



Nuove sostanze psicoattive (...e non solo)- strategie analitiche per la loro determinazione

**Attività di ricerca del centro collaborativo «Tossicologia
Forense Università Cattolica del S. Cuore»**

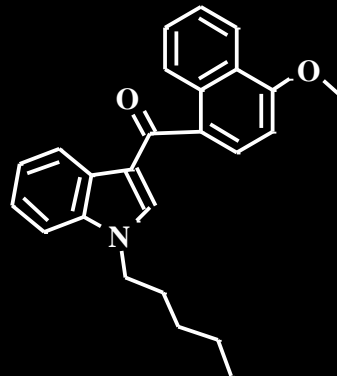
Sabina Strano Rossi – Referente Centro collaborativo



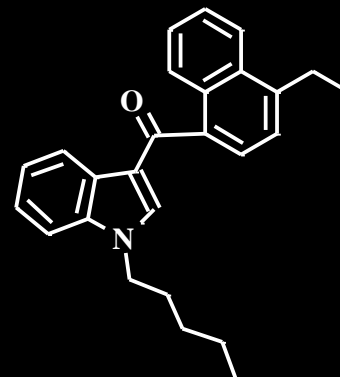
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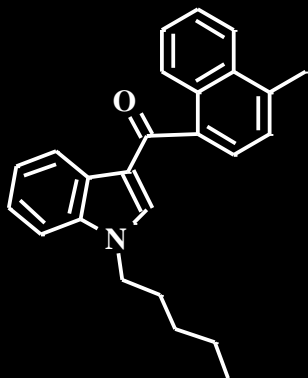
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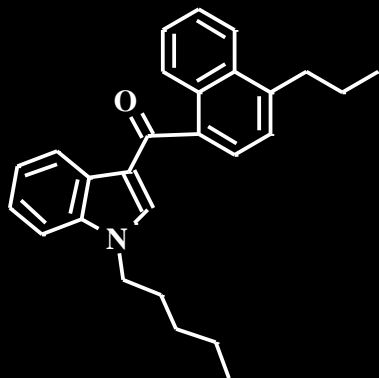
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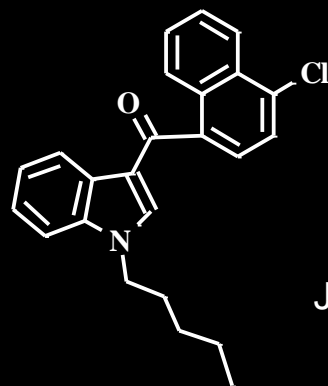
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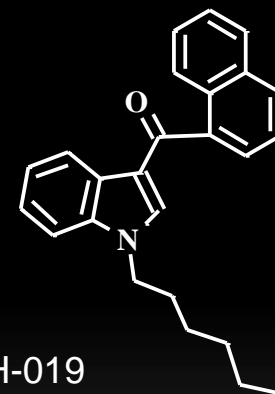
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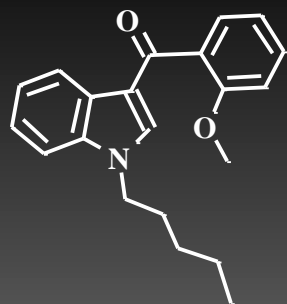
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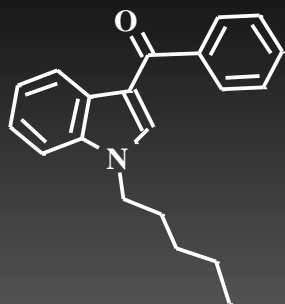
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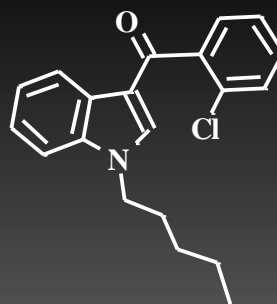
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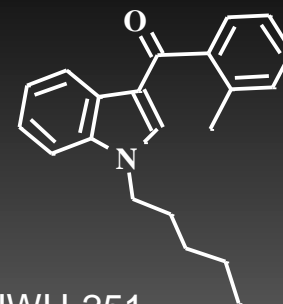
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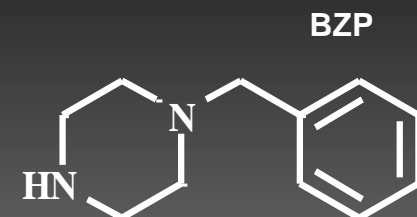
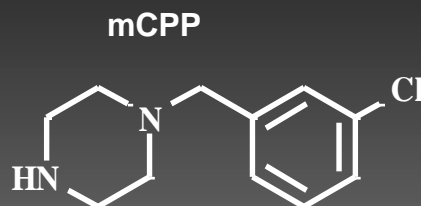
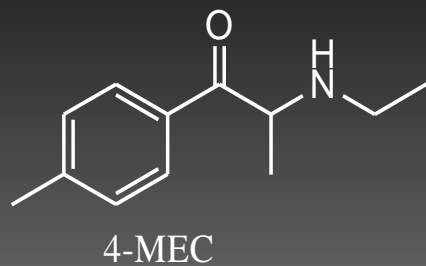
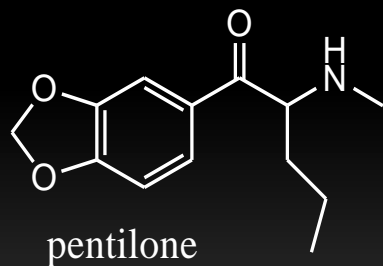
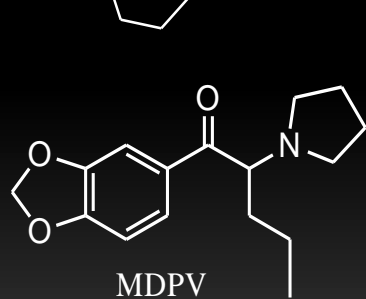
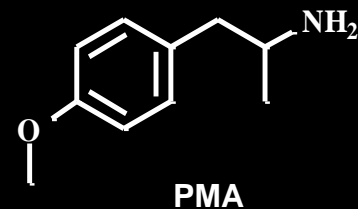
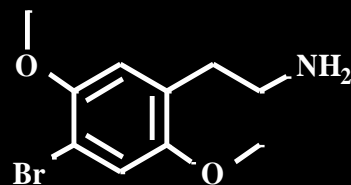
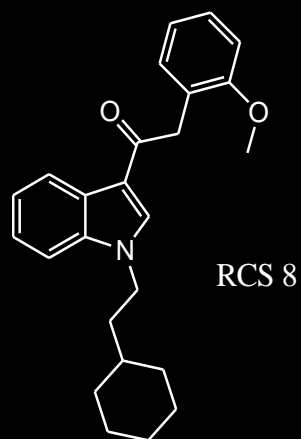
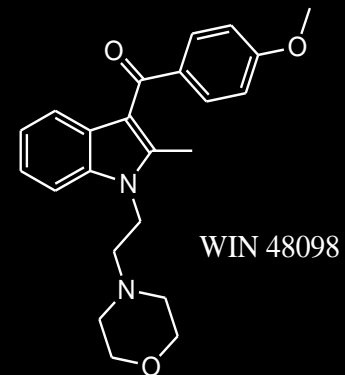
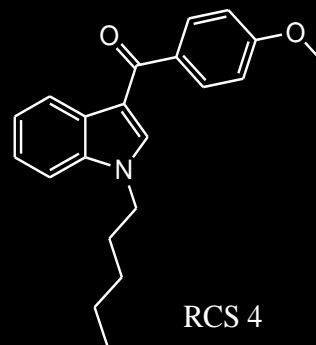
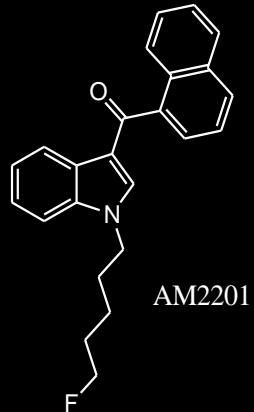
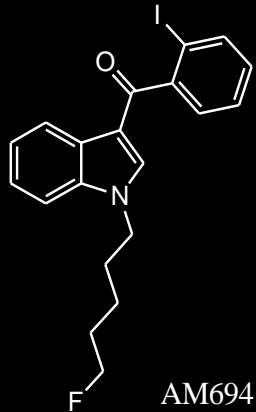
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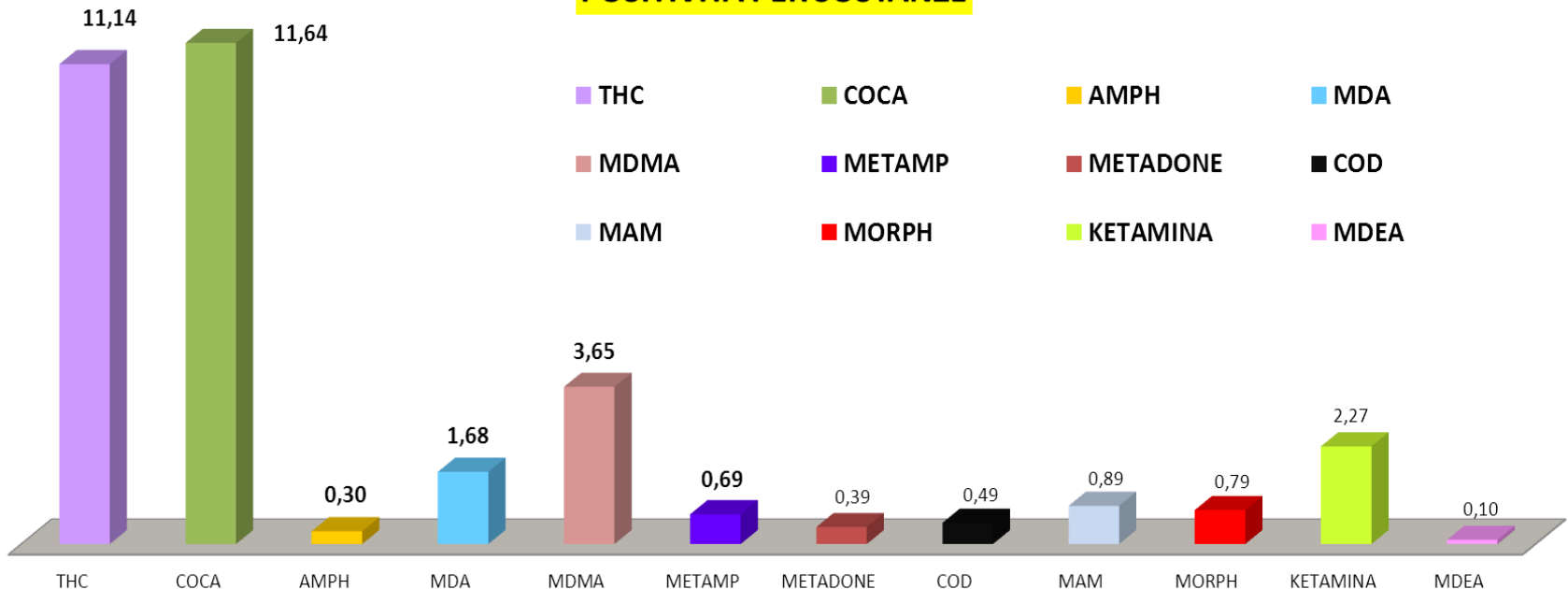
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Progetto Toxtest Studio sperimentale su sistemi di drug-testing rapidi

