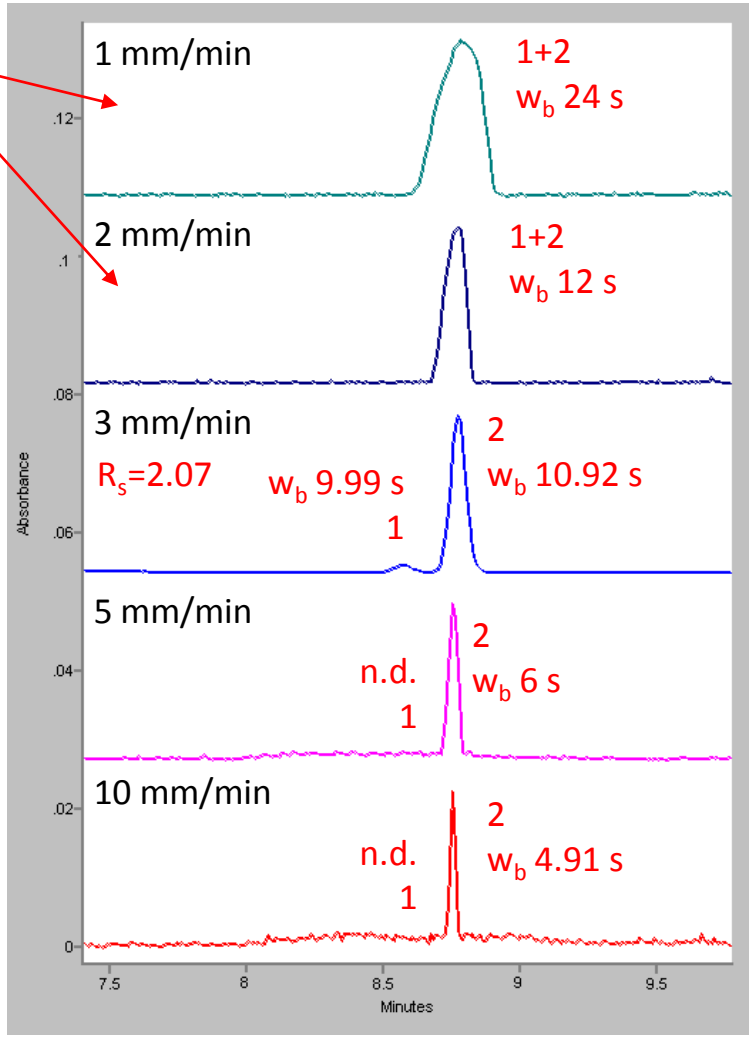
Extra-column band broadening

LC-PDA



	LC-PDA	LC-FTIR
R_s	2.01	2.07

LC-FTIR

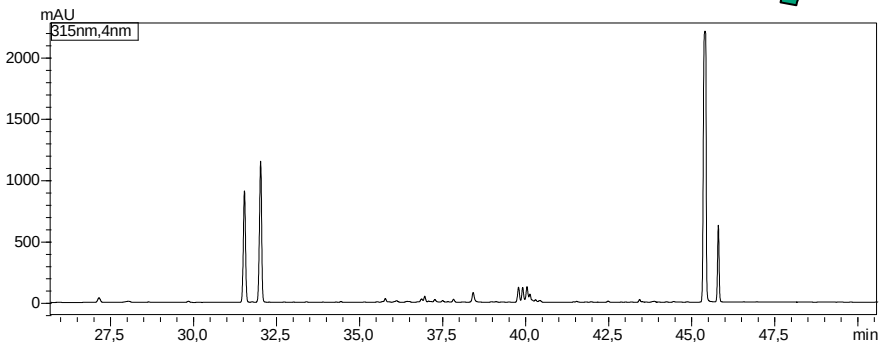


When all the parameters are equal absorbance intensity is inversely proportional to the disc speed

