New Drugs - Update sulle nuove sostanze psicoattive – NSP

Aggiornamento tecnico scientifico e strategia nazionale di intervento

Venezia Mestre, 29 Ottobre 2013

Il Laboratorio di Igiene Ambientale e Tossicologia Forense (LIATF) e le Nuove Sostanze Psicoattive

Giampietro Frison

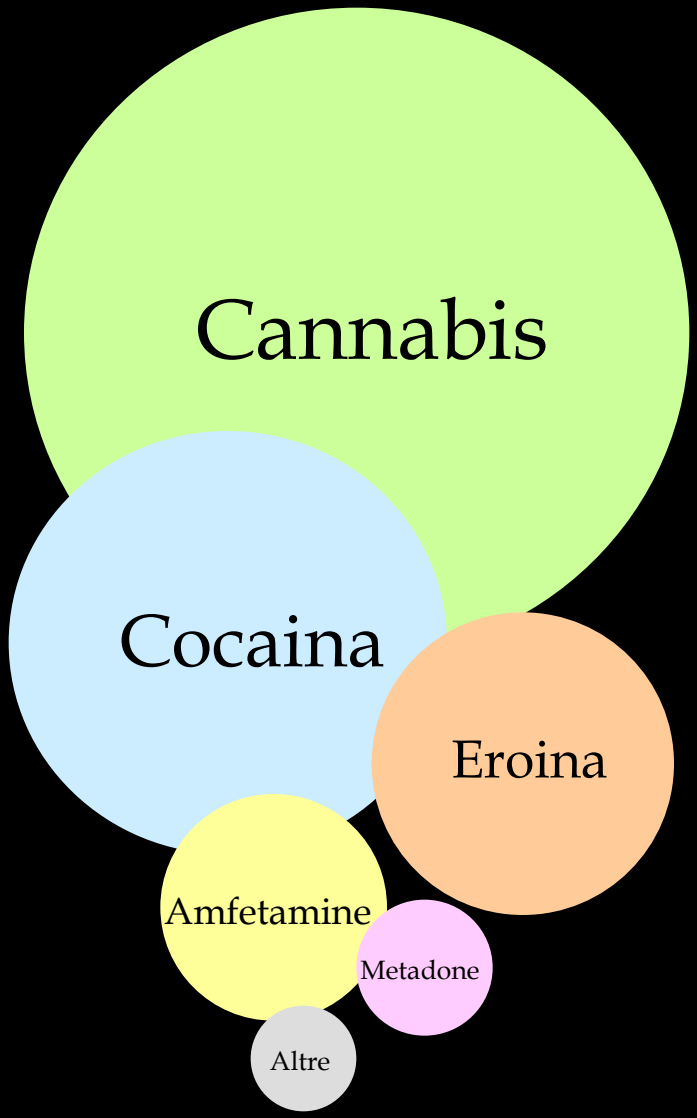
Laboratorio di Igiene Ambientale e Tossicologia Forense

Dipartimento di Prevenzione

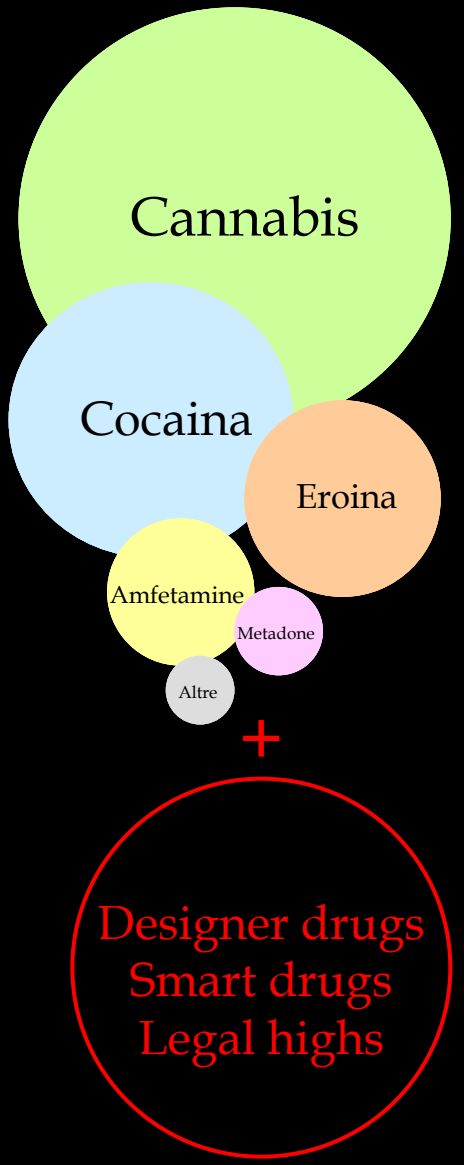
Azienda ULSS 12 Veneziana

Da droghe “classiche” a “nuove” droghe

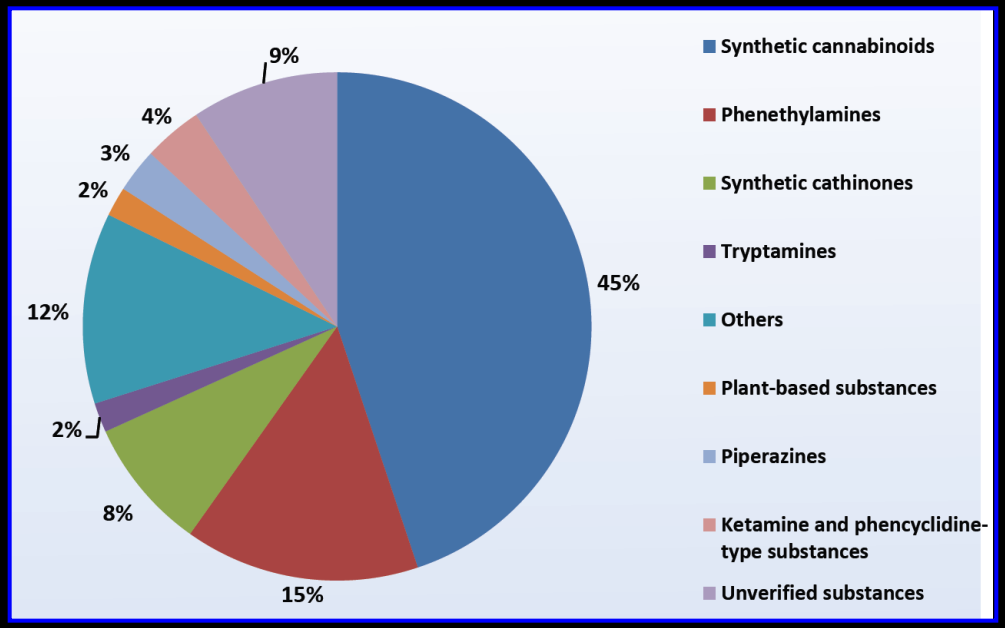
Fino a qualche anno addietro ……

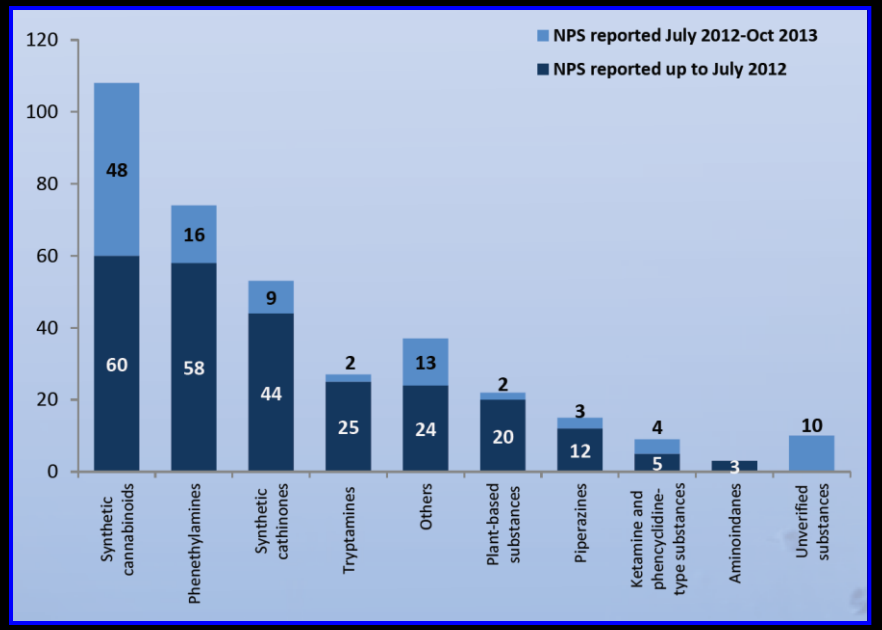


Da qualche anno a questa parte ……



L'aumento delle nuove sost. psicoattive





Nel corso del 2013 si è assistito ad un notevole incremento del numero di segnalazioni di NPS (oltre 350 NPS segnalate da 89 stati membri).
La maggioranza delle NPS è costituita da Cannabinoidi sintetici (170, 40%), Feniletilammine (89, 21%), e Catinoni (73, 17%).

United Nations Office on Drugs and Crime (UNODC)
Early Warning Advisory on New Psychoactive Substances (NPS) - October 2013 update

LIATF e Nuove Sost. Psicoattive (2010)

REGIONE DEL VENETO
AZIENDA ULSS 12 VENEZIANA
DIPARTIMENTO DI PREVENZIONE
Laboratorio di Igiene Ambientale e Tossicologia Forense

in collaborazione con LGC Standards
Excellence through measurement

SEMINARIO

Nuove Sostanze Psicoattive
Nuove Modalità di Consumo
Nuove Esigenze di Controllo

Giovedì 27 maggio 2010
ore 14.00



Ospedale dell'Angelo, Mestre (VE)

Aula Viola
Padiglione Rama



Programma

***Ore 14.00**: Apertura lavori e saluto degli organizzatori*

*Nuove sostanze psicoattive: Spice, N-Joy, Beta-cheto-amfetamine, MDAI, Piperazine, etc.**
***Ric Treble** (LGC Forensics)*

I nuovi mezzi di diffusione: Smart-shops e Internet
***Domenico Diano** (LGC Standards)*

Aspetti normativi delle nuove sostanze in Europa e in Italia
***Giampietro Frison** (LIATF, AULSS12)*

Coffee Break

*Nuove sfide ai laboratori di Tossicologia Clinica e Forense**
***Keith Williams** (LGC Standards)*

*MDAI: la nuova Ecstasy? **
***Andreas Sieg** (LGC GmbH)*

*Validazione di metodi analitici in Tossicologia Forense**
***Ric Treble** (LGC Forensics)*

**relazione in lingua inglese*

La partecipazione al Seminario è gratuita

Destinatari: medici di pronto soccorso, medici legali, medici del lavoro, medici dei servizi per le dipendenze patologiche, medici specialisti in farmacologia e tossicologia clinica, chimici, biologi, tecnici di laboratorio, tecnici della prevenzione.

Per motivi organizzativi è necessario comunicare la partecipazione via fax (041-2608732) o via email (giampietro.frison@ulss12.ve.it) compilando l'apposita scheda di adesione.
Su specifica richiesta verrà rilasciato un attestato di partecipazione.

Ric Treble lavora per LGC (*The Laboratory of the Government Chemist*) dal 1972. Dal 1990, dopo aver lavorato in molte aree di laboratorio, ricopre il ruolo di Capo della Sezione Antidroga di LGC, e successivamente anche della sezione di testing per i settori civili e militari. Attualmente è Scientific Advisor sia per LGC Forensics, con particolare focus nei settori DNA e droghe di abuso, sia per LGC Standards, dove collabora per l'identificazione dei requisiti degli standard di riferimento per le droghe legali.
E' membro del *Technical Committee of the UK's Advisory Council on the Misuse of Drugs* e del *United Kingdom Steering Group on Legally Defensible Workplace Drug Testing*. Grazie alla notevole esperienza in ambito qualità, dopo aver collaborato per il *United Kingdom Accreditation Service (UKAS)*, ha organizzato numerosi corsi di formazione in ambito qualità per il Regno Unito e per molti paesi europei, pubblicando numerosi articoli correlati.

Domenico Diano inizia la propria attività nel 2001 come Product Specialist per il settore della emocoagulazione e chimica clinica. Assunto il ruolo di Field Engineering, ha collaborato negli studi di procedure sperimentali in coagulazione, in particolar modo nello studio di una nuova metodica di analisi per la Sepsi e per le mutazioni congenite di Antitrombina III, ottimizzando le fasi operative strumentali ed i reagenti collegati. Nel 2008 passa ad LGC Standards estendendo la sua attività a tutti i settori analitici in genere, con particolare focus a quelli Farmaceutico, Fitochimico, Forense e Ambientale.

Giampietro Frison è stato, sino a novembre 2009, Dirigente Chimico presso l'Unità Operativa di Tossicologia Forense e Antidoping dell'Azienda Ospedaliera di Padova. Da dicembre 2009 è Dirigente Chimico presso il Laboratorio di Igiene Ambientale e Tossicologia Forense del Dipartimento di Prevenzione – Azienda ULSS 12 Veneziana. I suoi ambiti di interesse riguardano la Tossicologia Forense, Clinica, Postmortale, Analitica con particolare riferimento a: determinazioni di alcol etilico, sostanze psicoattive, farmaci in campioni biologici (urina, sangue, saliva, umor vitreo, formazioni pilifere, tessuti) in tema di diagnosi delle intossicazioni acute e croniche di rilevanza clinica e forense, disabilità e idoneità alla guida, idoneità al lavoro, mediante impiego di varie tecniche estrattive (LLE, SPE, SPME) ed analitiche (Immunochimiche, Spettrofotometriche, Cromatografiche-Spettrometriche di Massa). E' autore di svariate pubblicazioni in riviste scientifiche nazionali e internazionali e membro del Gruppo Tossicologi Forensi Italiano, della International Association of Forensic Toxicologists (TIAFT), della European Society of Hair Testing.

Keith Williams inizia la carriera in LGC come capo della divisione Analytical Toxicology nel 1997. Acquisisce un'esperienza di oltre 20 anni sulle analisi delle droghe in matrici biologiche presso il Dipartimento di Medicina Legale, Università di Dundee (Responsabile tossicologo) e Dipartimento di Medicina Legale e delle Scienze, Università di Glasgow (tossicologo). Ha una vasta esperienza in cromatografia e spettrometria di massa per la rilevazione di sostanze stupefacenti. Keith ha messo a punto, validato e applicato nuove metodologie per l'analisi di molti analiti in molti tipi di matrice tra cui capelli e saliva. E' collaboratore per il tribunale in numerose occasioni sia per accusa che per difesa, per le analisi e l'interpretazione di alcol e droghe nei liquidi corporei. Attualmente è Key Account Manager e Product Manager all'interno di LGC Standards per la divisione Forense, Fitochimica e Clinica.

Andreas Sieg ha iniziato la sua carriera nel 1999 in LGC, come Chimico dedicato alla sintesi delle impurezze farmaceutiche per MIKROMOL, acquisita dal gruppo LGC nel 2003, diventando Responsabile delle attività di analisi nel 2001. Nel 2006 assume il ruolo di Responsabile della Qualità per poi ricoprire quello di Product Manager supervisionando la qualità analitica di circa 2600 molecole essenziali per le attività del settore farmaceutico.

Per informazioni 041 260 8746 - 8773
Per informazioni su come arrivare è possibile consultare il sito:
http://www.ulss12.ve.it/ (vedi: sedi ospedaliere, Mestre)

Il LIATF e le nuove sostanze psicoattive











Aumento di sequestri giudiziari (polveri, compresse, capsule, ...)

Aumento di casi di intossicazione acuta

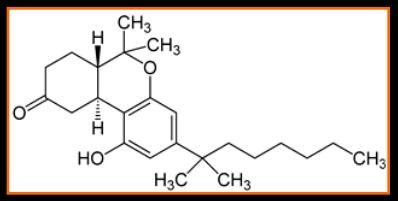
Necessità di identificare celermente le nuove sost. psicoattive, pur con scarsa disponibilità di standards (p/m) di riferimento





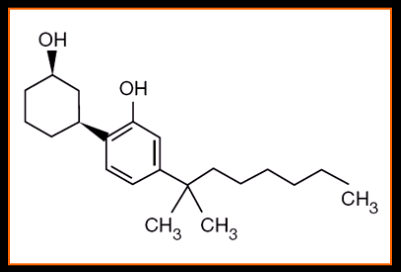

Cannabinoidi sintetici (Spice drugs)

DIBENZOPYRANES: "classic" cannabinoids, Δ9-THC analogues



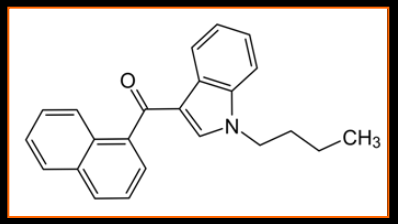
Hebrew University of Jerusalem, 1960 - **HU210**
Nabilone, Dronabinol

CYCLOHEXYLPHENOLS and alkyl- analogues



CP59,540 – CP47,497 and alkyl- analogues
Pfizer, 1980

AMINOALKYLINDOLES: JWH series



John W. Huffmann, Clemson University, South Carolina, 1990

1. Naphthoylindoles **JWH-018, JWH-073, JWH-398**
2. Naphthylmethylindoles
3. Naphthopyrroles
4. Naphthylmethylindenes
5. Phenylacetylindoles **JWH-250**

Cannabinoidi sintetici analizzati al LIATF

- ✓ JWH-018
- ✓ su matrice vegetale (1-3%)
- ✓ in polvere (5–70%)
- ✓ puro (97%, rep. 900 g)





Cannabinoidi sintetici analizzati al LIATF

Maggio 2012





- JWH-018





- JWH-122
- JWH-307

Cannabinoidi sintetici analizzati al LIATF



✓ **B-52 plus**

Intensive Shot

profumatore per ambienti a base di foglie e fiori secchi con aromi.

Tenere fuori dalla portata dei bambini.
Non ingerire. Non inalare.
In caso di ingestione consultare il medico.

Uso: svuotare il contenuto in una ciotola e posizionarla in armadi,cassetti o in auto. Prodotto ideale per profumare piccoli ambienti.

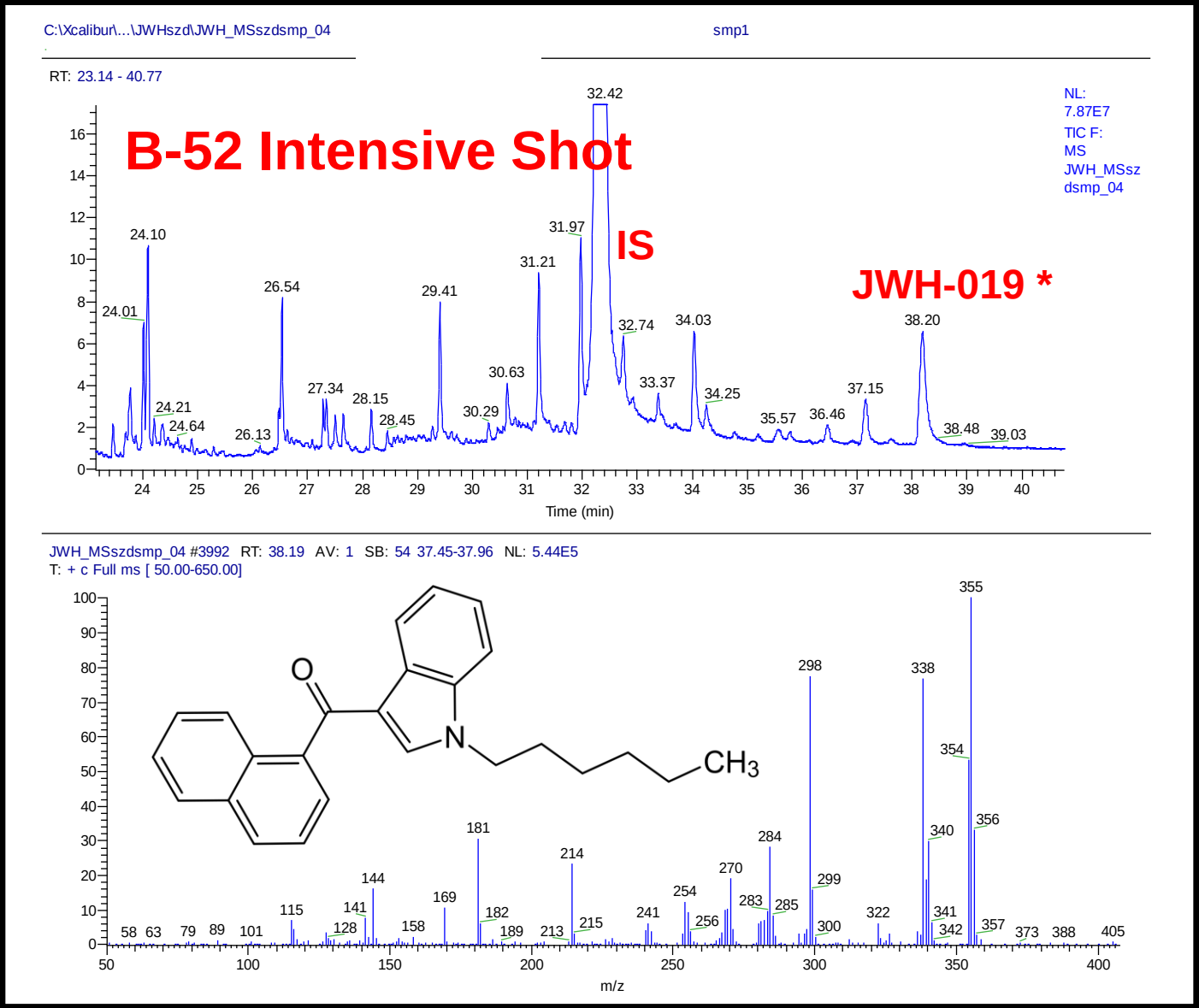
Si declina ogni responsabilità in caso di usi diversi da quelli descritti in etichetta.