Positivi alla guida sotto l'effetto

POSITIVITÀ PER SOSTANZE



NSP: su 400 campioni
1 benzilpiperazina
1 MDPV
1 4-MEC

Progetto Smart Stop

attivazione di misure di controllo degli esercizi commerciali che vendono prodotti contenenti sostanze pericolose per la salute

Attività del Laboratorio di Tossicologia Forense:

- Identificazione di NSP in sequestri di sostanze incognite
- Identificazione di Steroidi anabolizzanti, stimolanti ed altre sostanze non riportate in etichetta in integratori alimentari.
- Collaborazione con il RIS di Roma, Colonnello Ripani, Maggiore Peluso, Tenente Colonnello Rossi

Come identificare le nuove sostanze?

TEST Preliminari



- ✓ SPOT test: molte NPS reagiscono (Liebermann, Marquis,) ma NON tutte

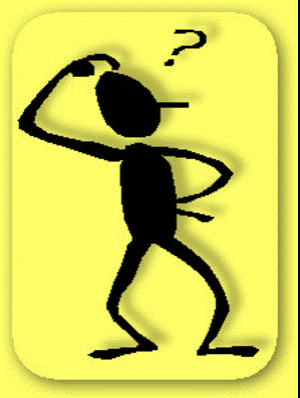


- ✓ On site kits: solo per alcune NPS



Come identificare con certezza le nuove sostanze?

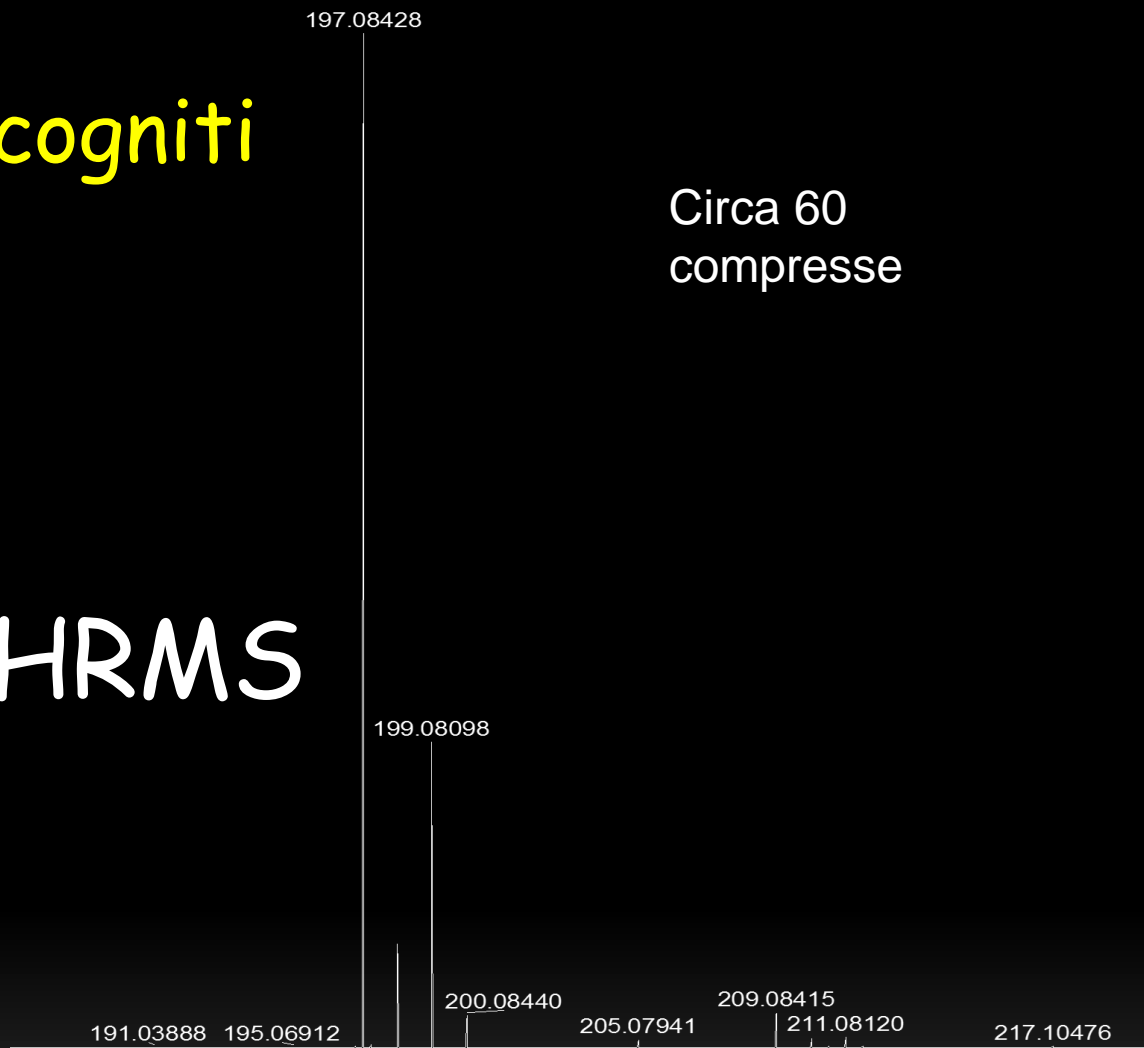
- ✓ GC o HPLC (tempo di ritenzione)
- ✓ MS (spettro di massa)
- ✓ GC/MS (tempo di ritenzione + spettro di massa)
- ✓ HRMS (massa accurata) → Formula bruta
(C_xH_xN_xO_x...)
- ✓ HRMSn → massa accurata dei frammenti
- ✓ NMR → struttura molecolare



