TECNICHE CROMATOGRAFICHE ACCOPPIATE ALLA SPETTROMETRIA DI MASSA

Sostanze Psicoattive

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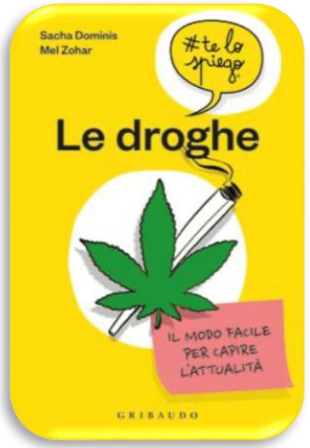


Che cosa è la droga?

Le droghe sono sostanze chimiche o derivate da piante che possono causare alterazioni fisiche, mentali ed emotive.

Sostanze che modificano sensazioni e comportamento

Droga: pianta o parte di pianta impiegata dopo essere stata opportunamente trattata.

Nel linguaggio corrente viene chiamata d. qualsiasi sostanza capace di modificare temporaneamente lo stato di coscienza o comunque lo stato psichico dell'individuo; rientrano pertanto fra le d. gli stupefacenti, gli allucinogeni, i barbiturici e gli altri psicostimolanti, nonché alcune sostanze atte ad aumentare le energie e il rendimento fisico, soprattutto nelle competizioni sportive. In tale quadro il termine d. è molto generico poiché comprende sostanze con proprietà diversissime e spesso indica in senso restrittivo composti esclusi per legge dal prontuario farmaceutico quali l'eroina, la cocaina, l'LSD. Solitamente si fa distinzione tra *d. leggere* e *d. pesanti* (oppure, per traduzione dell'ingl. hard e soft, tra d. dure e d. morbide), distinzione fondata soprattutto sulla considerazione dei danni che le varie d. possono produrre sull'organismo di chi ne fa uso, e sulla condizione di dipendenza che esse tendono a indurre.

PIACERI CHE DIVENTANO UNA SCHIAVITÙ

"Sono da considerarsi sostanze stupefacenti tutte quelle sostanze di origine naturale o sintetica che, agendo sul sistema nervoso centrale, provocano stati di dipendenza fisica e/o psichica, dando luogo a stati di tolleranza (bisogno di aumentare le dosi con l'aumento dell'abuso) ed in alcuni casi di dipendenza a doppio filo e cioè dipendenza dello stesso da più droghe"

World Health Organization

Classificazione

Criterio di preparazione

Criterio sintomatologico

Criterio legale

Testo Unico DPR 309/90 e successive *modificazioni*

aggiornato, da ultimo, con le modifiche apportate dal**D.M. 13 febbraio 2023**, a decorrere dal 10 marzo 2023.

Testo unico delle leggi in materia di disciplina degli stupefacenti e sostanze psicotrope, prevenzione, cura e riabilitazione dei relativi stati di tossicodipendenza

Tabella I

Oppio e derivati oppiacei (morfina, eroina, metadone ecc.)

Foglie di Coca e derivati

Amfetamina e derivati amfetaminici (ecstasy e designer drugs)

Allucinogeni (dietilammide dell'acido lisergico - LSD, mescalina, psilocibina, fenciclidina, ketamina , ecc.)

Tabella II
Cannabis

Tabella III
Barbiturici

Tabella IV
Benzodiazepine

Tabella dei medicinali

Nella Tabella dei medicinali sono inserite le sostanze attive che hanno attività farmacologica e pertanto sono usate in terapia e le relative preparazioni farmaceutiche. La tabella è suddivisa in cinque sezioni indicate con le lettere A, B, C, D ed E dove sono distribuiti i medicinali in relazione al decrescere del loro potenziale di abuso, nelle tabelle è anche indicato il regime di dispensazione.

Medicinali a base di morfina e sostanze analgesiche oppiacee

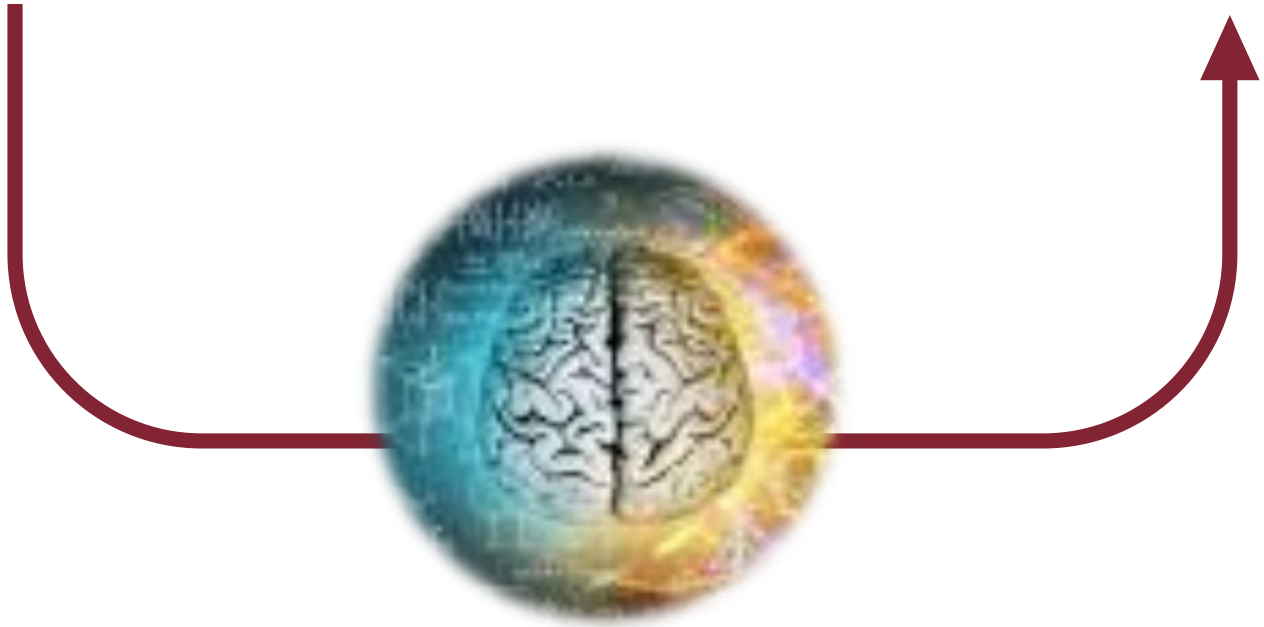
Medicinali di origine vegetale a base di Cannabis

Barbiturici

Benzodiazepine (diazepam, flunitrazepam, lorazepam ecc.)

Metabolismo delle sostanze psicoattive

Sostanza —Enzimi→ PRODOTTI **Metaboliti**

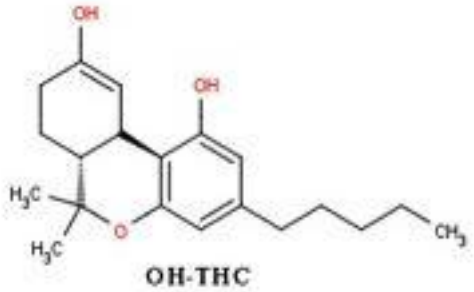


i prodotti del processo di trasformazione che subisce la sostanza inserita nel nostro sistema e che ha la funzione di renderla più facilmente assorbibile o eliminabile.

Il THC viene rapidamente metabolizzato nel fegato.

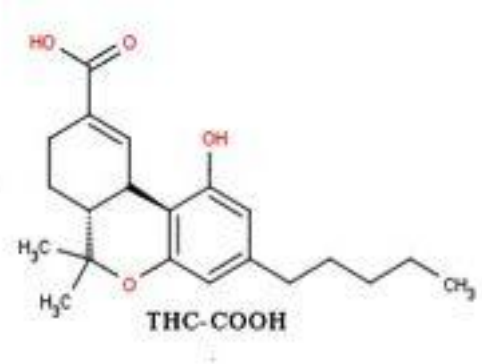
Cit-P450

11-idrossi tetraidrocannabinolo (OH-THC):
principale metabolita psicoattivo in grado di superare rapidamente la barriera encefalica



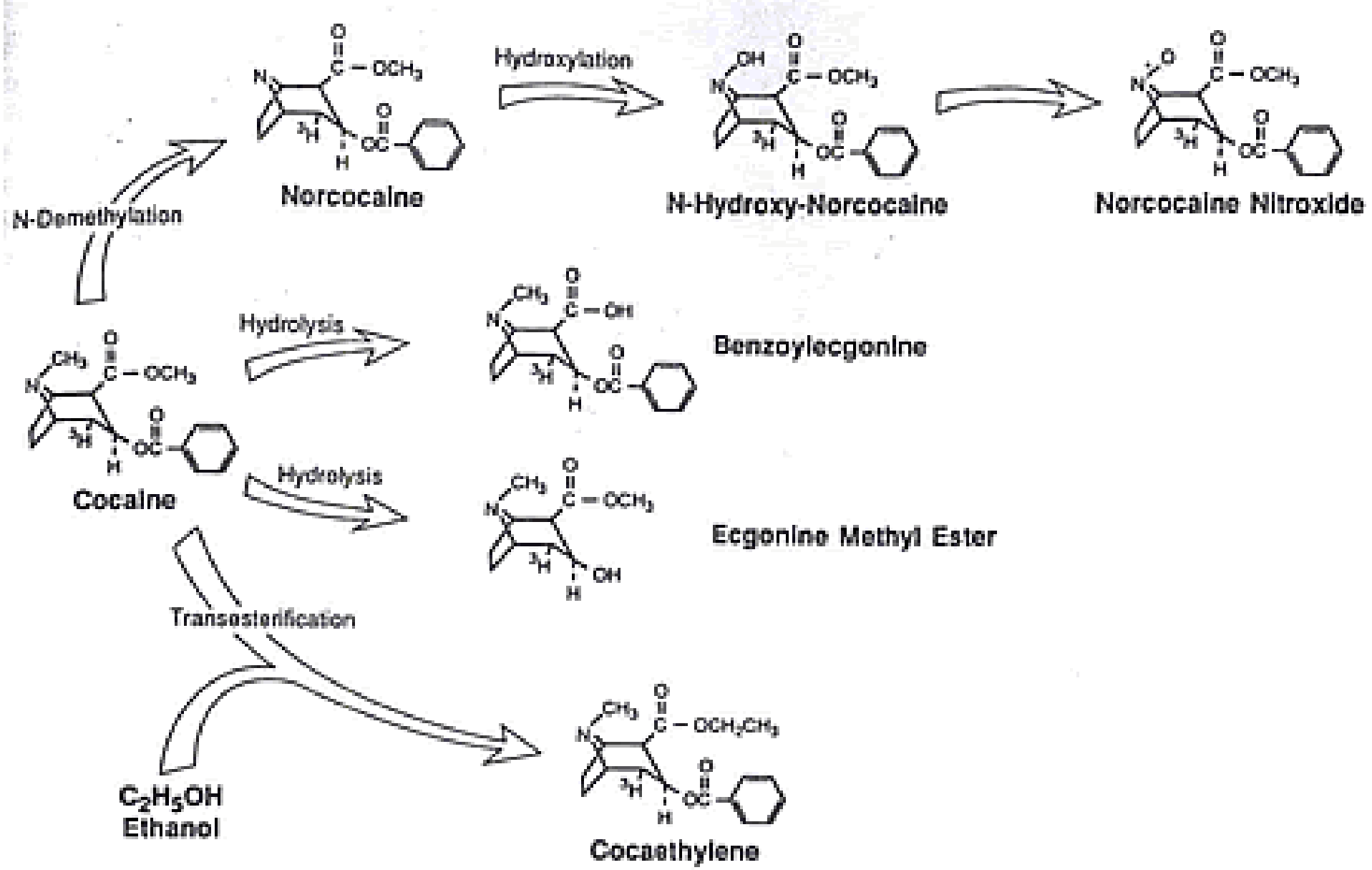
OH-THC

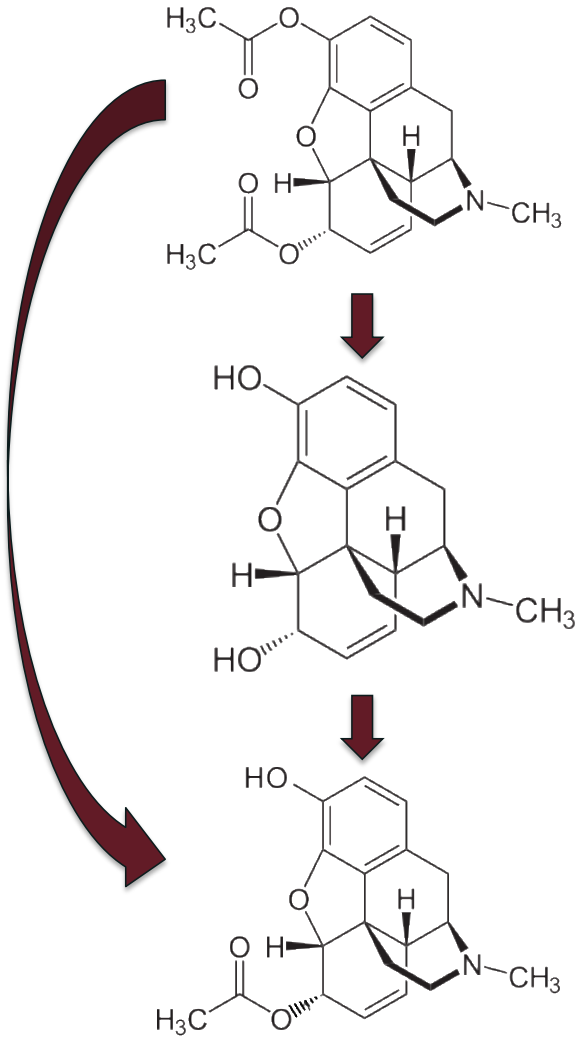
Deidrogenasi



THC-COOH

11-nor-9-carbossi-tetraidrocannabinolo (THC-COOH):
prodotto di ossidazione del OH-THC


Norcocaine
Hydroxylation
N-Hydroxy-Norcocaine
Norcocaine Nitroxide
N-Demethylation
Hydrolysis
Benzoylecgonine
Cocaine
Hydrolysis
Ecgonine Methyl Ester
Transesterification
C_2H_5OH
Ethanol
Cocaethylene



Diacetilmorfina
C21H23N05: 370.16489

Morfina

C17H19NO3: 286.14377
F1: 229.08580
F2:201.09019

6-Monoacetilmorfina (6-MAM)

C19H21NO4: 328.15433
F1: 211.07512
F2: 268.132970

Metaboliti

Biomarkers di assunzione

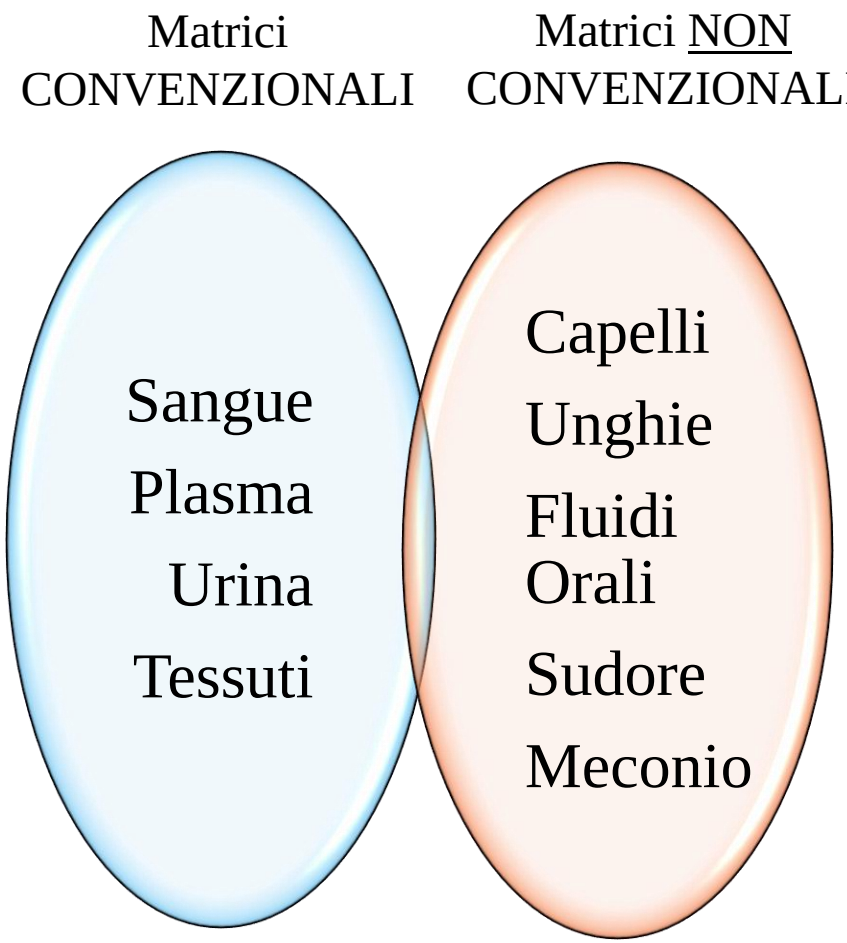
Tutte quelle sostanze che possono essere considerate analiti target nel metodo e che consentono di discriminare tra l'assunzione e il contatto passivo con la molecola parent

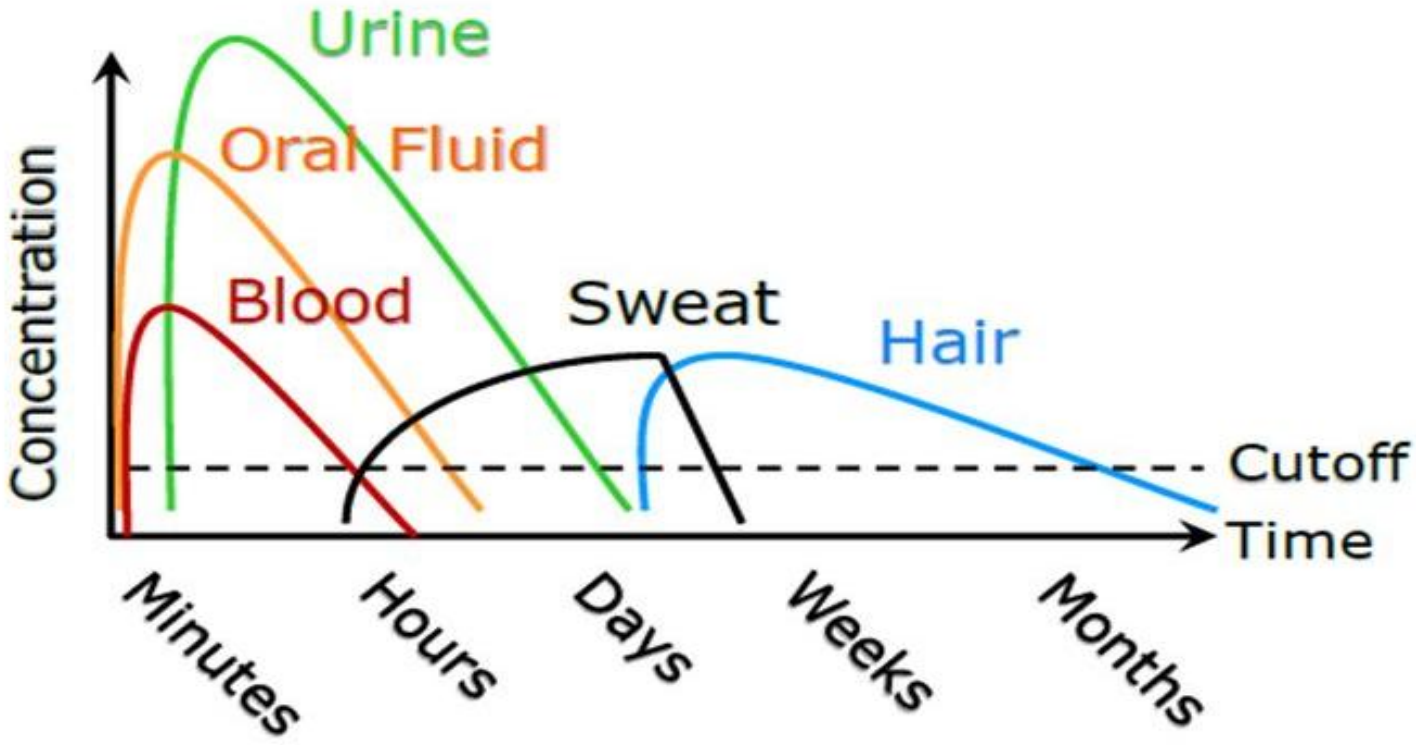
urina
feci
sudore
lacrime

Capelli
Sangue
Saliva

Come si può stabilire se il soggetto ha assunto sostanze psicoattive?