Prodotto impacchettato per volume soggetto a naturale diminuzione di peso.

Peso netto:2ge
PRODOTTO NON PER CONSUMO UMANO

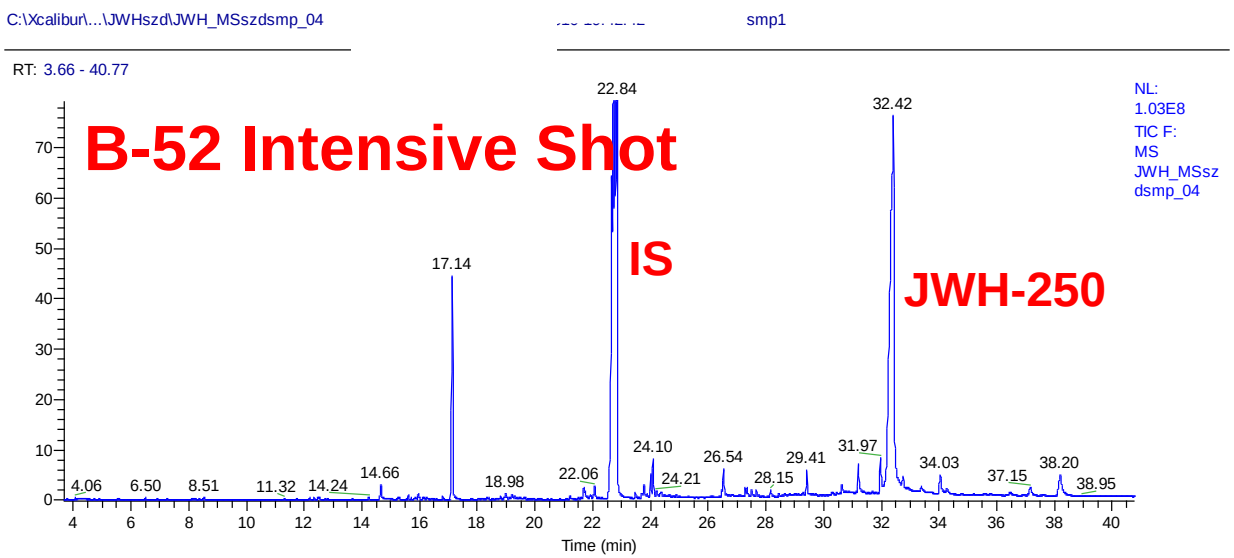
Distribuito da IDEA BRANDS MANAGMENT sro Praga

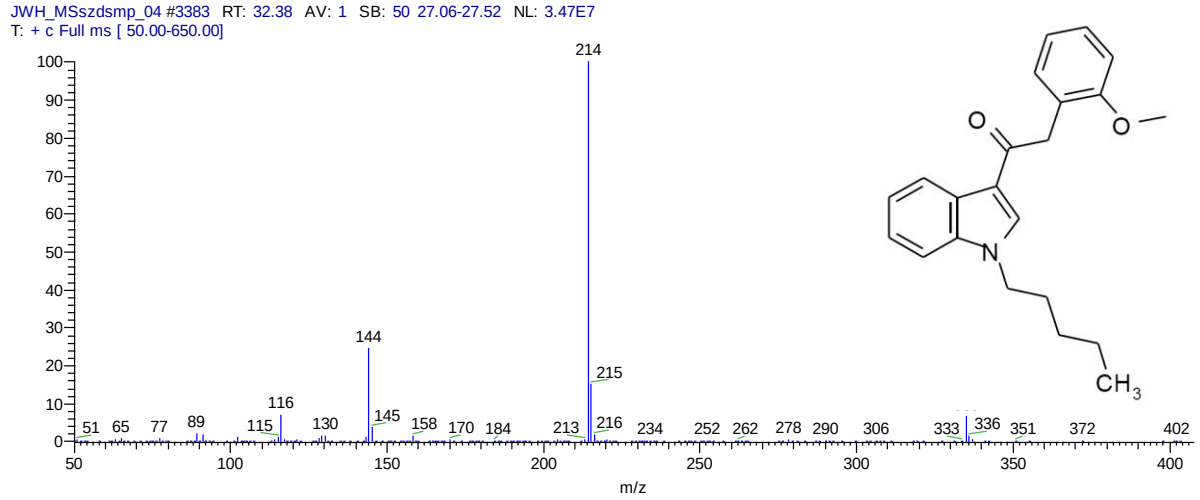
GC/MS di B-52 Intensive Shot



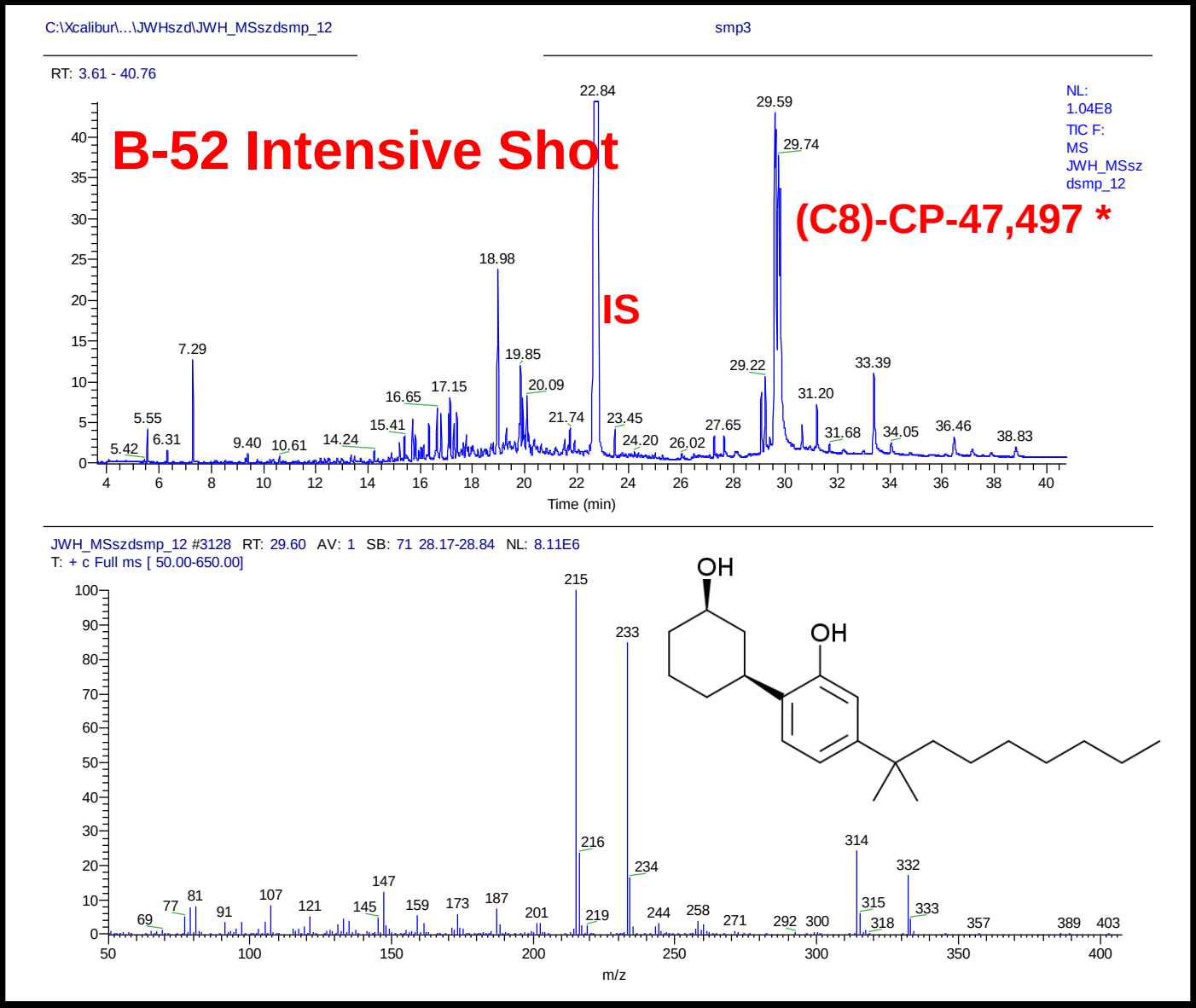
* Hexyl- omologue of JWH-018, contained in Sencation too

GC/MS di B-52 Intensive Shot





GC/MS di B-52 Intensive Shot



* 1,1 Dimethyl octyl- omologue of CP-47,497

Cannabinoidi sintetici analizzati al LIATF

Maggio 2012





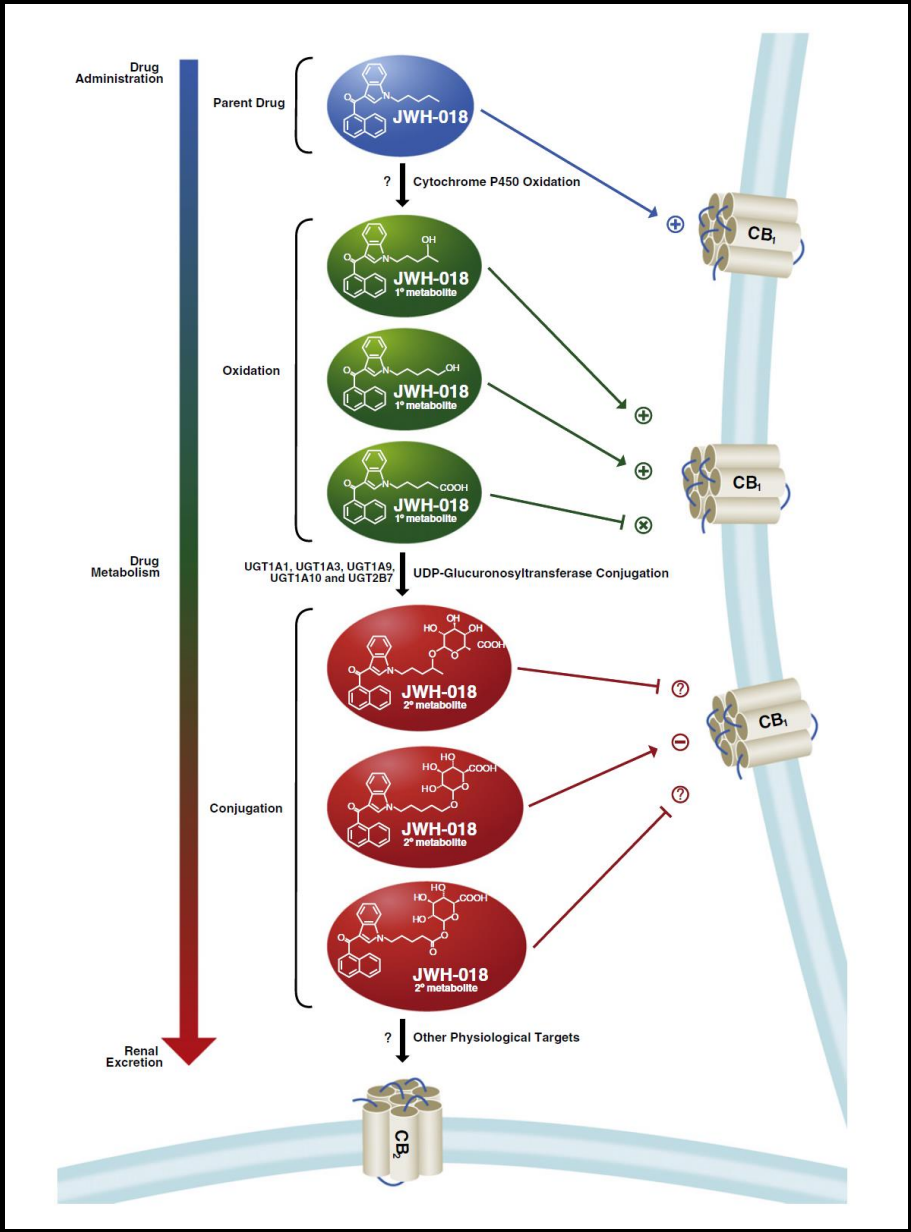
✓ Nelle stesse bustine 4 diversi Cannabinoidi sintetici!!!

JWH-018, JWH-073, JWH-015, JWH-302/250

Affinità per recettori CB1/CB2 del THC

	Δ-9-THC	HU-210	CP 47, 497	JWH-018	JWH-073	JWH-398	JWH-250
Famiglia/ gruppo	Dibenzopirano naturale	Dibenzopirano (Cannabinoide "classico")	Cicloesilfenolo	Naftoilindolo	Naftoilindolo	Naftoilindolo	Fenilacetilindolo/ benzoilindolo
Sottogruppo		Analogo del Δ9-THC		1-alchil-3-(1-naftoil) indolo	1-alchil-3-(1-naftoil) indolo	3-(4-alo-1-naftoil)indolo	1-pentil-3-fenilacetilindolo
Potenza e selettività	Riferimento. Agonista parziale del CB1	Agonista non-selettivo dei recettori CB1/CB2	Potente agonista selettivo dei recettori CB1	Agonista selettivo estremamente potente dei recettori CB2 (potente agonista anche dei CB1)	Potente agonista dei recettori CB1 (più debole agonista dei CB2)	Agonista non selettivo molto potente dei recettori CB1/CB2	Agonista selettivo dei recettori CB1 molto potente (agonista più debole dei recettori CB2)

Affinità JWH-018 per recettori CB1/CB2



- JWH-018 attivo su recettori CB1
- Metaboliti primari di JWH-018 attivi su recettori CB1
- Metaboliti THC inattivi!

Effetti avversi Cannabinomimetici

East Asian Arch Psychiatry, 2012

Acute mental disturbance caused by synthetic cannabinoid: a potential emerging substance of abuse in Hong Kong

Tung CK, Chiang TP, Lam M.

Clin Toxicol (Phila), 2011

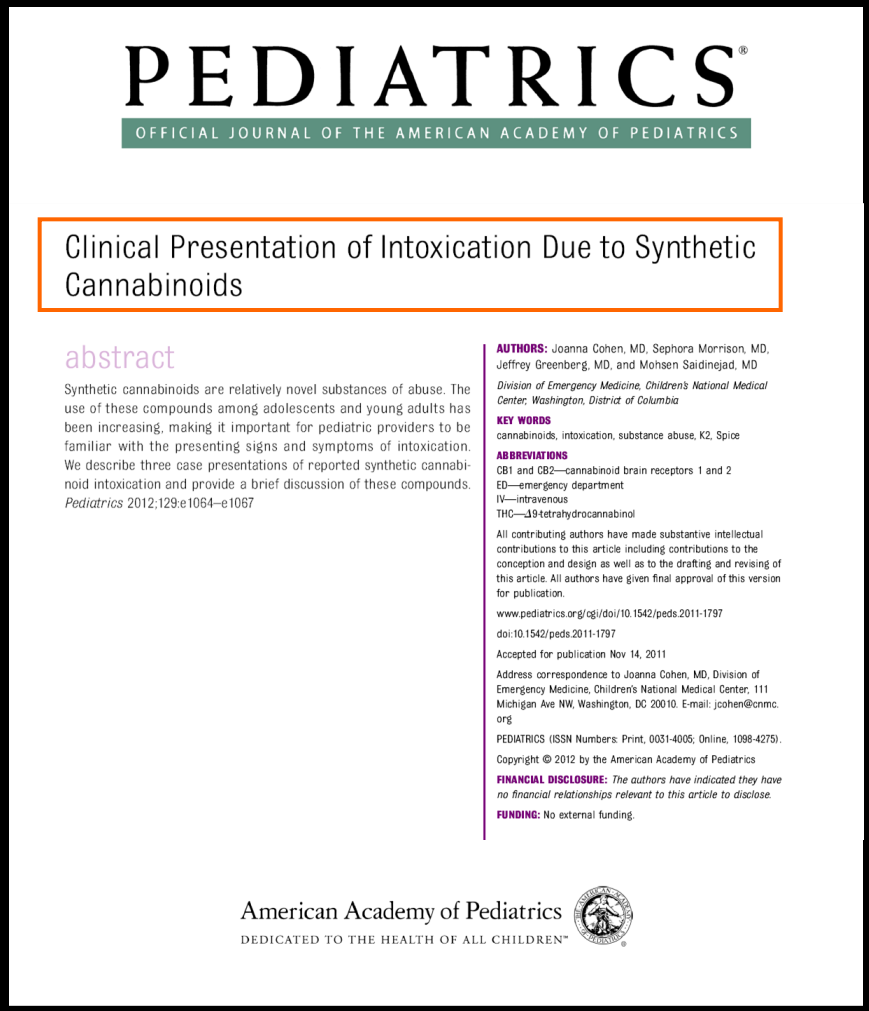
Severe toxicity following synthetic cannabinoid ingestion

Lapoint J, James LP, Moran CL, Nelson LS, Hoffman RS, Moran JH.

Pediatrics, 2011

Myocardial infarction associated with use of the synthetic cannabinoid K2

Mir A, Obafemi A, Young A, Kane C.



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OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

Clinical Presentation of Intoxication Due to Synthetic Cannabinoids

abstract

Synthetic cannabinoids are relatively novel substances of abuse. The use of these compounds among adolescents and young adults has been increasing, making it important for pediatric providers to be familiar with the presenting signs and symptoms of intoxication. We describe three case presentations of reported synthetic cannabinoid intoxication and provide a brief discussion of these compounds. *Pediatrics* 2012;129:e1064–e1067

AUTHORS: Joanna Cohen, MD, Sephora Morrison, MD, Jeffrey Greenberg, MD, and Mohsen Saidinejad, MD

Division of Emergency Medicine, Children's National Medical Center, Washington, District of Columbia

KEY WORDS

cannabinoids, intoxication, substance abuse, K2, Spice

ABBREVIATIONS

CB1 and CB2—cannabinoid brain receptors 1 and 2
ED—emergency department
IV—intravenous
THC—Δ9-tetrahydrocannabinol

All contributing authors have made substantive intellectual contributions to this article including contributions to the conception and design as well as to the drafting and revising of this article. All authors have given final approval of this version for publication.

www.pediatrics.org/cgi/doi/10.1542/peds.2011-1797

doi:10.1542/peds.2011-1797

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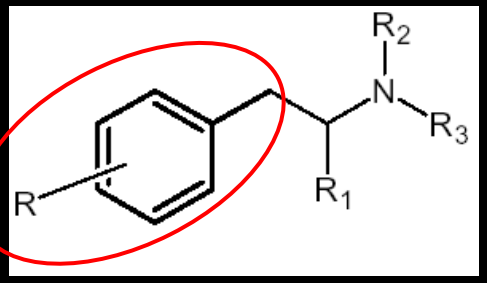
FINANCIAL DISCLOSURE: *The authors have indicated they have no financial relationships relevant to this article to disclose.*

FUNDING: No external funding.

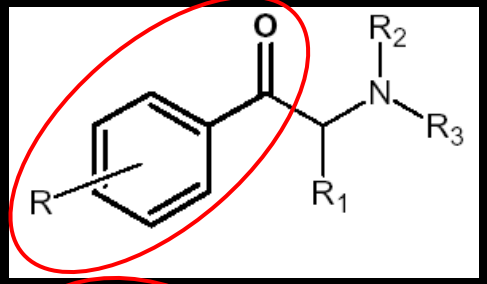
American Academy of Pediatrics

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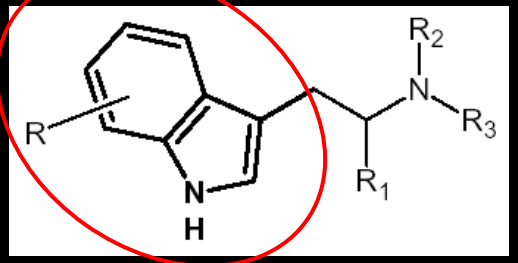
Amfetamino-simili



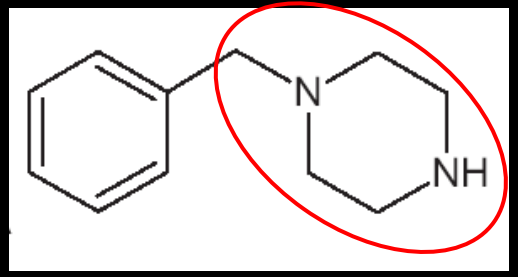
✓ Feniletil-ammine
(der. Amfetamina)



✓ Catinoni
(Beta-keto-amfetamine)



✓ Triptammine

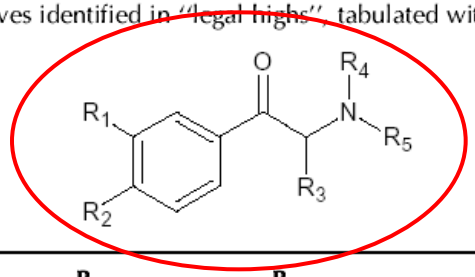


✓ Piperazine

Azioni
Stimolante
Entactogena
Allucinogena

Amfetamino-simili: Catinoni

Table 1. Structures of cathinone and its derivatives identified in "legal highs", tabulated with their MS base peaks



Name	R_1	R_2	R_3	R_4	R_5	Base peak [*m/z*]
Cathinone	H	H	CH_3	H	H	44
Methcathinone	H	H	CH_3	H	CH_3	58
Mephedrone	H	CH_3	CH_3	H	CH_3	
Methedrone	H	OCH_3	CH_3	H	CH_3	
Methylone	3,4-Methylenedioxy		CH_3	H	CH_3	
Flephedrone	H	F	CH_3	H	CH_3	
3,4-DMMC	CH_3	CH_3	CH_3	H	CH_3	
Ethcathinone	H	H	CH_3	H	C_2H_5	72
Buphedrone	H	H	C_2H_5	H	CH_3	
4-MEC	H	CH_3	CH_3	H	C_2H_5	
DMMC	H	H	CH_3	CH_3	CH_3	
Ethylone	3,4-Methylenedioxy		CH_3	H	C_2H_5	
Butylone	3,4-methylenedioxy		C_2H_5	H	CH_3	
Pentedrone	H	H	C_3H_7	H	CH_3	86
Pentylone	3,4-Methylenedioxy		C_3H_7	H	CH_3	
Diethylpropion	H	H	CH_3	C_2H_5	C_2H_5	100
Bupropion	Cl	H	CH_3	H	t-Butyl	(44)[a]
PPP	H	H	CH_3	Pyrrolidin-1-yl		98
MPPP	H	CH_3	CH_3	Pyrrolidin-1-yl		
MoPPP	H	OCH_3	CH_3	Pyrrolidin-1-yl		
MDPPP	3,4-Methylenedioxy		CH_3	Pyrrolidin-1-yl		
MPBP	H	CH_3	C_2H_5	Pyrrolidin-1-yl		112
MDPBP	3,4-Methylenedioxy		C_2H_5	Pyrrolidin-1-yl		
PVP	H	CH_3	C_3H_7	Pyrrolidin-1-yl		126
MDPV	3,4-Methylenedioxy		C_3H_7	Pyrrolidin-1-yl		
Naphyrone	Naphthyl[b]		C_3H_7	Pyrrolidin-1-yl		
PAPP	H	H	CH_3	Phthalimide		174

Intossicazioni acute da Catinoni

Forensic Sci Int, 2010

The detection of mephedrone (4-methylmethcathinone) in 4 fatalities in Scotland.