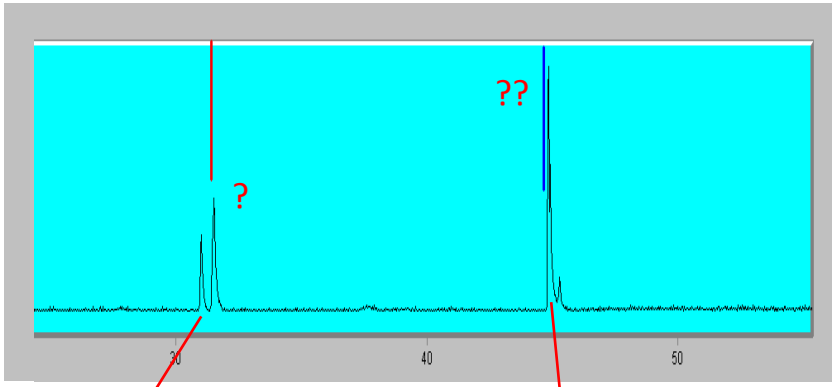
A Real Sample

Bergamot essential oil (non volatile residue)

LC-PDA



LC-FTIR



LC

Column: Ascentis Express C18 150 L × 4.6 mm I.D., 2.7 µm d.p.

Solvents: A(H_2O), B (MeOH) at 1.0 mL/min

Gradient: 0-5 min 0% B; 25 min 40% B, 45 min 90% B, 55 min 90% B

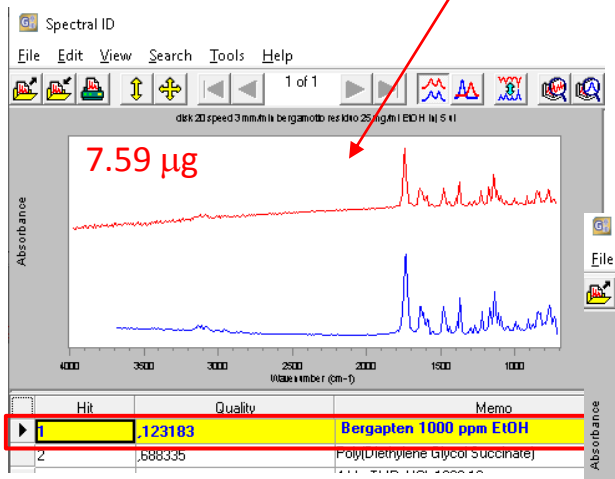
FTIR

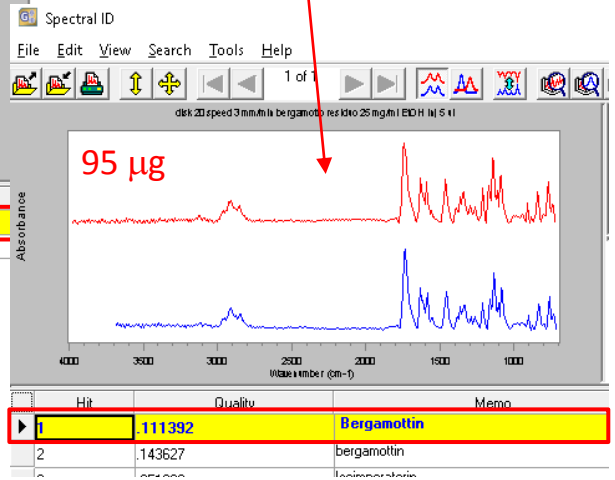
Nebulizer: 14 W

Cyclone: 180 ° C

Condenser: 3-7 ° C

Detection: MCT 4000-700 cm^{-1}





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- The “quantitative” issue.
- DiscovIR: how does it work?
- GC-FTIR and LC-FTIR Application fields.

DiscovIR-GC®

The Integrated Solution

Step 1	Step 2	Step 3
Separation (chromatography)	Detection DiscovIR	Identification by Proprietary Software