Esempi di Reperti incogniti



HRMS



Circa 60
compresse

Formula bruta: $C_{10}H_{13}N_2Cl$



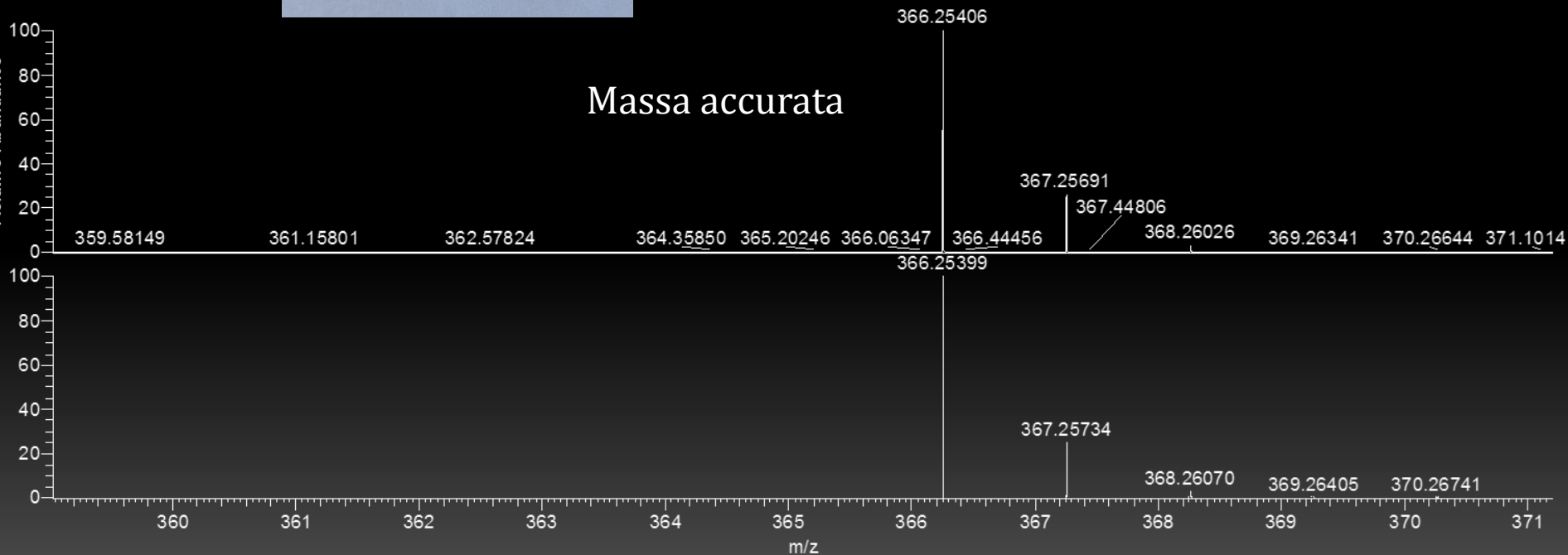
CPP



Segnalazione



Altri Esempi di Reperti incogniti, in collaborazione con il R.I.S.



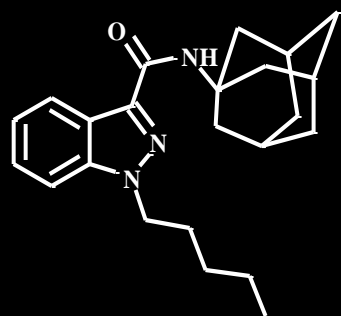
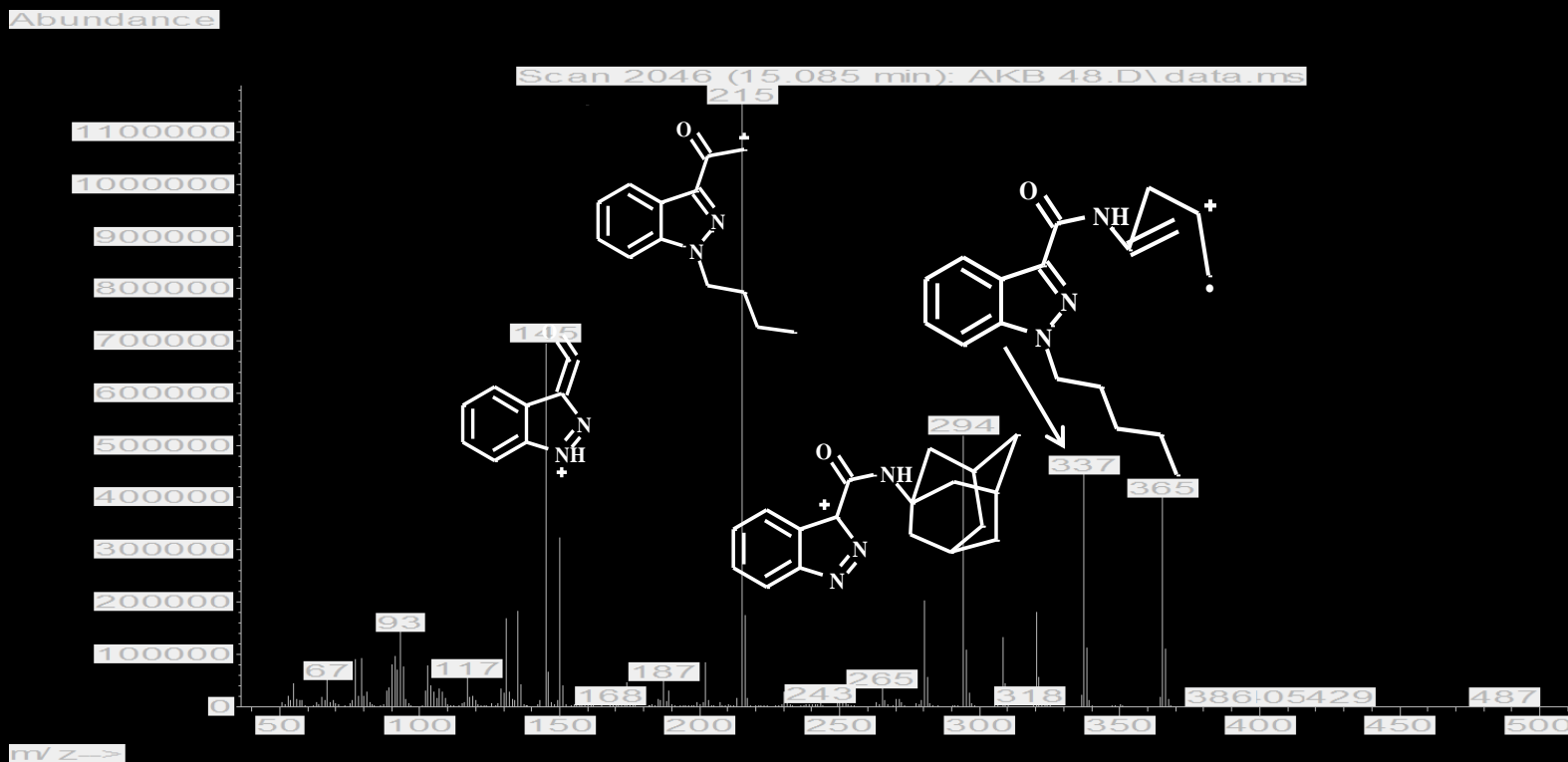
Formula bruta: $\text{C}_{23}\text{H}_{31}\text{N}_3\text{O}$

Frammentazione HRMSn



(...scarsa)