Matrici biologiche





Finestra di rivelabilità + Facilità del campionamento + stabilità

VALORE SOGLIA

Limite di concentrazione definito per stabilire se un campione è positivo o negativo

Procedura di controllo

Fermo

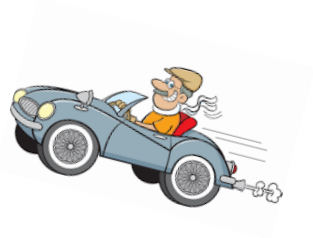
Autorità competenti FFO

Prelievo del campione da parte dell'operatore

Può essere effettuato direttamente dall'operatore

Test di screening

Valutazione di eventuale alterazione psicofisica da parte del personale sanitario



Test di screening **NEGATIVO**

Test di screening **POSITIVO**

Test di conferma

Deve essere effettuato da personale qualificato e presso laboratori certificati

Test di SCREENING

- **Servono per evidenziare e isolare i campioni positivi nel minor tempo possibile.**
- Metodi rapidi, semplice e applicabili anche da personale che non possiede conoscenze specifiche.
- Devono essere in grado di limitare l'errore, e soprattutto il numero di falsi negativi.

Generalmente si basano su test IMMUNOCHIMICI

Rispondono in base all'interazione antigene anticorpo

PRO

- Rapidi
- Semplici
- Economici

CONTRO

- SOLO Qualitativi
- n. di sostanza analizzabili LIMITATE
- Identifica CLASSI di sostanze
- Falsi positivi e falsi negativi
- Ha valenza esclusivamente clinica

- **Caratteristiche che prescindono dal tipo di matrice**

Test di CONFERMA

- **Generalmente condotti con tecniche cromatografiche accoppiate alla rivelazione condotta in spettrometria di massa**

- Sono analisi SENSIBILI e SPECIFICHE – Si ricercano i singoli analiti e non colo le classi di appartenenza
- I test di conferma hanno valenza amministrativa e medicolegale
- Risposta QUALITATIVA e QUANTITATIVA

Con la spettrometria di massa si riesce ad ottenere la cosiddetta *impronta digitale* della sostanza anche a concentrazioni molto basse, consentendo un risultato legalmente valido ed inequivocabile

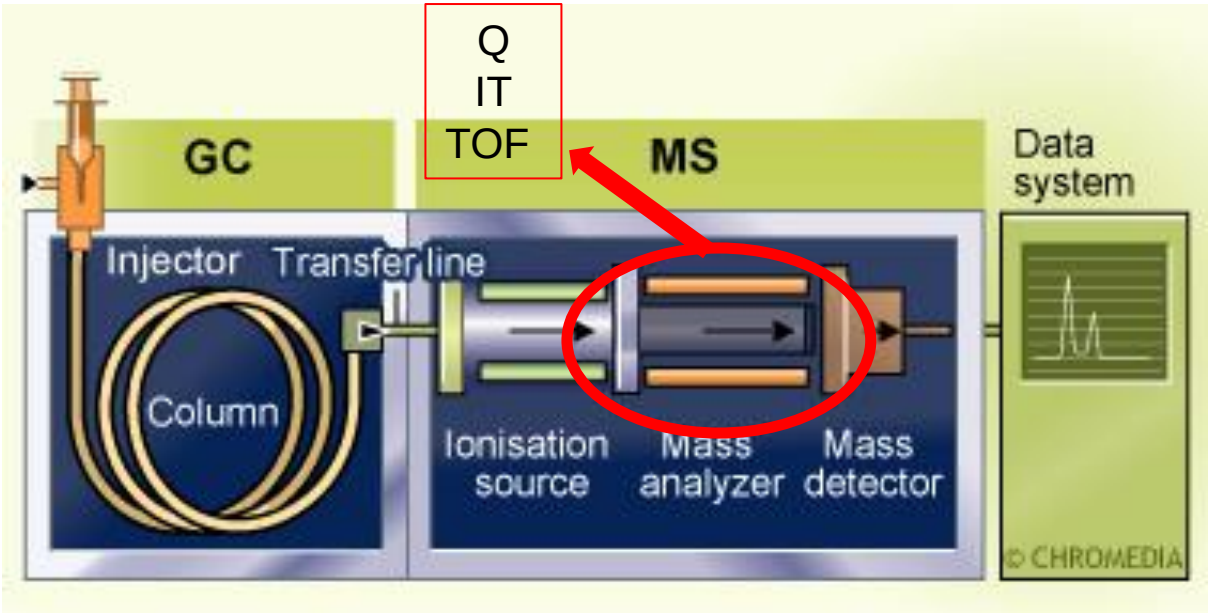
Ogni molecola reagisce in maniera differente e caratteristica in spettrometria di massa. Ciò si esprime sia in modalità di ionizzazione sia nel risultato della sua frammentazione. Ogni molecola frammenta in modo peculiare, definendo il pattern di frammentazione caratteristico.

Conferma GC-MS

Modalità di acquisizione:

Full scan (FS)
Ampio range m/z
Scansione lunga
Bassa sensibilità

Selected Ion Monitoring (SIM)
Range di m/z ristretto (o singolo)
Scansione breve
Alta sensibilità



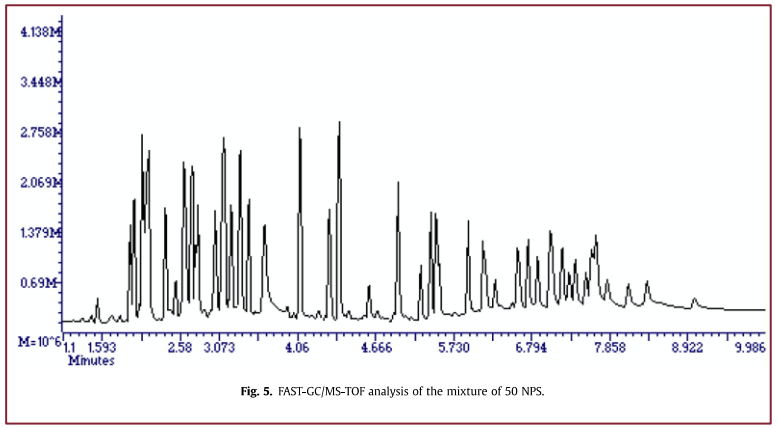
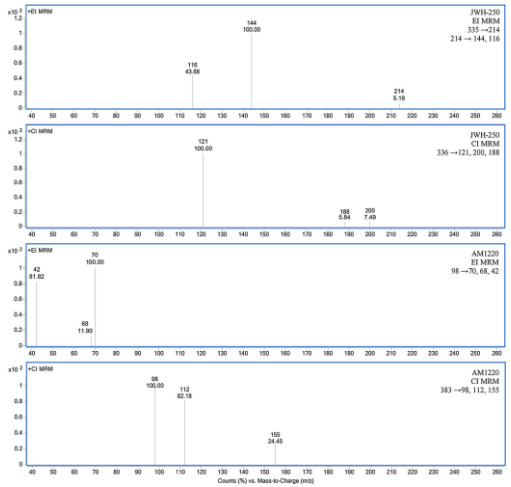


Fig. 5. FAST-GC/MS-TOF analysis of the mixture of 50 NPS.

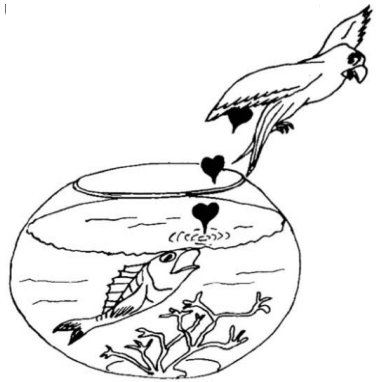


Identificazione:

- ✓ Tempo di ritenzione
- ✓ Spettro di massa
  - Picco BASE
  - Frammenti
  - Rapporto tra i frammenti

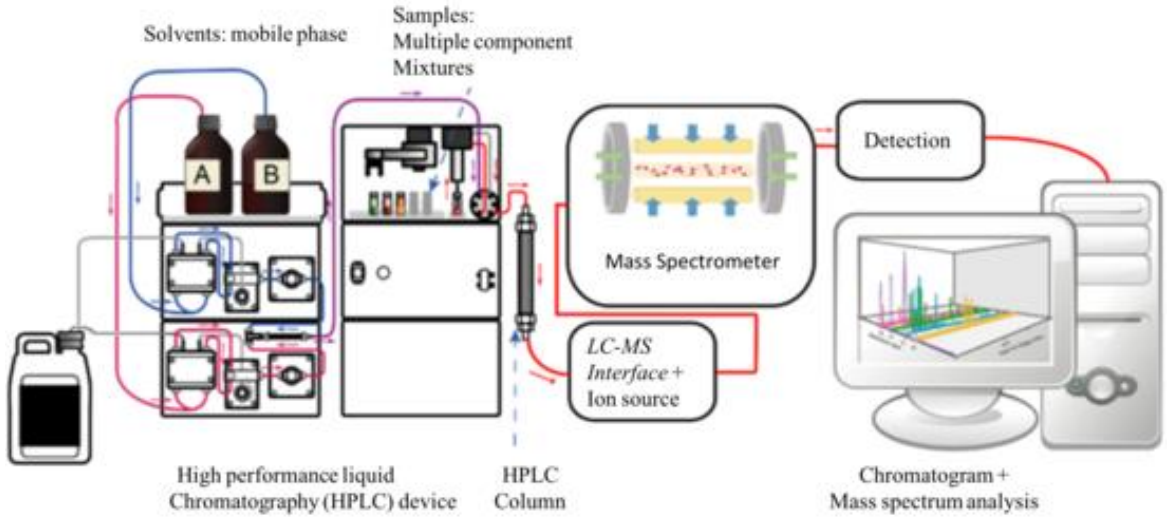
Standard **Banca Dati**

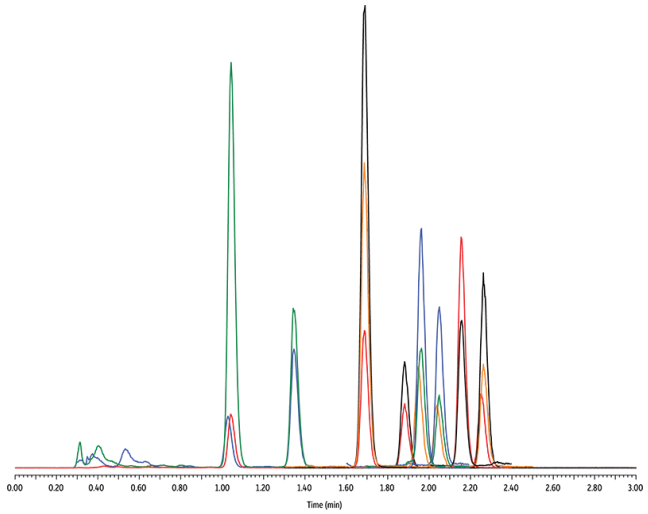
Conferma HPLC-MS(/MS)



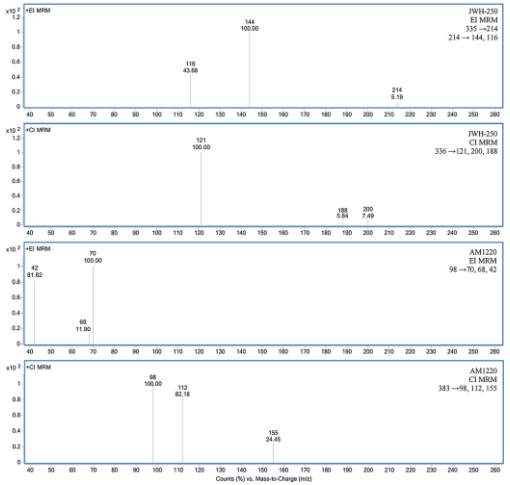
Identificazione:

- ✓ Tempo di ritenzione
- ✓ Spettro di massa
  - Transizione ione precursore/ione frammento
  - Rapporto tra le transizioni più intense





Standard **Banca Dati**



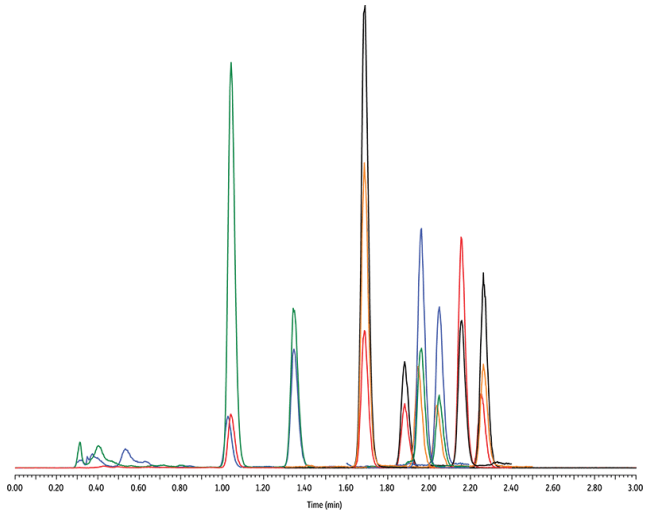
MS ***MS/MS*** ***MS^n***

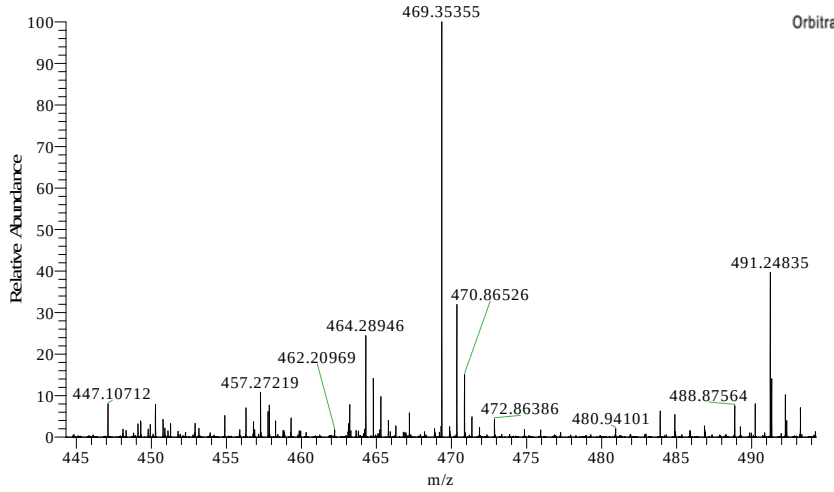
Targeted, Semi-Targeted, Suspect

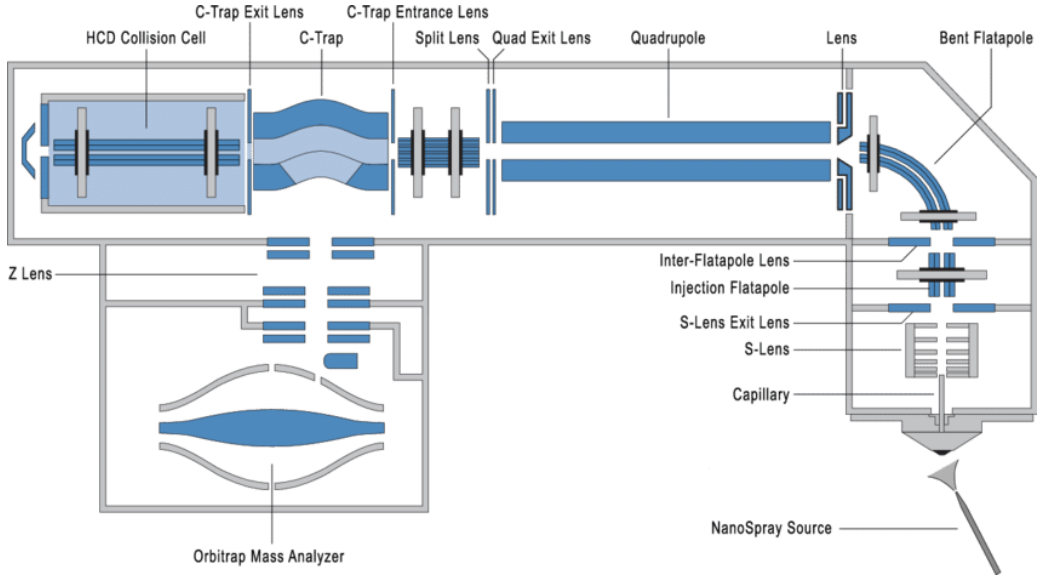
Conferma HPLC-HRMS(/MS)

Identificazione:

- ✓ Tempo di ritenzione
- ✓ Spettro di massa
  - Delta di Massa (massa esatta massa accurata)
  - Massa accurata dello ione parent
  - Massa accurata dei frammenti
  - Rapporto tra le intesintà dei frammenti







Modalità di Acquisizione:

- ✓ Full Scan
- ✓ HRMS/MS
- ✓ Data-Dependent Scan (FS-ddA)
- ✓ Selected Ion Monitoring

Targeted, Semi-Targeted, Suspect, Untargeted

Procedura analitica

Sub Campionamento

- Si preleva l'aliquota di campione necessaria a svolgere l'analisi,

Lavaggio o decontaminazione

- Procedura necessaria su campioni solidi in particolare i campioni cheratinici
- Rimuove le possibili contaminazioni esterne

Estrazione

- Gli analiti vengono estratti dalla matrice:
- Diluizione
- Estrazione con solvente
- Precipitazione proteica
- Tecniche combinate

Clean-up

- LLE
- SPE
- Tecniche miniaturizzate

Analisi

- GC–MS(/MS)
- LC–MS (/MS)
- HPLC-HRMS (/MS)

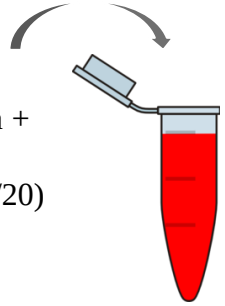
Qualche esempio di protocolli analitici

Drugs' extraction fron Blood

Protein precipitation

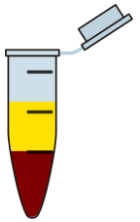
- Time reduction and automation
- Improve clean-up
- New trends: PP plates

250 µL of plasma + 750 µL of ANC/MeOH (80/20) storage at -20°



5' at -20°

15' at 4000 RPM



Supernatant

5' at 12000 RPM

100 µL supernatant + 100 µL H2O

UHPLC-HRMS/MS

Blood analysis

Sample preparation → Protein precipitation (PPT)

- BetaBasic18 column 150x2,1 mm with 5 µm particles
- Phases: - H2O 0,1% HCOOH
  - ACN 0,1% HCOOH

Liquid Chromatography

Dionex UltiMate 3000 Rapid Separation LC system Thermo Fisher Scientific equipped with 100 µL loop and degasser

Mass Spectrometry

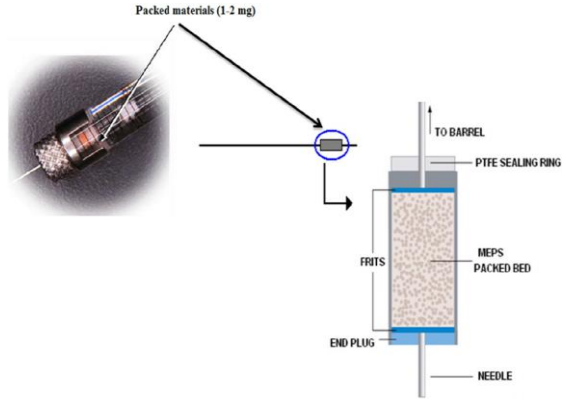
Q-Exactive-Orbitrap Thermo Fisher Scientific with H-ESI source →

H-ESI source operating condition:

- Spray Voltage 3.30 kV
- Capillary temperature 320 ° C
- H-ESI temperature 320 ° C
- Sheat gas (Azoto) 55 unità
- Auxiliary gas 20 unità