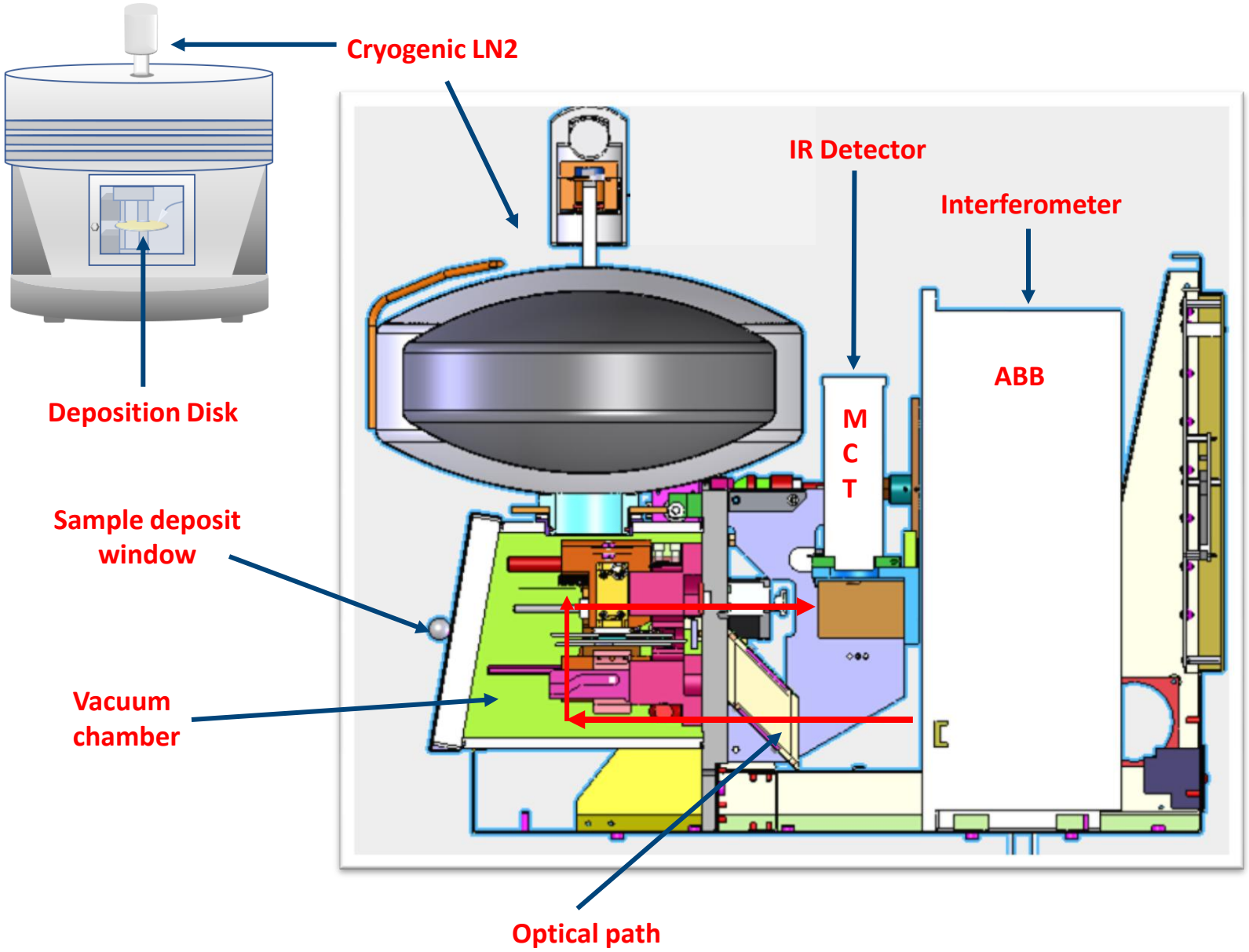
- Fully Automated Operation
- Real-Time Chromatography & Spectral Data
- Solid Phase Transmission IR Spectra
- Identification of unknowns in complex samples
- Compound identification through library searching

Instrument installed in Prof. Mondello's Lab

The GC was raised of 14 cm

A SilTite µ-union was installed in order to allow an higher GC temperature without leaks