Torrance H, Cooper G.

Am J Drug Alcohol Abuse, 2012

"Bath salt" ingestion leading to severe intoxication delirium: two cases and a brief review of the emergence of mephedrone use.

Kasick DP, McKnight CA, Klisovic E.

J Anal Toxicol, 2012

Three fatal intoxications due to methylone.

Pearson JM, Hargraves TL, Hair LS, Massucci CJ, Clinton Frazee C 3rd, Garg U, Pietak BR.

Forensic Sci Int, 2012

"Legal highs" toxicity in the clinical and medico-legal aspect as exemplified by suicide with bk-MBDB administration.

Rojek S, Kłys M, Strona M, Maciów M, Kula K.

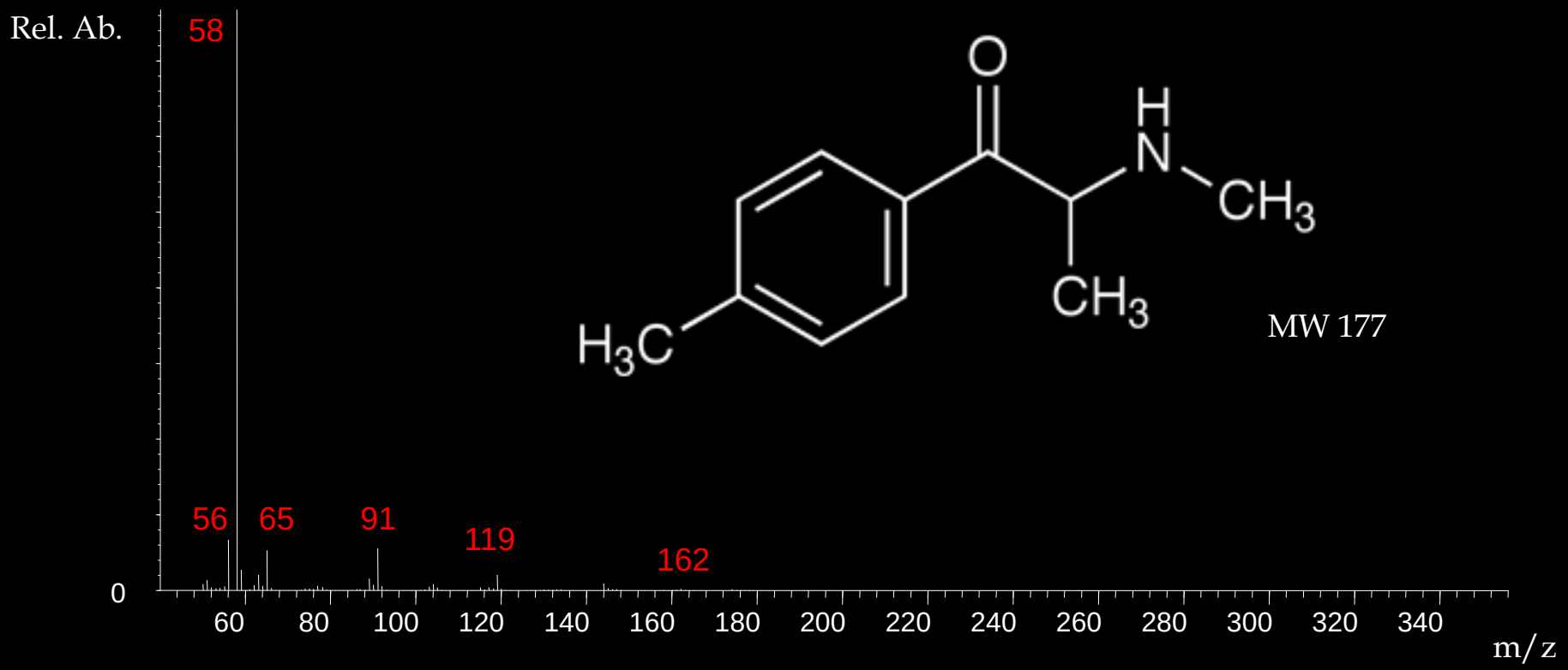
Strategia analitica LIATF per la caratter. strutturale dei nuovi amfetamino-simili

GC-MS
di p. a. non derivatizzati

GC-MS
dopo deriv. con 3Cl-etil-cloroformiato

UHPLC-HRMS
(Orbitrap)

GC/MS - Mefedrone

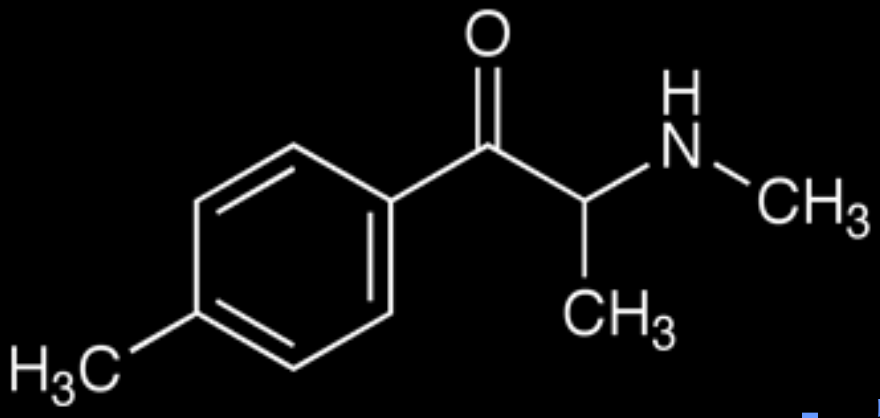


GC/MS

Agilent 7890 II - 5975
Full Scan EI (40-450 u)
Inj. 1 µl, 250C, split / splitless (1 min)
Carrier gas (He), 1 ml/min

Agilent HP-5MS Ultra Inert column
30 m x 0.25 mm x 0.25 µm
50C (0.5 min), 200C a 30C/min, 300C (5 min) a 10C/min
Interf. 280C, EMV + 300 V

Mefedrone deriv. con 3ClEtClfor



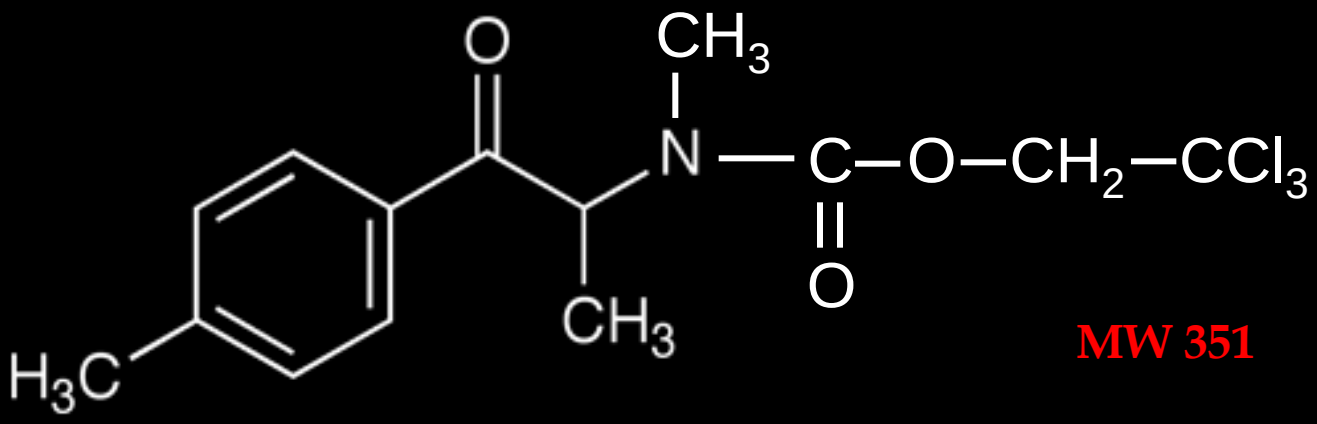
MW 177

+ Cl—CO—O—CH_2—CCl_3

50 µL 2,2,2 - 3ClEtClfor : Ethyl acetate 3 : 7

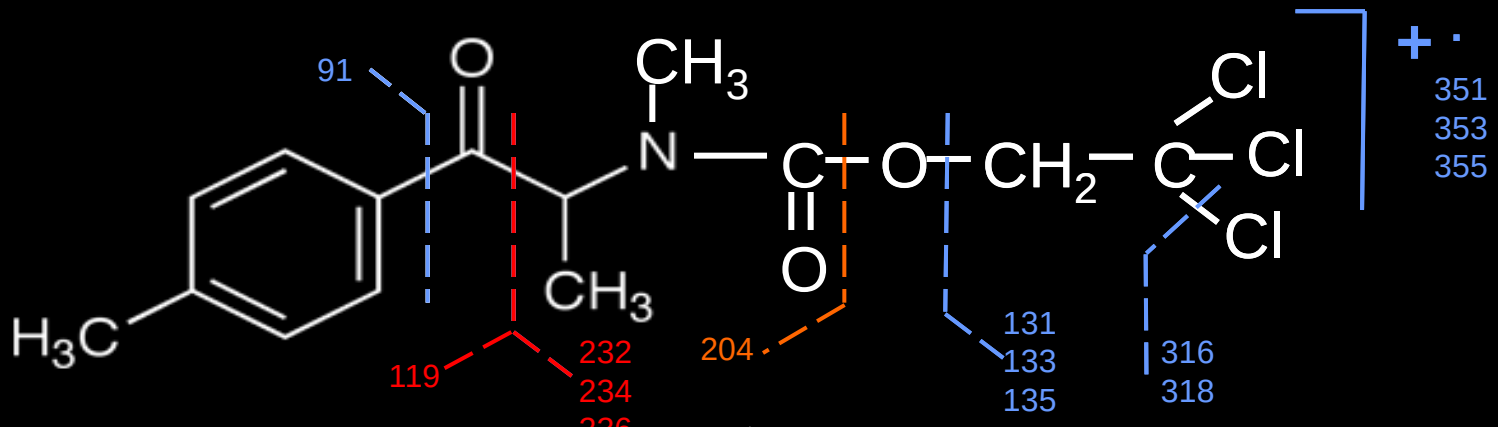
80 ° C, 15 min, to dryness

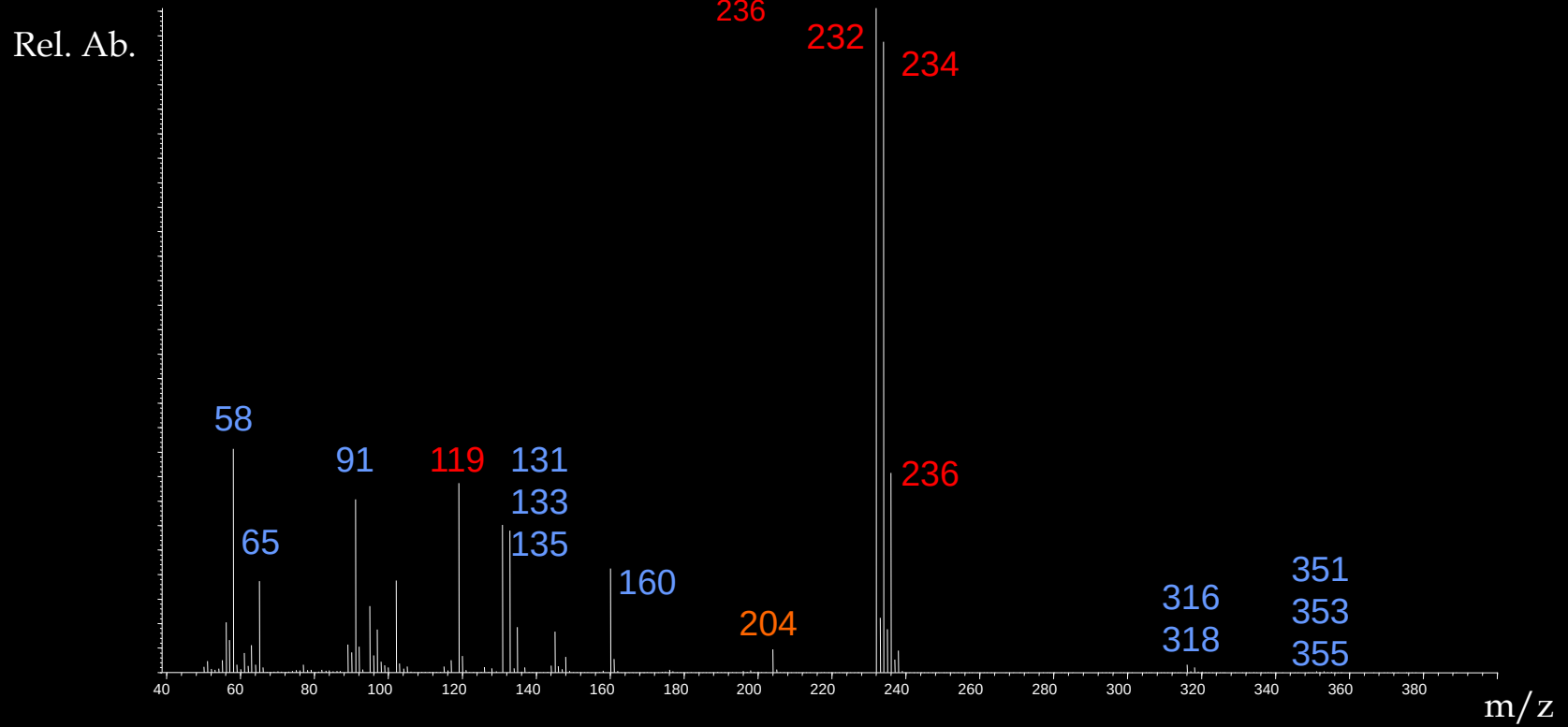
Residue reconst. with 50 µL Ethyl acetate



MW 351

GC/MS di Mefedrone - 3ClEtClfor





G. Frison et al. *Rapid Commun. Mass Spectrom.* 2011, **25**, 387–390

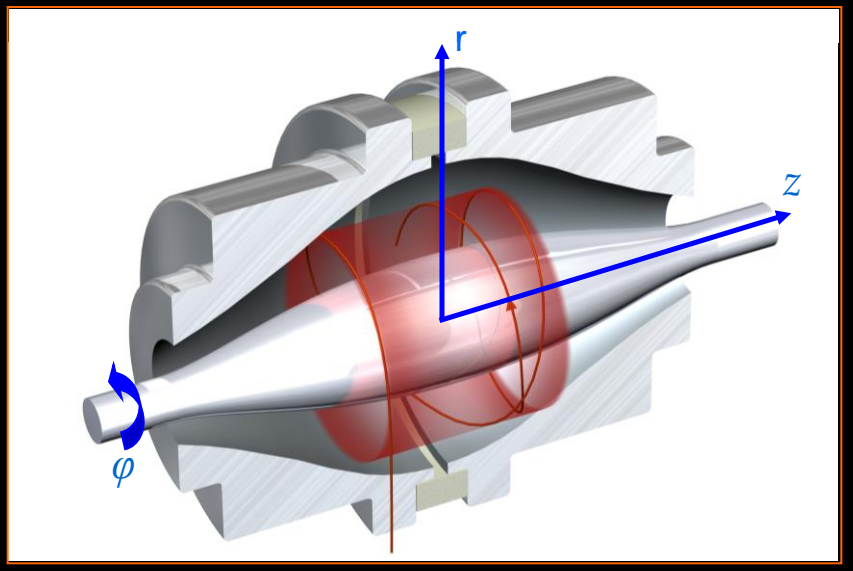
Drog@news – Tecniche analitiche (09.03.11)



UHPLC/HR-Orbitrap-MS

- ✓ Elevata accuratezza di misura di massa: < 5ppm (e.c.), < 2ppm (i.c.)
- ✓ Elevato potere risolvente: 100.000 a *m/z* 200
- ✓ Full Scan and "all ions" MS/MS



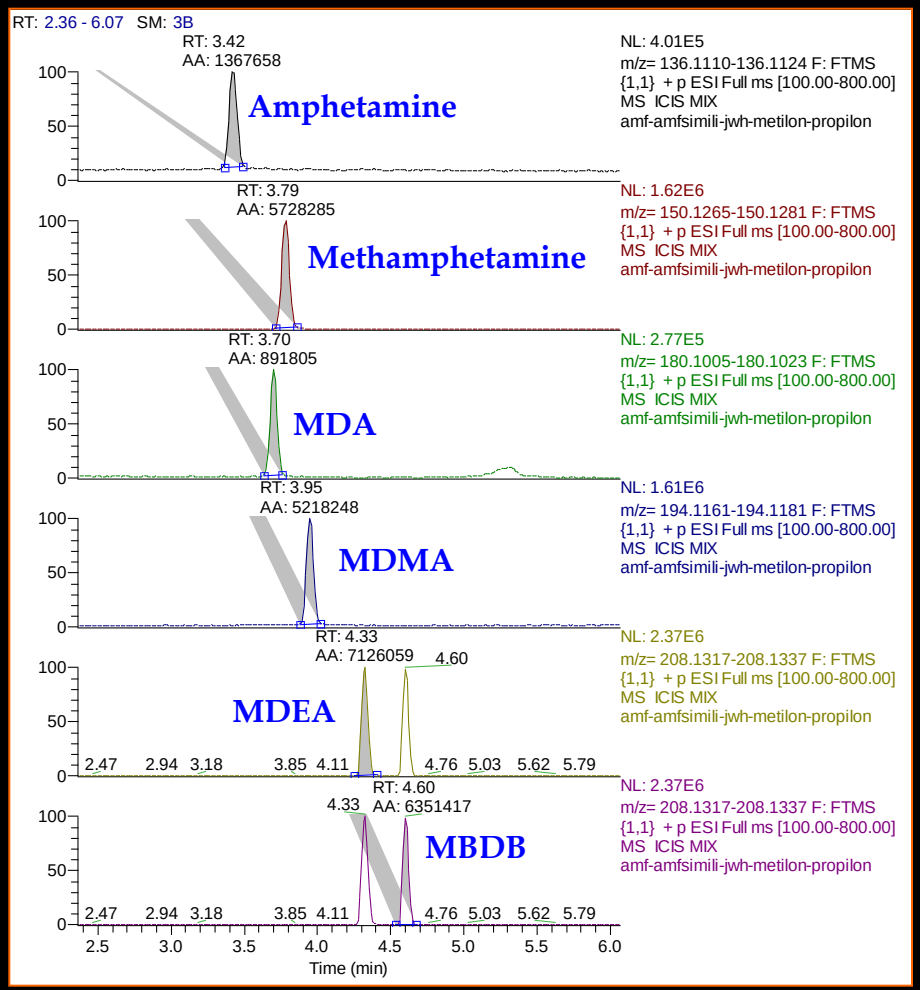


Composizione elementare e caratterizzazione strutturale di nuove sostanze psicoattive in assenza di standard di riferimento!!!