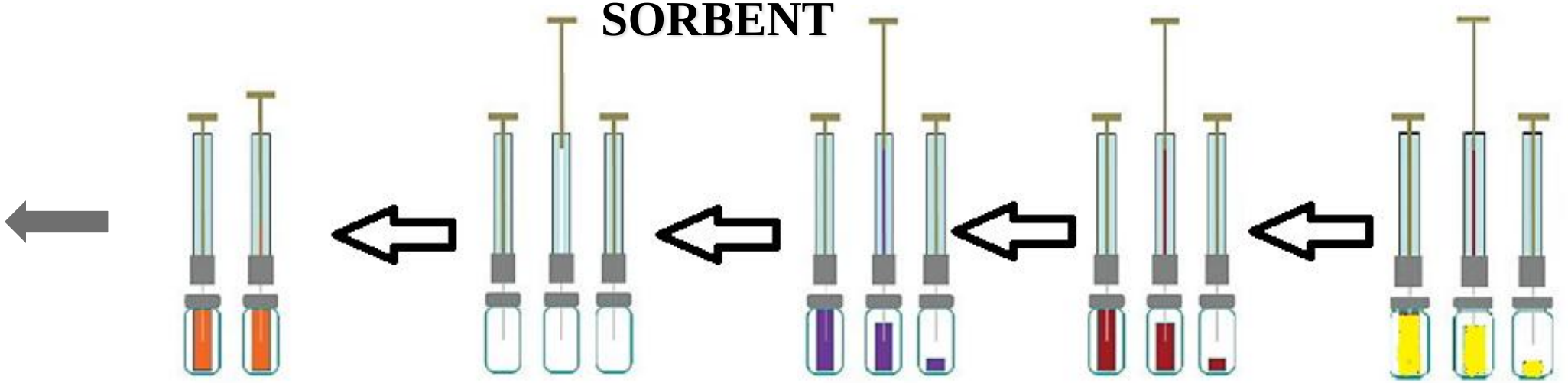
Drugs' extraction from Oral Fluid

MEPS



✓Few mg of stationary phase
✓Reduction of sample volume to 10-100 µL
✓Reduction of organic solvent needed

MICRO EXTRACTION BY PACKED SORBENT



HPLC-MS/MS

ELUITION: 100 µL of MeOH 10 mM HCOOH

WASHING: 250 µL of H_2O/MeOH 90/10 (v/v)

LOADING: Sample

CONDITIONING: 250 µL of H_2O/MEOH 75/25 (v/v)

ACTIVATION: 250 µL of MeOH

Oral Fluid analysis

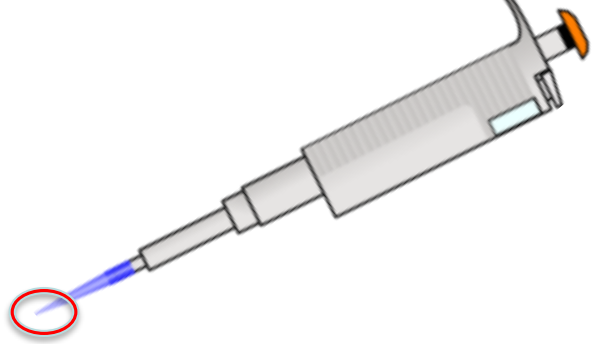
➢90 µL of OF mixed with 60 µL H2O, 50 µL of MeOH with ISs
➢Sonication (6’)
➢Centrifugation for 5’ at 10,000 × g

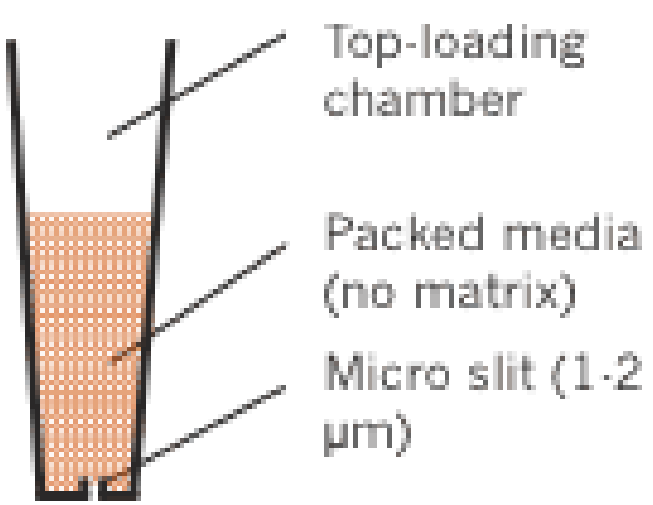
Chromatography: Micro-Lc Pump Perkin-Elmer serie 200; Col. Brownlee SPP packed with core-shell particles (2.7 µm, 2.1 x 150 mm); mobile phases: MeOH:AcN (80:20, v:v) 5mM HCOOH (A) and water 5 mM HCOOH (B);
Mass Spec: API 2000 AB-Sciex
ESI+,urboionspray, MRM
Total time 15 min

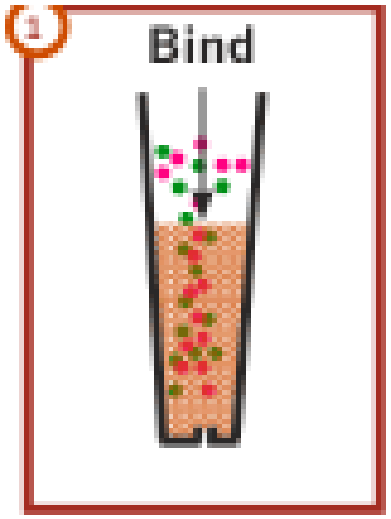
❖BIN activation	250 µl MeOH x 3 cycles
❖Conditioning	250 µl H_20:MeOH 90:10 x 2 cycles
❖Loading	5 aspire/dispense cycles
❖Washing	100 µl of H_20:MeOH (90:10)
❖Eluition	100 µl MeOH/HCOOH 10mM
❖Injection	6 µl in LC-MS/MS system.

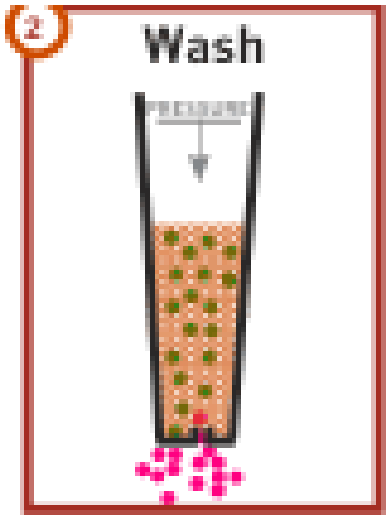
Drugs' extraction from Urine

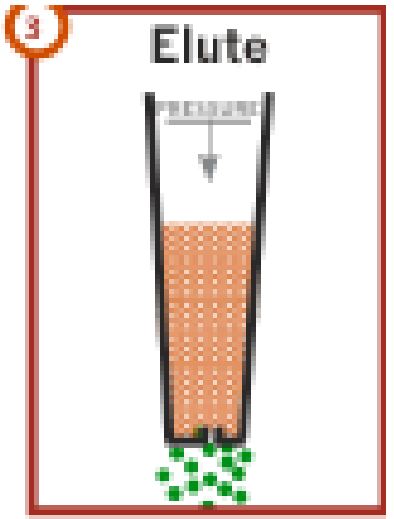
MEPS











Conditioning
100 µl H_20:MeOH (90:10)

100 µL urine + IS

100 µl H_20

100 µl MeOH
HCOOH 10mM

Urine analysis

HYDROLYSIS
enzimatic with β-Glucoronidase from E.Coli

HPLC-MS/MS
MRM- 5 window

Chromatography: Micro-Lc Pump Perkin-Elmer serie 200; Col. Brownlee SPP packed with core-she particles (2.7 µm, 2.1 x 150 mm); mobile phases: MeOH:can 5mM HCOOH (A) and water 5 mM HCOOH (B);
Mass Spec: API 2000 AB-Sciex
ESI+,urboionspray, MRM
Total time 15 min

CLEAN-UP
SPE

Drugs' extraction from hair

PLE

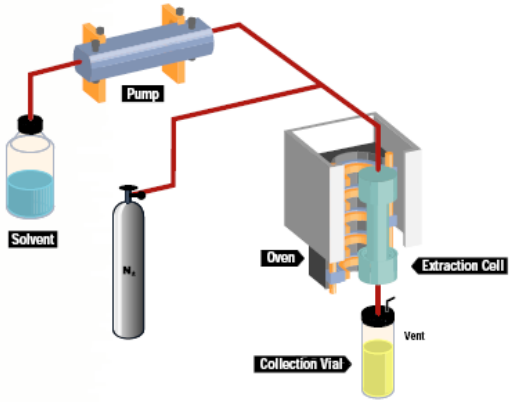
- T: 150° C
- P: 100 barr
- Extraction solvent: H_2O/2-Propanol 80/20 pH3,5
- Cycles: 1

9000 rpm
5 min

5 mL

Clean-up

UHPLC-HRMS/MS

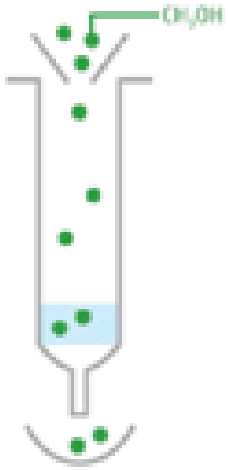


- Rapid extraction from sample with high temperature and pressure solvents

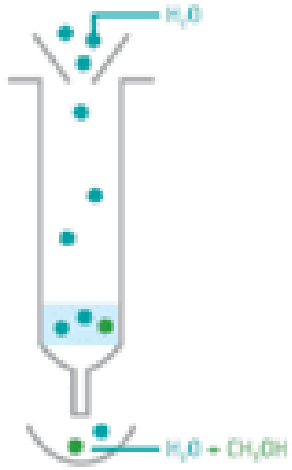
- Features:
  - ✓Reduced use of organic solvents: water is the main extractant solvent (PHWE)
  - ✓Different classes in one process
  - ✓Automated process- reprodicibility
  - ✓Reduced analysis time

Clean- up SPE

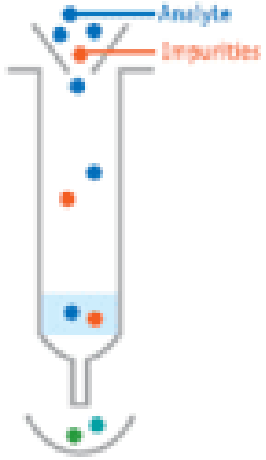
- Time reduction
- Improve selectivity
- Miniaturization, reduction of sample volume
- New trends: MISPE, SPME, MEPS, monolith spin extraction



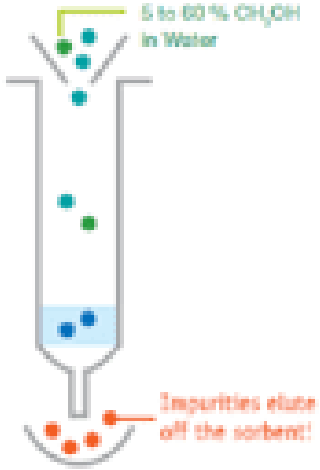
ACTIVATION: 1 mL of MeOH



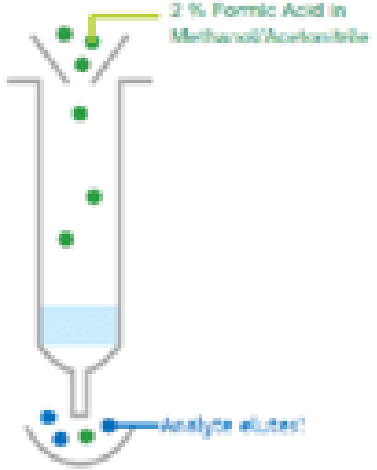
CONDITIONING: 1 mL of H_2O/MEOH 80/20 (v/v)



LOADING: Sample



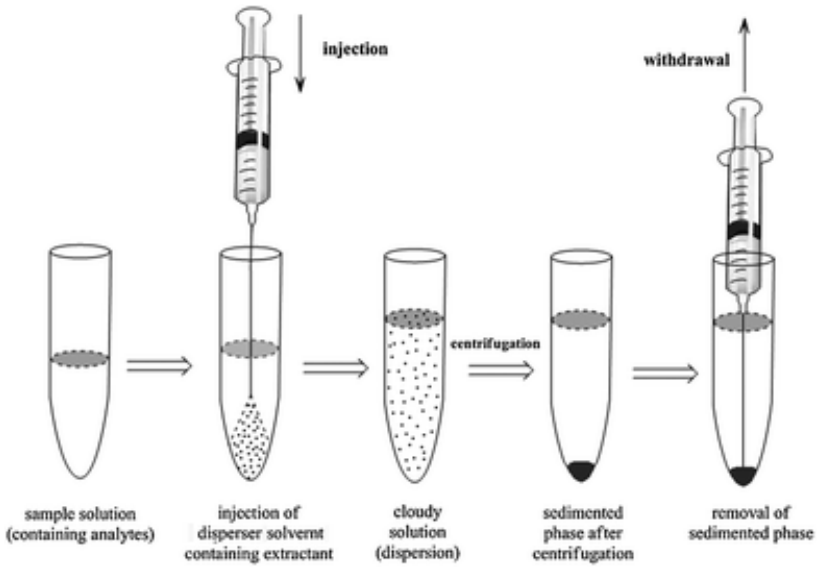
WASHING: 1 mL of H_2O



ELUITION: 500 µL of MeOH 10 mM HCOOH

UHPLC-HRMS/MS

Clean- up dLLME



- 5000 µL from the ASE extract
- 20 µL NaOH 1M
- 5000 µL H_2O milliQ
- 500 µL buffer $(HCO_3)^-/(CO_3)^{2-}$ pH 11
- 1,2 g NaCl

→ 500 µL 2-Propanol + 200 µL $CHCL_3$

Evaporate and reconstitute with 200 µL of MeOH/H_2O 50/50 (v/v)

→ **UHPLC-HRMS/MS**

Miniaturized Liquid-Liquid Extraction Technique

Exploits a ternary mixture of solvents
- Water Solvent
- Extraction Solvent
- Disperser Solvent

Rapid injection of a mixture of disperser solvent and extraction solvent

↓

Cloudy solution is formed

↓

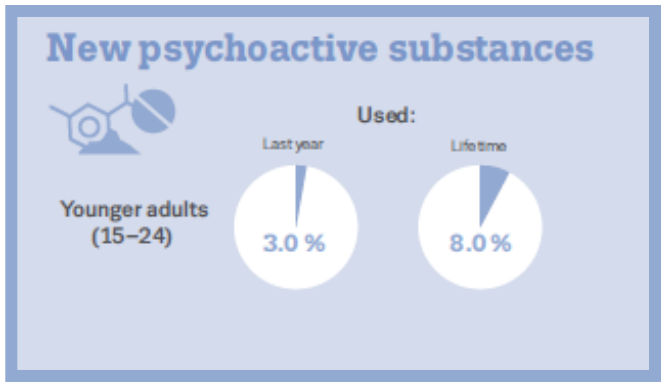
Centrifugation and sedimentation

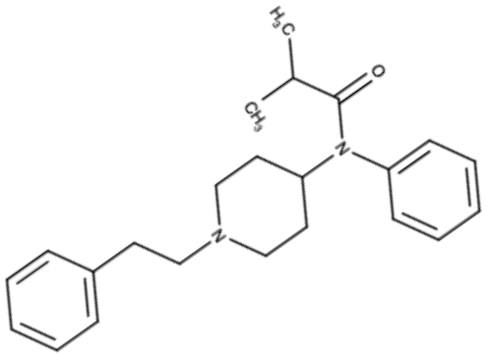
↓

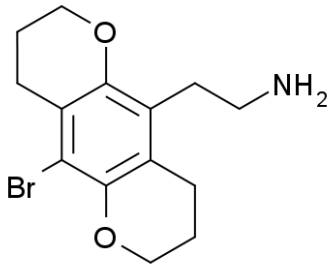
Store the organic phase containing the target analites

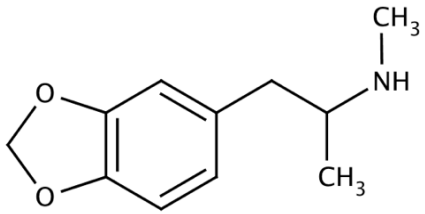
…Nuove Sostanze Psicoattive

New Psychoactive Substances - NPS









«Il termine Nuove Sostanze Psicoatttive (NPS) è definite da UNODC come “sostanze da abuso, sia in forma pura che come preparazione, che non rientra nella lista del Single Convention on Narcotic Drugs (1961) o nella Convention on Psychotropic Substances (1971), ma che rappresentano un rischio effettivo per la salute pubblica»

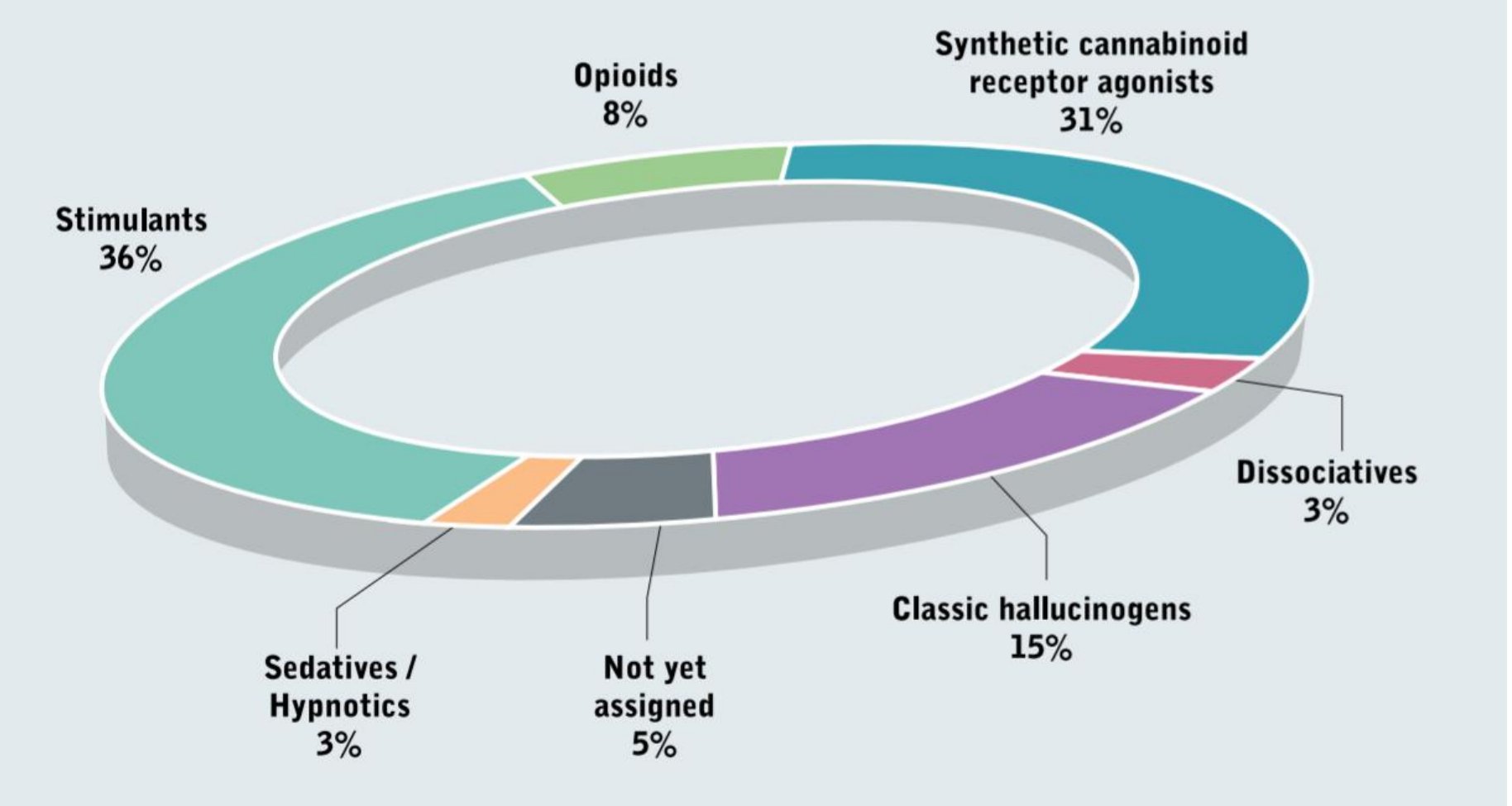
Situazione legale:

Sono legislate dalle normative dei singoli paesi → **DPR 309/90** → **Early warning system (EWS)**

Approccio generico: attenzionare gli analoghi e i precursori

950 NPS sono state riportate fino a Dicembre al sistema EWS

NPS - Classificazione

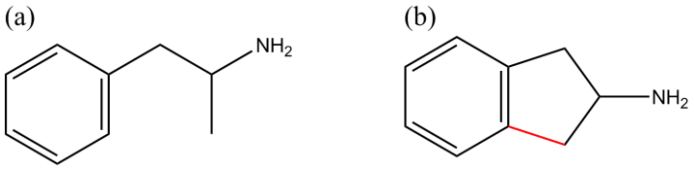


Classification fornita da United Nations Office on Drugs and Crime - UNODC

NPS - Classificazione

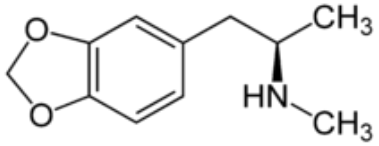
Classification fornita da United Nations Office on Drugs and Crime - UNODC

NPS - Classification



amphetamine (a) and 2-aminoindane (2-AI) (b)

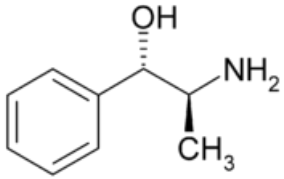
Theaminoindanes were reported to possess significant bronchodilating and analgesic properties. Potent effects on the release and re-uptake of serotonin were also observed. As a result of the latter these substances have been sold as NPS for their ability to produce empathogenic and entactogenic effects of serotonin releasing drugs, such as MDMA.