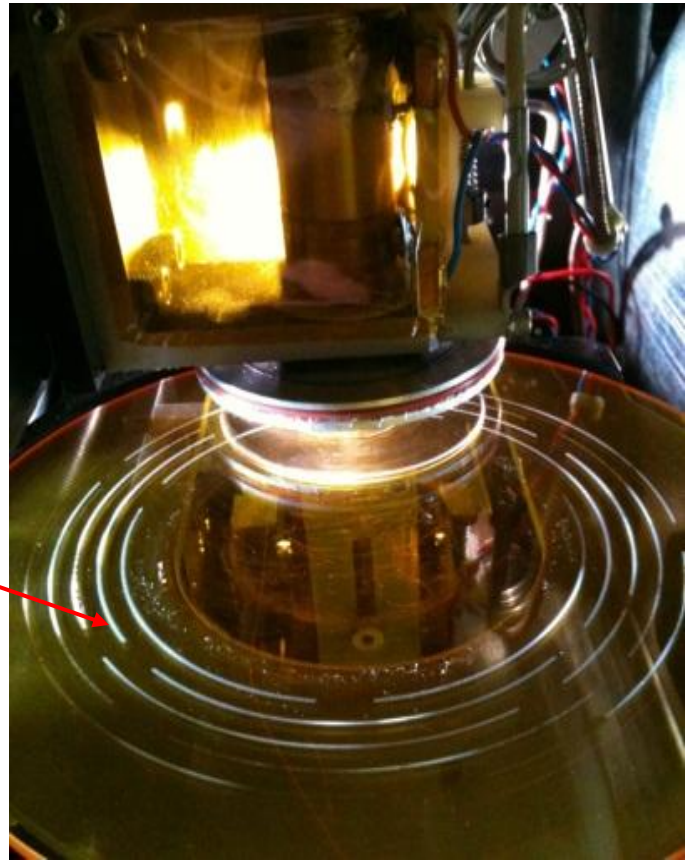
DiscovIR


Cryogenic LN2
Deposition Disk
IR Detector
Interferometer
ABB
M
C
T
Sample deposit window
Vacuum chamber
Optical path

DIRECT DEPOSITION – SOLID PHASE

Features:

- Mid-IR transparent 4000 – 700 cm^{-1}
- tunable speed 10-3 mm/min
- Disk Temp: - 50°C to ~ 100°C
- Flow rates: 0.25 to 1.5 mL/min
- Transmission IR analysis on the solid deposit
- Re-usable after solvent cleaning
- Unattended overnight runs/10 hrs



The ZnSe disk is under vacuum to remove moisture & CO2 interference

The Coupling of LC and FTIR

Over the past years, the coupling of liquid chromatography (LC) and Fourier-transform infrared spectrometry (FT-IR) has been pursued by means of different types of interfaces

→ achieve specific detection/identification of sample constituents

INTERFACES

Flow cell (on-line approach)

The column effluent pass directly through a flow cell and the IR absorption is continuously recorded.

Features

- Solvents suited for LC have many absorption bands in the IR region
- The path length of the flow cell has to be limited
- Low LC flow rates are allowed

Solvent removal (semi on-line approach)

Eluent is eliminated by the interface; compounds are deposited on a substrate prior to the collection of IR spectral data.