Spettro in GC-MS



M⁺ 365

+NMR

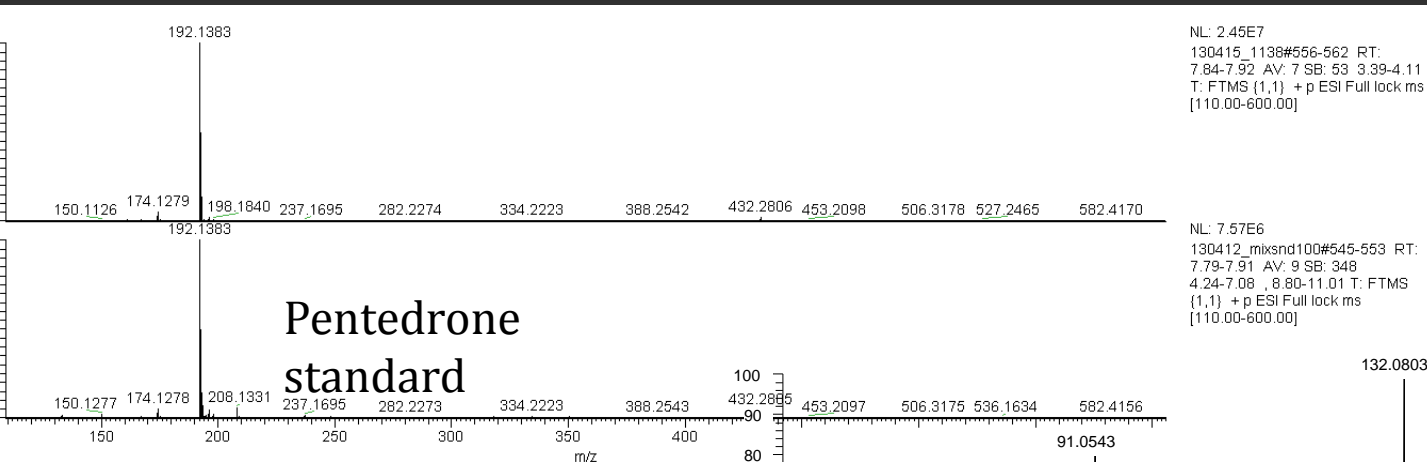


AKB48 o Apinaca



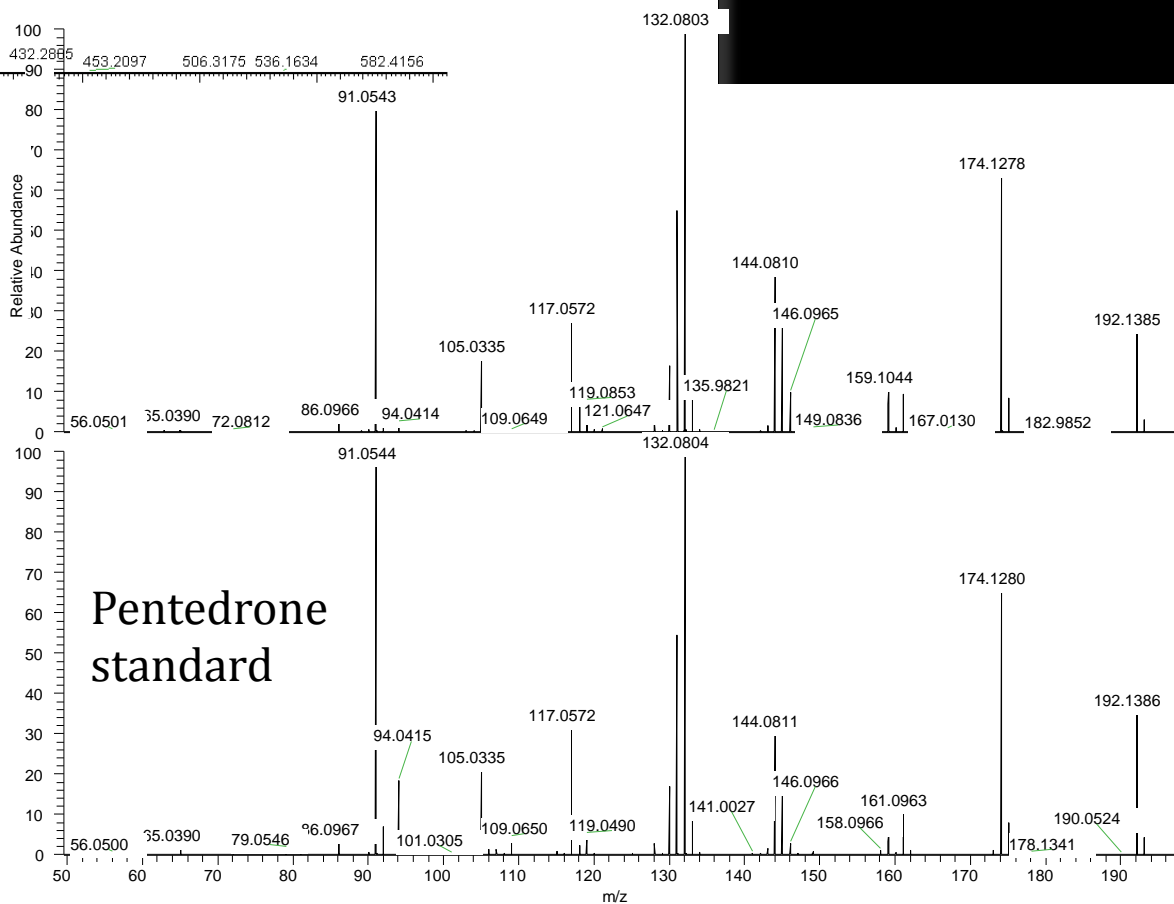
Segnalazione

Identificazione contro standard

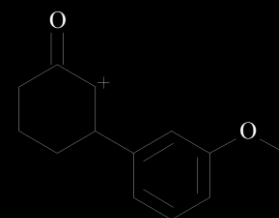
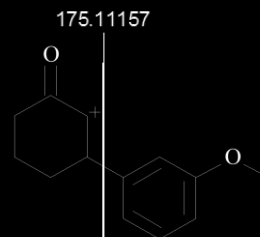
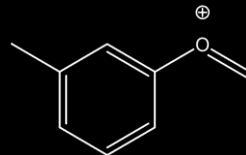
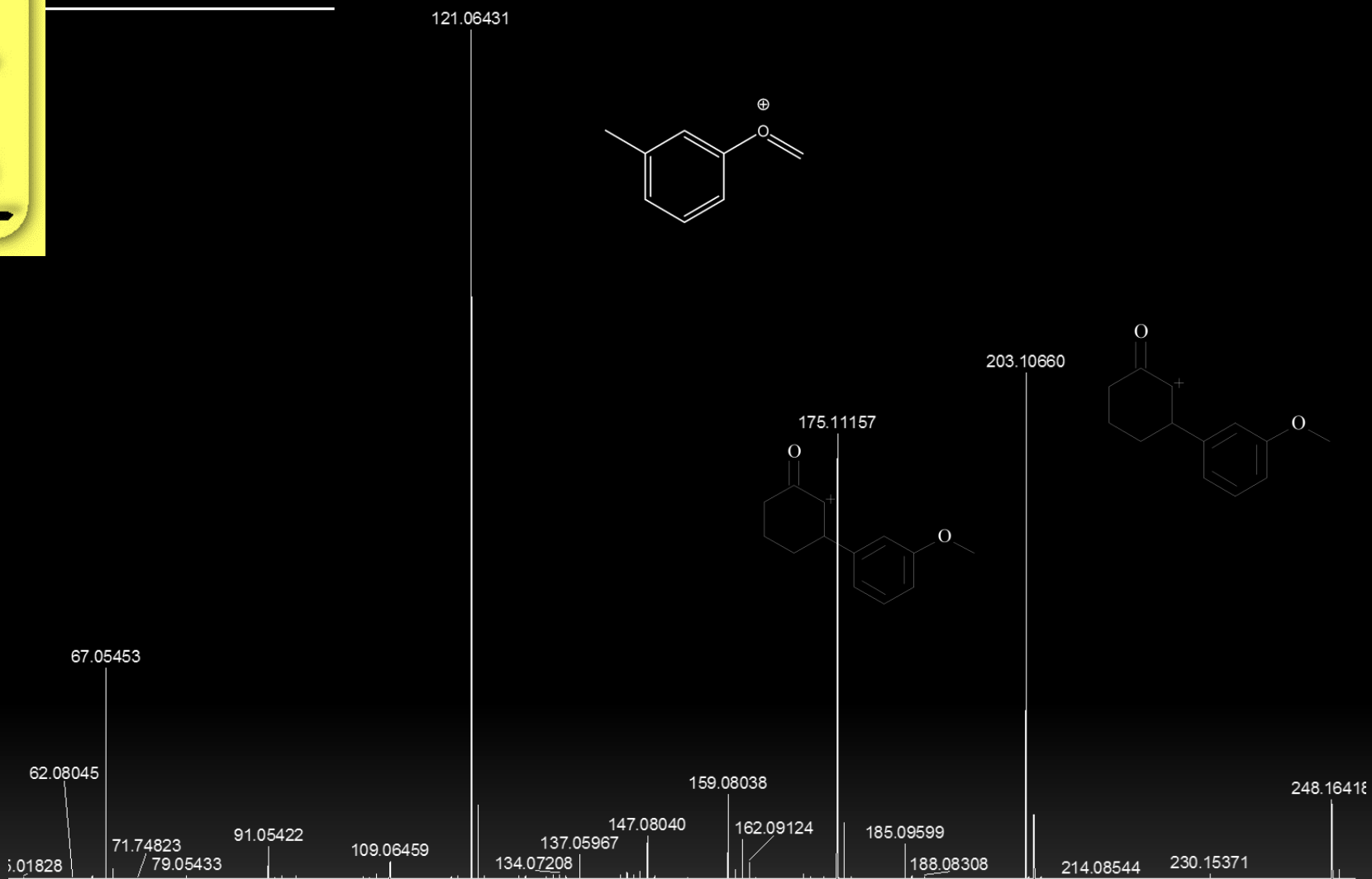


Pentedrone
standard

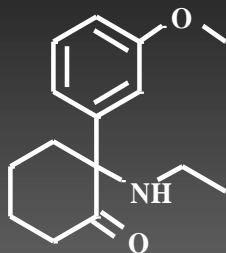
Formula bruta: $C_{12}H_{17}NO$
 MH^+ 192.1383