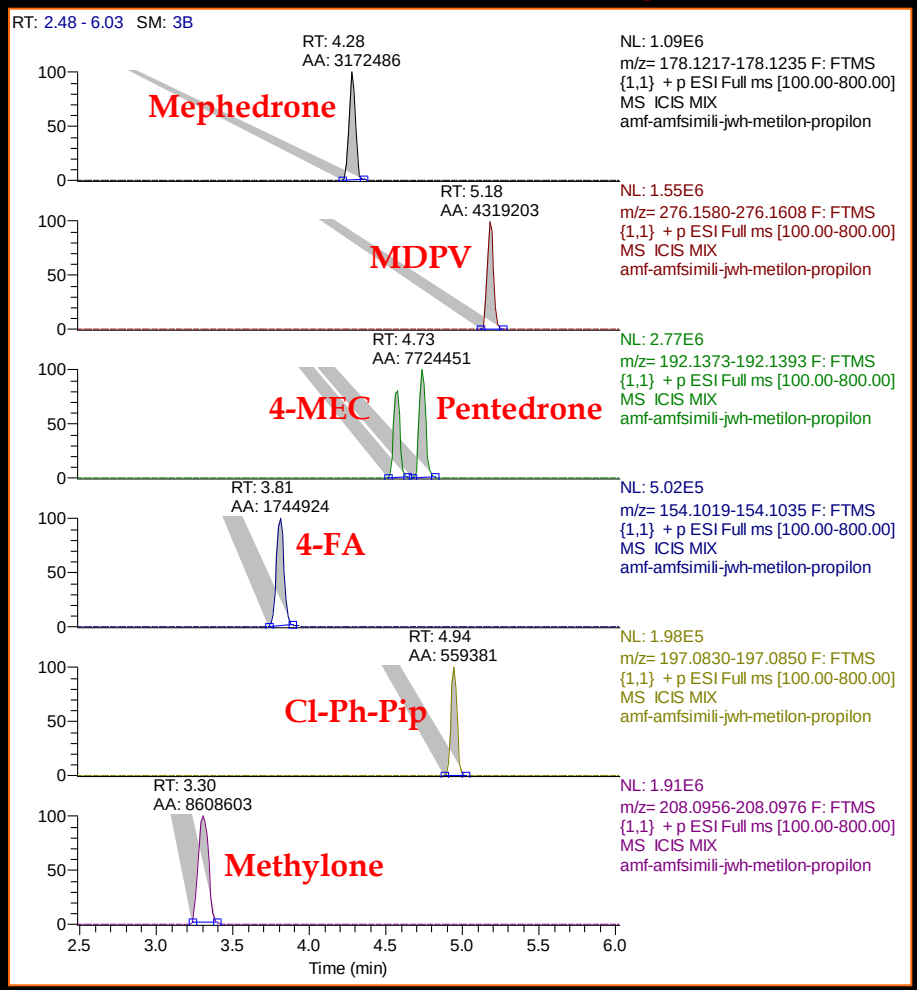
U-HPLC / HR-Orbitrap-MS e designer drugs

Efficiente separazione cromatografica degli analiti

Amfetamine "Classiche"

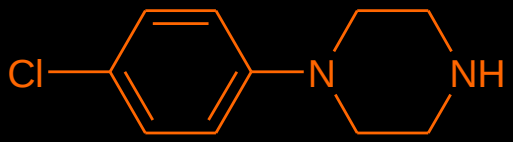


Nuove A-R drugs

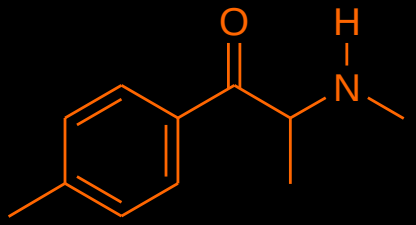


U-HPLC / HR-Orbitrap-MS e designer drugs

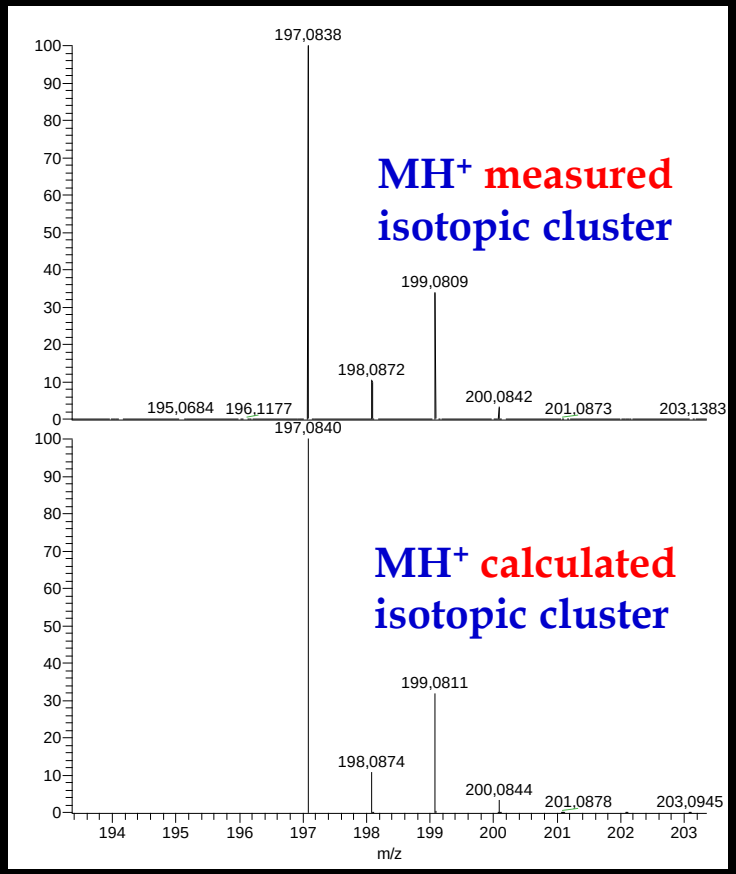
Confronto pattern isotopici MH^+ sperimentali e calcolati

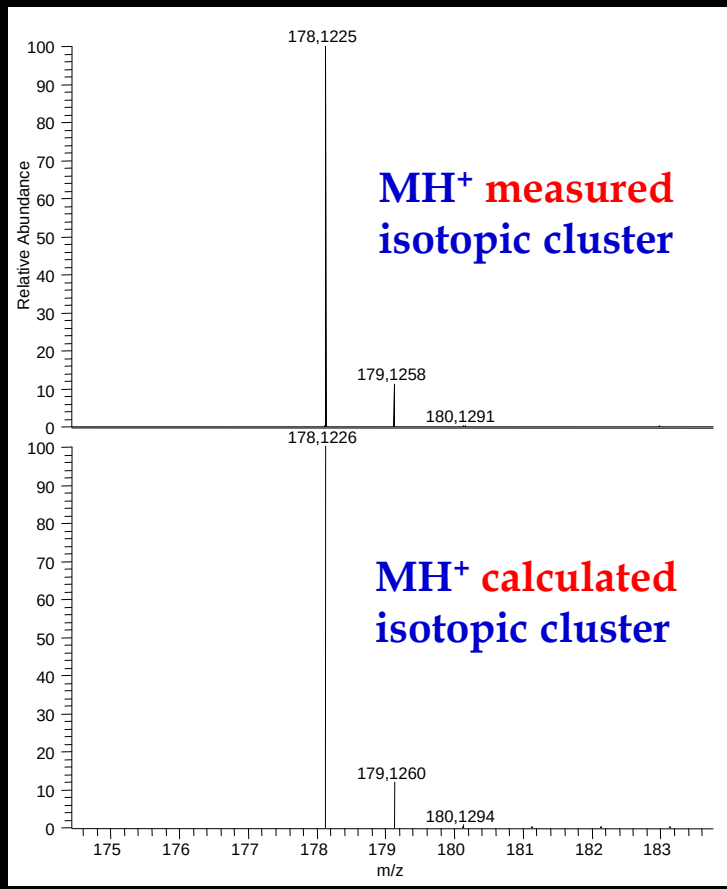


Cl-PhPip



Mephedrone

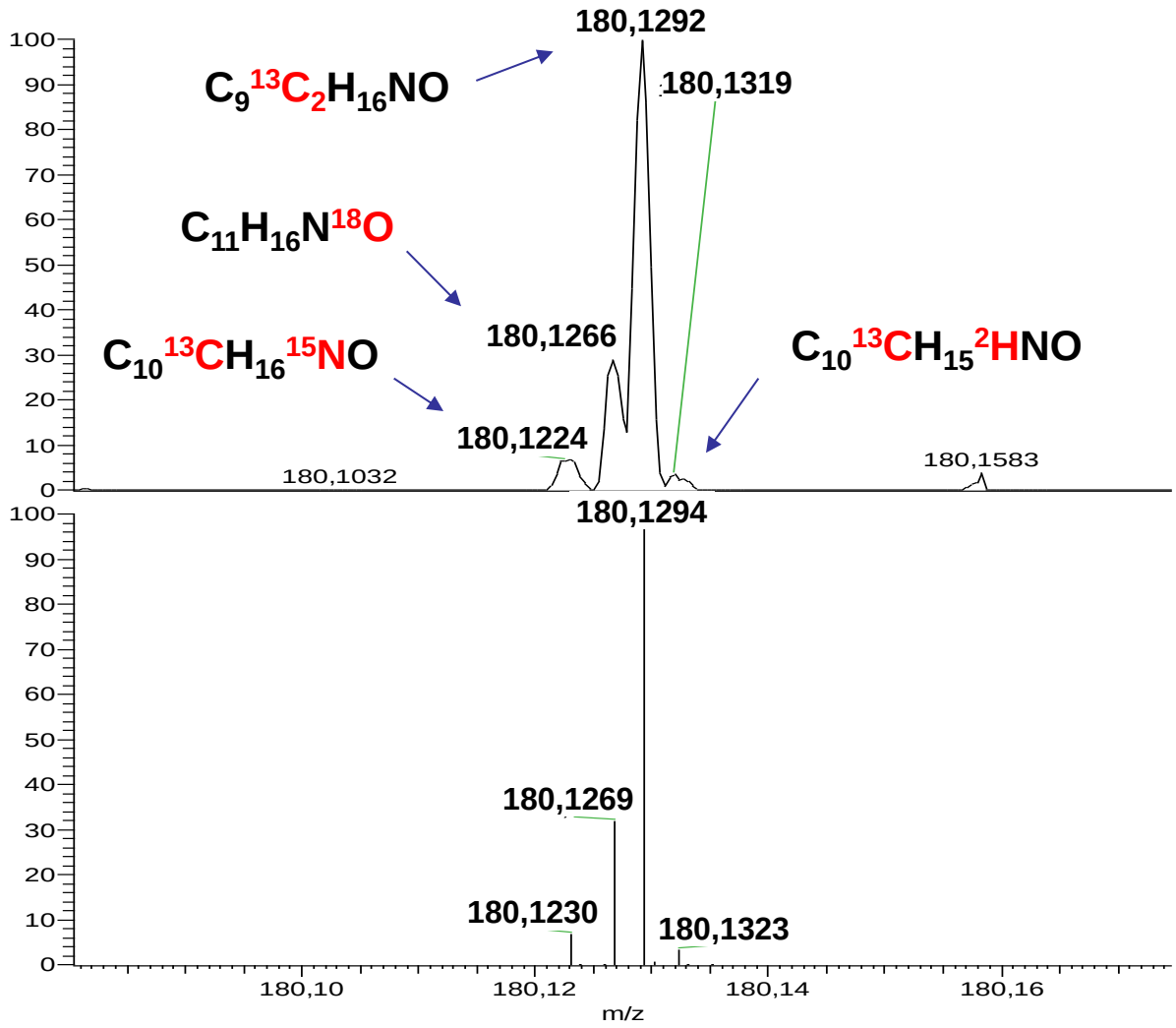




U-HPLC / HR-Orbitrap-MS e designer drugs

Isotopic Fine Structure di (M+1), (M+2), (M+3), ...

Mephedrone: cluster isotopico M+2 (0.5%M+0)



NL:
2,88E4
20110330AMFSIMILI-MIX1-1000_100K#290-294 RT: 4,30-4,36
AV: 5 SB: 31 3,97-4,14 , 4,53-4,79
T: FTMS {1;1} + p ESI Full ms
[100,00-800,00]

Misurato a 100.000 RP

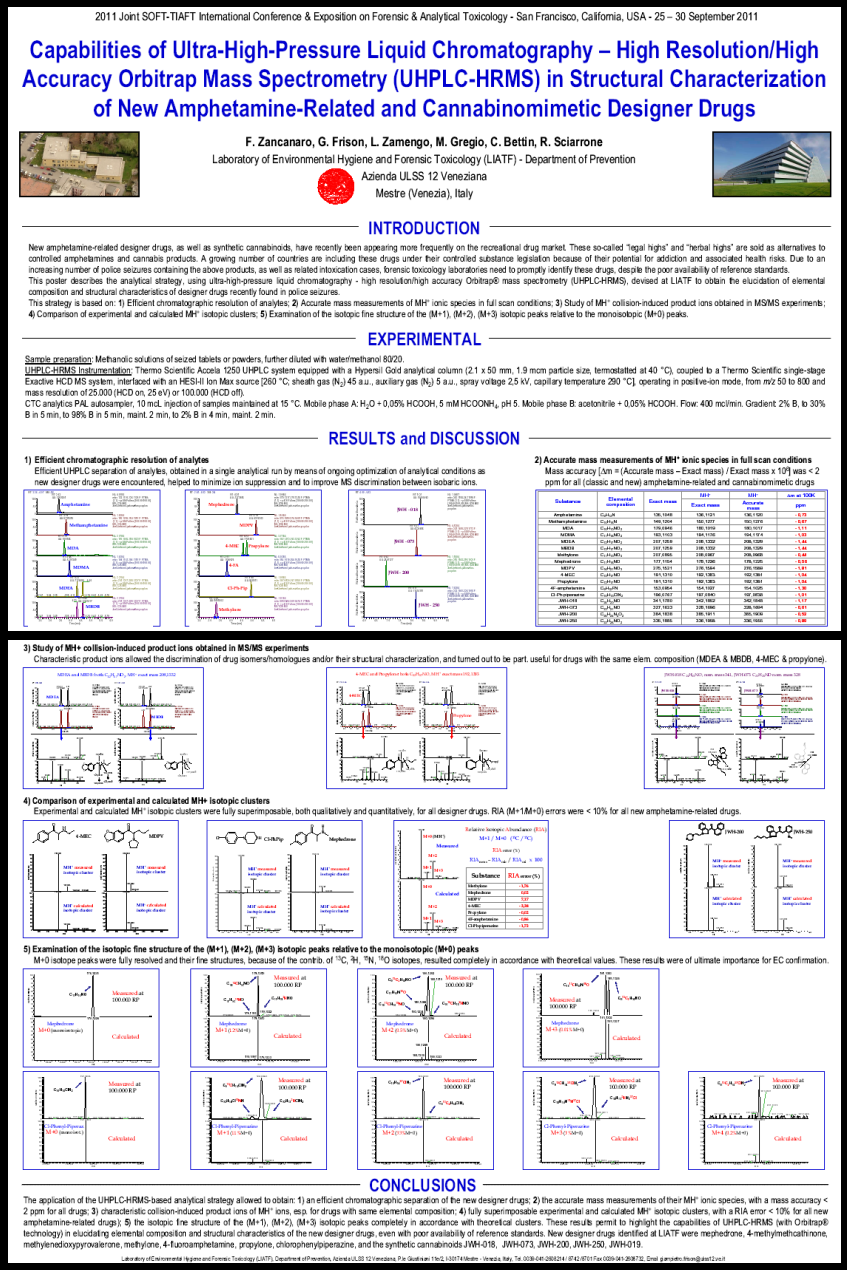
NL:
5,67E3
$C_{11}H_{15}NO$ +H:
$C_{11}H_{16}N_1O_1$
pa Chrg 1

Calcolato

Meeting SOFT-TIAFT 2011



✓Society of Forensic Toxicologists (SOFT) – International Association of Forensic Toxicologists (TIAFT) 2011 Joint Meeting



2011 Joint SOFT-TIAFT International Conference & Exposition on Forensic & Analytical Toxicology - San Francisco, California, USA - 25 – 30 September 2011

Capabilities of Ultra-High-Pressure Liquid Chromatography – High Resolution/High Accuracy Orbitrap Mass Spectrometry (UHPLC-HRMS) in Structural Characterization of New Amphetamine-Related and Cannabinomimetic Designer Drugs

F. Zancanaro, G. Frison, L. Zamengo, M. Gregio, C. Bettin, R. Sciarrone
Laboratory of Environmental Hygiene and Forensic Toxicology (LIATF) - Department of Prevention
Azienda ULSS 12 Veneziana
Mestre (Venezia), Italy

INTRODUCTION

New amphetamine-related designer drugs, as well as synthetic cannabinoids, have recently been appearing more frequently on the recreational drug market. These so-called "legal highs" and "herbal highs" are sold as alternatives to controlled amphetamines and cannabis products. A growing number of countries are including these drugs under their controlled substance legislation because of their potential for addiction and associated health risks. Due to an increasing number of police seizures containing the above products, as well as related intoxication cases, forensic toxicology laboratories need to promptly identify these drugs, despite the poor availability of reference standards.
This poster describes the analytical strategy, using ultra-high-pressure liquid chromatography - high resolution/high accuracy Orbitrap® mass spectrometry (UHPLC-HRMS), devised at LIATF to obtain the elucidation of elemental composition and structural characteristics of designer drugs recently found in police seizures.
This strategy is based on: **1)** Efficient chromatographic resolution of analytes; **2)** Accurate mass measurements of MH^+ ionic species in full scan conditions; **3)** Study of MH^+ collision-induced product ions obtained in MS/MS experiments; **4)** Comparison of experimental and calculated MH^+ isotopic clusters; **5)** Examination of the isotopic fine structure of the (M+1), (M+2), (M+3) isotopic peaks relative to the monoisotopic (M+0) peaks.

EXPERIMENTAL

Sample preparation: Methanolic solutions of seized tablets or powders, further diluted with water/methanol 80/20.
UHPLC-HRMS Instrumentation: Thermo Scientific Accela 1250 UHPLC system equipped with a Hypersil Gold analytical column (2.1 x 50 mm, 1.9 mcm particle size, termostatted at 40 °C), coupled to a Thermo Scientific single-stage Exactive HCD MS system, interfaced with an HESI-II Ion Max source [260 °C; sheath gas (N_2) 45 a.u., auxiliary gas (N_2) 5 a.u., spray voltage 2.5 kV, capillary temperature 290 °C], operating in positive-ion mode, from *m/z* 50 to 800 and mass resolution of 25.000 (HCD on, 25 eV) or 100.000 (HCD off).
CTC analytics PAL autosampler, 10 mcL injection of samples maintained at 15 °C. Mobile phase A: H_2O + 0,05% HCOOH, 5 mM $HCOONH_4$, pH 5. Mobile phase B: acetonitrile + 0,05% HCOOH. Flow: 400 mcl/min. Gradient: 2% B, to 30% B in 5 min, to 98% B in 5 min, maint. 2 min, to 2% B in 4 min, maint. 2 min.

RESULTS and DISCUSSION

1) Efficient chromatographic resolution of analytes
Efficient UHPLC separation of analytes, obtained in a single analytical run by means of ongoing optimization of analytical conditions as new designer drugs were encountered, helped to minimize ion suppression and to improve MS discrimination between isobaric ions.

2) Accurate mass measurements of MH^+ ionic species in full scan conditions
Mass accuracy [Δm = (Accurate mass – Exact mass) / Exact mass x 10^6] was < 2 ppm for all (classic and new) amphetamine-related and cannabinomimetic drugs

Substance	Elemental composition	Exact mass	MH+ Exact mass	MH+ Accurate mass	Δm/m 100K ppm
Amphetamine	$C_9H_{13}N$	135.1048	136.1121	136.1120	0.70
Methamphetamine	$C_{10}H_{15}N$	149.1204	150.1277	150.1276	0.67
MDA	$C_{10}H_{13}NO_2$	179.0946	180.1019	180.1017	1.11
MDMA	$C_{11}H_{15}NO_2$	193.1103	194.1176	194.1174	1.03
MDEA	$C_{12}H_{17}NO_2$	207.1259	208.1332	208.1329	1.44
MBDB	$C_{11}H_{15}NO_2$	193.1259	208.1332	208.1329	1.44
Methylone	$C_{11}H_{13}NO_3$	207.0895	208.0967	208.0968	0.48
Mephedrone	$C_{11}H_{15}NO$	177.1154	178.1226	178.1225	0.56
4-MEC	$C_{12}H_{17}NO$	191.1310	192.1383	192.1382	0.52
4-MEC	$C_{12}H_{17}NO$	191.1310	192.1383	192.1382	0.52
Pregabalin	$C_8H_{17}NO_2$	159.1310	160.1383	160.1381	1.04
4F-amphetamine	$C_9H_{12}FN$	153.0954	154.1027	154.1025	1.30
Cl-Propoxyvone	$C_{15}H_{22}ClON_2$	296.0787	297.0860	297.0858	0.81
JWH-018	$C_{24}H_{23}NO$	341.1780	342.1852	342.1848	1.17
JWH-073	$C_{24}H_{23}NO$	227.1623	228.1696	228.1694	0.81
JWH-200	$C_{23}H_{24}N_2O_2$	364.1838	365.1911	365.1908	0.82
JWH-250	$C_{22}H_{23}NO_2$	336.1885	336.1958	336.1955	0.86

3) Study of MH^+ collision-induced product ions obtained in MS/MS experiments
Characteristic product ions allowed the discrimination of drug isomers/homologues and/or their structural characterization, and turned out to be part, useful for drugs with the same elem. composition (MDEA & MBDB, 4-MEC & propylone).

4) Comparison of experimental and calculated MH^+ isotopic clusters
Experimental and calculated MH^+ isotopic clusters were fully superimposable, both qualitatively and quantitatively, for all designer drugs. RIA (M+1/M+0) errors were < 10% for all new amphetamine-related drugs.