Features

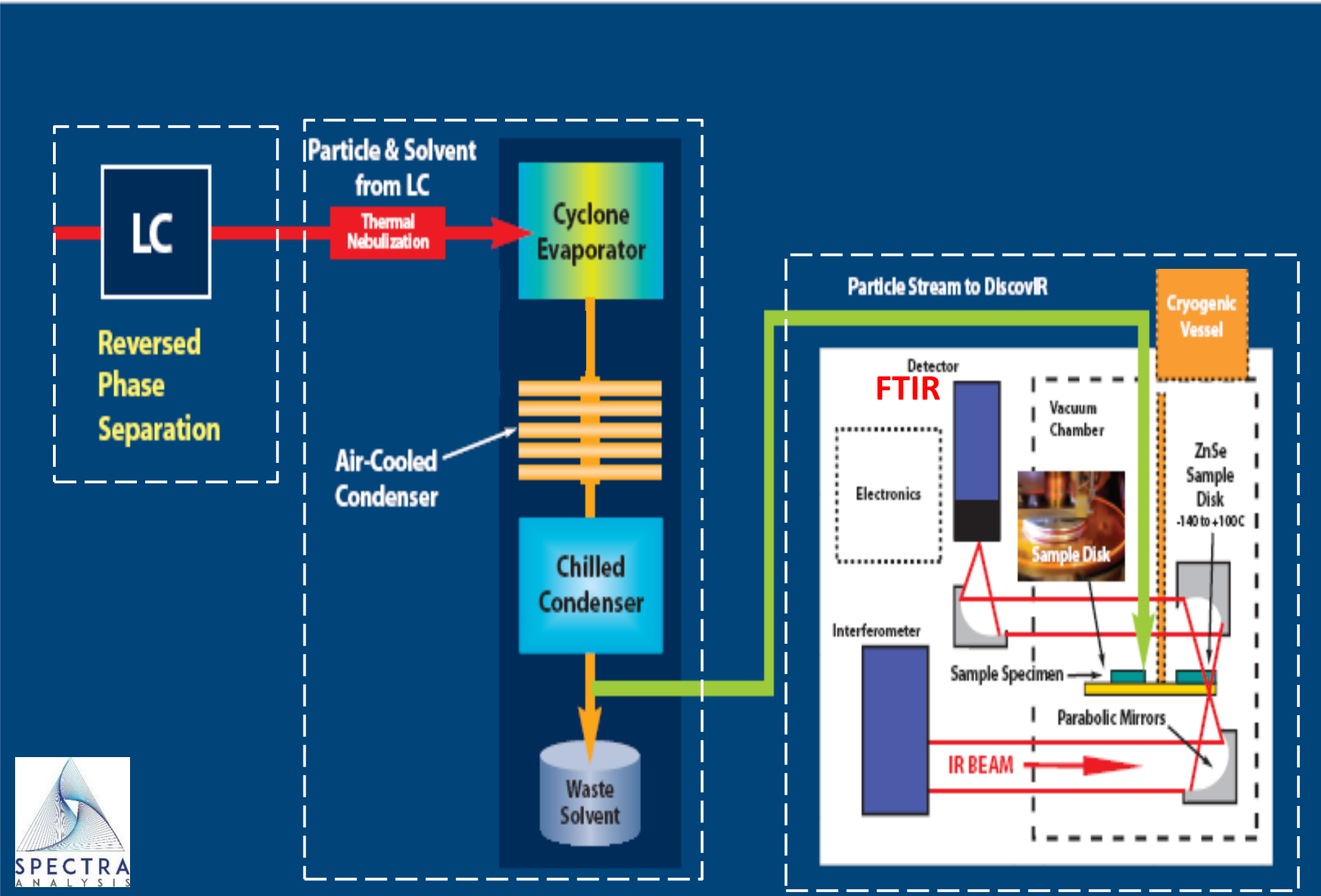
- IR spectra can be recorded independently from the LC conditions
- Concentrated deposits may be obtained
- Analyte spots can be analyzed repeatedly

DiscovIR-LC ®

Step 1	Step 2	Step 3
Separation (chromatography) →	De-solvation Detection Hyphen & DiscovIR (PATENT: US 8695813 B2)	→ Identification by Proprietary Software

- Fully Automated Operation
- Real-Time Chromatography & Spectral Data
- Solid Phase Transmission IR Spectra
- All LC Solvents: water, AcN, methanol, THF, chloroform.
- HPLC: Isocratic or Gradient; NP; LC.
- GPC/SEC: TCB at high temp.