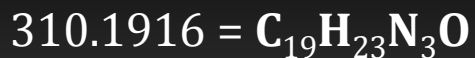
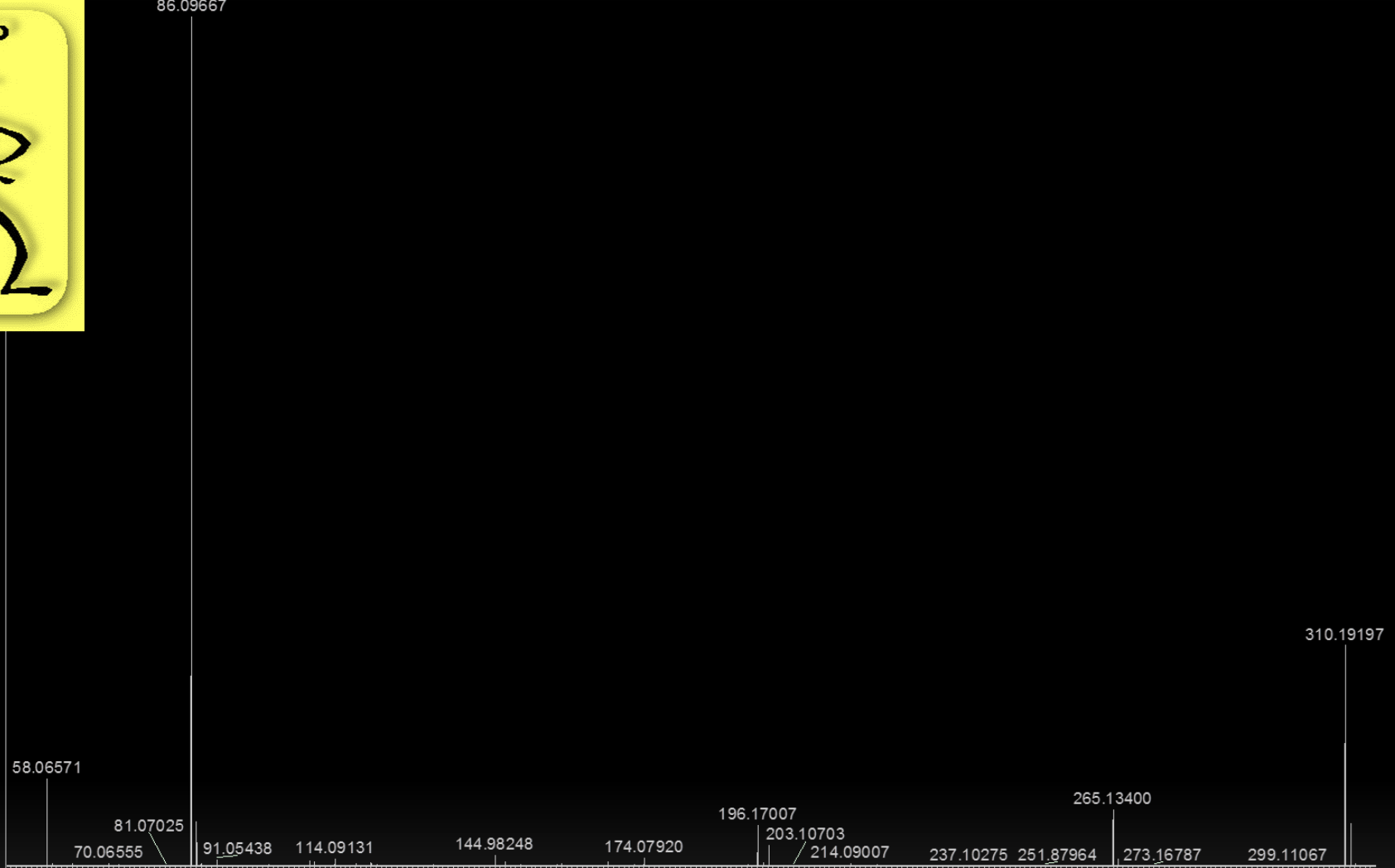
Pentedrone
standard



MH⁺ 248.1642 C₁₅H₂₁NO₂



methoxietamina



Benzidamina (Tantum verde, tantum rosa)

LA DENUNCIA DEI CENTRI ANTIVELENI DI MILANO-NIGUARDA E PAVIA

Bevono per sbaglio lavanda vaginale,
boom di intossicate. Sotto accusa lo spot

*Lo spot tv avrebbe tratto in inganno le consumatrici.
E ora si pensa di cambiare il colore della confezione*

...Benzydamine has been misused
recreationally. In overdosages it acts
a delirant and CNS stimulant....

Il campione biologico



▪ Sangue



▪ Parent drug + metaboliti

▪ Urine

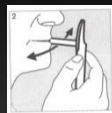
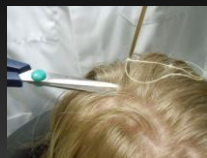


▪ **Metaboliti >> parent**

▪ Matrici alternative
(capelli, fluido orale)



▪ Parent drug (>> metaboliti)



Progetto VARD - Violence and date-rape drugs



DFSA

‘...occurs when a person (male or female) is subjected to sexual act(s) while they are incapacitated or unconscious due to the effect(s) of ethanol, a drug and/or other intoxicating substance, and as a result unable to resist or consent to such acts...’

(Costrizione ad atti sessuali non-consensuali favoriti da notevole riduzione o completa perdita di coscienza, causate dalla surrettizia somministrazione o talvolta volontaria assunzione di xenobiotici ad effetto neurodepressivo)



Guidelines for the Forensic analysis of drugs facilitating sexual assault and other criminal acts- United Nations Office on Drugs and Crime UNODC (Vienna, 2011)

DFSA – sostanze incapacitanti

- ✓ GHB, GBL, 1,4-BD, valerolactone
- ✓ Benzodiazepines
 - ✓ Alprazolam, Bromazepam, Chlordiazepoxide, Clobazam, Clonazepam, Clorazepate, Clotiazepam, Cloxazolam, Diazepam, Estazolam, Flunitrazepam, Loprazolam, Lorazepam, Lormetazepam, Medazepam, Midazolam, Nitrazepam, Nordiazepam, Oxazepam, Phenazepam, Prazepam, Temazepam, Tetrazepam, Triazolam
- ✓ Z-drugs (hypnotics)
 - ✓ Zaleplon, Zolpidem, Zopiclone
- ✓ Antihistamines and others
 - ✓ Antihistamines: Brompheniramine, Cetirizine, Chlorpheniramine, Cyclobenzaprine, Diphenhydramine, Doxylamine, Hydroxyzine, Niaprazine Others: Aceprometazine, Alimemazine, Amitriptyline, Chloral hydrate, Clonidine, Clozapine, Cyamemazine, Dextromethorphan, Haloperidol, Meprobamate, New Antidepressants, Oxomemazine, Valproic acid
- ✓ Barbiturates
 - ✓ Amobarbital, Barbital, Pentobarbital, Phenobarbital, Secobarbital
- ✓ Opiates and Opioids (licit narcotic analgesics)
 - ✓ Codeine, Dihydrocodeine, Hydromorphone, Methadone, Morphine, Oxycodone
- ✓ Street drugs and traditional drugs of abuse
 - ✓ Cannabinoids: THC, **Synthetic cannabinomimetics**
 - ✓ Opiates: Heroin, Morphine
 - ✓ Cocaine: Cocaine and crack cocaine
 - ✓ Amphetamines: Amphetamine, PMA, MBDB, MDA, MDEA, MDMA, Methamphetamine
- ✓ Others
 - ✓ Atropine, Ayahuasca, **Cathinone and cathinon derivatives**, Hallucinogenic mushrooms,
 - ✓ Kava-kava, Ketamine, LSD, Mescaline, Phencyclidine, **Piperazine group**, Poppers, Salvinorine A,
 - ✓ Scopolamine
- ✓ Ethanol

Rape Drugs are out there.