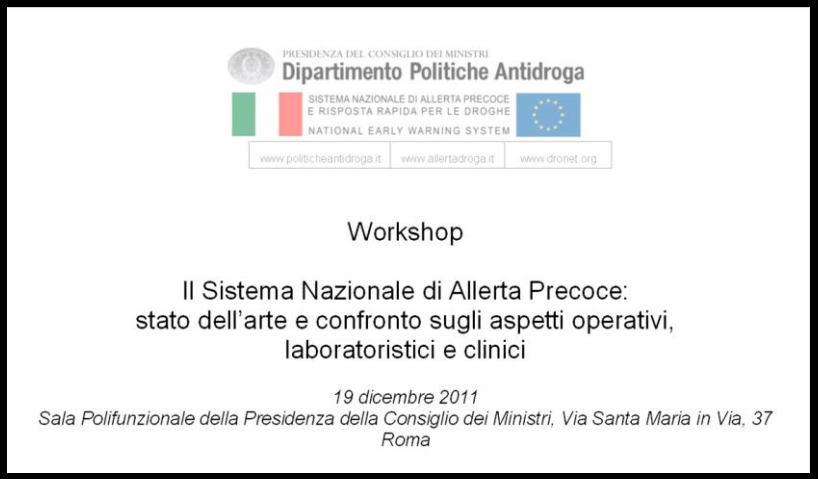
5) Examination of the isotopic fine structure of the (M+1), (M+2), (M+3) isotopic peaks relative to the monoisotopic (M+0) peaks
M+0 isotope peaks were fully resolved and their fine structures, because of the contrib. of ^{13}C, ^{2}H, ^{15}N, ^{17}O isotopes, resulted completely in accordance with theoretical values. These results were of ultimate importance for EC confirmation.

CONCLUSIONS

The application of the UHPLC-HRMS-based analytical strategy allowed to obtain: **1)** an efficient chromatographic separation of the new designer drugs; **2)** the accurate mass measurements of their MH^+ ionic species, with a mass accuracy < 2 ppm for all drugs; **3)** characteristic collision-induced product ions of MH^+ ions, esp. for drugs with same elemental composition; **4)** fully superimposable experimental and calculated MH^+ isotopic clusters, with a RIA error < 10% for all new amphetamine-related drugs); **5)** the isotopic fine structure of the (M+1), (M+2), (M+3) isotopic peaks completely in accordance with theoretical clusters. These results permit to highlight the capabilities of UHPLC-HRMS (with Orbitrap® technology) in elucidating elemental composition and structural characteristics of the new designer drugs, even with poor availability of reference standards. New designer drugs identified at LIATF were mephedrone, 4-methylmethcathinone, methylenedioxypyrovalerone, methylone, 4-fluoroamphetamine, propylone, chloropheny lpiperazine, and the synthetic cannabinoids JWH-018, JWH-073, JWH-200, JWH-250, JWH-019.

Laboratory of Environmental Hygiene and Forensic Toxicology (LIATF), Department of Prevention, Azienda ULSS 12 Veneziana, Via Giustiniani 1/bis/2, I-30174 Mestre - Venezia, Italy. Tel. 0039-041-2608241 / 8742-67011 Fax 0039-041-2608732. Email: giampietro.frison@ulss12.ve.it

Workshop Dip. Pol. Antidroga – Dic. 2011



PRESIDENZA DEL CONSIGLIO DEI MINISTRI
Dipartimento Politiche Antidroga
SISTEMA NAZIONALE DI ALLERTA PRECOCE E RISPOSTA RAPIDA PER LE DROGHE
NATIONAL EARLY WARNING SYSTEM
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Workshop

Il Sistema Nazionale di Allerta Precoce:
stato dell'arte e confronto sugli aspetti operativi,
laboratoristici e clinici

19 dicembre 2011
Sala Polifunzionale della Presidenza della Consiglio dei Ministri, Via Santa Maria in Via, 37
Roma

- ✓ Dipartimento Politiche Antidroga
- Workshop
- "Il Sistema Nazionale di Allerta Precoce: Stato dell'arte e Confronto sugli Aspetti Operativi, Laboratoristici e Clinici"

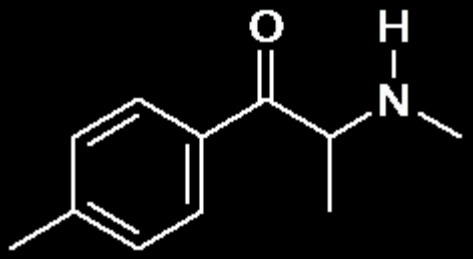
Programma

Sessione plenaria	
Moderatore: Giovanni Serpelloni	
11.30 - 11.45	Il NEWS: gli aspetti strategici, le reti delle collaborazioni locali, nazionali ed europee e la collaborazione con il Ministero della Salute (Giovanni Serpelloni)
11.45 – 12.00	Il Sistema Nazionale di Allerta Precoce: principali attività e risultati raggiunti (Claudia Rimondo, Catia Seri)
12.00 – 12.15	Aspetti analitici delle nuove sostanze d'abuso individuate dal NEWS (Teodora Macchia)
12.15 – 12.30	Nuove sostanze e nuove tipologie di consumo: attività clinica e aspetti operativi del NEWS nel 2010-2011 (Carlo Locatelli)
12.30 – 12.50	Ricerca e identificazione di composti farmacologicamente attivi e potenzialmente pericolosi in prodotti di libero commercio: risultati del progetto "Smart search" (Franco Tagliaro)
12.50 – 13.10	Diagnosi di laboratorio in urgenza: l'evoluzione nel 2010-2011 (Pietro Papa)
13.10 – 14.00	Colazione di lavoro

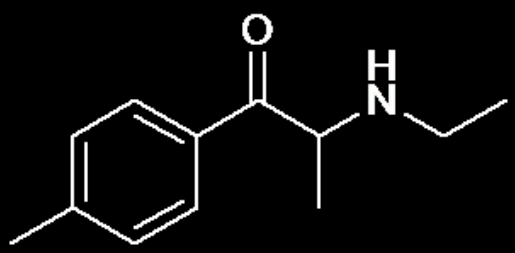
Sessione pomeridiana I – gruppo dei laboratori	
Moderatore: Teodora Macchia	
14.00 – 14.20	Possibilità analitiche offerte dalla spettrometria di massa ad elevata risoluzione/elevata accuratezza nella caratterizzazione strutturale di nuove sostanze psicoattive ad azione amfetamino-simile (Giampietro Frison)
14.20 – 14.40	Studi di metabolismo: applicazioni per la rilevazione di nuove sostanze *in vivo* (Sabina Strano Rossi)
14.40 – 15.00	Analisi di composti chirali di interesse farmaceutico, tossicologico e forense per mezzo di tecniche miniaturizzate (Salvatore Fanali)

Coordinamento aspetti bio-tossicologici	Coordinamento operativo	Coordinamento aspetti clinico-tossicologici
Istituto Superiore di Sanità	Regione del Veneto, Azienda ULSS 20 Verona, Dipartimento delle Dipendenze	I.R.C.C.S. Fondazione S. Maugeri Centro Antiveleni Pavia

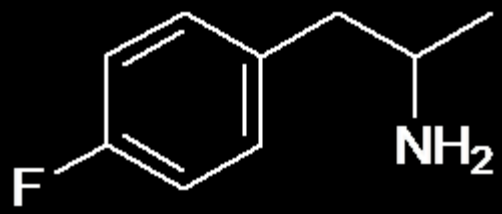
Nuove "A-R drugs" identificate al LIAFT



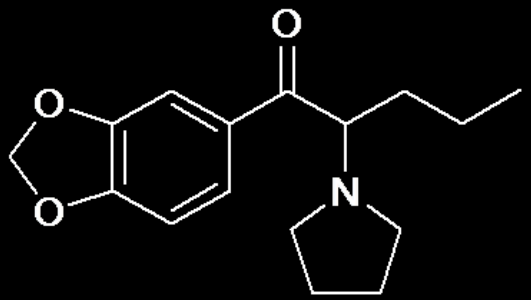
Mefedrone



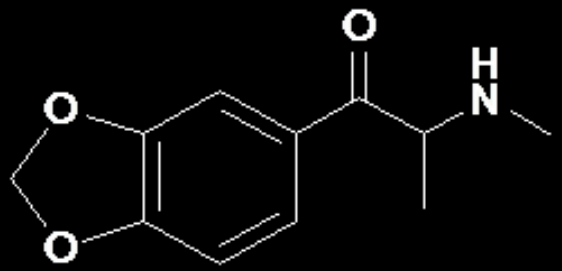
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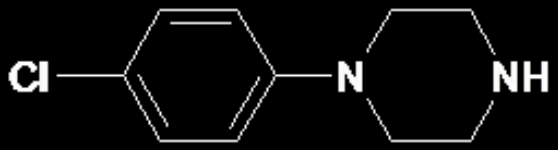
4-FA



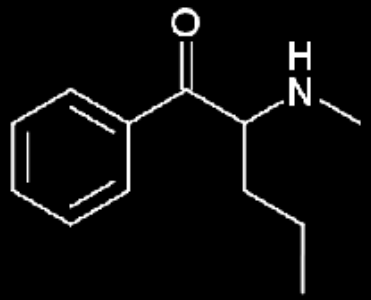
MDPV



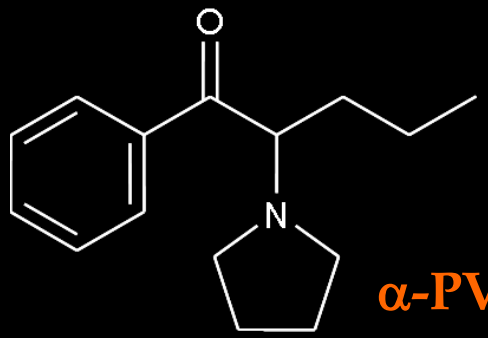
Metilone



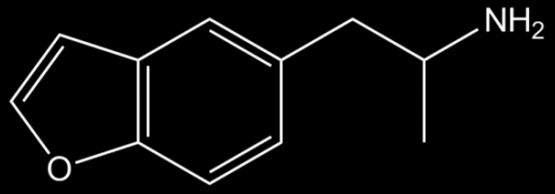
Cl-Fenil-Piperazina



Pentedrone

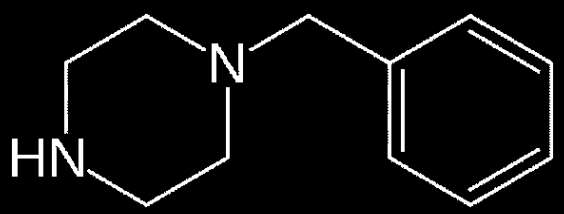


α-PVP

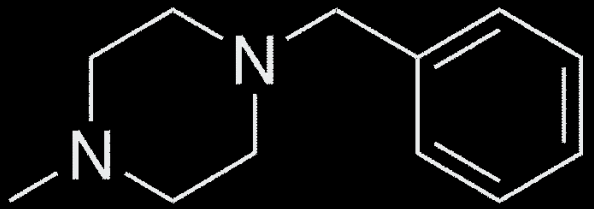


5-APB

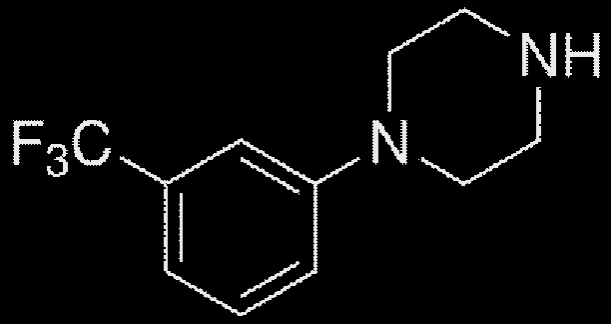
Nuove "A-R drugs" identificate al LIAFT



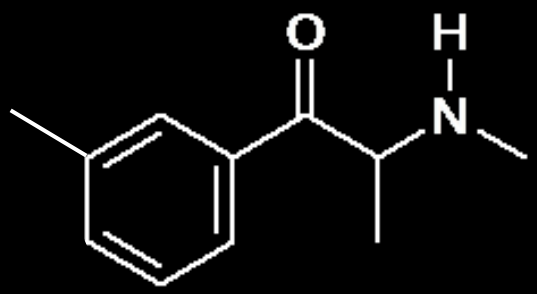
1-Benzil-Piperazina



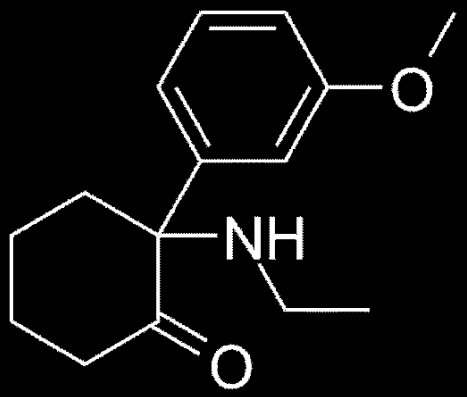
1-Benzil-4-Metil-Piperazina



1-(-3-Trifluorometil)-Benzil-Piperazina



3-MMC

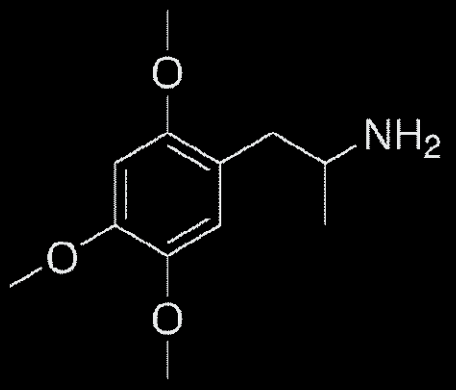


Metossietamina

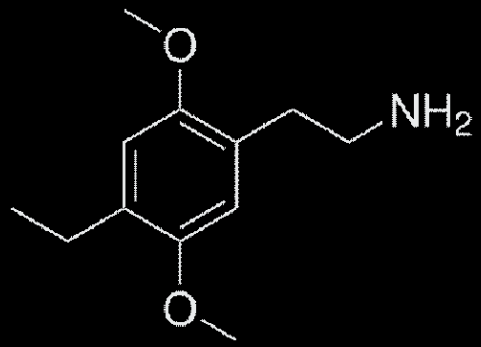


25I-NBOMe

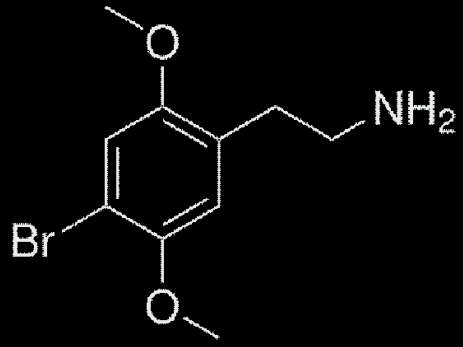
Nuove "A-R drugs" identificate al LIAFT



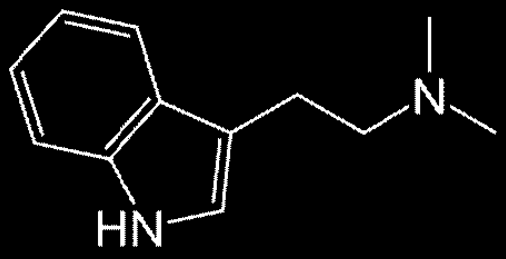
TMA-2



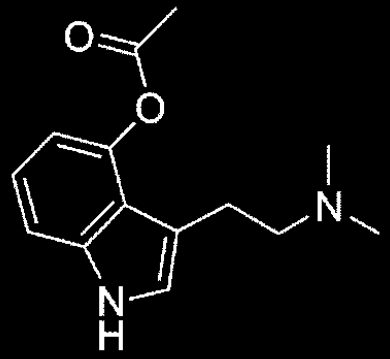
2C-E



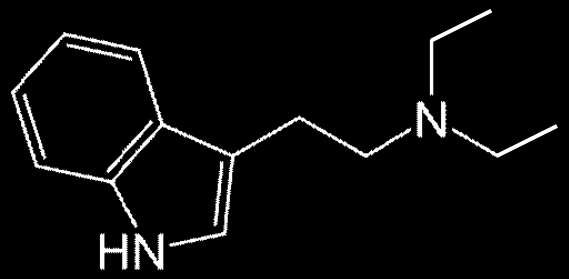
2C-B



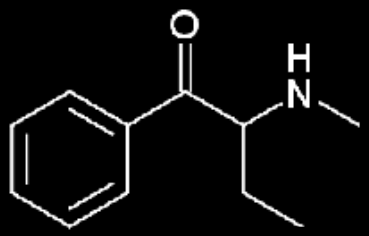
DMT



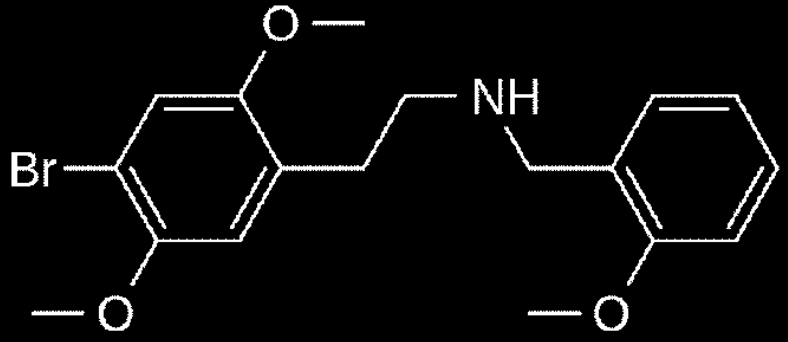
4-AcO-DMT



DET



Bufedrone



25B-NBOMe

25B-NBOMe / 25I-NBOMe

The Sydney Morning Herald
New South Wales

'He wanted to fly': father says son made a fatal mistake

June 7, 2013 Read later



SIDNEY, Australia – 7 June 2013. A 17 year-old jumped from a balcony to his death after ingesting a new psychoactive substance believed to be either 25B-NBOMe or 25I-NBOMe. According to his family, the teenager began acting erratically as soon as he arrived home, removing all his clothes and appeared to be in some kind of drug-induced psychosis."He took off his clothes and said he wanted to fly", reported the family. He obtained the substance from a classmate who told him it was LSD, however, the substance is reported to be widely available on the internet for a price under USD 2. Police say there have been other recent cases of teenagers and young adults acting recklessly after ingesting the NPS 25B-NBOMe and 25I-NBOMe, including a number of teenagers who stripped naked and ran through the streets of Sydney's north shore earlier this year.

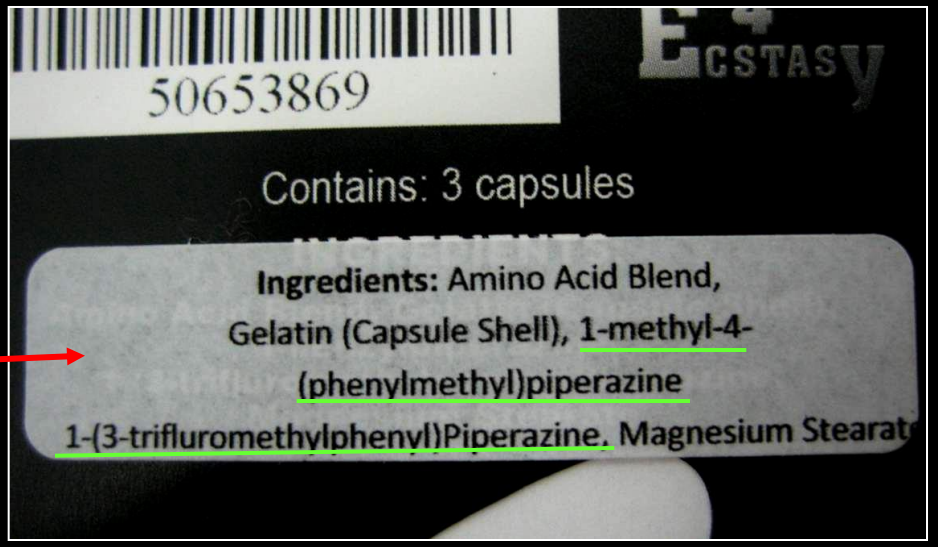
Cosa si acquista / si consuma veramente?

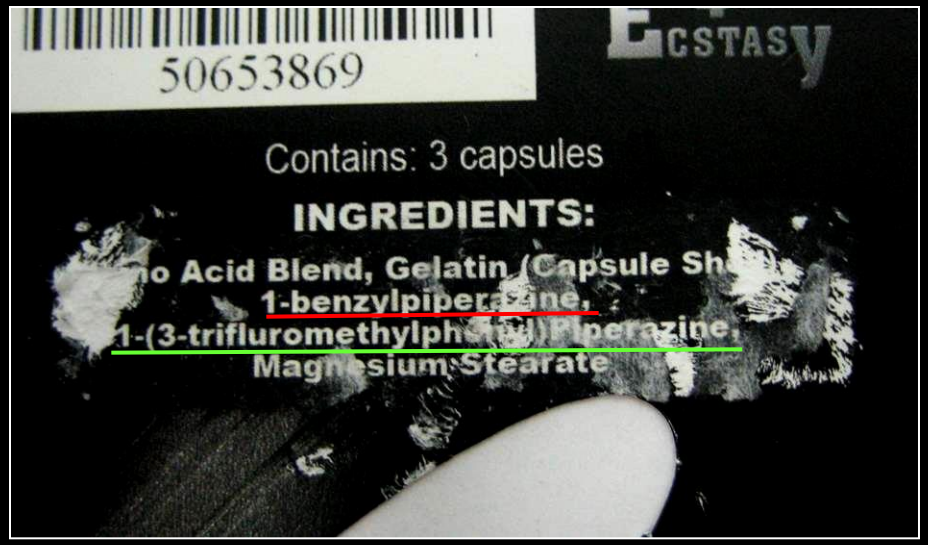
Recente sequestro ed analisi presso il LIATF

Blisters cont. 3 capsule









LIATF – Rapporto di Analisi

REGIONE DEL VENETO – AZIENDA ULSS 12 VENEZIANA – DIPARTIMENTO DI PREVENZIONE
Laboratorio di Igiene Ambientale e Tossicologia Forense
Piazzale San Lorenzo Giustiniani, 11/E - 30174 Mestre Venezia
Tel. 041.2608746 – Fax 041.2608732

CONCLUSIONI

Di seguito si riportano i dati conclusivi delle determinazioni analitiche eseguite. In particolare, a fianco della quantità assoluta di principio attivo espressa in milligrammi, viene riportato il corrispondente numero di Dosi Medie Singole (DMS) e l'esito del confronto con il Quantitativo Massimo Detenibile (QMD), ai sensi del D.P.R. 309/90 e D.M. 11 Aprile 2006.