DiscovIR-LC

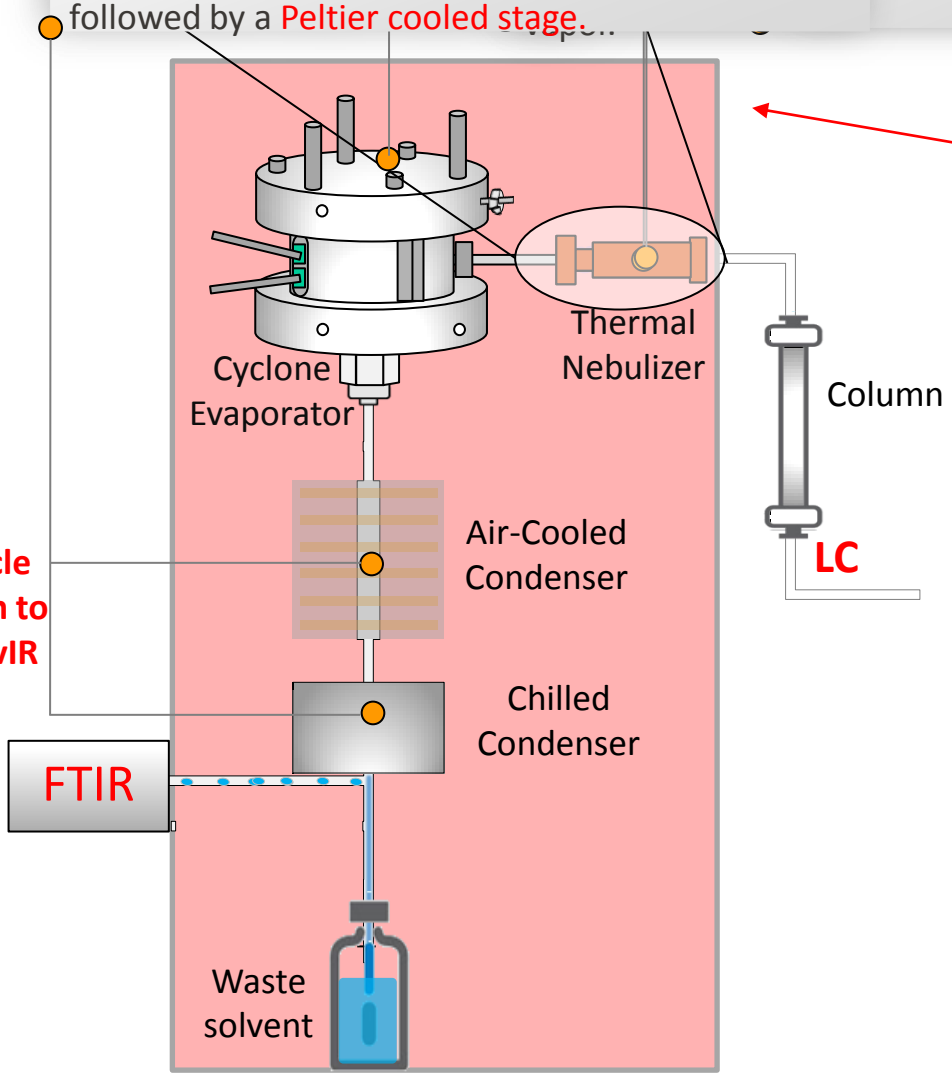

LC
Reversed Phase Separation
Particle & Solvent from LC
Thermal Nebulization
Cyclone Evaporator
Air-Cooled Condenser
Chilled Condenser
Waste Solvent
Particle Stream to DiscovIR
Cryogenic Vessel
Detector
FTIR
Vacuum Chamber
Electronics
ZnSe Sample Disk
-140 to +100C
Sample Disk
Interferometer
Sample Specimen
Parabolic Mirrors
IR BEAM

holds the droplets of
e wall.
goes to dryness
ne-to-surface ratio
that it is dragged
he exiting solvent

n conversion
bulizer back
h-speed jet
id droplets

Dual-stage Condenser: After ejection from the cyclone, the solvent vapor is removed by diffusion to, and condensation on, the cooled condenser walls.

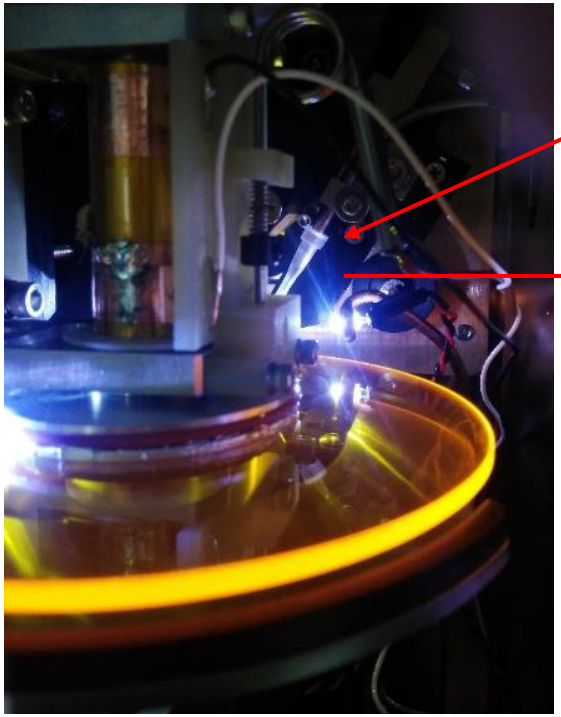
The condenser consists of an air cooled stage followed by a Peltier cooled stage.