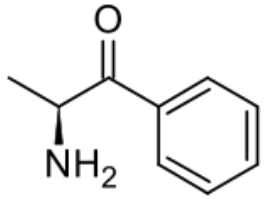


Katrom

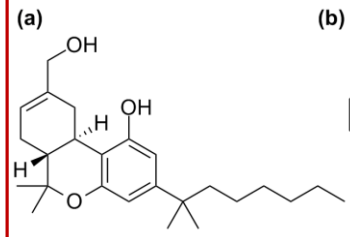
Kath

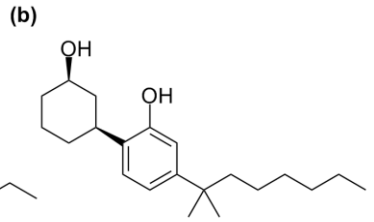
Internet surveys conducted by the EMCDDA in 2008 and 2011 revealed that kratom is one of the most widely offered NPS.

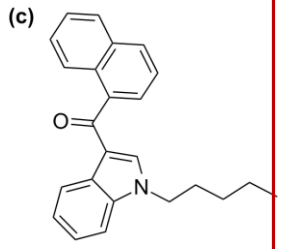




Since 2004, the composition of 'herbal highs' products seems to have substantially changed and noe include potent new psychoactive compounds known as synthetic cannabinoids.



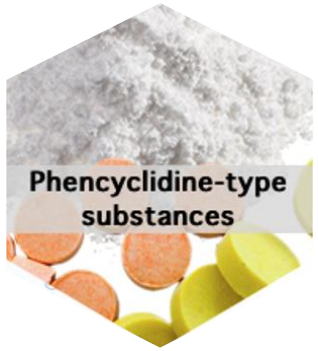




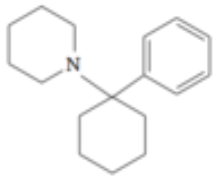
(a) HU-210; *(b) CP-47,497;* *(c) JWH-018*

While cannabis and THC are controlled under the international drug control treaties, none of the synthetic cannabinoids are currently under international control. However, several have been subject to control measures at the national level.

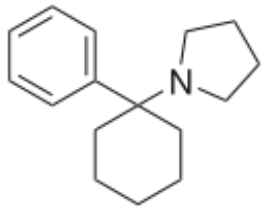
NPS - Classification



Phencyclidine-type substances show structural similarity to phencyclidine (PCP) and ketamine. Phencyclidine-type substances act as central nervous system stimulants, or dissociatives.

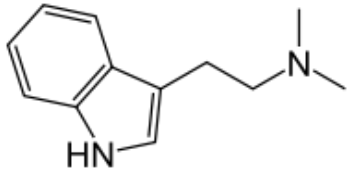


PCP

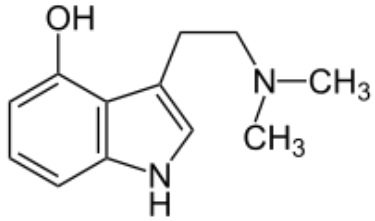


PHP

Tryptamine and its derivatives that have been reported as NPS are indolealkylamine molecules. Most are psychoactive hallucinogens found in plants, fungi and animals .

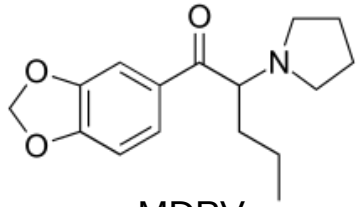


DMT

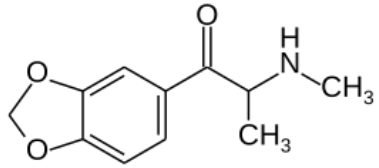


Psilocin

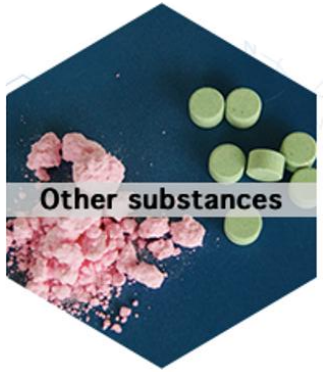
Synthetic cathinones are *β*-keto phenethylamines and chemically similar to amphetamine and methamphetamine.



MDPV

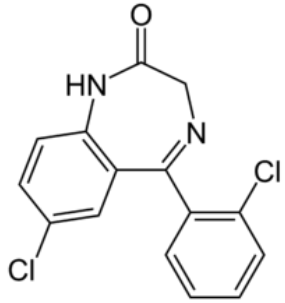


Methylone



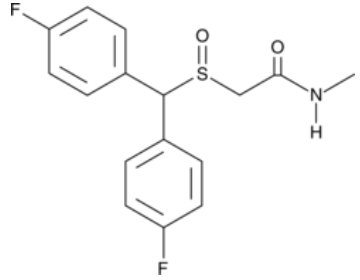
Sedatives / Hypnotics:

Delorazepam

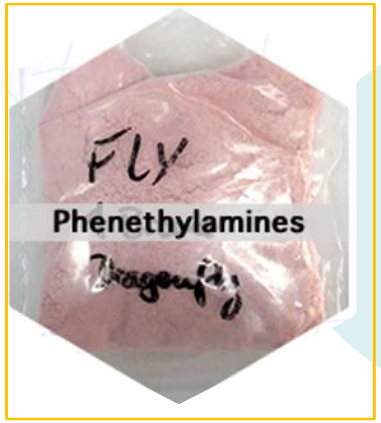


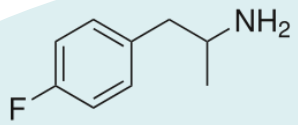
Stimulants

Modafiendz

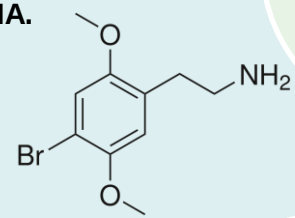


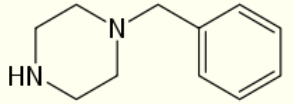
Fenetilammine Piperazine e Opioidi sintetici



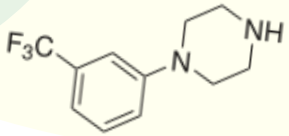


Phenethylamines refer to a class of substances with documented psychoactive and stimulant effects and include amphetamine, methamphetamine and MDMA.



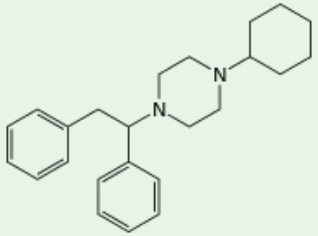


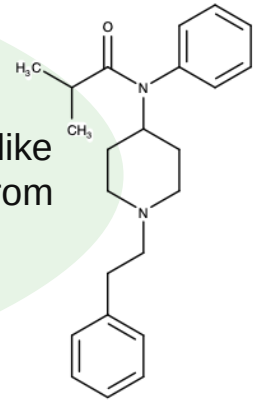
Piperazines have been described as 'failed pharmaceuticals', as some had been evaluated as potential therapeutic agents by pharmaceutical companies but never brought to the market.Most piperazines act as central nervous system stimulants. In some cases, they can also act as opioids.



Synthetic Opioids

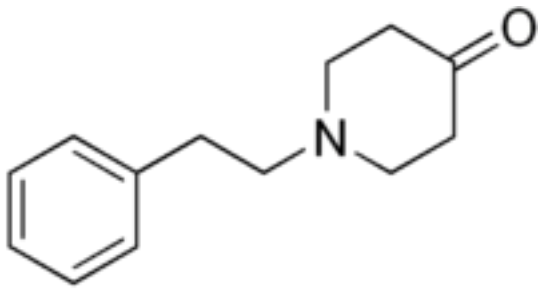
Synthetic opioids refer to a category of novel psychoactive substances (NPS) that are either known to be opiates or have opiate-like effects. These are not naturally occurring substances, although they have effects related to the naturally occurring drugs from several species of the opium poppy plant.



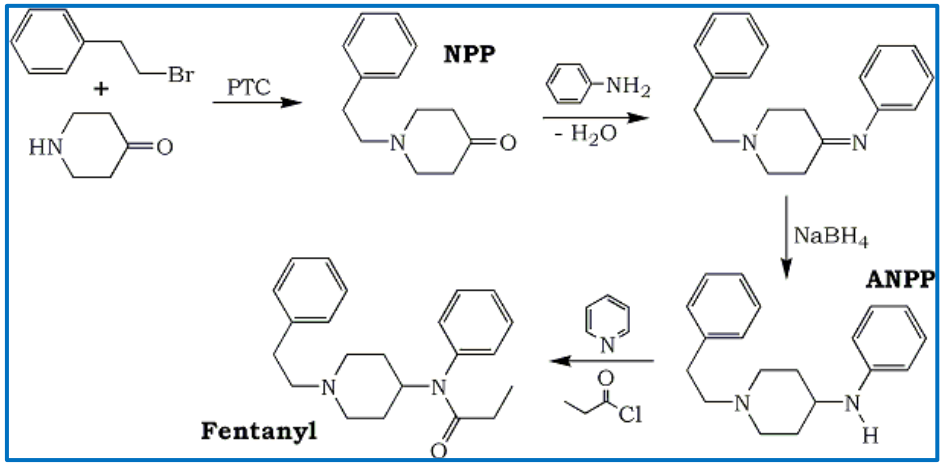


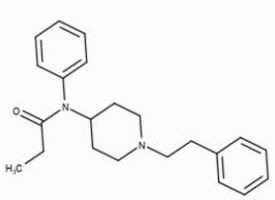
FENTANYL e I suoi derivati

Precursore

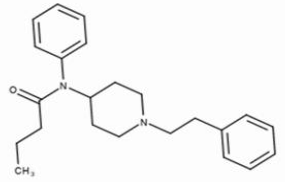


N-Phenethyl-4-piperidinone
(4-ANPP)

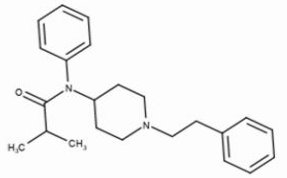




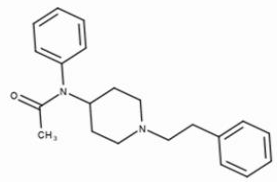
Fentanyl



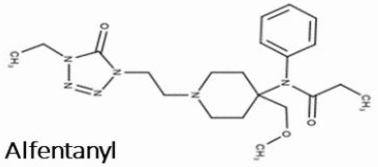
Butyrfentanyl



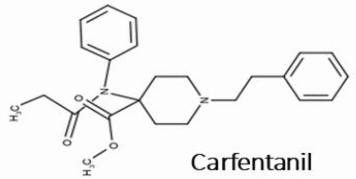
Isobutyrylfentanyl



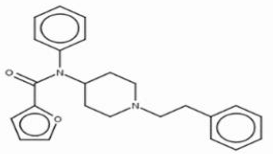
Acetylfentanyl



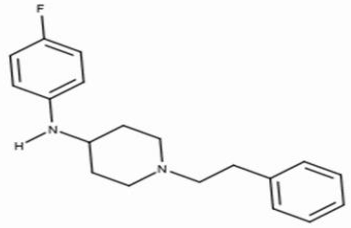
Alfentanyl



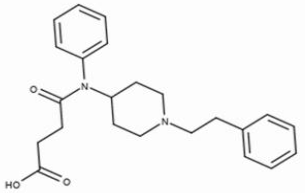
Carfentanil



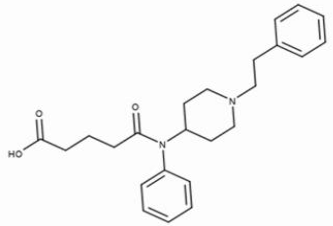
Furanylfentanyl



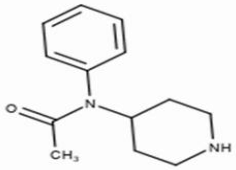
Despropionyl para-Fluorofentanyl



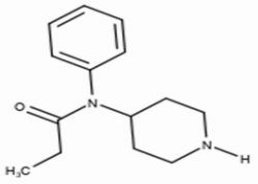
Butyryl fentanyl carboxy metabolite



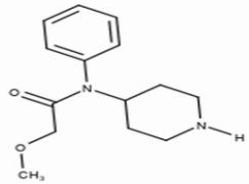
Valeryl fentanyl carboxy metabolite



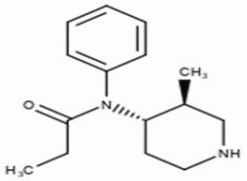
Acetyl norfentanyl



Norfentanyl

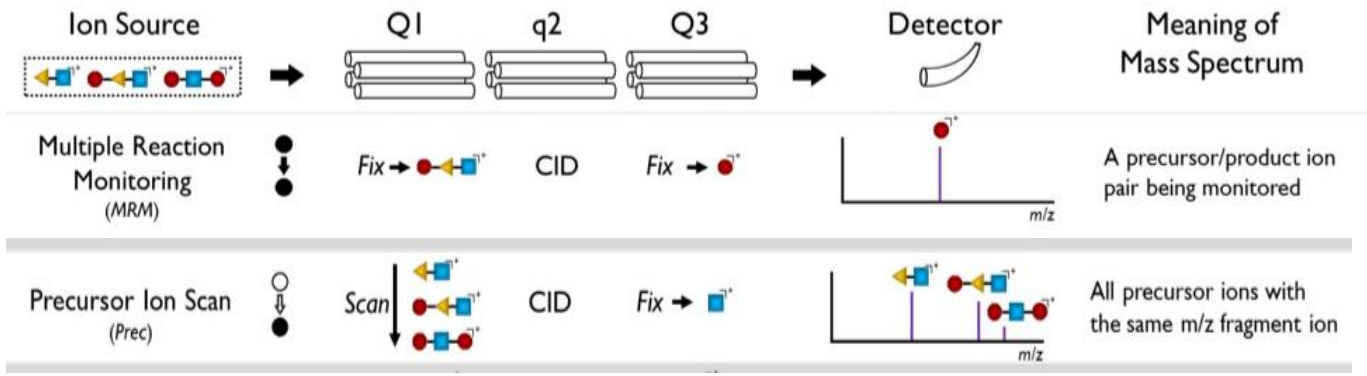


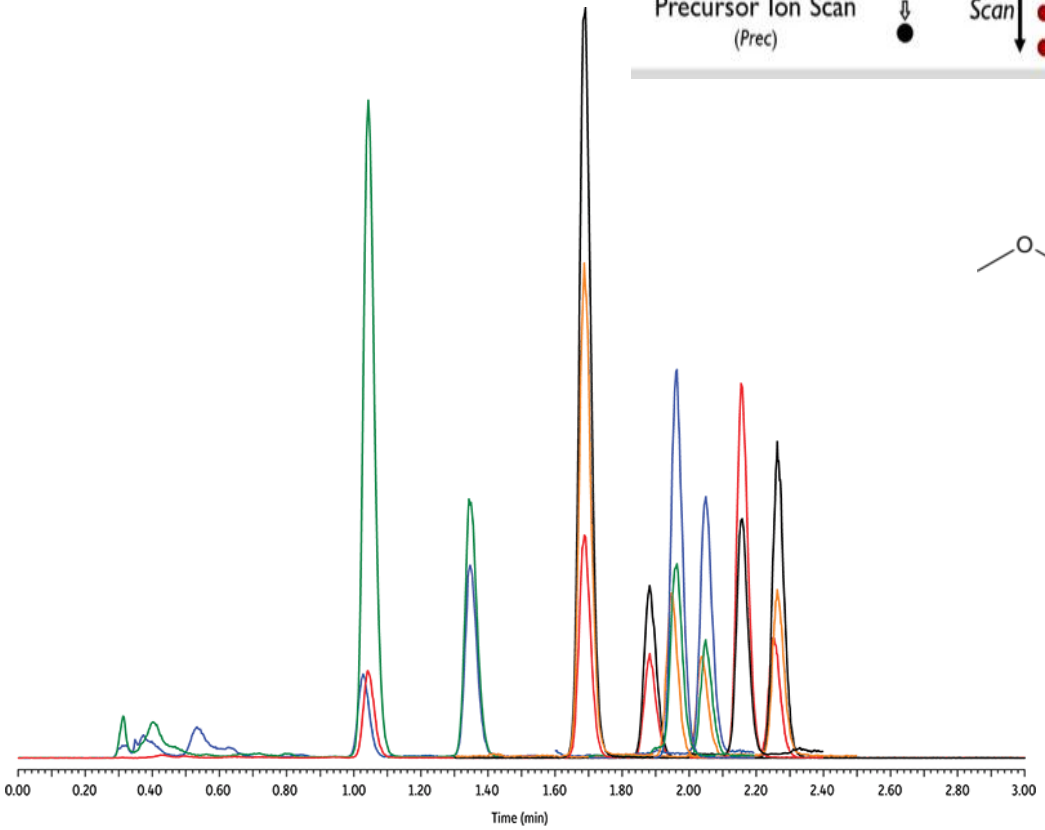
Methoxyacetyl norfentanyl

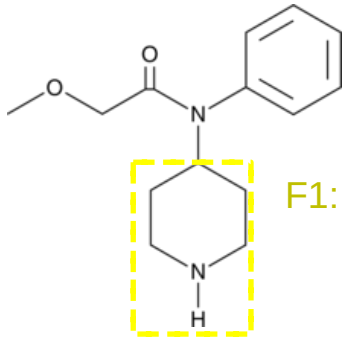


(±)-*trans*-3-methyl Norfentanyl

FENTANYLS - Analytical techniques: LC-MS/MS







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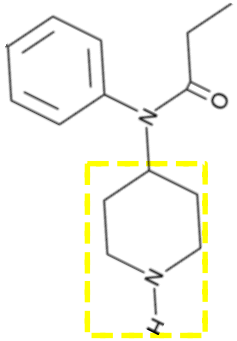
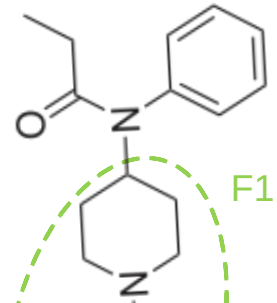
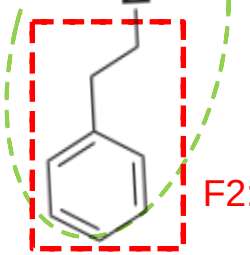