ID Reperto	Principio Attivo	Principio Attivo (mg)	DMS (n°)	Confronto con QMD	DMS (QMD) (mg)
Reperto 1	3-MMC	-	-	-	-
Reperto 2 (2/1-2/2)	MDMA	133 ± 10	INF. a 1 Dose	INFERIORE	150 (750)

Commenti Conclusivi:

Le determinazioni analitiche qualitative eseguite sul materiale relativo al **Reperto 1**, del peso netto pari a 0.566 g, hanno accertato la presenza del principio attivo stupefacente **3-Metilmetcatinone (3-MMC)**, sostanza ad azione amfetamino-simile appartenente alla famiglia dei catinoni, inclusa in Tabella I del DPR309/90 con Decreto 29 Dicembre 2011 e successive modifiche (analogo di struttura derivante dal 2-amino-1fenil-1-propanone).
Per tale principio attivo non sono attualmente definiti Limiti di Legge.

Il materiale relativo al **Reperto 2 (2/1-2/2)**, del peso netto totale pari a 0.492 g, contiene il principio attivo stupefacente **MDMA**, per una quantità assoluta pari a 133 ± 10 mg. Ai sensi del D.M. 11 Aprile 2006, tale quantità risulta **INFERIORE** alla Dose Media Singola, e **INFERIORE** al Quantitativo Massimo Detenibile.

Segnalazioni LIATF al DPA

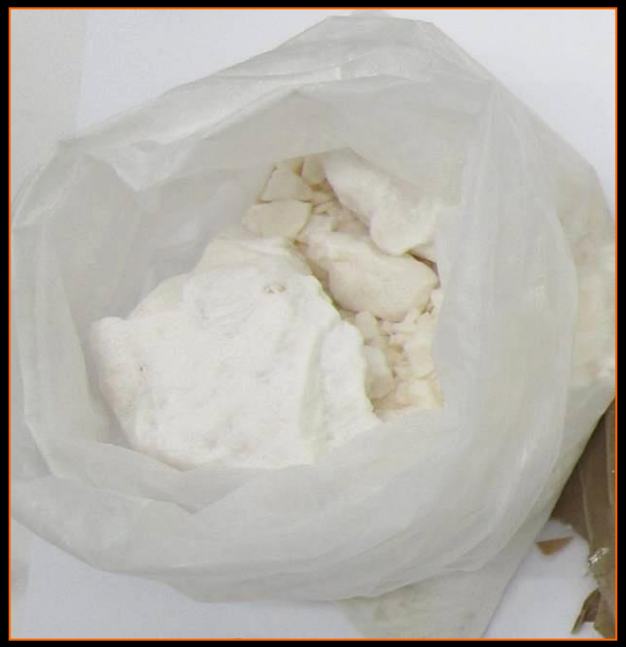
N.	Periodo	NSP segnalate	Note
1	Luglio 2010	Mefedrone	Polveri (15 g)
2	Luglio 2010	Clorofenilpiperazina (CPP)	n. 7 compresse
3	Gennaio 2011	3,4 Metilendiossipirovalerone (MDPV) e 4-Metiletcatinone (4-MEC)	Polveri (0.6 g)
4	Agosto 2011	Metorfano	Polvere di Eroina (3 kg) Eroina 31%, Metorfano 14%
5	Agosto 2011	Mefedrone, MDPV), 4-MEC, 3,4 Metilendiossimetcatinone (Metilone), 4-Fluoroamfetamina (4-FA), JWH-018	Circa 300 reperti costituiti da polveri e agglomerati di polveri, capsule, compresse, matrici vegetali, oggetti con tracce di sostanze
6	Novembre 2011	α-Pyrrolidinopentiophenone (α-PVP) e 4-MEC	Polveri (5 g)
7	Giugno 2012	Alto tenore di Tetraidrocannabinolo (THC)	Reperti vari
8	Luglio 2012	(2-aminopropil)benzofurano (APB)	Compressa (0.303 g)
9	Maggio 2013	APB, Pentedrone, 4-MEC, 25I-NBOMe, Metossietamina, Benzilpiperazina, 1-benzil-4-metilpiperazina e 1(-3-trifluorometil) piperazina	n. 8 compresse, n. 46 capsule 1 cartoncino, polveri (ca. 20 g)

Allertadroga.it – National Early Warning System



L'adulterante Destrometorfano

- ✓ Nel 2012-13 numerosi reperti in sequestro a base di Eroina contenenti Destrometorfano
- ✓ Nel 2012 sequestrato ed analizzato al LIATF circa ½ Kg di destrometorfano puro



JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, 2012
doi: 10.1111/j.1556-4029.2012.02133.x
Available online at: onlinelibrary.wiley.com

CASE REPORT

TOXICOLOGY

Barry K. Logan,[1,2] Ph.D.; Jillian K. Yeakel,[1] M.S.F.S.; Gary Goldfogel,[3] M.D.; Michael P. Frost,[4] M.D.; Greg Sandstrom,[5] A.A.; and Dennis J. Wickham,[6] M.D.

Dextromethorphan Abuse Leading to Assault, Suicide, or Homicide

ABSTRACT: Dextromethorphan is a commonly encountered antitussive medication which has found additional therapeutic use in the treatment of pseudobulbar disorder and as an adjunct to opiate use in pain management. Dextromethorphan at high doses has phencyclidine-like effects on the NMDA receptor system; recreational use of high doses has been found to cause mania and hallucinations. The toxicology and pharmacology of the drug in abuse are reviewed, and the historical literature of adverse psychiatric outcomes is assessed. Five new cases of dextromethorphan intoxication that resulted in assault, suicide, and homicide are reported, together with the corresponding toxicology results. Blood concentrations ranged from 300 to 19,000 μg/L. These results are compared with typical concentrations reported in therapeutic use and impaired driving cases. Based on these findings, dextromethorphan should be considered as a potential causative agent in subjects presenting with mania, psychosis, or hallucinations, and abusers are at risk for violent and self-destructive acts.

KEYWORDS: forensic science, forensic toxicology, dextromethorphan, psychosis, suicide, intoxication

Dextromethorphan is an over-the-counter antitussive medication present in many brands of cough, cold, and flu preparations, such as Robitussin, Coricidin, Benylin, and others (1). It is also the subject of renewed therapeutic interest as an adjunct to opiate use in pain management (2) and has been proposed as a possible rapidly acting antidepressant (3). The antitussive effects are believed to be mediated through the sigma receptor group, which has affinity for opioid antitussives such as codeine (4). Both dextromethorphan and its major metabolite dextrorphan have agonist activity at the phencyclidine (PCP)-1 receptor in the *N*-methyl-D-aspartate (NMDA) complex. When taken in significant excess, dextromethorphan produces hallucinogenic and dissociative effects similar in nature to PCP and ketamine. In the drug user community, dextromethorphan goes by the names Red devils, Robo, Skittles, Triple C's, Dex, and DXM. Dextromethorphan metabolism is mediated through the isoenzyme group CYP2D6, which is subject to genetic polymorphism, which may result in impaired metabolism and accumulation of the drug, leading to toxicity (5).

There is a significant Internet drug subculture regarding the recreational use of dextromethorphan, discussing and promoting the intoxicating and hallucinogenic effects of the drug, including out-of-body experiences which can be achieved by increasing dose levels through four "plateaus" to achieve the ultimate dissociative high (6). These plateaus, which have been related to dose amounts by user self-reports, correspond to different levels of altered consciousness. The first plateau (1.5–2.5 mg/kg) is described by users as causing mild stimulant and perceptual effects similar to MDMA (Ecstasy) (http://www.dextroverse.org/whatis.html, accessed May 29, 2011). The second plateau (2.5–7.5 mg/kg) may cause effects similar to those experienced with the co-ingestion of marijuana and ethanol including a more profound impairment of perceptual, cognitive, and motor functions than either drug alone (7). Mild hallucinations allegedly begin at this dosing level. In the third plateau (7.5–15 mg/kg), users report experiencing strong hallucinations, described as similar to low doses of ketamine. The fourth and highest plateau (>15 mg/kg) is described as the highest level, and effects are reportedly comparable to high-dose ketamine use. At this level users claim to have experienced complete mind/body dissociation (6). This dissociative state can result in violent behavior causing harm to the user or to bystanders. Diagnosis of the condition may be based on history, self-report, and circumstantial evidence. As demonstrated in the cases reported here, blood toxicological concentrations may also be helpful in assessing the degree of ingestion.

We describe a series of cases in which individuals abusing dextromethorphan became involved in violent or harmful behaviors. In contrast to previous reports, blood concentrations of dextromethorphan were measured to provide context to toxicological measurements made in future cases. Following the case reports, we review and discuss the various adverse psychological effects of the drug including psychosis, dissociation, and mania described in the literature since the 1960s.

Methods

The cases described were submitted in the course of routine criminal or death investigations and submitted for toxicological

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[2]NMS Labs, 3701 Welsh Road, Willow Grove, PA.
[3]Whatcom County Medical Examiner's Office, Bellingham, WA.
[4]Addiction Medicine, 706 Fayette Street, Conshohocken PA.
[5]Kitsap County Coroner's Office, Bremerton, WA.
[6]Clark County Medical Examiner's Office, Vancouver, WA.

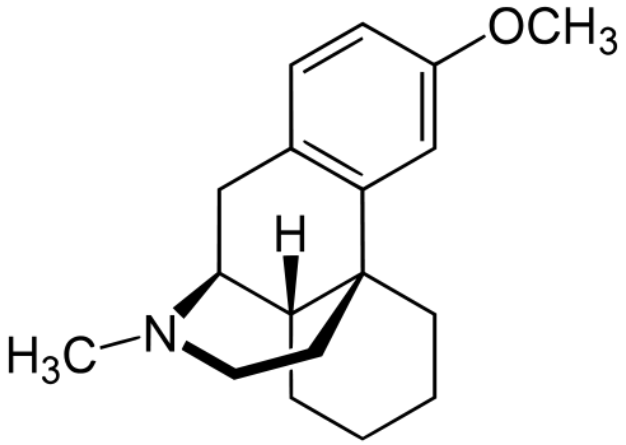
Received 19 Jan. 2011; and in revised form 1 June 2011; accepted 8 July 2011.

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L'adulterante Destrometorfano

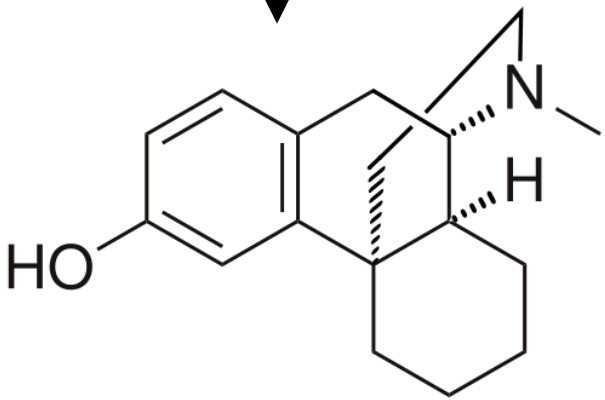
Destrometorfano

- A dosi terapeutiche (10-20 mg/4h): Antitussivo
- Ad alte dosi (sing. dose 250-1500 mg): Allucinogeno dissociativo!!!
- Effetti alluc. simili a Fenciclidina o Ketamina
- Effetti causati dal metabolita Destrorfano
- Potente agonista recett. N-metil-D-aspartato (NMDA)



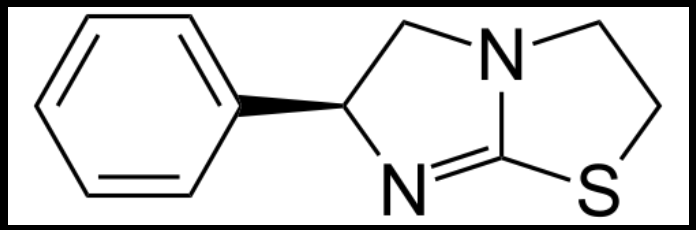
Destrometorfano

Isoforma CYP2D6
Citocr. P450

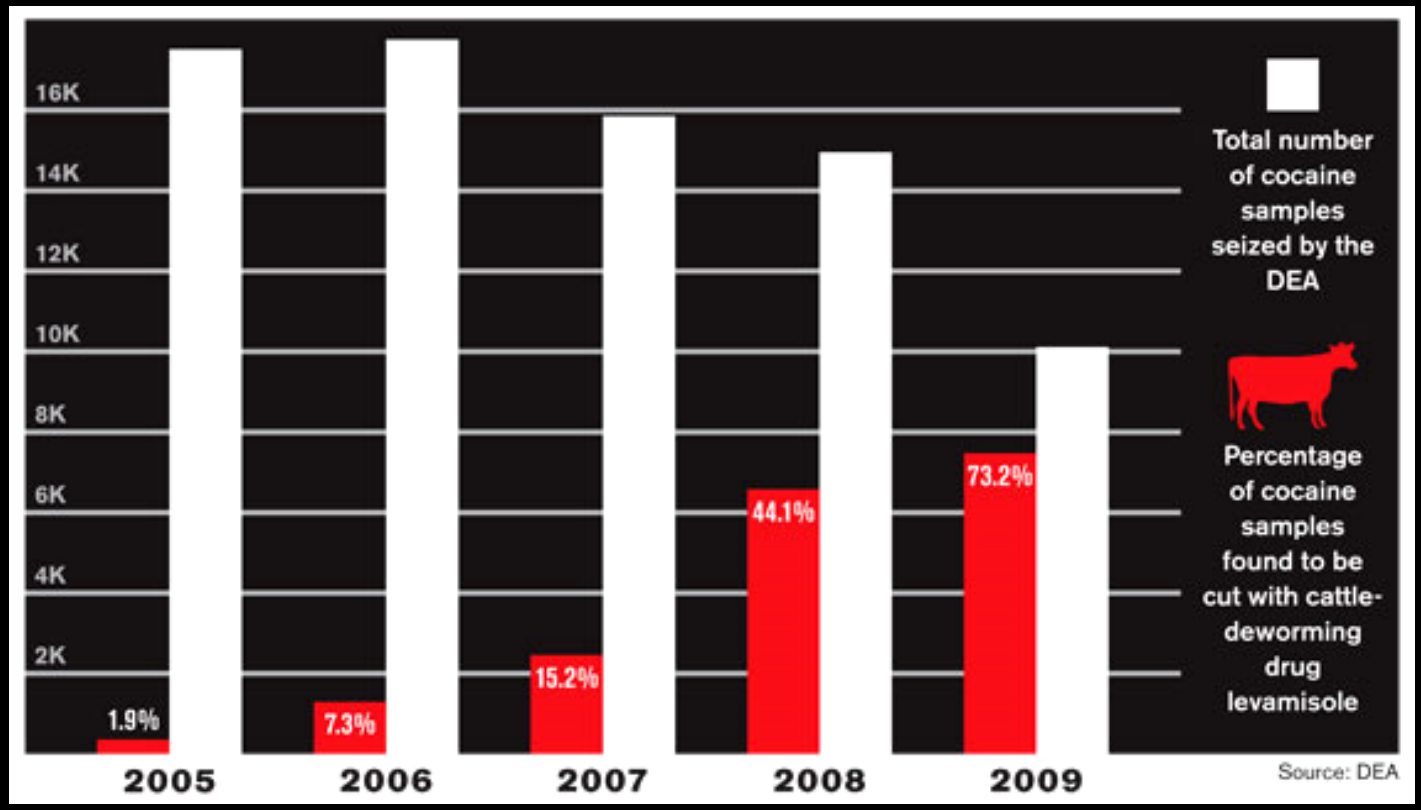


Destrorfano

L'adulterante Levamisolo

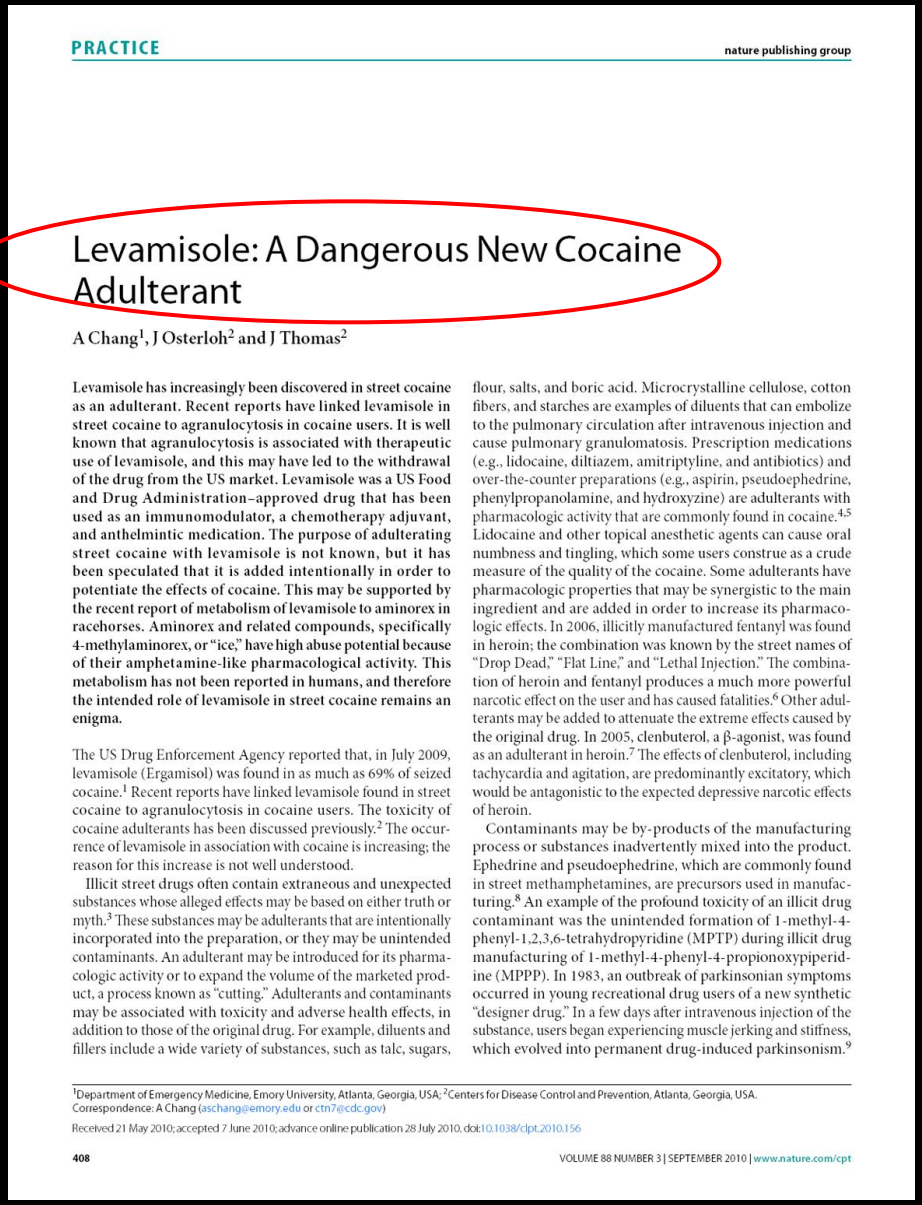


- ✓ Immunomodulatore, adiuv. chemioterapico (ritirato nel 1999)
- ✓ Antielmintico (antiparassitario) in veterinaria



- ✓ Negli USA circa 70-80% cocaina cont. levamisolo
- ✓ Elevate percentuali anche in Italia
- ✓ Numerosi casi evidenziati anche al LIATF

Perché Levamisolo addiz. a Cocaina?



PRACTICE nature publishing group

Levamisole: A Dangerous New Cocaine Adulterant

A Chang[1], J Osterloh[2] and J Thomas[2]

Levamisole has increasingly been discovered in street cocaine as an adulterant. Recent reports have linked levamisole in street cocaine to agranulocytosis in cocaine users. It is well known that agranulocytosis is associated with therapeutic use of levamisole, and this may have led to the withdrawal of the drug from the US market. Levamisole was a US Food and Drug Administration–approved drug that has been used as an immunomodulator, a chemotherapy adjuvant, and anthelmintic medication. The purpose of adulterating street cocaine with levamisole is not known, but it has been speculated that it is added intentionally in order to potentiate the effects of cocaine. This may be supported by the recent report of metabolism of levamisole to aminorex in racehorses. Aminorex and related compounds, specifically 4-methylaminorex, or "ice," have high abuse potential because of their amphetamine-like pharmacological activity. This metabolism has not been reported in humans, and therefore the intended role of levamisole in street cocaine remains an enigma.