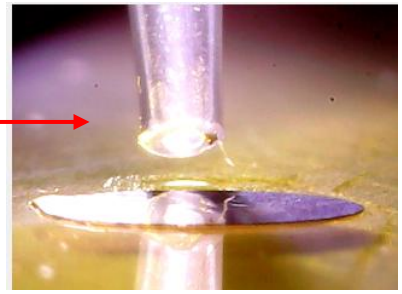
Desolvation process

Hyphen Interface

Solute-deposition Disk



deposition tip



- Re-usable after solvent cleaning
- Transmission IR on the solid deposit
- Mid-IR transparent 4000–700 cm^{-1}
- Flow rates: 0.25 to 1.5 mL/min
- Sensitivity: ≈µg, component-dependent
- Tunable speed 1-10 mm/min
- Disk Temp: - 10 to 100 ° C

De-solvation Interface Parameters

- Thermal Nebulizer Voltage

Under LC gradients it should be <4 W than ½ the energy required for evaporation of the highest boiling point solvent (16 Watt for water/MeOH)

- Cyclone Temperature

The cavity inner surface should be >100 ° C the boiling point of the fluid stream to cause the droplets to “film boil” (150-200 ° C for water/MeOH gradients)

- Condenser Temperature

The Peltier second stage should be near the temperature of the lowest freezing point solvent components (3-7 ° C for water/MeOH gradients)

- Potential market and Spectra positioning (industrial sectors, conference presentations, scientific activities, R%D with Professor Luigi Mondello from the University of Messina).

- What makes DiscovIR solid-deposition FTIR technology a unique identification tool?

- Which benefits are to be gained by the online hyphenation GC(LC)-MS-FTIR (parallel detection)?

- Presentation of the FFNSC GC-MS-FTIR Library (flavour and fragrance natural and synthetic compounds) based on GC-EI MS with Linear Retention Indices (LRI) and solid state FTIR spectra.

- The “quantitative” issue.

- DiscovIR: how does it work?

- GC-FTIR and LC-FTIR Application fields.

GC-FTIR Application Fields

- Agricultural/Food
  - Identification of Fatty Acids Metyhyl Esters (FAMEs)
  - Identification of Positional Isomers (Flavour&Fragrance)

- Forensic Drug Chemistry (Drugs of abuse)
  - Amphetamine Analysis
  - N-BOMB, Solaris, Smiles, Wizard
  - N-BOMB Isomers
  - BATH SALTS ANALYSIS
  - FENTANYL ANALYSIS
  - FLUOROMETHCATHINONE
  - SYNTHETIC CANNABINOIDS JWH-018
  - SYNTHETIC CANNABINOIDS JWH-250 Isomers
  - SYNTHETIC CANNABINOIDS JWH-203
  - SYNTHETIC CANNABINOIDS Phenylethylamines

- Explosives
  - Identification of explosive components and isomers
  - Chemical Weapons (OPCW) test mixture

LC-FTIR Application Fields

- LC-FTIR General
- Agricultural/Food
  - Beverages, Flavor
  - 1% mixture components
  - Analysis of a Real Sample
- Pharmaceutical
  - Polymeric Excipients in Drug Delivery
  - Oxidative Degradation of PEG
- Chemicals
  - Polymer blends
  - Copolymer Compositional Drift Measurement
  - Characterization of Hot-Melt Adhesive

Conference Presentations (1/3)

1. **42nd International Symposium on Capillary Chromatography and 15th GC × GC Symposium (ISCC&GC × GC 2018)**, Riva del Garda, Italy, 13-18 May 2018
Utczás M. , Trovato E., Alibrando F., Vento F. , L. Mondello
Gas chromatography coupled with condensed phase FTIR: a novel and reliable technique for flavor and fragrance analysis
Oral Communication

2. **42nd International Symposium on Capillary Chromatography and 15th GC × GC Symposium (ISCC&GC × GC 2018)**, Riva del Garda, Italy, 13-18 May 2018
Utczás M., Carson W.W., Mondello L.
Univocal identification of flavor and fragrance compounds using GC-MS (TOF)/condensed phase FTIR with an extensive library
Poster Communication