F1: 84.08144



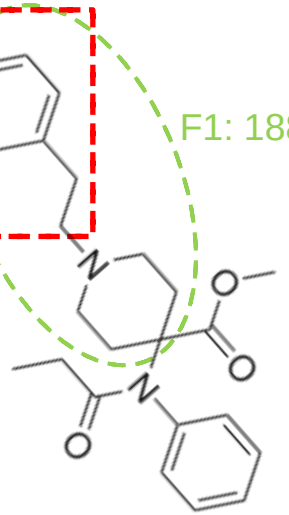
F1: 188. 14333



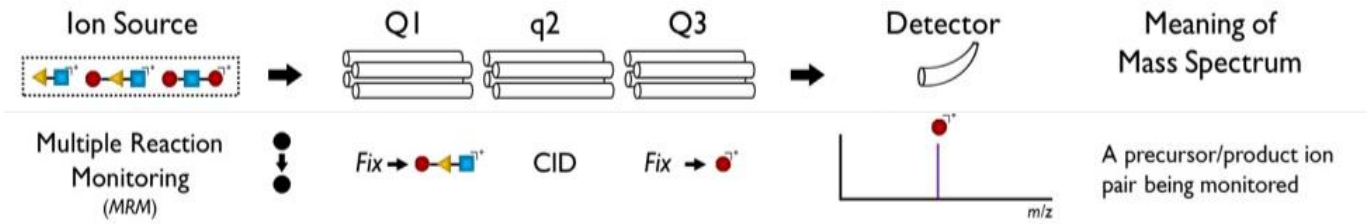
F2: 105.07033

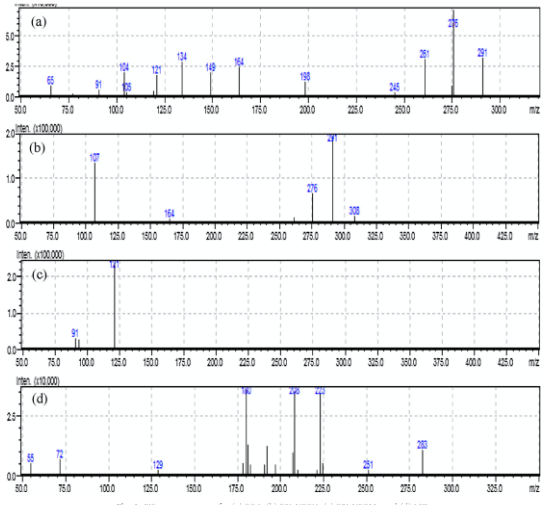
F2: 105.07033

F1: 188. 14333

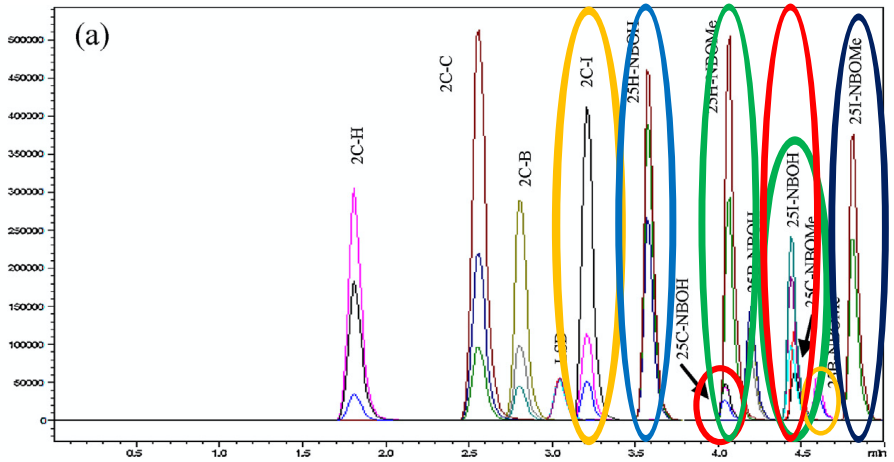


HPLC-MS(/MS) - Applicazioni





1. Ricerca delle più opportune condizioni operative dello spettrometro di massa



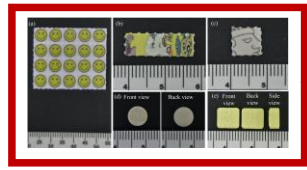
2. Messa a punto di un efficiente metodo cromatografico

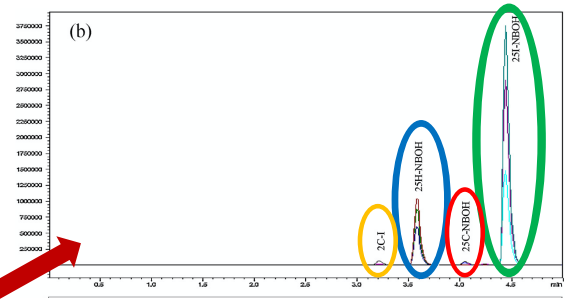
Forensic Science International 301 (2019) 394–401
Contents lists available at ScienceDirect
Forensic Science International
journal homepage: www.elsevier.com/locate/forsciint

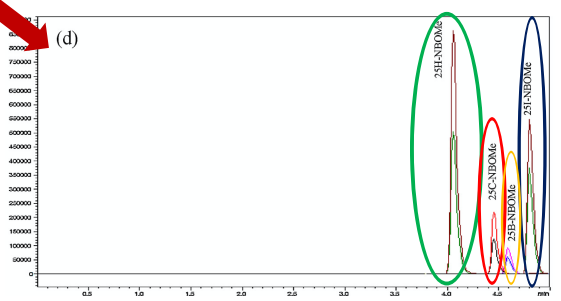
Simultaneous analysis of 2Cs, 25-NBOHs, 25-NBOMes and LSD in seized exhibits using liquid chromatography–tandem mass spectrometry: A targeted approach

Xue Wei Sarah Chia[a,*], Mei Ching Ong[a], Yuan Yuan Cheryl Yeo[a], Yanqing Joanne Ho[a], Edelia Izzati Binte Ahmad Nasir[a], Lin-Li Jaime Tan[a], Puay Yun Chua[b], Tiong Whei Angeline Yap[a], Jong Lee Wendy Lim[a]

[a] Illicit Drugs Laboratory, Health Sciences Authority, 11 Outram Road, Singapore 169078, Singapore
[b] Department of Chemistry, Faculty of Science, National University of Singapore, 6 Science Drive 2, Singapore 117546, Singapore



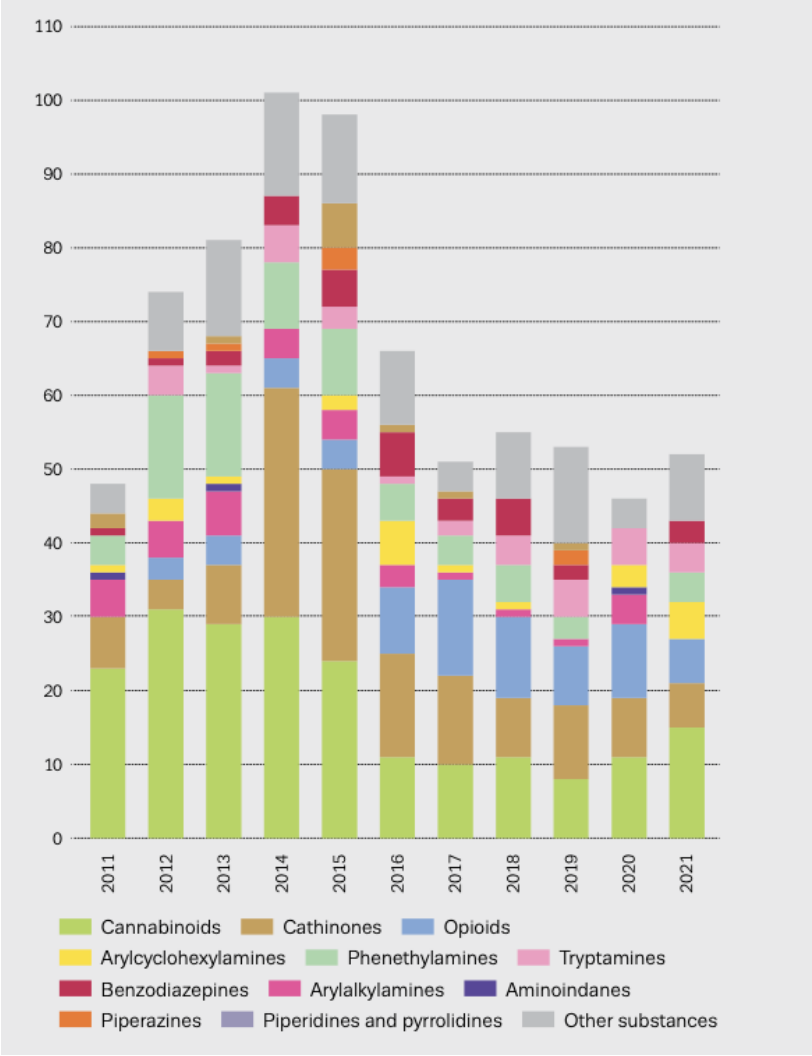




Identificazione:

- ✓ Tempo di ritenzione
- ✓ DUE Transizioni ione precursore/ione frammento

Considerando la varietà di NPS continuamente immesse sul mercato, l'identificazione di nuovi composti nei sequestri o nei campioni biologici è una vera sfida



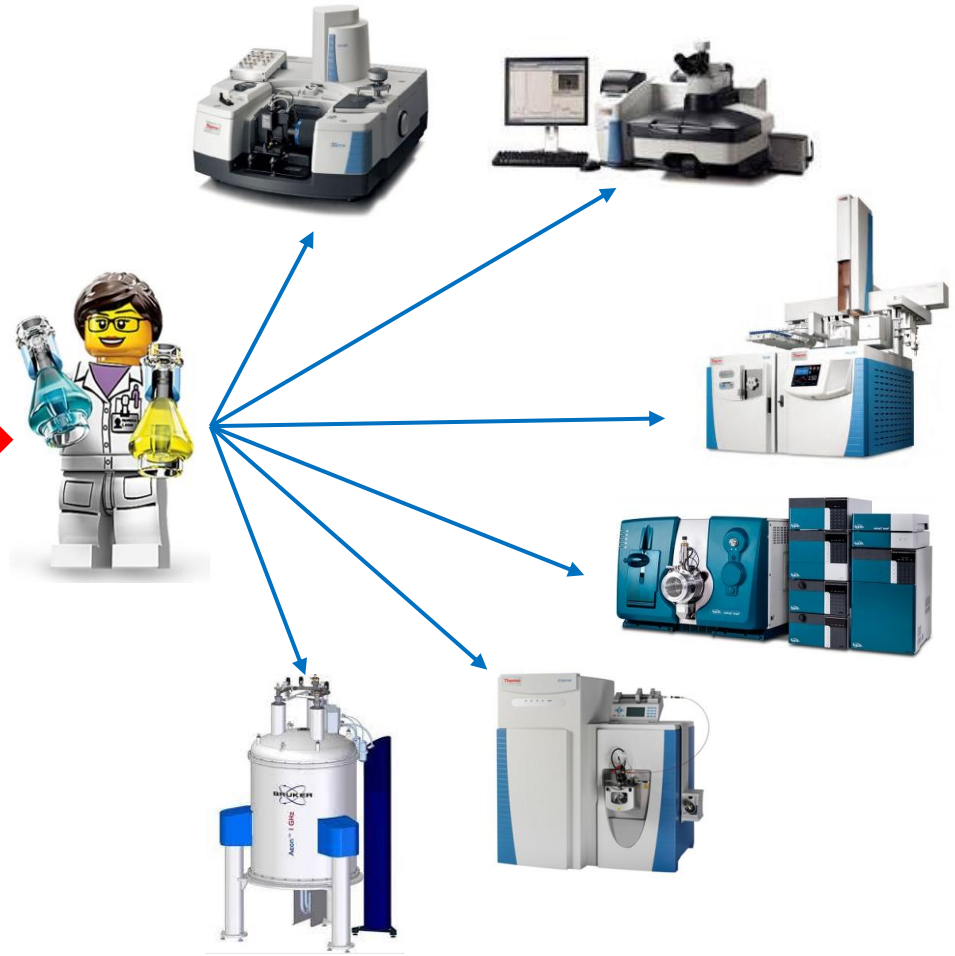
Number and categories of new psychoactive substances reported to the EU Early Warning System for the first time, 2011–21

Esempio di pericolosità dei nuovi oppioidi sintetici. In immagine sono riportate le dose con uguale effetto sul SNC

La spettrometria di massa ad alta risoluzione (HRMS) è uno strumento essenziale per lo screening e l'identificazione di nuove NPS

Strumenti a disposizione

Complementarietà delle diverse tecniche analitiche



RISULTATO

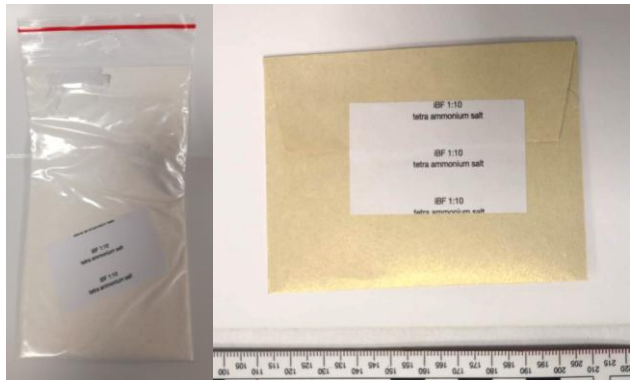
Espresso in termini di

- Identificazione

(o Caratterizzazione)

- Quantificazione

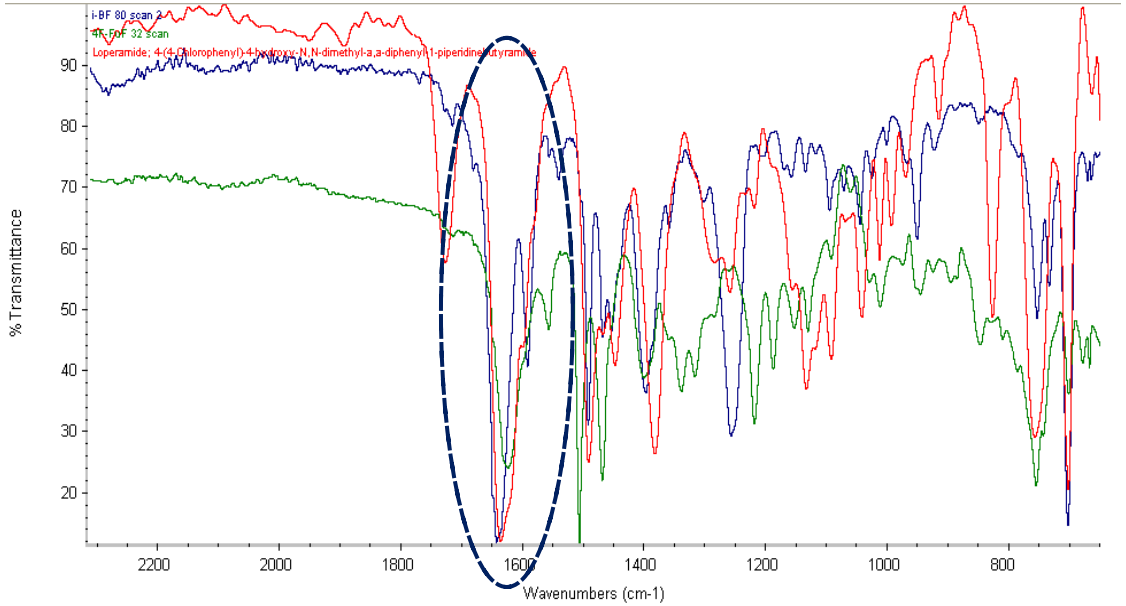
Caso reale



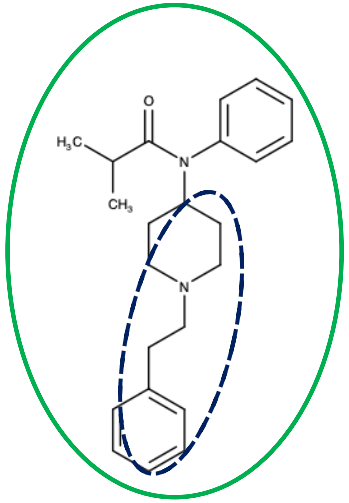
Dicembre 2019
Nord Italia
Sequestro di una spedizione postale da mittente già precedentemente attenzionato

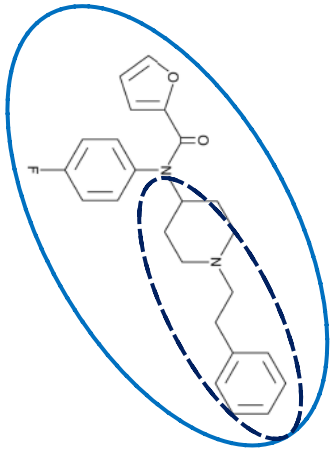
La confezione della spedizione riporta una etichetta che suggerisce la presenza di un derivato del fentanyl

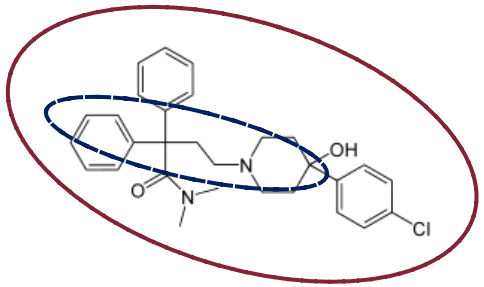
Tecniche spettroscopiche

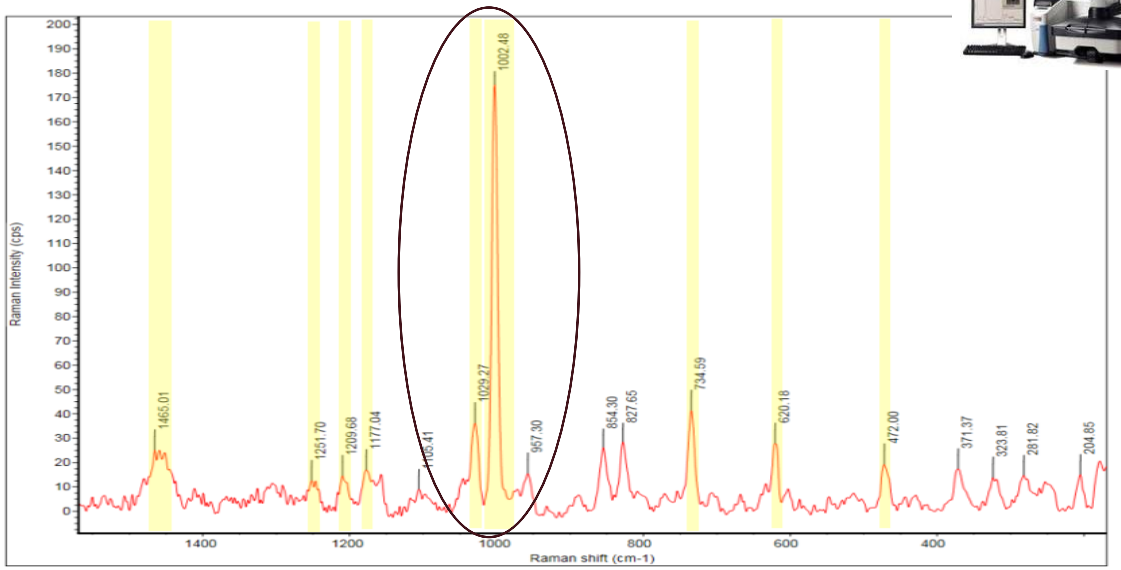


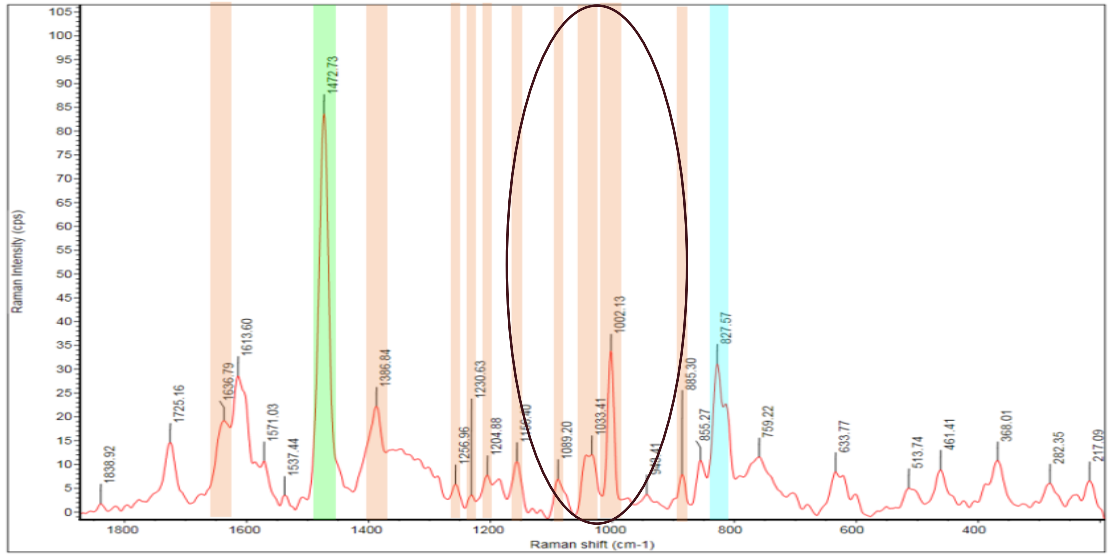
IR spectra of Furanyl Fentanyl (Blue), Isobutyryl Fentanyl (Green) and Loperamide (Red)