The US Drug Enforcement Agency reported that, in July 2009, levamisole (Ergamisol) was found in as much as 69% of seized cocaine.[1] Recent reports have linked levamisole found in street cocaine to agranulocytosis in cocaine users. The toxicity of cocaine adulterants has been discussed previously.[2] The occurrence of levamisole in association with cocaine is increasing; the reason for this increase is not well understood.

Illicit street drugs often contain extraneous and unexpected substances whose alleged effects may be based on either truth or myth.[3] These substances may be adulterants that are intentionally incorporated into the preparation, or they may be unintended contaminants. An adulterant may be introduced for its pharmacologic activity or to expand the volume of the marketed product, a process known as "cutting." Adulterants and contaminants may be associated with toxicity and adverse health effects, in addition to those of the original drug. For example, diluents and fillers include a wide variety of substances, such as talc, sugars, flour, salts, and boric acid. Microcrystalline cellulose, cotton fibers, and starches are examples of diluents that can embolize to the pulmonary circulation after intravenous injection and cause pulmonary granulomatosis. Prescription medications (e.g., lidocaine, diltiazem, amitriptyline, and antibiotics) and over-the-counter preparations (e.g., aspirin, pseudoephedrine, phenylpropanolamine, and hydroxyzine) are adulterants with pharmacologic activity that are commonly found in cocaine.[4,5] Lidocaine and other topical anesthetic agents can cause oral numbness and tingling, which some users construe as a crude measure of the quality of the cocaine. Some adulterants have pharmacologic properties that may be synergistic to the main ingredient and are added in order to increase its pharmacologic effects. In 2006, illicitly manufactured fentanyl was found in heroin; the combination was known by the street names of "Drop Dead," "Flat Line," and "Lethal Injection." The combination of heroin and fentanyl produces a much more powerful narcotic effect on the user and has caused fatalities.[6] Other adulterants may be added to attenuate the extreme effects caused by the original drug. In 2005, clenbuterol, a β-agonist, was found as an adulterant in heroin.[7] The effects of clenbuterol, including tachycardia and agitation, are predominantly excitatory, which would be antagonistic to the expected depressive narcotic effects of heroin.

Contaminants may be by-products of the manufacturing process or substances inadvertently mixed into the product. Ephedrine and pseudoephedrine, which are commonly found in street methamphetamines, are precursors used in manufacturing.[8] An example of the profound toxicity of an illicit drug contaminant was the unintended formation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) during illicit drug manufacturing of 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP). In 1983, an outbreak of parkinsonian symptoms occurred in young recreational drug users of a new synthetic "designer drug." In a few days after intravenous injection of the substance, users began experiencing muscle jerking and stiffness, which evolved into permanent drug-induced parkinsonism.[9]

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Received 21 May 2010; accepted 7 June 2010; advance online publication 28 July 2010. doi:10.1038/clpt.2010.156

- ✓ Marker tracciante il mercato clandestino?
- ✓ Confondere i cani antidroga?
- ✓ Migliorare le caratteristiche chimico-fisiche della cocaina?
- ✓ Inibizione MAO e CMT: > catecolamine nelle sinapsi > azione cocaina!
- ✓ Metabolismo a Aminorex (amfetaminosimile, stimolante centrale) > azione cocaina!!

"Levamisole: another reason not to use cocaine"

- Agranulocytosis and other consequences due to use of illicit cocaine contaminated with levamisole
- Cutaneous Vasculopathy Associated with Levamisole-adulterated Cocaine
- Levamisole-induced occlusive necrotising vasculitis in cocaine abusers: An unusual cause of skin necrosis and neutropenia
- Palpable purpura complicated by streptococcal toxic shock syndrome resulting in limb necrosis and amputation: a case of levamisole and cocaine coingestion
- Vasculopathy, hematological, and immune abnormalities associated with levamisole-contaminated cocaine use
- Necrotizing peripheral vasculitis/vasculopathy following the use of cocaine laced with levamisole
- Purpura, cutaneous necrosis, and antineutrophil cytoplasmic antibodies associated with levamisole-adulterated cocaine
- Levamisole-induced necrosis of skin, soft tissue, and bone: case report and review of literature
- Necrosis of ears after use of cocaine probably adulterated with levamisole

“Levamisole: another reason not to use cocaine”











Aumento di THC nella Cannabis

Research article

Drug Testing and Analysis

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(www.drugtestinganalysis.com) DOI 10.1002/dta.1515

Variability of cannabis potency in the Venice area (Italy): A survey over the period 2010–2012

Luca Zamengo,* Giampietro Frison, Chiara Bettin and Rocco Sciarrone

Cannabis is the most widely used illicit substance globally, with an estimated annual prevalence in 2010 of 2.6–5.0% of the adult population. Concerns have been expressed about increases in the potency of cannabis products. A high tetrahydrocannabinol (THC) content can increase anxiety, depression, and psychotic symptoms, and can increase the risk of dependence and adverse effects on the respiratory and cardiovascular systems in regular users. The aim of this study was to report statistical data about the potency of cannabis products seized in the north-east of Italy, in a geographical area centred in Venice and extending for more than 10 000 km^2 with a population of more than two million, by investigating the variability observed in THC levels of about 4000 samples of cannabis products analyzed over the period 2010–2012. Overall median THC content showed an increasing trend over the study period from about 6.0% to 8.1% (6.2–8.9% for cannabis resin, 5.1–7.6% for herbal cannabis). The variation in the THC content of individual samples was very large, ranging from 0.3% to 31% for cannabis resin and from 0.1 to 19% for herbal cannabis.

Median CBN:THC ratios showed a slightly decreasing trend over the study period, from 0.09 (2010) to 0.03 (2012), suggesting an increasing freshness of submitted materials. Median CBD:THC ratios also showed a decreasing trend over the study from about 0.52 (2010) to 0.18 (2012), likely due to the increase in submissions of materials from indoor and domestic cultivation with improved breeding methods. **Copyright © 2013 John Wiley & Sons, Ltd.**

Keywords: cannabis potency; Venice (Italy); seized materials; THC; CBD; CBN

Introduction

Cannabis is the most widely used illicit substance globally, with an estimated annual prevalence in 2010 of 2.6–5.0% of the adult population (between 119 million and 224 million users aged 15–64 years).[1] Some recent data[2] from Western and Central Europe indicate a stable or decreasing trend in cannabis use, especially among young adults, also mirrored in school surveys and partly influenced by the declining trends in tobacco smoking among young people or the replacement of cannabis with other drugs. In Italy, in the years 2010–2012, a decrease from 3.0% to 1.8% in the use of cannabis for the population aged 15–64, has been reported, along with a minor increase (+0.3%) for the population in the scholastic range (15–19 years).[3]

On the contrary, a dramatic increase in illicit cultivation of cannabis plants in Western and Central Europe on an industrial scale, mainly indoors, and with the involvement of organized criminal groups has been also reported.[4] According to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), the rise of cannabis production within the European Union has resulted in the increasing displacement of imported cannabis resin by home-produced herbal cannabis products. Indeed, a decreasing trend in seizures of cannabis resin was observed in many countries in Europe, while seizures of cannabis herb increased in some countries in recent years with trends varying between countries.[4] In Italy, from 2010 to 2011, a significant increase of herbal cannabis seizures (+98%) was reported, along with a minor variation for cannabis resin (+0.2%).[3]

The rise of indoor cultivation of cannabis is often related to an increase in cannabis potency, which is reflected in the published data only to a limited extent.[1] Concerns have been expressed about increases in the potency, expressed as delta-9-tetrahydrocannabinol (THC) content in per cent by weight of cannabis products.[5–8] A high THC content can increase anxiety, depression, and psychotic symptoms, and can increase the risk of dependence and adverse effects on the respiratory and cardiovascular systems in regular users.[9] Regular cannabis use in adolescence might also adversely affect mental health in young adults, with the strongest evidence for an increased risk of psychotic symptoms and disorders.[9]

However, published data concerning the diffusion of high potency cannabis varieties and the level of increase in potency are poor worldwide. Two reviews on cannabis potency mention that the data on this issue are still not adequate and further research is required.[10,11]

The United Nations Office on Drugs and Crime (UNODC) has pointed out several problems with the comparison of THC level data from different countries or periods, such as differences in the classification of cannabis and in the analytical method, or a lack of randomness in sampling.[12]

In forensic studies of cannabis, potency has routinely been described by simply reporting the THC content in per cent by weight. However, the role of other major cannabinoids such as cannabidiol (CBD) and cannabinol (CBN), may also be relevant.

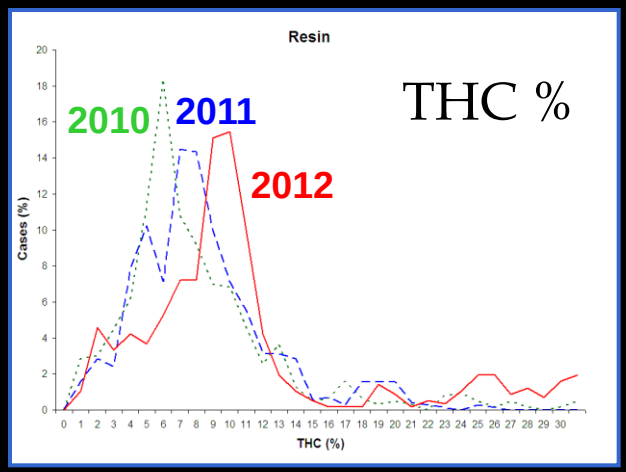
In particular, CBN, which is not present in fresh materials,[13,14] is regarded as a primary degradation product of THC. Thus, the

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- ✓ Analisi di 4000 preparati della Cannabis (hashish e marijuana), sequestrati nel periodo 2010-2012 nelle province di Venezia, Treviso, Rovigo e Belluno
- ✓ Contenuto di THC: 6,0% nel 2010
  6,5% nel 2011
  8,1% nel 2012
- ✓ < CBN, < CBD
- ✓ > Coltivazioni locali e indoor

LIATF – Analisi di campioni biologici





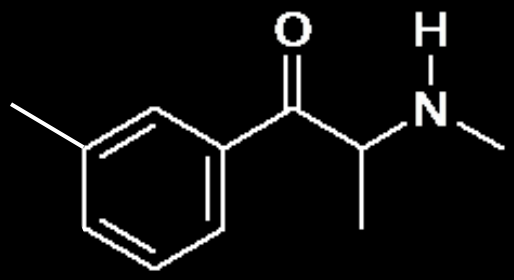




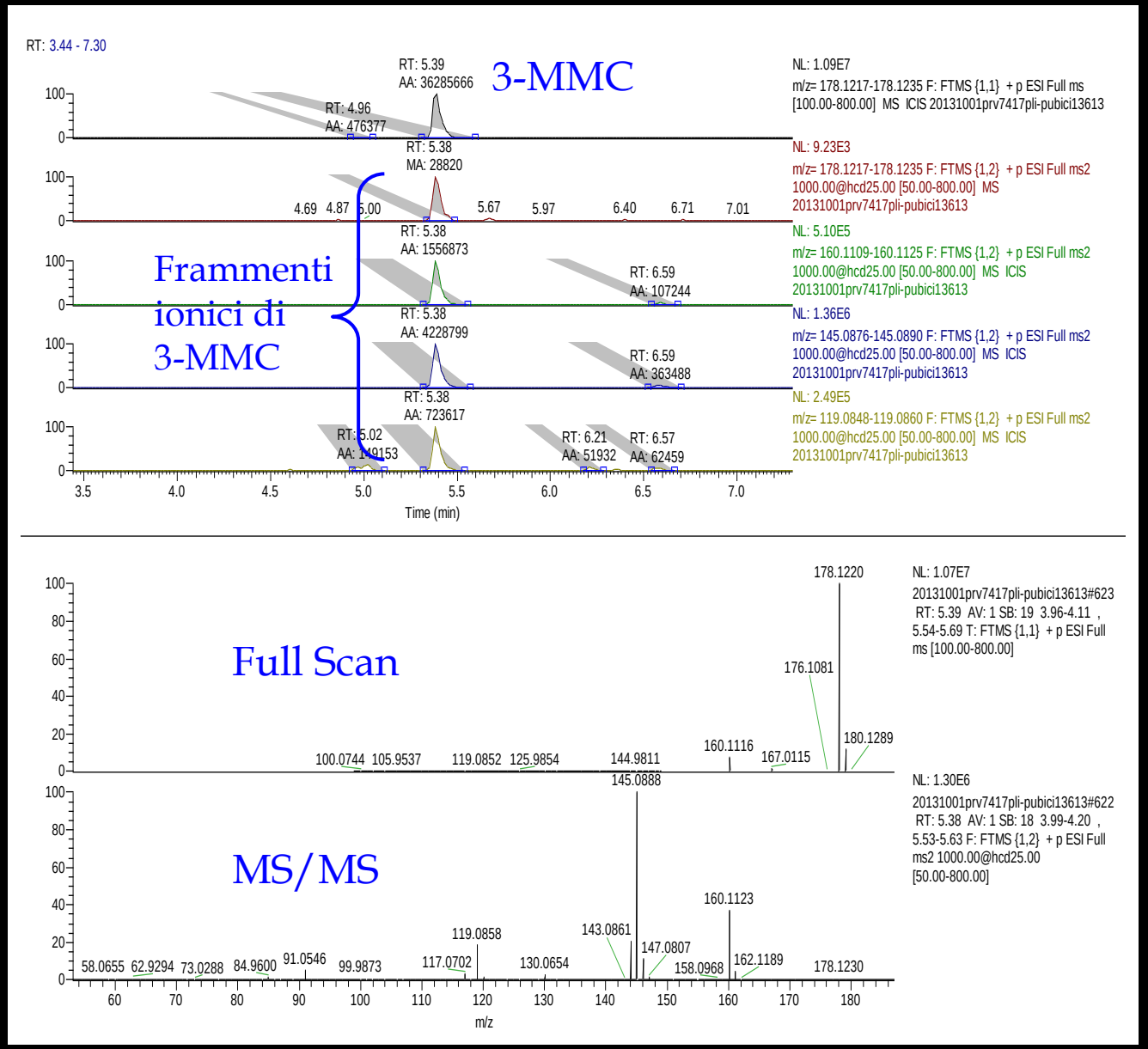
✓ Ambiti applicativi

- Determinazione alcol, farmaci, sostanze psicoattive in campioni biologici
- Disabilità alla guida
- Idoneità alla guida
- Idoneità al lavoro
- Reati sessuali facilitati da sostanze psicoattive
- Tossicologia post-mortale
- Altri ambiti applicativi tossicologico-forensi

3-MMC in peli pubici di assuntore

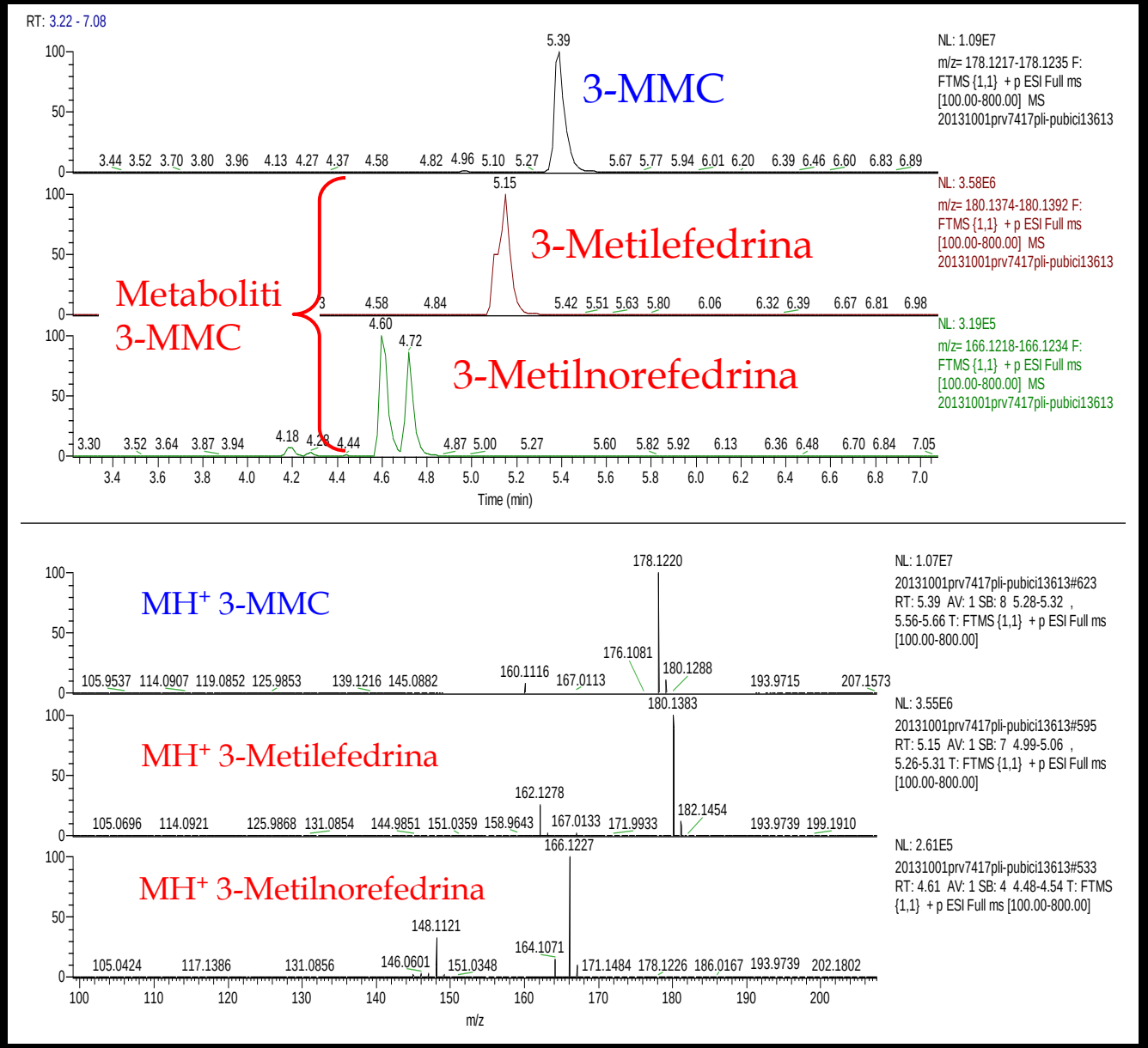


- Campione di peli pubici di soggetto abituale assuntore di 3-MMC
- Analisi campione con UHPLC/HRMS
- Identificazione di 3-MMC nei peli pubici

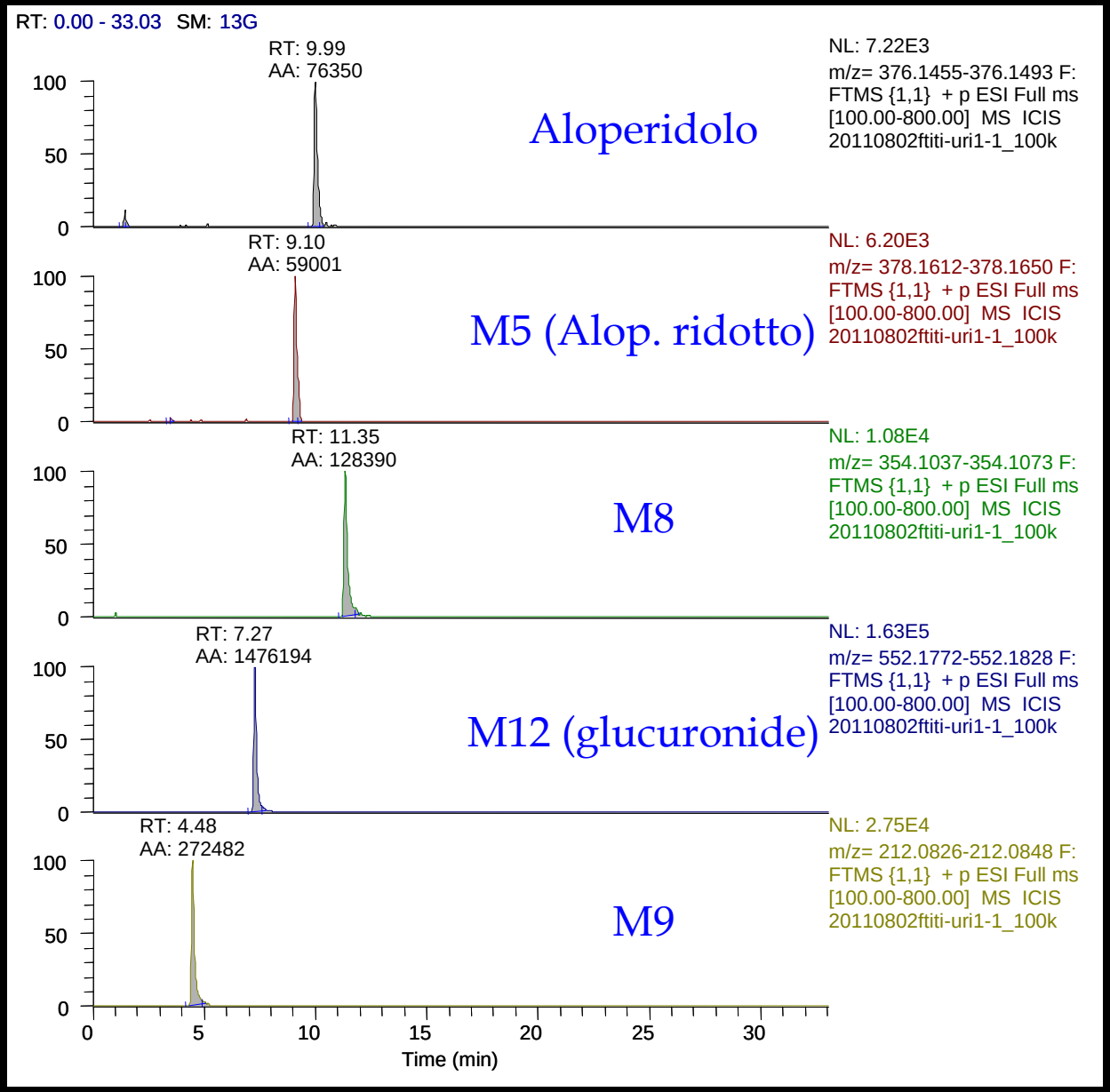


Metaboliti di 3-MMC nei peli pubici

- Identificazione dei metaboliti del 3-MMC nei peli pubici del soggetto:
  3-Metilefedrina
  3-Metilnorefedrina
- Distinzione tra contaminazione esterna della matrice biologica ed assunzione della sostanza psicoattiva