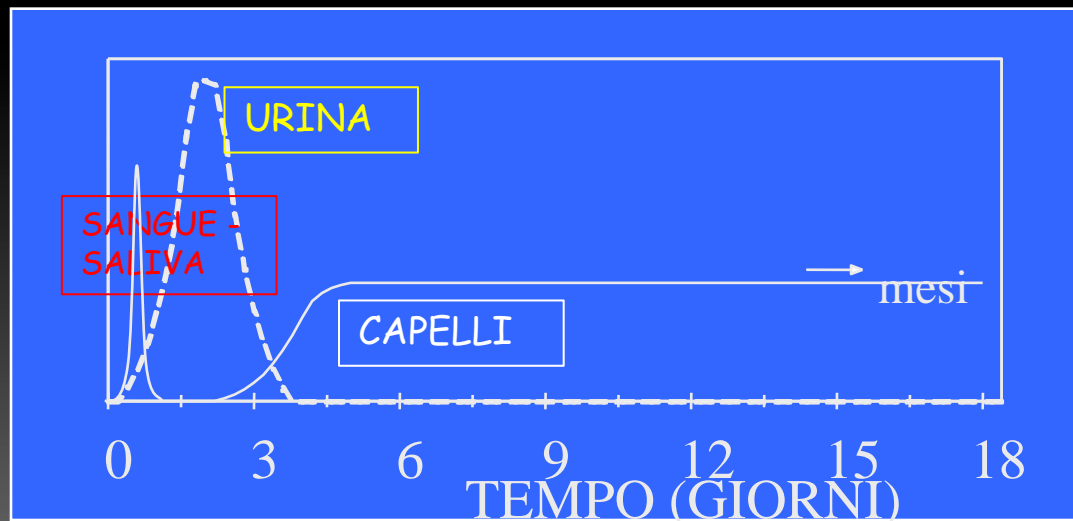


Are they in here?

Il campione biologico



- ✓ **L'urina è la matrice biologica di prima scelta nell'analisi delle sostanze incapacitanti e d'abuso**
- ✓ Nel caso in cui i fatti siano molto antecedenti al prelievo: capelli
 - ✓ Aspettare almeno tre settimane.
 - ✓ Effettuare il prelievo in modo da distinguere la sezione prossimale



Finestre temporali di rilevabilità

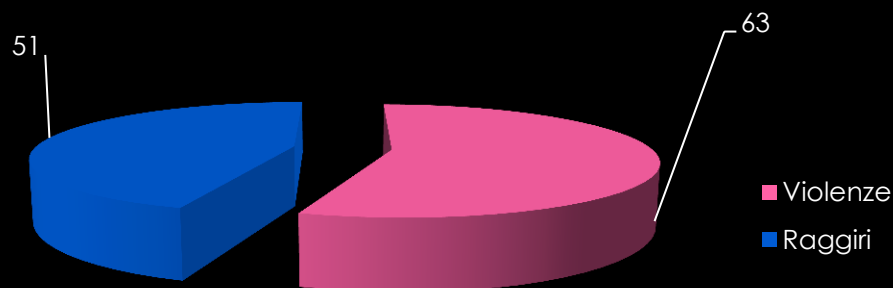
Zolpidem metaboliti



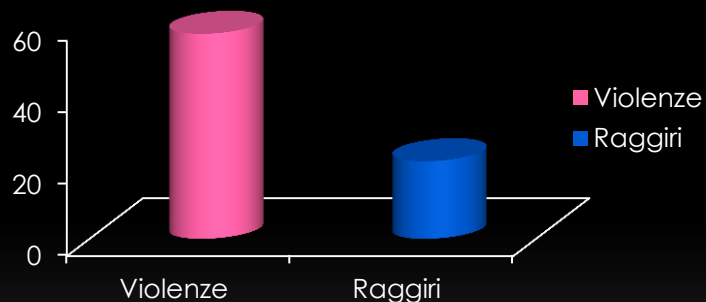
UHPLC-MS/MS and UHPLC-HRMS identification of zolpidem and zopiclone main urinary metabolites and method development for their toxicological determination. S. Strano-Rossi, et al. *Drug Test. Anal.* 2013 Mar 19. doi: 10.1002/dta.1470.

Risultati: 80 soggetti, 49 violenze, 31 raggiri

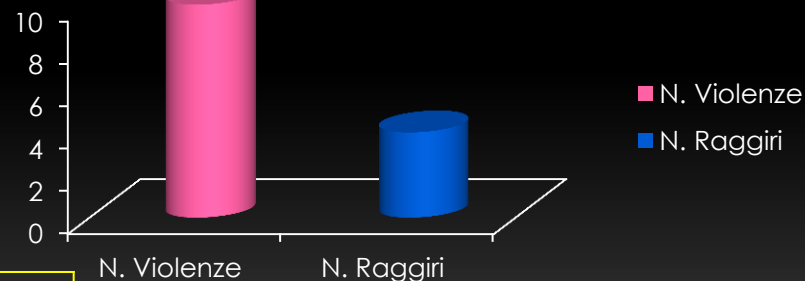
% Positività generale



% Positività per alcol



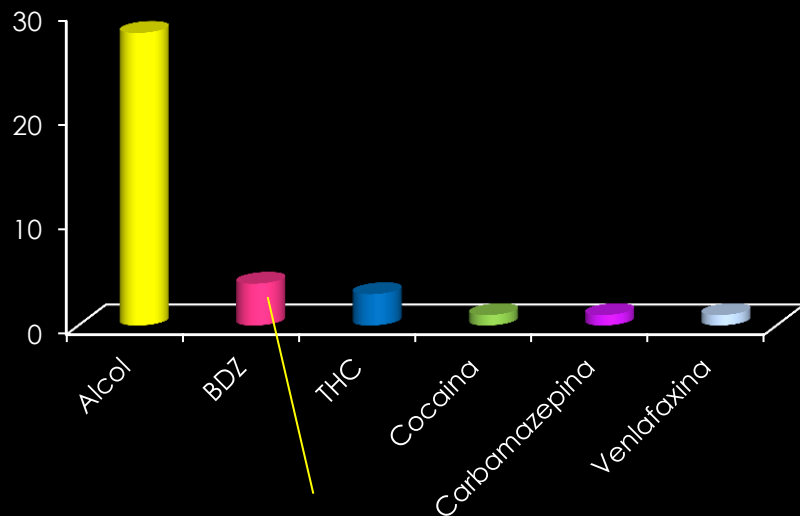
alcol+ altre sost



2 + BDZ
1 + Z
3 + THC
2 + coca
1 + carbamazepina
1 + venlafaxina

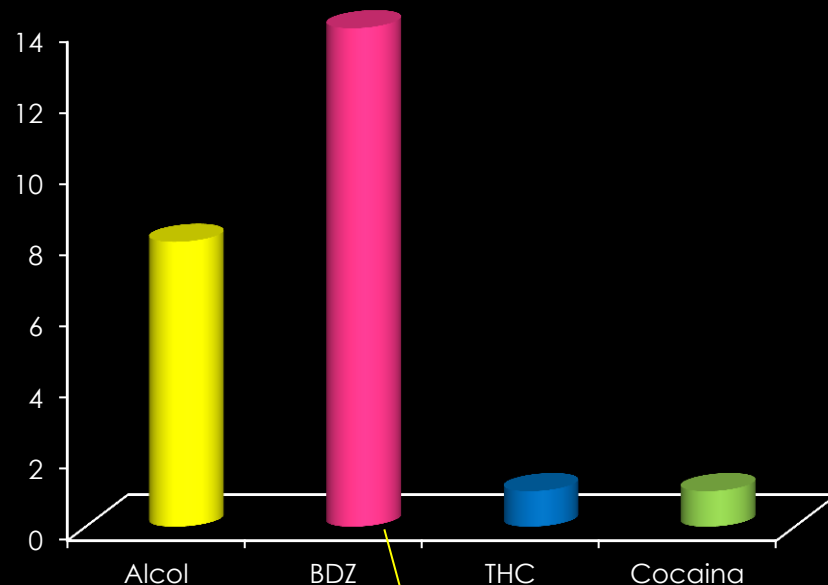
3 + BDZ
1 + THC

Sostanze rilevate nelle violenze




Temazepam + Nordiazepam + Oxazepam + Zolpidem e metaboliti
Clonazepam e 7NHClonaz + Lorazepam
Lorazepam + Delorazepam
Zolpidem e metab + Clonazepam e 7NHClonaz

Sostanze rilevate nei raggiri



2 casi lorazepam
7 casi di Clonazepam e/o 7NHClonaz
Lorazepam + Delorazepam
2 casi Zolpidem e metab
Bromazepam
Triazolam + metab

Come studiare il metabolismo?