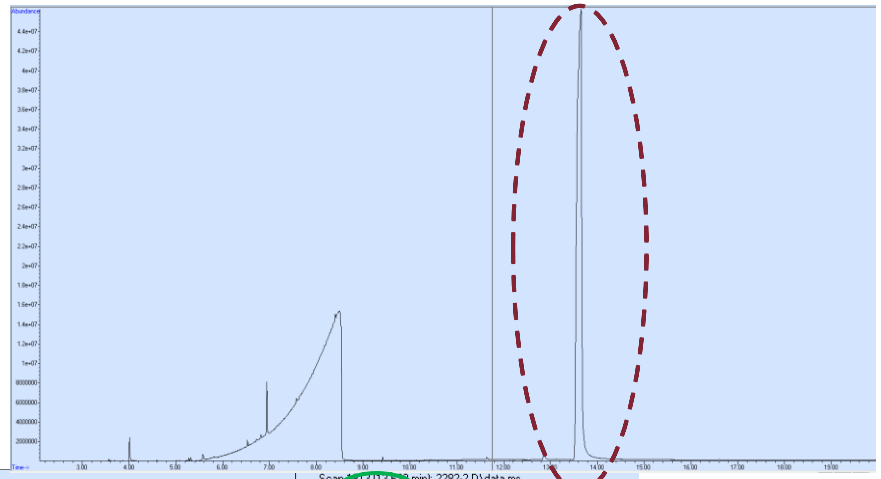


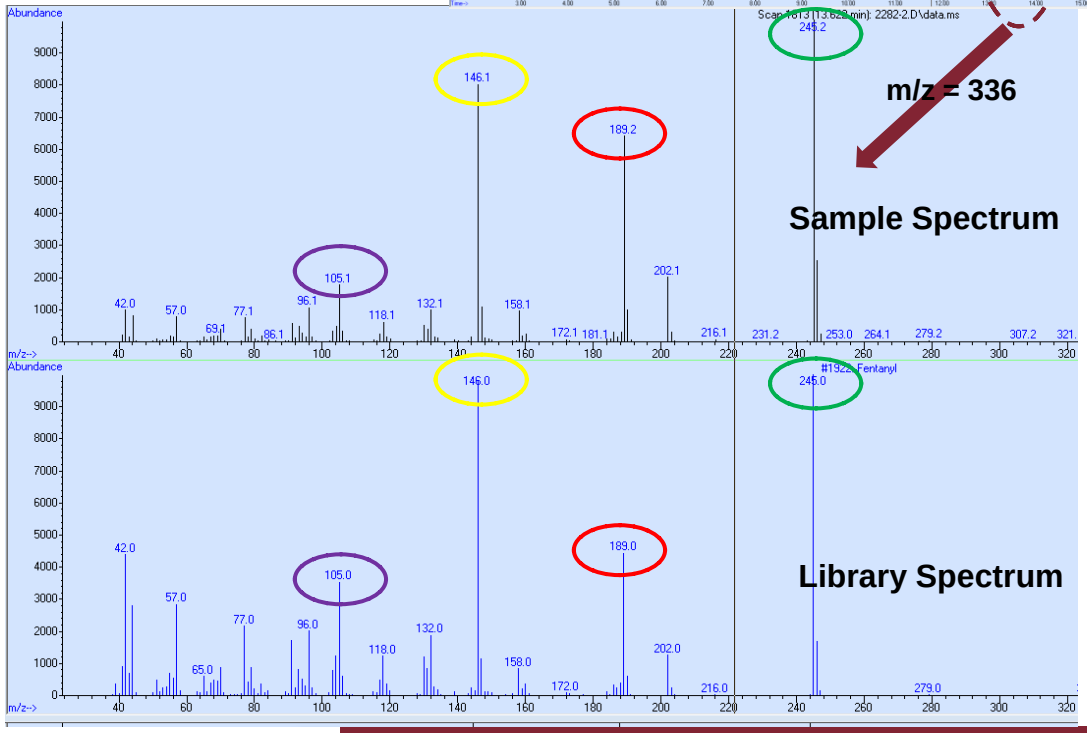


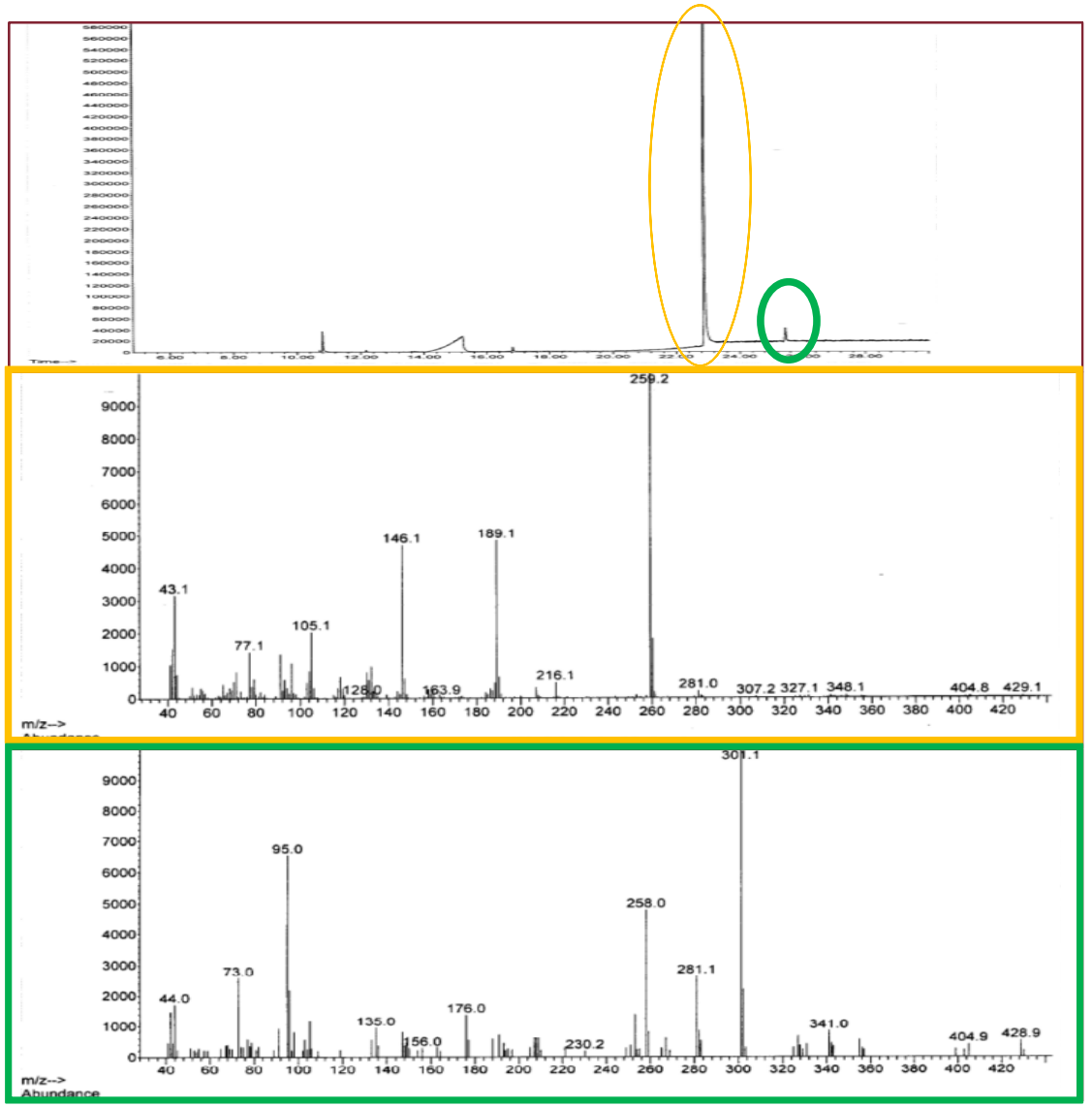




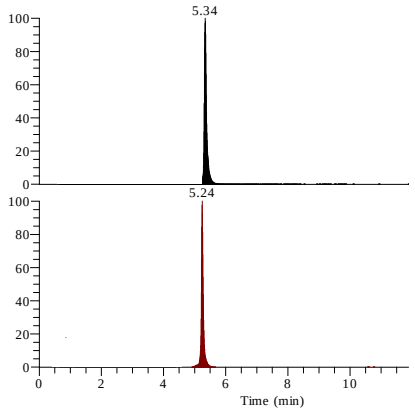
Analisi GC-MS

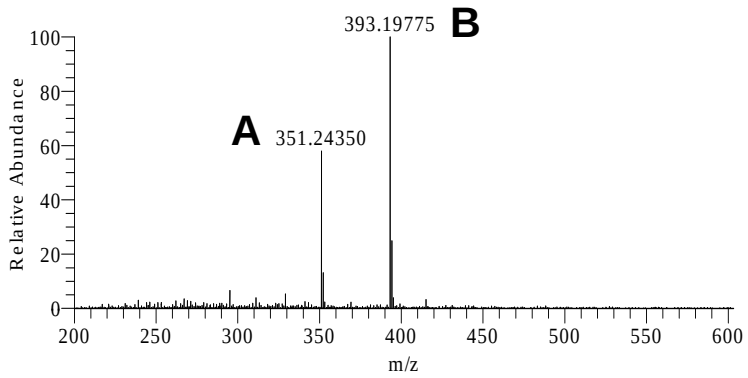


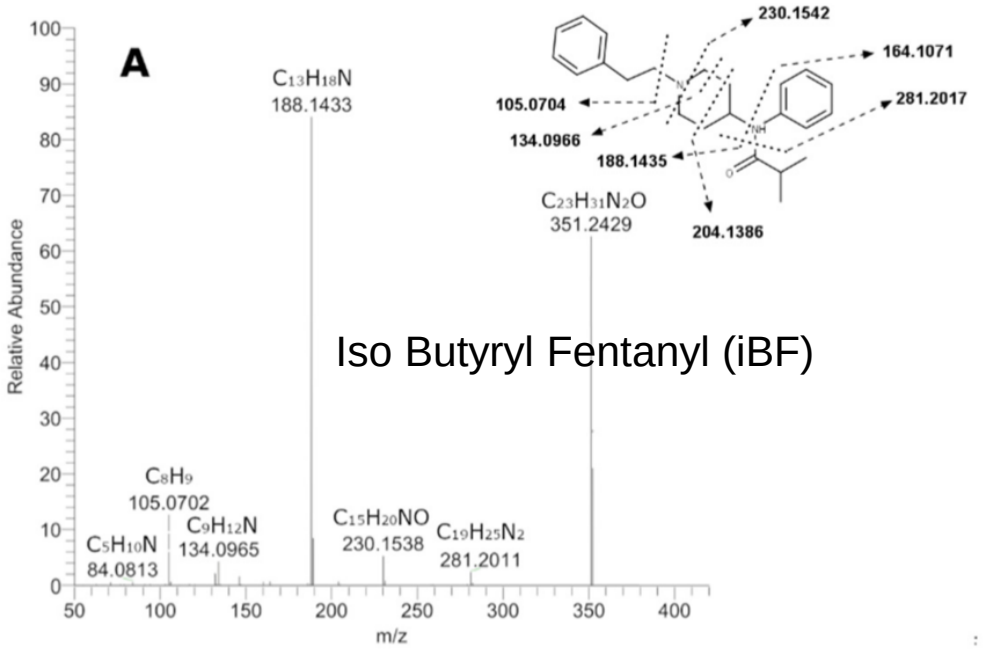




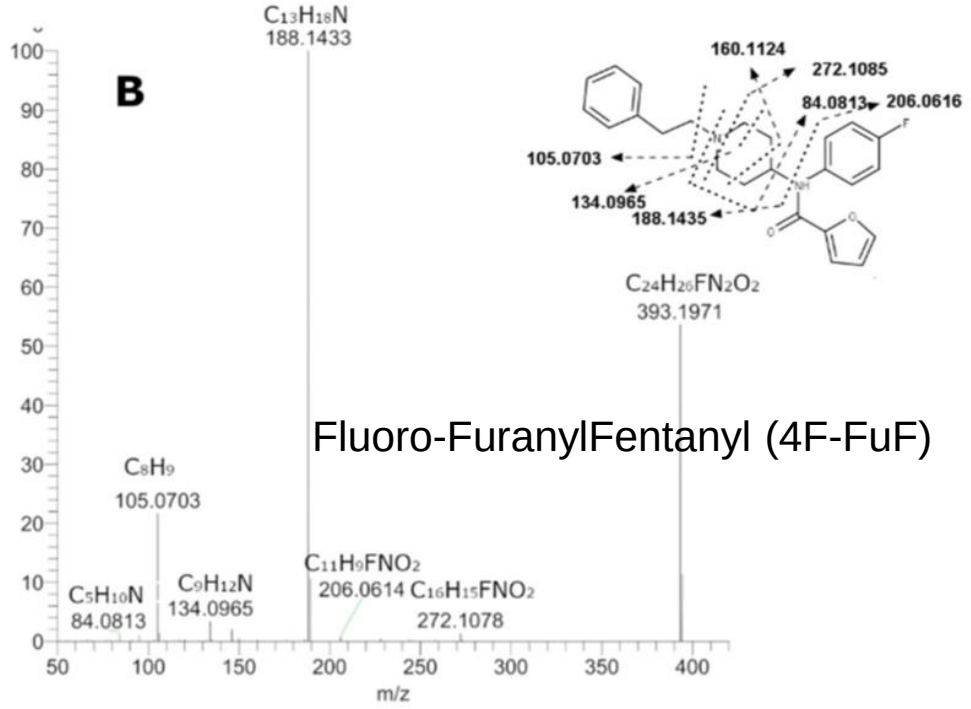
Analisi HPLC-HRMS/MS







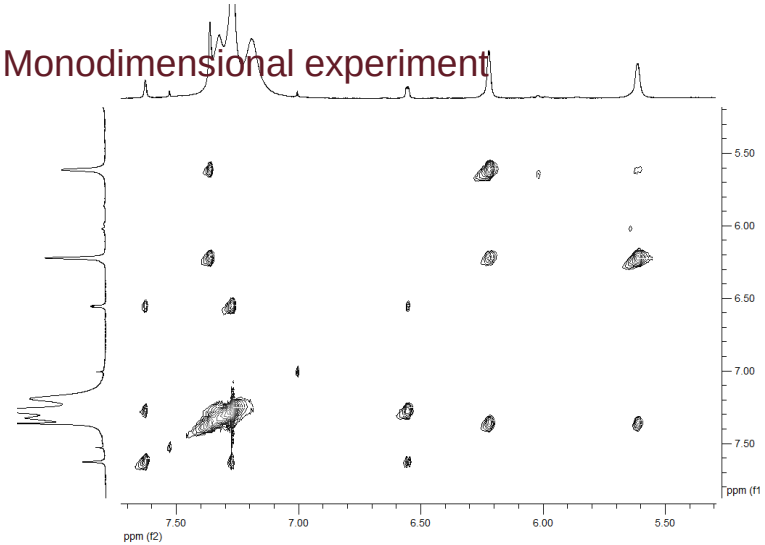
Iso Butyryl Fentanyl (iBF)



Fluoro-FuranylFentanyl (4F-FuF)

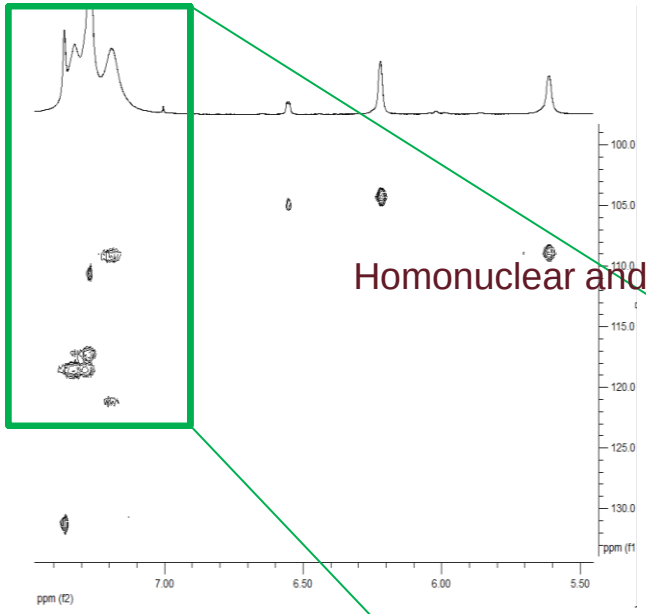
Analisi NMR

Homonuclear Monodimensional experiment

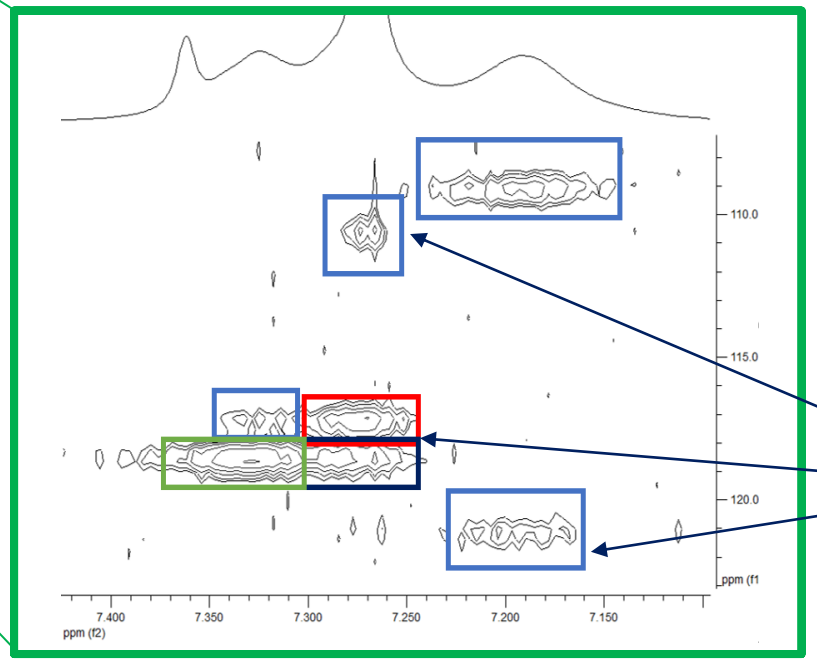


^{1}H-^{1}H TOCSY spectrum of 4F-FuF

Homonuclear and Heteronuclear Bidimensional experiment



^{1}H-^{13}C HSQC spectrum of 4F-FuF



^{1}H-^{13}C HSQC spectrum of 4F-FuF (enlargement). In the picture are evidenced the seven (7) non-equivalent CH groups belonging to 6 terms aromatic rings.

resounances

STRUCTURE

Risultato

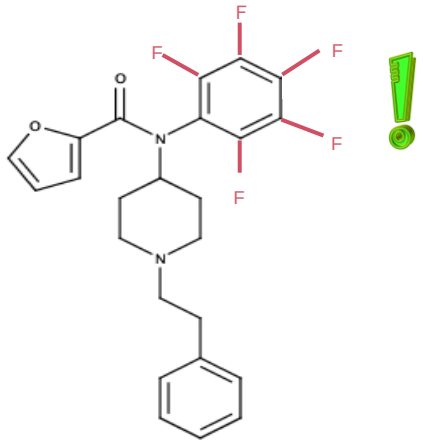
HighResNPS

HighResNPS.com is a crowd-sourced mass spectral database for HR-MS screening of New Psychoactive Substances (NPS).