3. **49th International Symposium on Essential Oils (ISEO)**, Niš, Serbia, 13-16 September 2018
Utczás M., Trovato E., Alibrando F., Vento F., Mondello L.
Novel analytical tool for a univocal flavor and fragrance identification: Gas chromatography coupled with condensed phase FTIR and TOF mass spectrometry
Poster Communication

4. **37th Informal Meeting on Mass Spectrometry,** Fiera di Primiero (TN), Italy, 5-8 May 2019
Utczás M., Trovato E., Mondello L.
Application of a Novel Analytical Tool for Flavor and Fragrance Analysis: Gas Chromatography Coupled with Condensed Phase FTIR
Oral communication

Conference Presentations (2/3)

5. 48th International Symposium on High-Performance Liquid Phase Separations and Related Techniques - HPLC 2019, Milan, Italy, 16-20 June 2019
Salerno T., Donato P., Mondello L.
Solute-deposition LC-FTIR of Furocoumarin Isomers
Oral communication

6. 57th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Birmingham, UK, 2-6 September 2019
Frison G., Zamengo L., Tedeschi G., Trovato E., Salerno T., Mondello L.
Differentiation of the isomers isomephedrone and mephedrone by GC-MS after 2,2,2-trichloroethyl chloroformate derivatization, LC-HRAM Orbitrap™ MS, and GC-FTIR
Poster communication

7. 57th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Birmingham, UK, 2-6 September 2019
Salerno T., Donato P., Frison G., Utczás M., Trovato E., Mondello L.
Solute-deposition GC-FTIR and LC-FTIR as highly discriminating technologies for forensic applications
Poster communication

8. 57th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Birmingham, UK, 2-6 September 2019
Salerno T., Donato P., Frison G., Mondello L.
Solid-deposition GC-FTIR for Reliable Identification of NPS on Seized Drugs and Human Urine Samples
Oral communication

Conference Presentations (3/3)

9. **57th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT)**, Birmingham, UK, 2-6 September 2019
Zamengo L., Frison G., Bettin C., Zancanaro F., Trovato E., Mondello L.
Characterization of 4-chloroethcathinone (4-CEC) by GC-MS, GC-MS after 2,2,2-trichloroethyl chloroformate derivatization, LC-HRAM OrbitrapTM MS, and Solid Deposition GC-FTIR
Poster Communication

10. **XXVIII Congress of the Analytical Chemistry Division,** Bari, Italy, 22-26 September 2019
Salerno T., Donato P., Mondello L.
Potential of on-line LC-FTIR hyphenated tecnique as a reliable tool for identification
Oral communication

11. **Incontri di scienze delle separazioni,** Napoli, Italy, 28-29 Novembre 2019
Salerno T., Donato P., Mondello L.
Identification of New Psychoactive Substances in Seized Materials by Means of GC-FTIR Technique
(Abstract submitted for oral communication)

12. **Incontri di scienza delle separazioni**, Napoli, Italy, 28-29 November 2019
Salerno T., Donato P., Mondello L.
The Direct Linkage of LC and Solid Phase IR Spectroscopy: Filling the Gap in Identification of Unknowns
(Abstract submitted for poster communication)

Upcoming Conferences

Gulfood 2020, Dubai, United Arab Emirates, 15-20 February 2020

Pittcon 2020 Conference&Expo, Chicago, Illinois (US), March 1-5 2020

Analytica 2020: 27th International Trade Fair for Laboratory Technology, Analysis, Biotechnology and Analytical Conference, Munich, Germany, 31 March-3 April 2020

44nd International Symposium on Capillary Chromatography and 17th GC × GC Symposium (ISCC&GC × GC 2018), Riva del Garda, Italy, 24-29 May 2018

50th International Symposium on High-Performance Liquid Phase Separations and Related Techniques - HPLC 2020, San Diego, CA (US), 20-25 June 2020

2nd Global Congress on Chemistry and Catalysis (CGC-2020), Osaka, Japan, June 22-23 2020 (Mass Spectroscopy & Chromatography, Forensic & Clinical Chemistry)

58th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Cape Town, South Africa, 1-6 September 2020