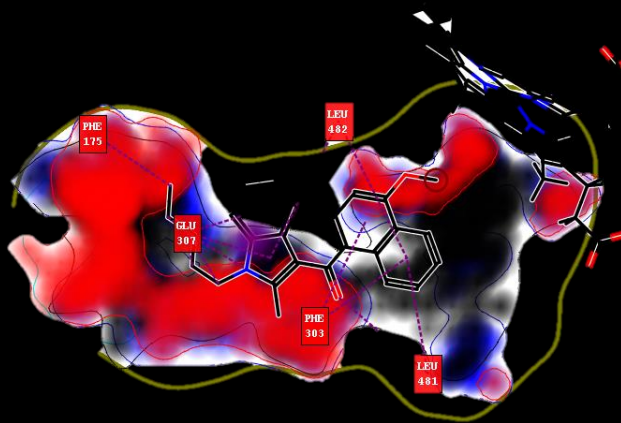
- Studi umani  (problemi etici)
- Studi su animali (problemi logistici ed etici)
- **Studi *In vitro* (utilizzando colture cellulari, frazioni microsomiali epatiche, enzimi ricombinanti)**



- **Studi *In silico* (predicendo tramite software i metaboliti e identificandoli in dati sperimentali)**

Studi *In silico*

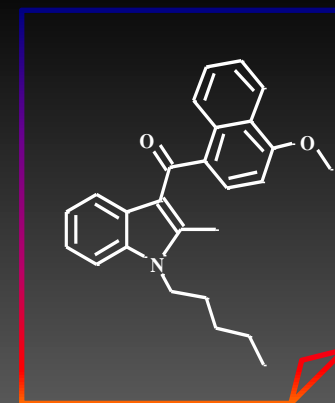
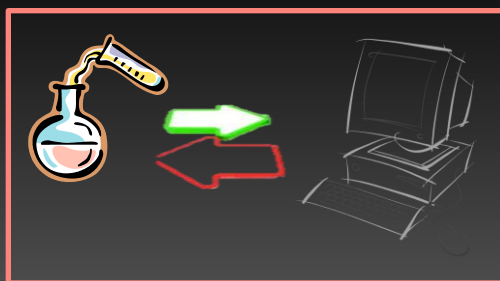
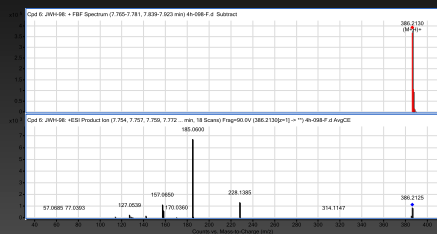
- **MetaSite** è un programma software che predice le biotrasformazioni metaboliche di fase I mediate dal citocromo P450.



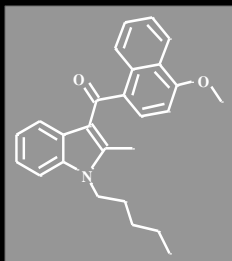
Il software attribuisce i più probabili siti di metabolismo (SoM), basandosi sulla sua struttura molecolare (sulla reattività dei siti), sulla sua possibile interazione con l'enzima (sull'ingombro sterico), e predice i principali metaboliti che possono quindi formarsi in diversi tessuti (fegato, pelle, cervello, polmoni).

Combinazione studi *in vitro/in silico* per l'interpretazione di datafiles in MS/MS

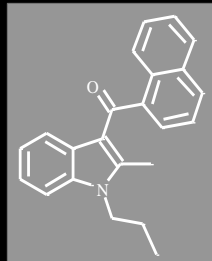
- **Mass-MetaSite** è un nuovo approccio per l'identificazione automatica dei metaboliti da datafiles di Cromatografia Liquida – Spettrometria di massa, riducendo i tempi di analisi manuale da varie ore a pochi minuti per composto. Il programma è in grado di assegnare le strutture chimiche ad ogni picco cromatografico rilevato, basandosi sulla massa accurata e sulla frammentazione ottenuta in MS/MS della molecola substrato e dei metaboliti



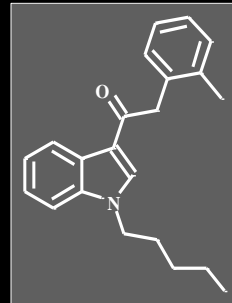
Studio Pilota



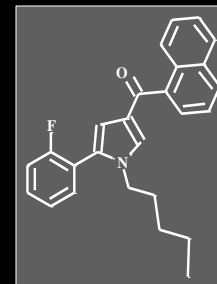
JWH-098



JWH-015

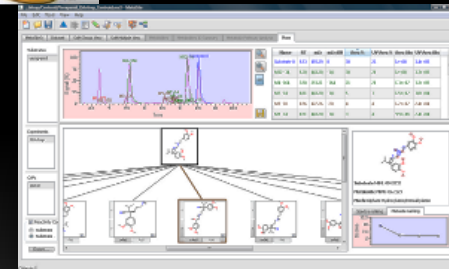


JWH-251



JWH-307

- ✓ Studiare metabolismo in vitro (su fettine di fegato di ratto)
- ✓ Analisi in HR con QTOF
- ✓ Uso MassMetaSite per una rapida elucidazione degli spettri ed attribuzione delle strutture dei metaboliti

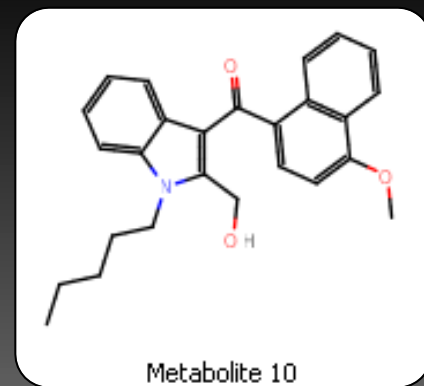
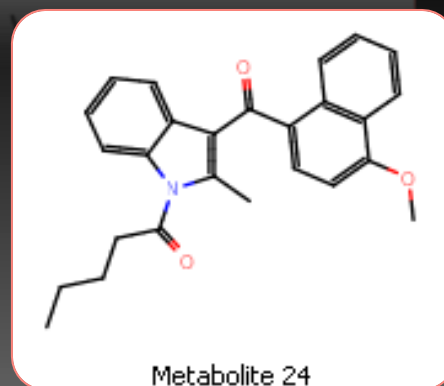
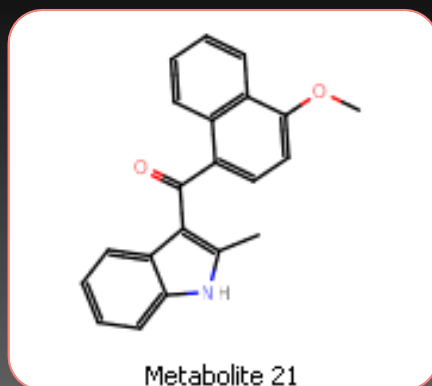
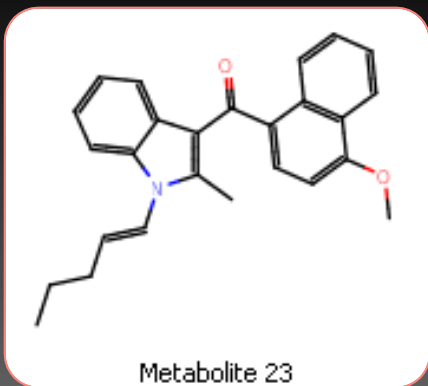
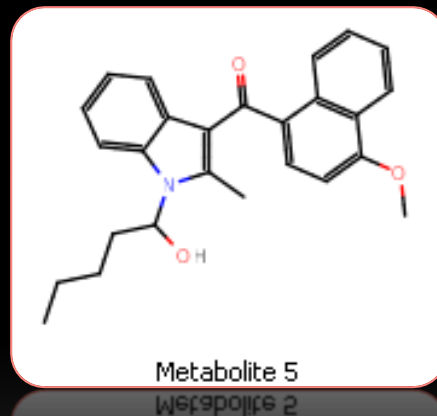
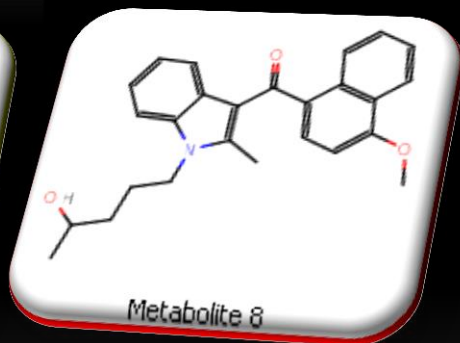
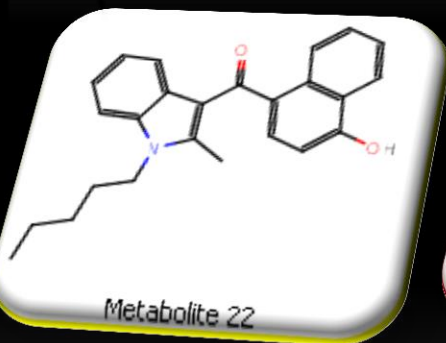
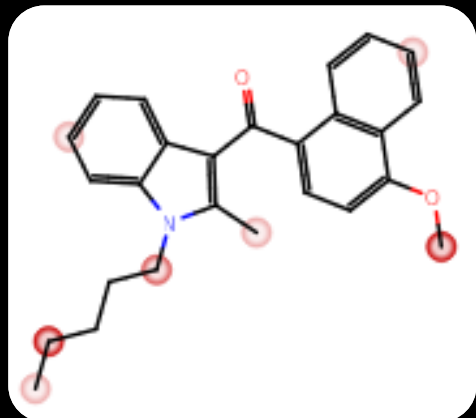