The database can be used for direct searches on compound names and exact mass of precursor and/or fragment ions. Also, the database can be downloaded and converted to a suspect library for **Agilent** QTOF MS, **Bruker** QTOF MS, **Waters** QTOF MS, **Sciex** QTOF MS, **Thermo** Orbitrap MS and **Shimadzu** QTOF MS.

HighResNPS.com currently contains more than **6,200** entries (where more than **2,200** entries are unique).

HighResNPS.com is only intended for a closed user group and not open to the general public.



Aggiornamento
Giugno 2025

Forensic Chemistry 21 (2020) 100283

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journal homepage: www.sciencedirect.com/journal/forensic-chemistry

Multi-analytical characterization of 4-fluoro-furanyl fentanyl in a drug seizure

Flaminia Vincenti [a,b], Flavia Pagano [c], Camilla Montesano [a,*], Fabio Sciubba [a], Maria Enrica Di Cocco [a], Adolfo Gregori [d], Fabiana Di Rosa [d], Livia Lombardi [d], Manuel Sergi [c], Roberta Curini [a]

[a] Sapienza University of Rome, Department of Chemistry, 00185 Rome, Italy
[b] Sapienza University of Rome, Department of Public Health and Infectious Disease, 00185 Rome, Italy
[c] University of Teramo, Faculty of Bioscience and Technology for Food, Agriculture and Environment, 64100, Italy
[d] Carabinieri, Department of Scientific Investigation (RIS), 00191 Rome, Italy

ARTICLE INFO

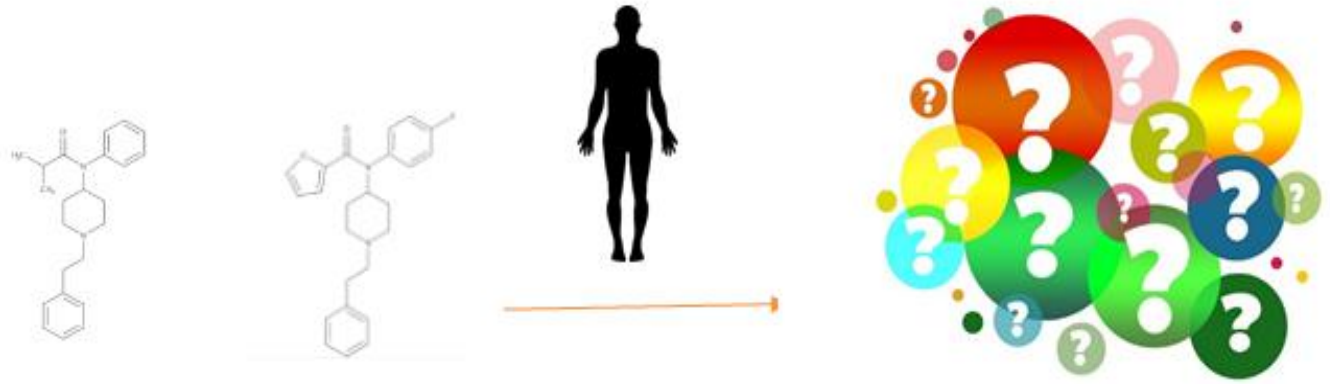
Keywords:
Fentanyls
IR
Raman spectroscopy
GC–MS
LC–HRMS, NMR

ABSTRACT

In recent years, the synthesis and introduction of Novel Psychoactive Substances (NPS) into the illicit market has reached alarming levels. More than 730 compounds have been identified by the European Monitoring Center for Drugs and Drug Addiction (EMCDDA). Among the newest NPS, synthetic opioids deserve special attention, in particular fentanyl and its analogues that in 2018 covered more than 70% of the world demand for opioids, with thousands of fatal events in USA. In this context it is of significant importance to have the right tools to identify the most recent NPS exploiting the synergy between different analytical techniques.

Two synthetic opioids, belonging to the family of fentanyl derivatives were found in the same seizure occurred in Italy in 2019. IsobutyrylFentanyl (iBF) and 4-Fluoro-Furanylfentanyl (4F-FuF) were identified. A comprehensive elucidation of 4F-FuF by means of several analytical techniques, namely IR, Raman, GC–MS, LC–HRMS/MS and NMR, is provided in this work.

In Italy, as a result of the present study, a formal notification of 4F-FuF to the national early warning system was sent and notification to EMCDDA ensued.



E se non fosse stata sequestrata in tempo?

Quali sono i suoi effetti collaterali?

Come possiamo capire se un soggetto la ha assunta?

Qualcuno la ha già assunta?

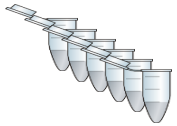
Cosa può fare il SSN per contrastare gli effetti indesiderati?

Identificazione del Patway metabolico di una sostanza

In Silico

Informazioni preliminari
Metaboliti putativi

In Vitro



Il metabismo vero e proprio viene simulato per selezionare i prodotti più probabili

In Vivo

Conferma delle informazioni ottenute da silici e vitro

Tutti i campioni sono analizzati e processati assieme.

PRO:

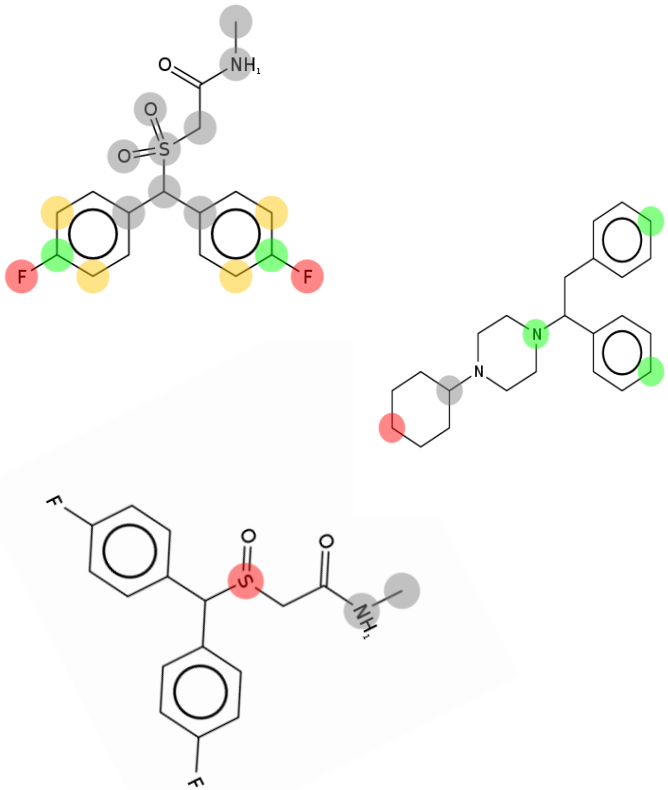
- Non trascuro nulla
- Informazione più ricca possibile
- Non escludo risultati frutto di uno solo dei passaggi

CONTRO:

- Più l'informazione è ricca più è complessa la sua elaborazione

UHPLC-HRMS/MS

Studi in silico

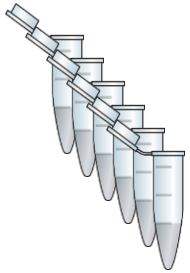


Colore assegnato	Valori di NOR
ROSSO	0,66 < NOR < 1
ARANCIO	0,33 < NOR < 0,66
VERDE	0,15 < NOR < 0,33
BIANCO	0 < NOR < 0,15
GRIGIO	Pochi dati

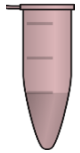
Molti studi si avvalgono di programmi di chimica farmaceutica computazionale con lo scopo di riuscire a trovare le masse sulle quali concentrare l'indagine; considerando che la tecnica principe per l'identificazione dei metaboliti è la cromatografia, gas o liquida, accoppiata alla spettrometria di massa (GC-MS o LC-MS), bisogna prima di tutto conoscere la massa degli analiti. Sono stati usati molti programmi allo scopo, ma quelli più utilizzati sembrano essere Meteor e MetaSite. Questi programmi vengono configurati in modo da coprire tutti i possibili pathway metabolici che una molecola può intraprendere, arrivando anche a superare il centinaio di composti. Una volta trovati i derivati metabolici se ne calcolano le masse, che andranno ricercate con gli opportuni analizzatori.

Studi in vitro

500 µl
1x10^6 cell/ml
20µM



- Blank incubation sample
- Positive incubation sample (Morfina)
- 6 topi per iBF + 6 topi per 4F-FuF



15000g 5 min

Prelievo surnatante

UHPLC-HRMS/MS

Solitamene, il campione viene preparato partendo da una soluzione della sostanza da analizzare, stabilizzandone, se necessario il pH mediante soluzioni tampone. Ottenuti i risultati delle cellule microsomiali si utilizzano anche le cellule epatiche, sia in sospensione, che le cellule platable. A seconda dei risultati ottenuti con le cellule microsomiali si decidono i tempi di prelievo, solitamente si tende ad avere 3 o 4 tempi, si utilizzano composti di controllo per verificare l'effettiva attività delle cellule. Gli epatociti vengono allora immersi nel medium. Al termine della procedura si ha cura di prelevare la surnatante senza andare a disturbare il pellet depositato sul fondo della provetta.

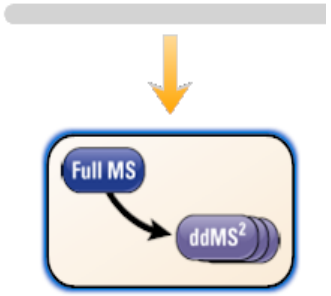
Analisi UHPLC-HRMS/MS

- **Spettrometro di Massa:** Q-Exactive-Orbitrap Thermo Fisher Scientific

- **Sorgente:** H-ESI

Condizioni operative sorgente H-ESI:

- Polarity: Positive
- Spray Voltage: 3.30 kV
- Capillary temperature: 320 ° C
- H-ESI temperature: 320 ° C
- Sheat gas (Nitrogen) 55 unit
- Auxiliary gas 20 unit



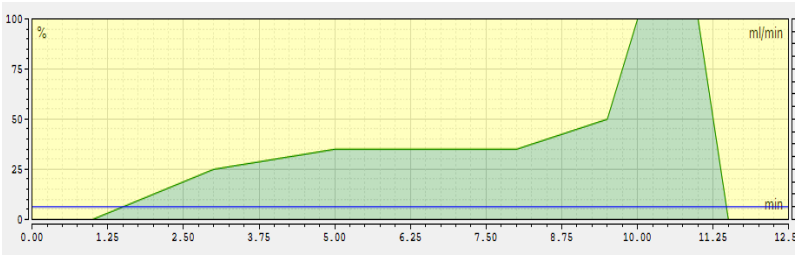
Full scan

MS/MS

Pattern di frammentazione

Inclusion lists

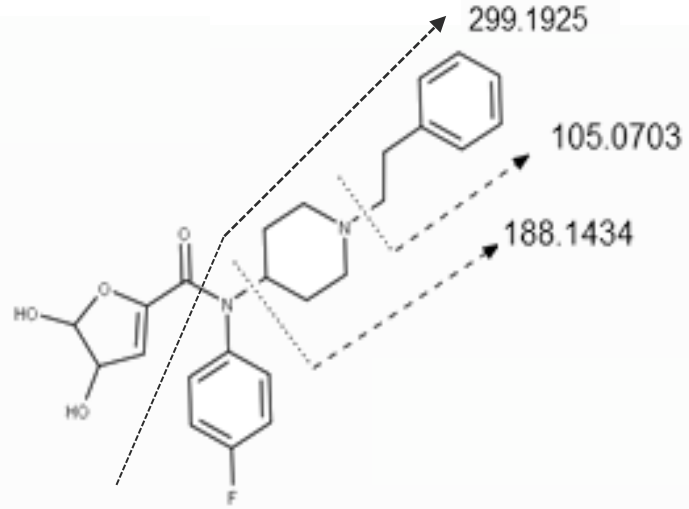
- ***Colonna:*** *ACE Excel 2 C18-PFP 100 x 2.1 mm (1.6 um particles)*
- ***Fasi:*** A *H2O 0,1% HCOOH 10mM NH4COOH*
  B *50:50 MeCN: MeOH 0,1% HCOOH*
- ***Flusso:*** *0.500 mL/min*



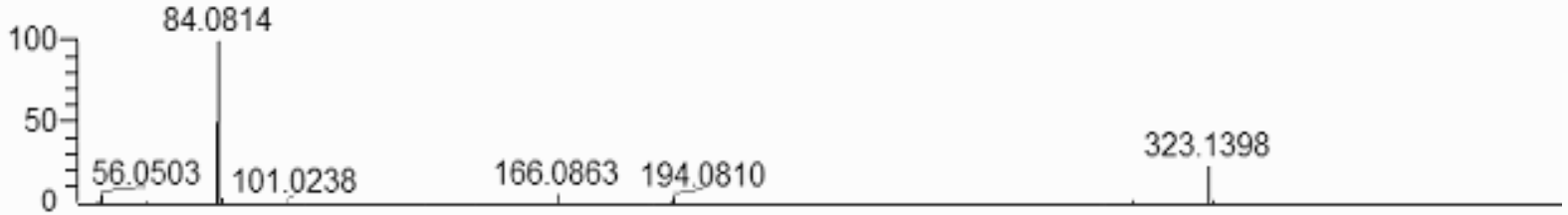
Identificazione del Ptway metabolico di 4F-FuF

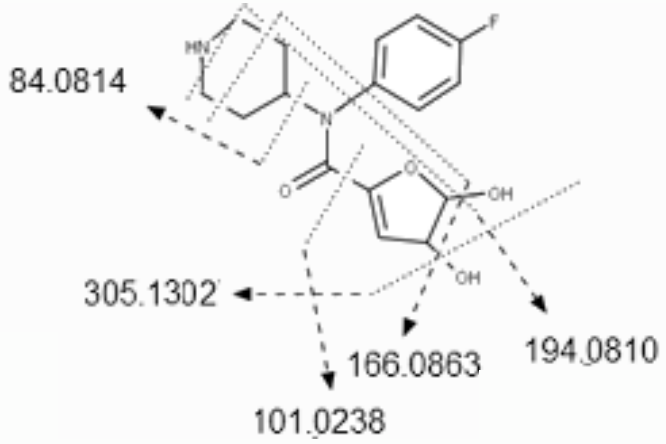
The main metabolite was the dihydrodiol derivative resulting from epoxidation of furan, followed by hydration, which was further N-dealkylated to produce the second most relatively intense metabolites in vivo, whereas N-dealkylation of the parent compound prevailed in vitro.

Dihydrodiol derivative



Dihydrodiol formation + N-Dealkylation

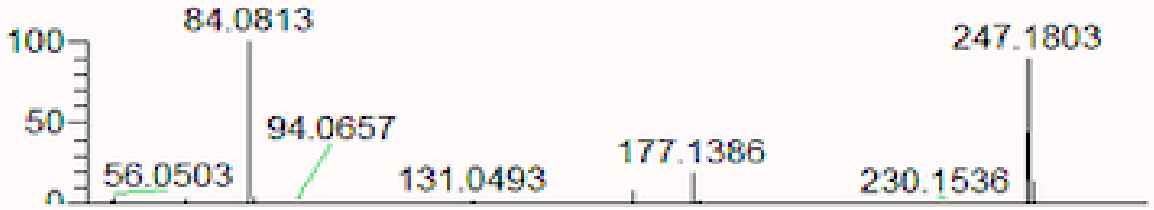


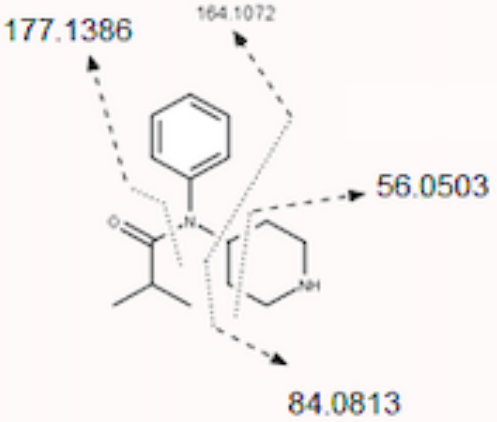


Identificazione del Ptway metabolico di iBF

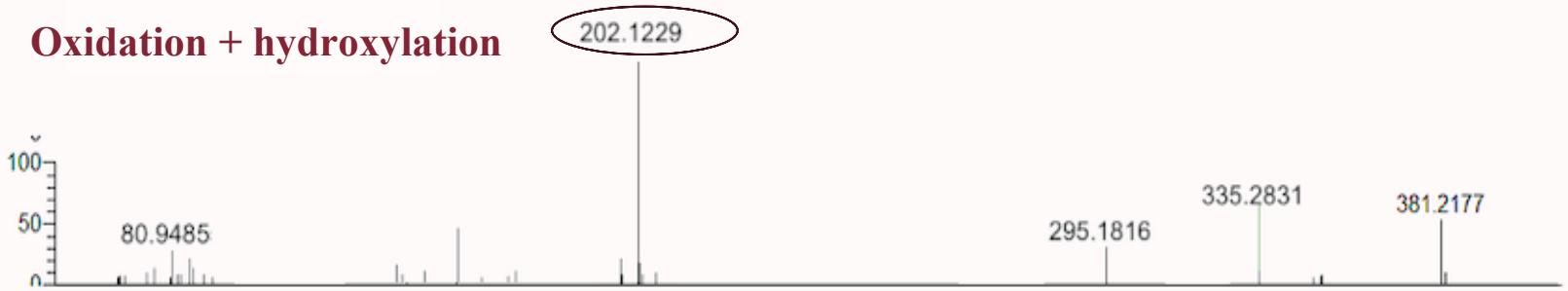
N-dealkylated metabolite was the relatively most intense but hydroxylation and subsequent carbonylation of the parent compound was also a main transformation.