- ✓ Valutare la concordanza tra la predizione teorica ed i metaboliti rilevati
- ✓ Sviluppo di metodi per la ricerca dei corretti metaboliti nei campioni biologici

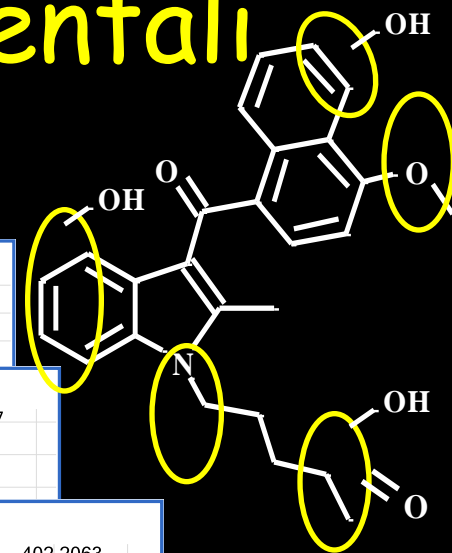
Es: JWH 098

Siti più probabili di metabolismo

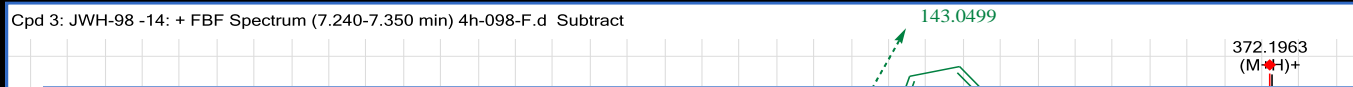
...e **principali metaboliti** predetti



JWH098: risultati sperimentali



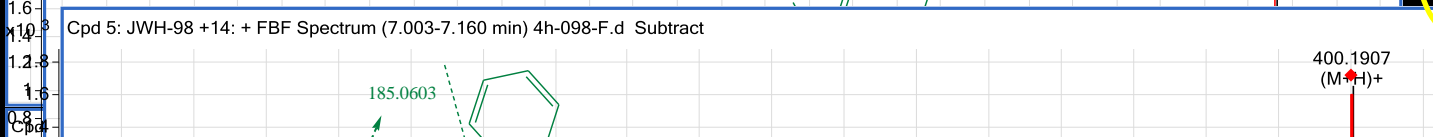
Cpd 3: JWH-98 -14: + FBF Spectrum (7.240-7.350 min) 4h-098-F.d Subtract



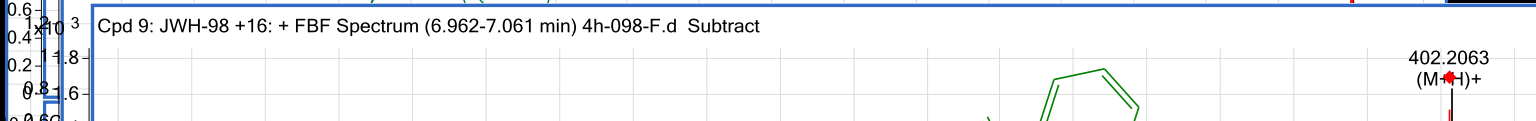
Cpd 1: JWH-98 -70: + FBF Spectrum (6.716-6.745 min) 4h-098-F.d Subtract



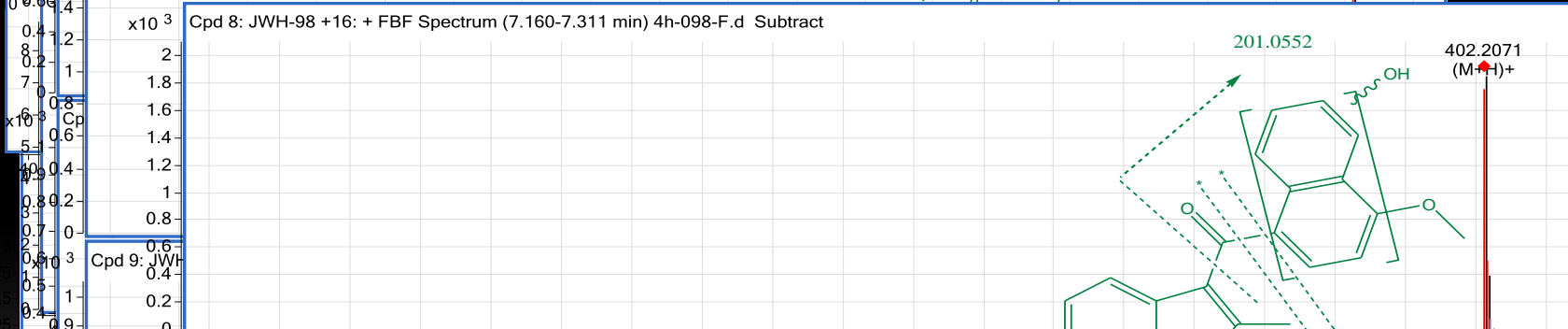
Cpd 5: JWH-98 +14: + FBF Spectrum (7.003-7.160 min) 4h-098-F.d Subtract



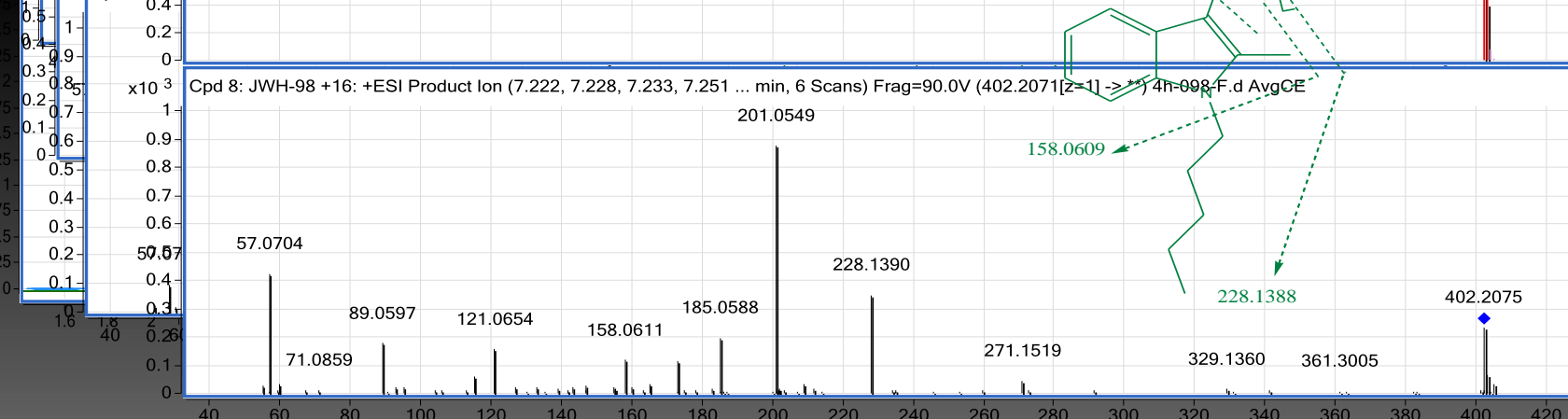
Cpd 9: JWH-98 +16: + FBF Spectrum (6.962-7.061 min) 4h-098-F.d Subtract



Cpd 8: JWH-98 +16: + FBF Spectrum (7.160-7.311 min) 4h-098-F.d Subtract



Cpd 9: JWH-98 +16: +ESI Product Ion (7.222, 7.228, 7.233, 7.251 ... min, 6 Scans) Frag=90.0V (402.2071 [Z=1] -> *) 4h-098-F.d AvgCE



CONCLUSIONI

Esempi di attività di ricerca di un centro collaborativo del sistema di allerta rapida sulle droghe

- ✓ Possibilità di identificare con certezza le nuove sostanze psicoattive nei reperti non biologici tramite strumenti estremamente sofisticati, ma anche con l'ausilio di strumentazioni "classiche"
- ✓ *Possibilità di identificare le NSP nei fluidi biologici, anche se metabolizzate, grazie anche a studi di predizione dei metaboliti In silico e la successiva identificazione dei metaboliti tramite software ad hoc (MassMetaSite)*

...passi avanti nella lotta all'avvento sul mercato clandestino di nuove sostanze



The word "Grazie" is written in a cursive, handwritten style in blue ink. A single horizontal line is drawn underneath the word, extending from the start of the 'G' to the end of the 'e'.

- Dipartimento Politiche Antidroga
- Prof. Marcello Chiarotti
- Prof. Vincenzo Pascali
- Luca Anzillotti, Sara Odoardi, Erika Castrignanò
- **A tutti voi per l'attenzione!**