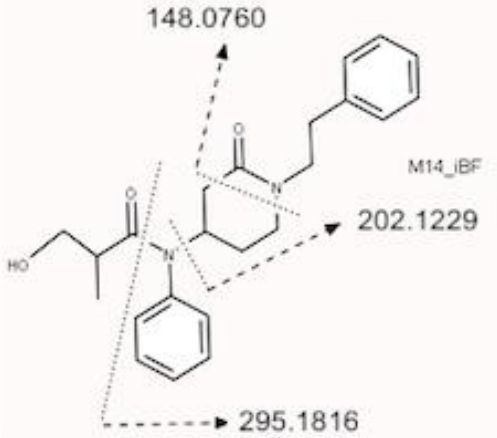
Oxidative N-dealkylation

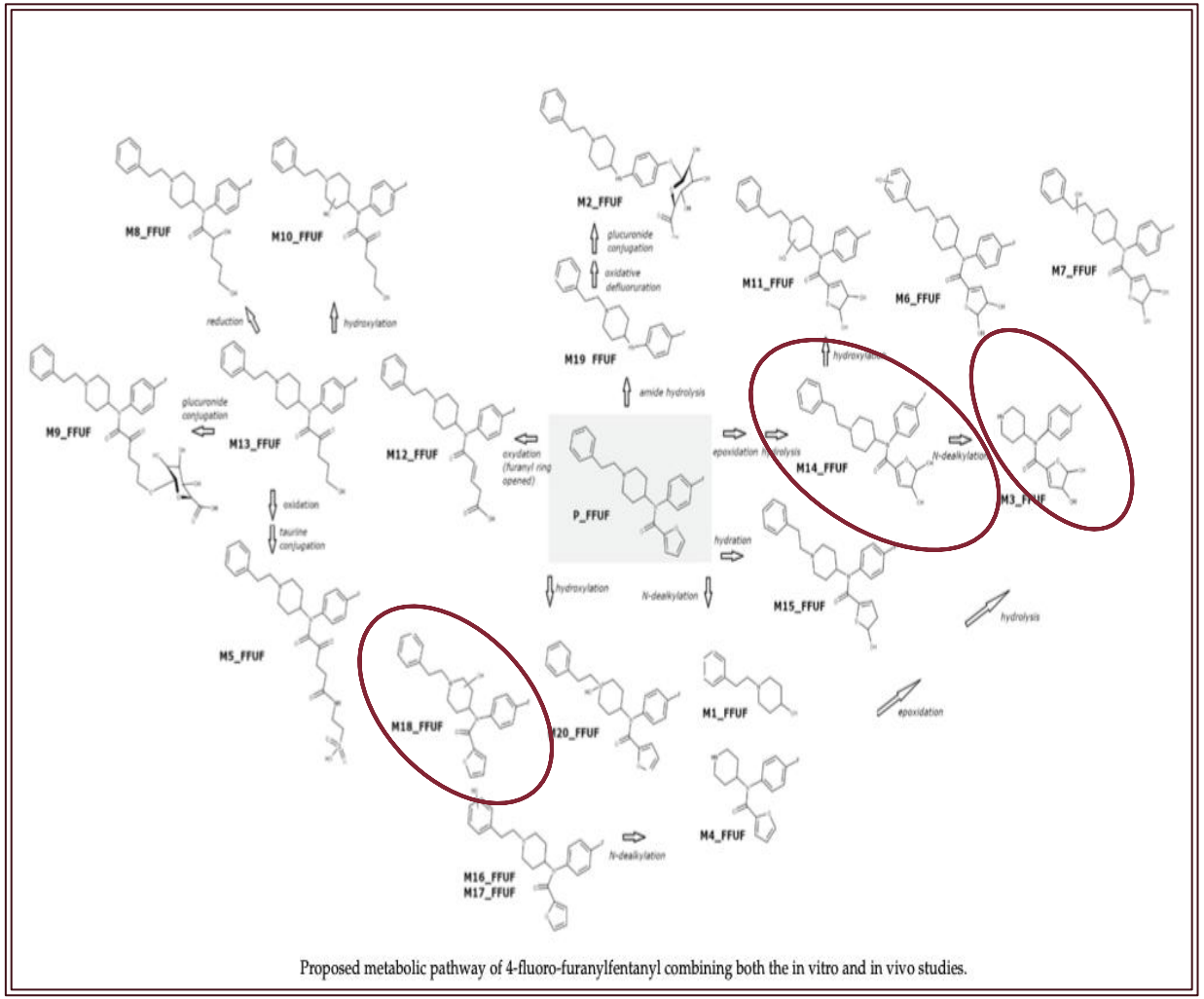




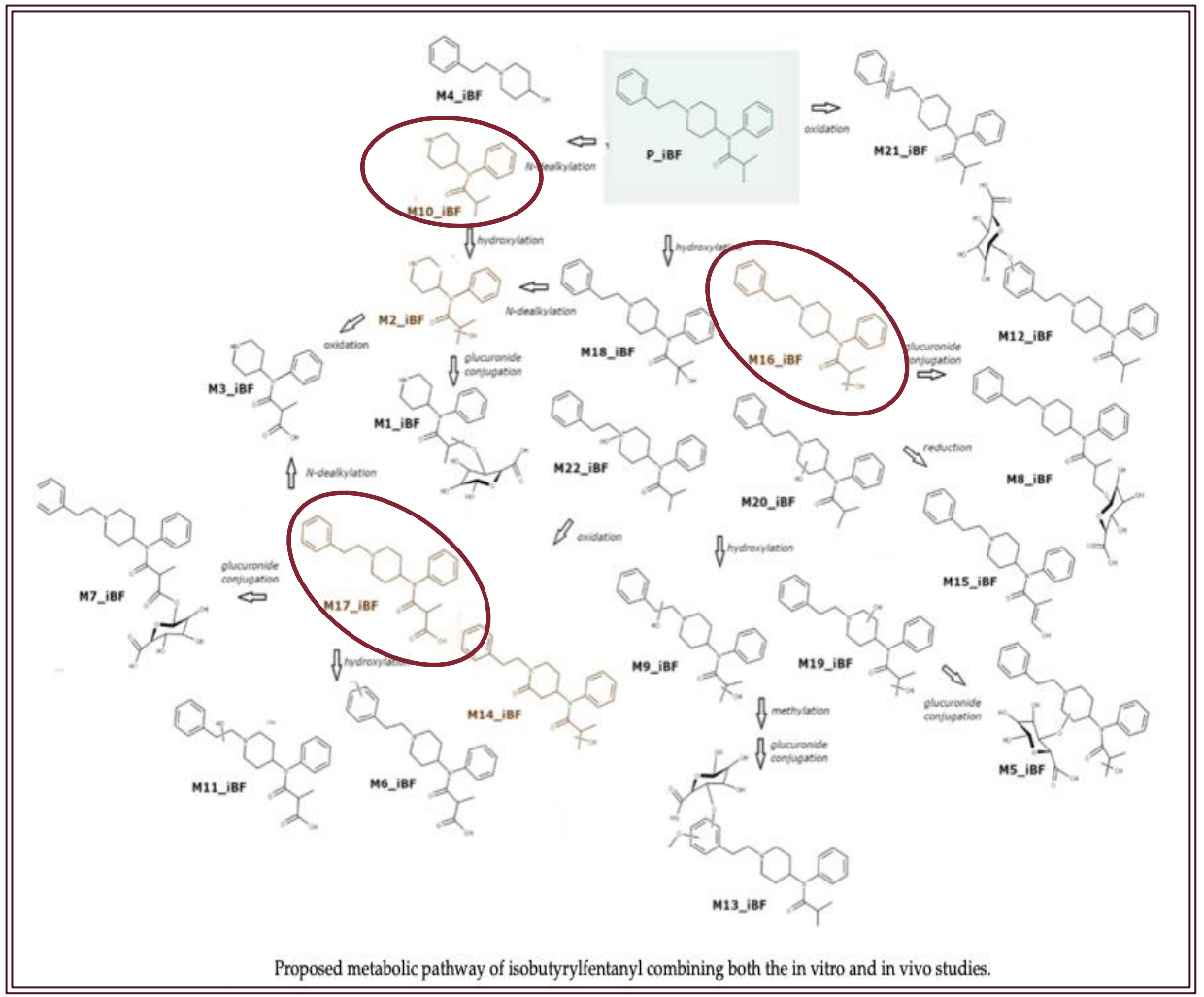
Oxidation + hydroxylation





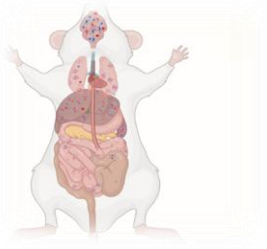


Proposed metabolic pathway of 4-fluoro-furanylfentanyl combining both the in vitro and in vivo studies.



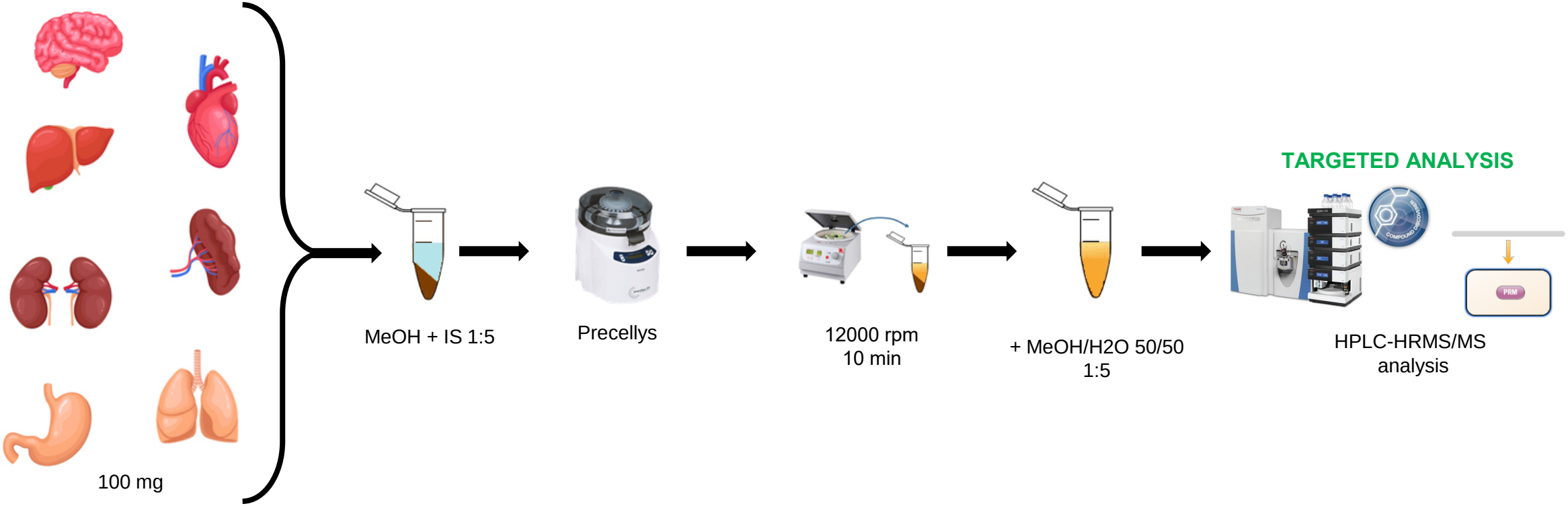
Proposed metabolic pathway of isobutyrylfentanyl combining both the in vitro and in vivo studies.

L'incubazione della sostanza con epatociti è un buon approccio per identificare i metaboliti di una sostanza e cercare quindi i biomarkers di assunzione da inserire nei futuri metodi targeted.



Studi farmaco-tossicologici

L'importanza degli studio farmaco tossicologici è duplice. Da un lato analizzando I tessuti siamo in grado non solo di capire se sono stati selezionati giusti biomarkers, dall'altra siamo in grado di stabilire gli eventuli organi bersaglio per la sostanza parent e e per I metabolite. In ultimo consente di studiare un piano di intervento rapido per I PS.



Implementazione software

Non sempre è possible eseguire il Met-ID così come è stato illustrato per diversi motivi:

- Costi
- Strumentazione
- Problemi etici

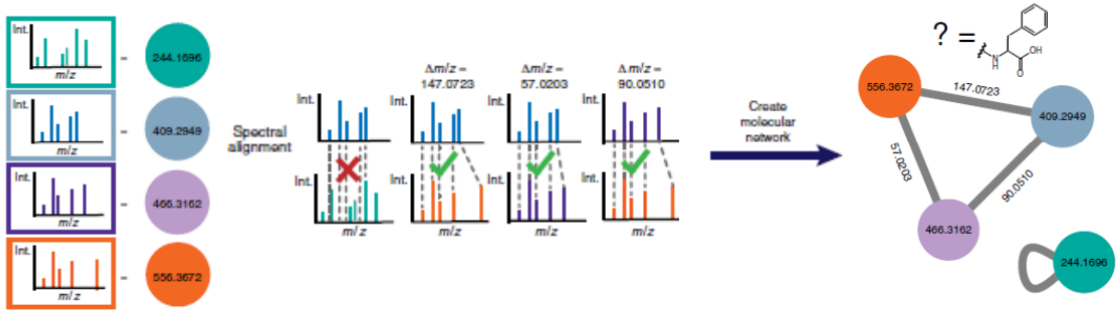
In Silico

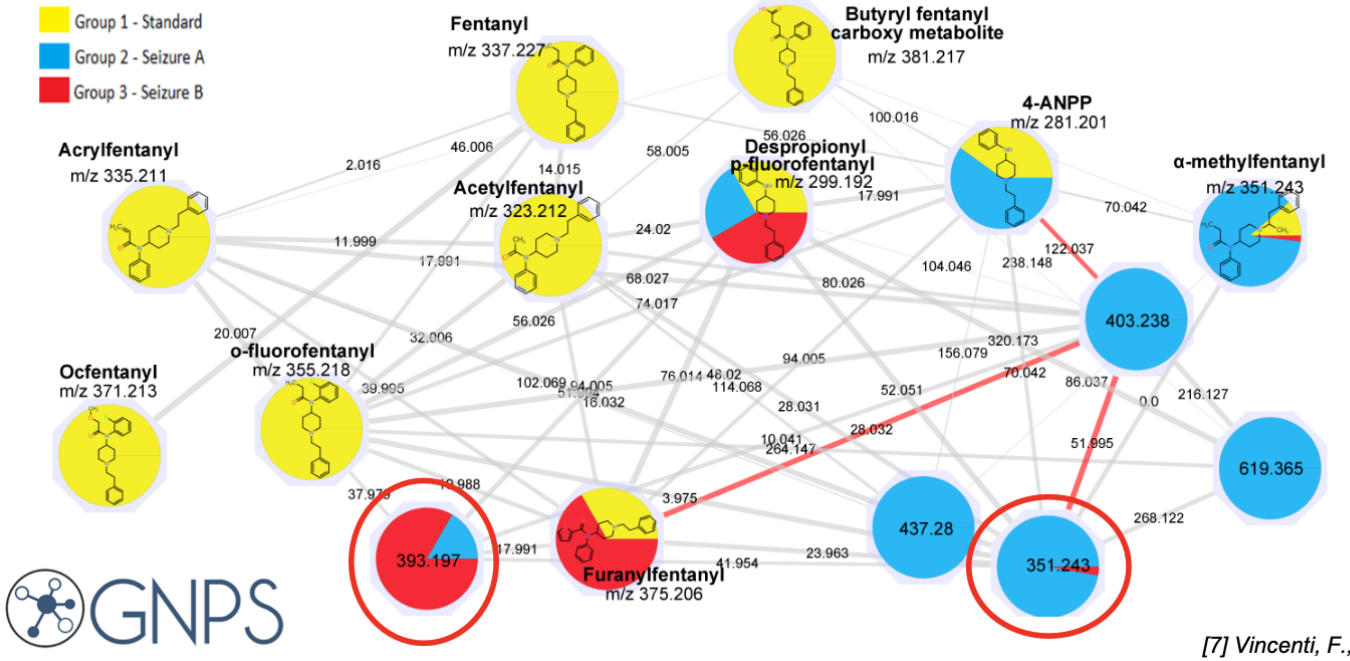
La strategia prevede che I risultati ottenuti dagli studi in silico vengano utilizzati per creare una inclusion list per analisi suspect.

Potrebbe non essere sufficiente…

Molecular networking

I molecular networks sono un modo di visualizzare lo spazio chimico presente in esperimenti MS/MS. Gli spettri di molecole appartenenti ad una determinata classe chimica vengono raggruppati in network molecolari perché presentano analogie.

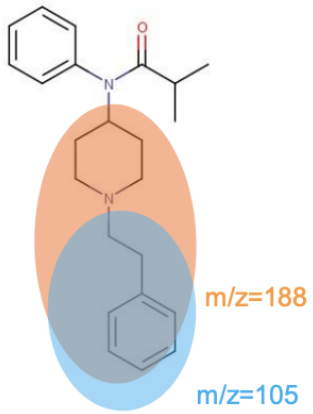




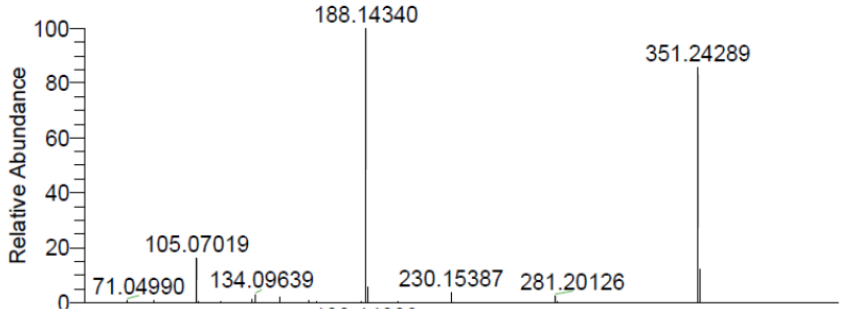
- ✓ Raccolta di spettri MS/MS di standard analitici appartenenti a classi note e creazione di «network standard» (nodi in giallo)
- ✓ Analisi di campioni incogniti sfruttando la molecular networking analysis; gli spettri che presentano analogie con quelli standard vengono messi in evidenza (nodi blu e rossi)

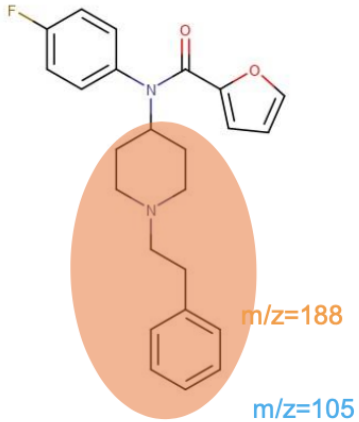
Identificazione di due nuovi analoghi del fentanyl in due sequestri

[7] Vincenti, F., Montesano, C., Di Ottavio, F., Gregori, A., Front. Chem. (2020) doi: 10.3389/fchem.2020.572952

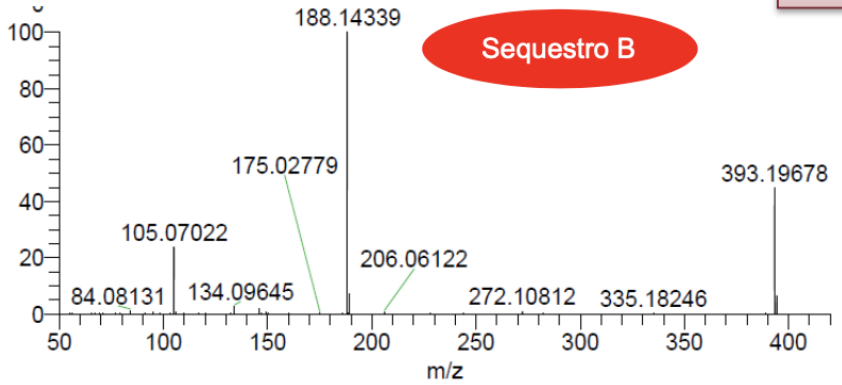


Sequestro A

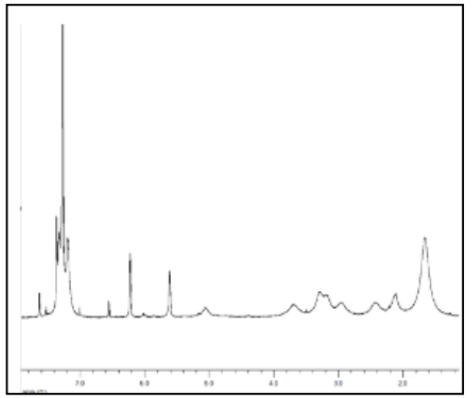




Sequestro B



CONFERMA ATTRAVERSO ANALISI NMR



[7] Vincenti, F., Montesano, C., Di Ottavio, F., Gregori, A., Front. Chem. (2020) doi: 10.3389/fchem.2020.5729

LC-HRMS è attualmente la tecnica di elezione per l'analisi di NPS

- Maggiore potere identificativo rispetto a MS tradizionale
- L'uso di strategie informatiche rende più facile l'identificazione di nuove sostanze
- Limite per nuove sostanze che non rientrano nelle classi note

Analisi Target e suspect screening

Possibilità di reinterrogare i campioni analizzati

Si tratta di uno screening limitato alle sostanze presenti nella libreria, inadatto alla rivelazione ed identificazione di nuove droghe

Analisi non target

Possibilità di identificare (putativamente) nuove sostanze

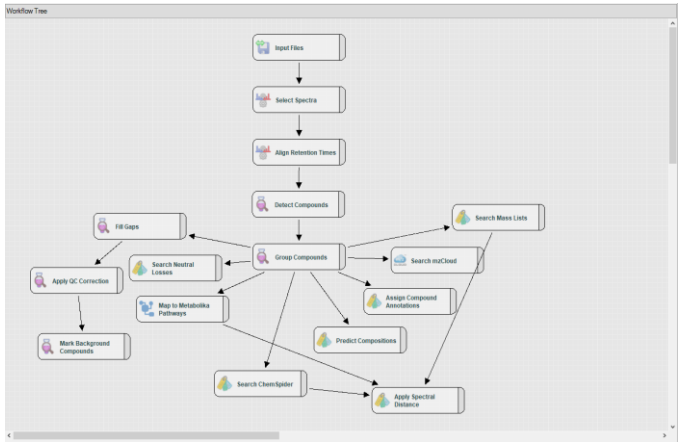
Possibilità di applicare diverse strategie informatiche post-acquisizione

Condivisione online dei dati spettrali di nuove sostanze individuate

- **Metabolomica**: studio del profilo metabolico di un organismo e delle sue possibili alterazioni a carico di fattori quali dieta, sesso, età, stress, condizioni fisiopatologiche.

Applicazioni

CHIMICA CLINICA: studio del profilo metabolico di **soggetti malati** rispetto ad un gruppo di controlli sani per la **diagnosi precoce di malattie**.



CHIMICA ANALITICA FORENSE: sviluppo di **nuovi metodi analitici** per l'identificazione di ***biomarkers*** endogeni dell'assunzione di NPS in matrici biologiche.

Nuovi oppioidi sintetici o NSOs