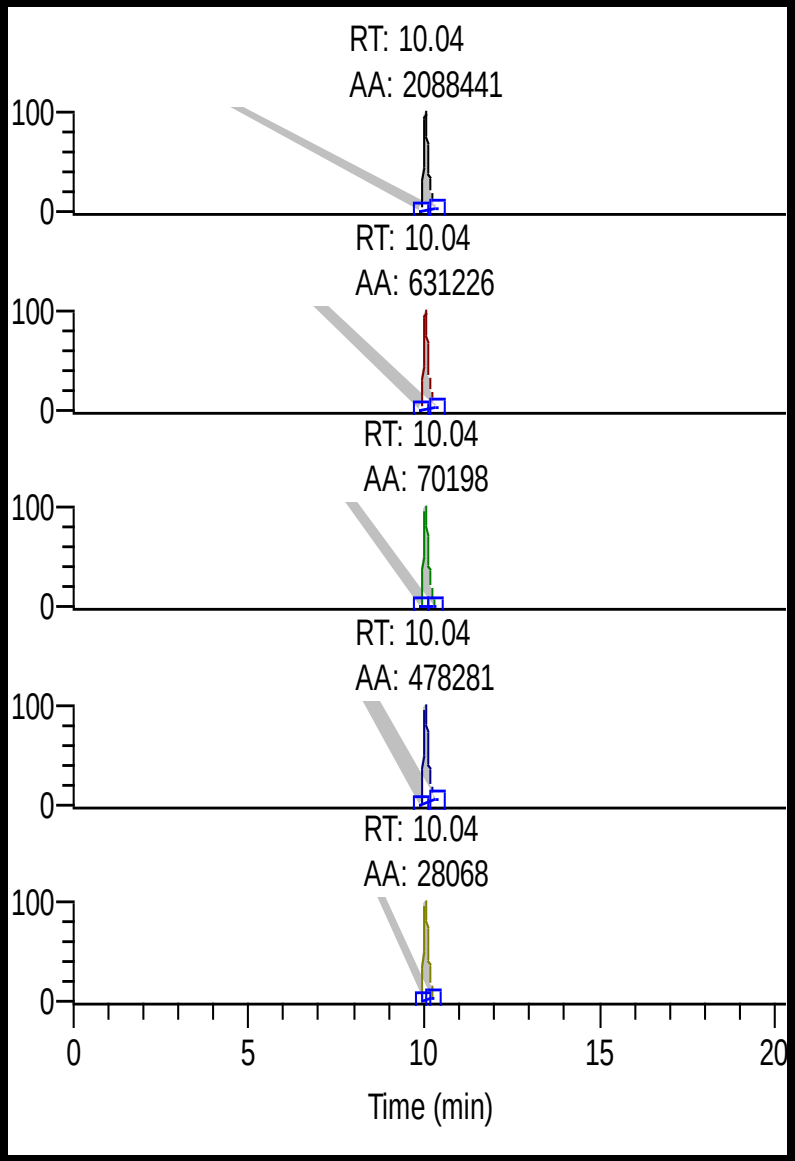
Intoss. da Aloperidolo in tre adolescenti

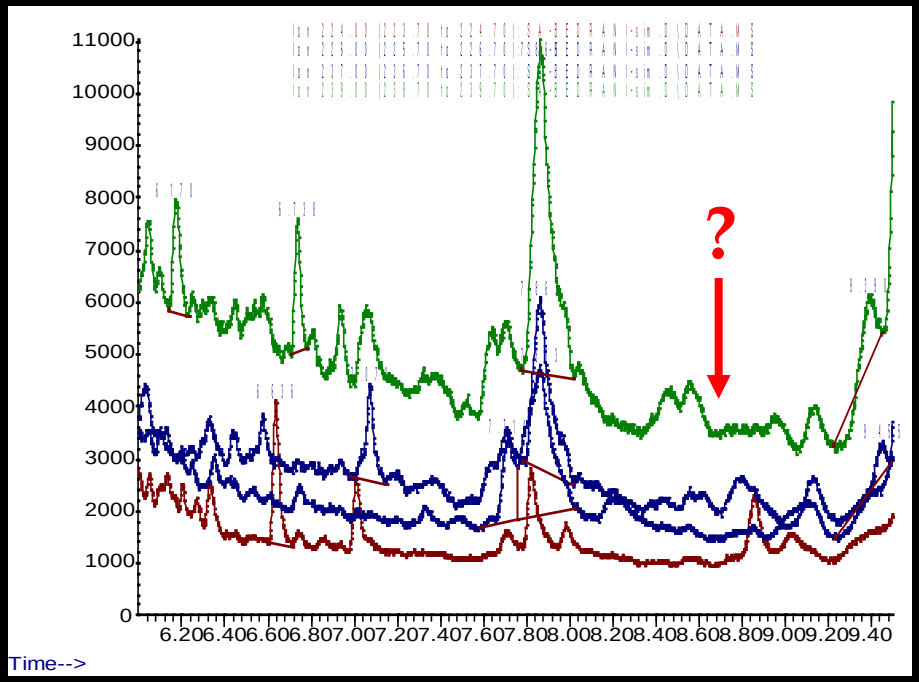


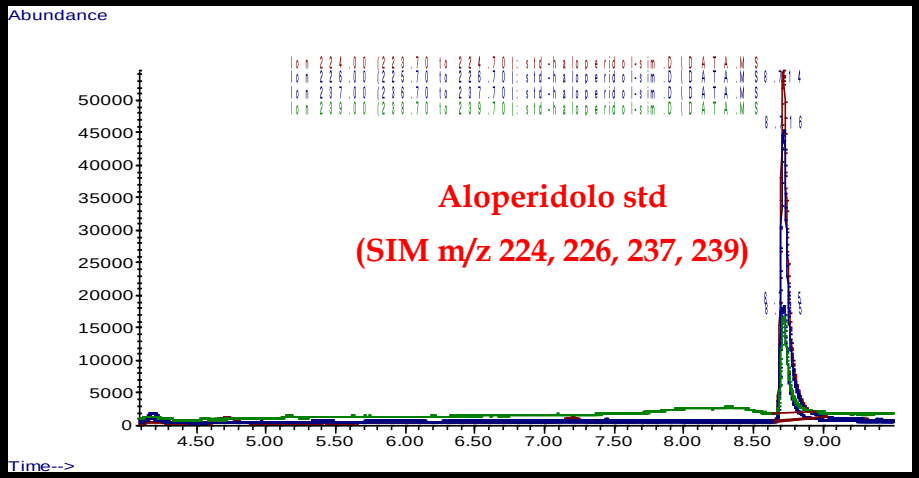
- ✓ Tre adolescenti assumono compresse comprate in strada
- ✓ Accesso a PS con disturbi neurologici (**sindrome extrapiramidale**)
- ✓ Analisi tox. (anche GC/MS) negative in tutti i soggetti
- ✓ Analisi di sangue e urina con UHPLC/HR-MS: **Aloperidolo e metaboliti** in tutti i soggetti!

UHPLC/HR-MS vs. GC/MS

Sangue







Dietilammide dell'acido lisergico (LSD)



UHPLC/HRMS – LSD in sangue e urina

42° Congresso Nazionale SIMLA – XV Giornate Medico Legali Romane ed Europee – Giornata GTFI – Roma, 20 giugno 2012

Determinazione di LSD, iso-LSD, nor-LSD, 2-oxo-3-idrossi-LSD in sangue e urina mediante U-HPLC/HR-MS in un caso di precipitazione volontaria



F. Zancanaro, G. Frison, S. Frasson, S. Zancaner, R. Sciarrone

Laboratorio di Igiene Ambientale e Tossicologia Forense (LIATF) e Servizio di Medicina Legale - Dipartimento di Prevenzione

Azienda ULSS 12 Veneziana

Mestre - Venezia



INTRODUZIONE



DAL 1887

IL GAZZETTINO

www.gazzettino.it

il Quotidiano del Nord Est

All'interno il fascicolo VENEZIA MESTRE

Mistero a Bibione, giovane nudo cade dal terzo piano sopra due auto: è grave

Un 28enne friulano trovato in una pozza di sangue

Era con due amici, ha attraversato una finestra di 40 centimetri

VENEZIA - Giallo a Bibione (Venezia): un giovane friulano è caduto dal terzo piano dell'appartamento di villeggiatura che aveva appena occupato per qualche giorno di vacanza con gli amici. Ieri intorno alle sette il giovane è stato trovato dai soccorritori completamente nudo, in una pozza di sangue: è gravissimo. Un volo di 13 metri, attutito da due auto che erano posteggiate dietro al complesso residenziale nel pieno centro della località turistica veneziana. I carabinieri indagano per capire cosa sia accaduto. Ipotizzato un fatto accidentale, ma al vaglio ci sono anche il tentato suicidio e un possibile omicidio. Per questo il magistrato ha ascoltato i due coetanei del ragazzo: «Abbiamo passato una serata tra amici - hanno raccontato i due giovani agli investigatori - ci siamo divertiti e poi siamo rientrati. Non sappiamo cosa possa essere successo a Giovanni». Da una prima sommaria ricostruzione, il ventottenne si sarebbe lanciato dalla finestra del bagno. Un manovra che per gli investigatori non era nemmeno tecnicamente facile. Il giovane infatti è volato dal terzo piano, attraversando una finestra del bagno che misura circa 40 centimetri di larghezza e un metro di altezza, ed è finito sul parcheggio che dà sul retro, ma a più di 7 metri di distanza dal muro, cadendo su due auto.

Dopo avere trascorso una serata in compagnia di amici, un giovane ventottenne è precipitato all'alba, completamente nudo, dalla finestra del bagno di un appartamento situato al terzo piano di un complesso residenziale, da un'altezza di circa 13 metri. E' atterrato su due automobili in sosta e, immediatamente soccorso, è stato ricoverato in ospedale in gravissime condizioni (nel riquadro a sinistra il relativo articolo di stampa). La dinamica dell'accaduto è subito apparsa relazionabile ad un lancio volontario dalla finestra, con possibile rincorsa, considerata la posizione delle auto colpite, situate a circa 6-7 metri dalla perpendicolare di caduta.

Il poster descrive la strategia analitica adottata per le analisi tossicologiche, essenzialmente basate sull'impiego di un sistema liquido-cromatografico ad ultra prestazioni accoppiato a spettrometria di massa Orbitrap ad elevata risoluzione (U-HPLC/HR-MS), ed i relativi risultati ottenuti. Nei campioni di sangue e urina, prelevati alcune ore dopo l'accaduto dai medici rianimatori, è stata determinata la presenza dell'allucinogeno LSD (dietilammide dell'acido lisergico) e di alcuni suoi metaboliti. Le tecniche MS ad elevata risoluzione hanno permesso di utilizzare un generico trattamento estrattivo dei campioni biologici, di effettuare una ricerca generica indeterminata senza preselezione di specie ioniche caratteristiche, di determinare l'LSD nei campioni biologici fino a concentrazioni di pochi pg/mL, e di identificare i suoi metaboliti anche in assenza di standard di riferimento.

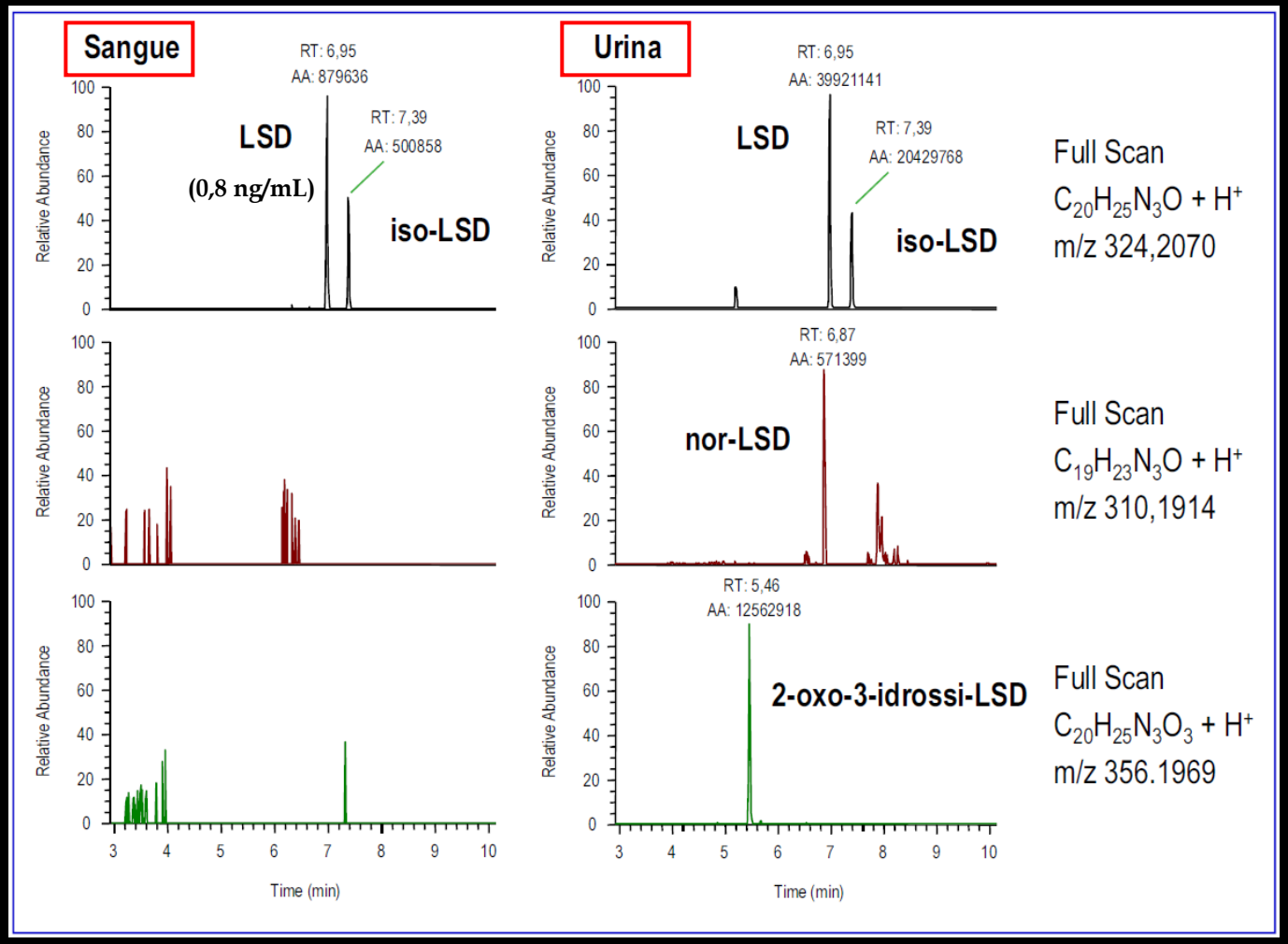
L'assunzione di LSD è stata ritenuta idonea a determinare la precipitazione volontaria, in relazione agli effetti farmacologici dispercettivi ed allucinatori (descritti in tabella 1) propri di tale xenobiotico. Il giovane è stato dimesso dopo un prolungato periodo di ricovero ospedaliero.

TABELLA 1 Typical sensory and psychological effects under the influence of a medium dose of LSD (100–200 μg p.o.)

Sensory alterations (visual, auditory, taste, olfactory, kinaesthetic)
Illusion
Pseudo-hallucination
Intensification of color perception
Metamorphosis-like change in objects and faces
Intense (kaleidoscopic or scenic) visual imagery with transforming content
Alterations of affectivity
Intensification of emotional experience: euphoria, dysphoria, anxiety, mood swing
Alterations of thinking
Less abstract and more imaginative thought
Broader and unusual association
Attention span shortened
Alterations of body perceptions
Change in body image
Unusual inner perception of bodily processes
Metamorphic alteration of body contours
Memory changes
Reexperiencing significant biographical memories
Hypermnesia
Age-regression
Mystical-type experiences

Da: Passie et al., CNS Neuroscience & Therapeutics 14 (2008), 295-314.

UHPLC/HRMS: LSD e metaboliti

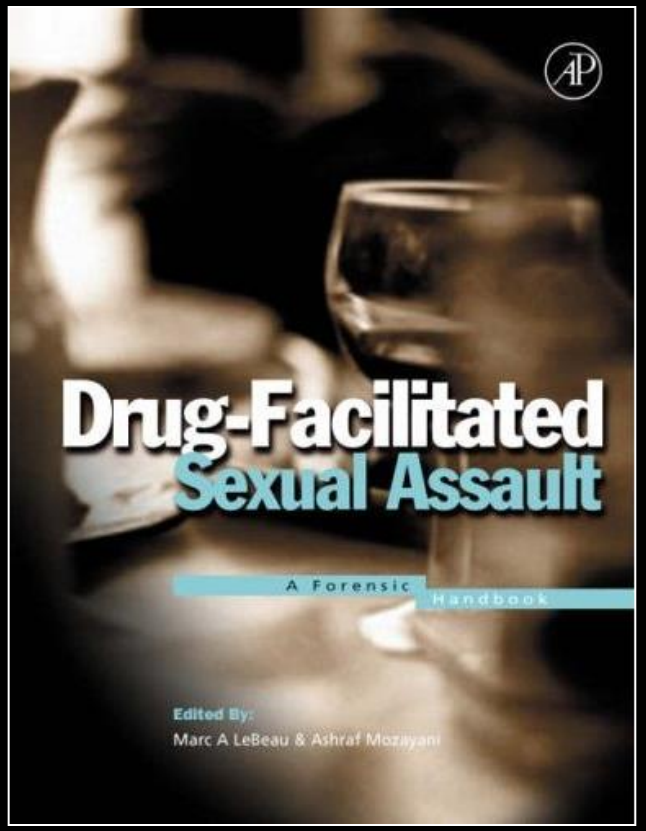


- ✓ UHPLC/HR-MS: identif. LSD e metaboliti senza std di riferimento
- ✓ Sensibilità analitica per LSD di pochi pg/mL
- ✓ Effetti dispercettivi ed allucinatori LSD > precipitazione volontaria

Drug-Facilitated Sexual Assault (DFSA)



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



DFSA con Z-drugs

- ✓ Zolpidem, Zopiclone, Zaleplon: ipnotici non BDZ
- ✓ Rapida azione, > amnesia retrograda, no screening IA
- ✓ Metodo UHPLC/HRMS per analisi urina in casi DFSA





Research article

Drug Testing and Analysis

Received: 30 November 2012 Revised: 24 January 2013 Accepted: 13 February 2013 Published online in Wiley Online Library

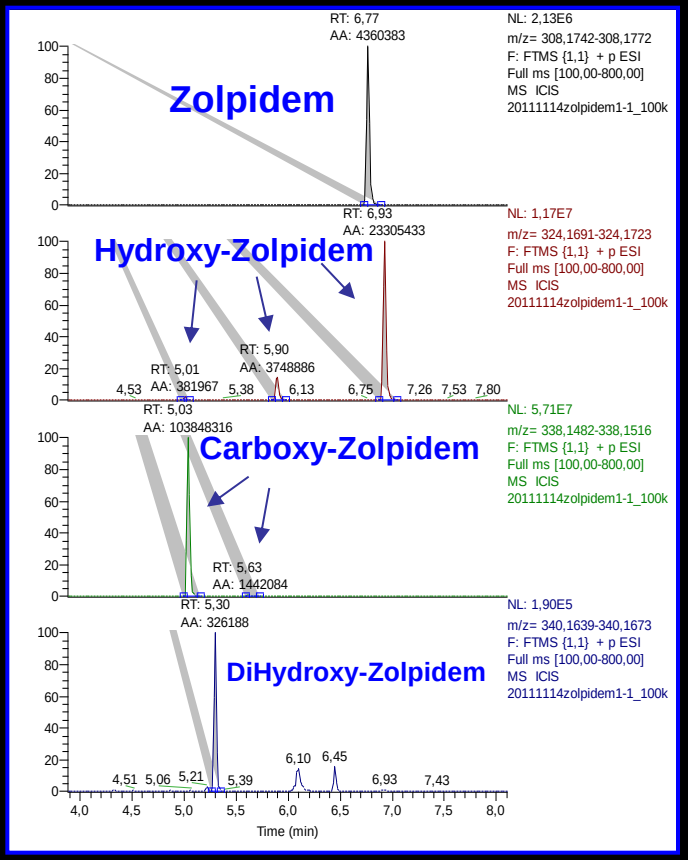
(www.drugtestinganalysis.com) DOI 10.1002/dta.1470

UHPLC-MS/MS and UHPLC-HRMS identification of zolpidem and zopiclone main urinary metabolites and method development for their toxicological determination

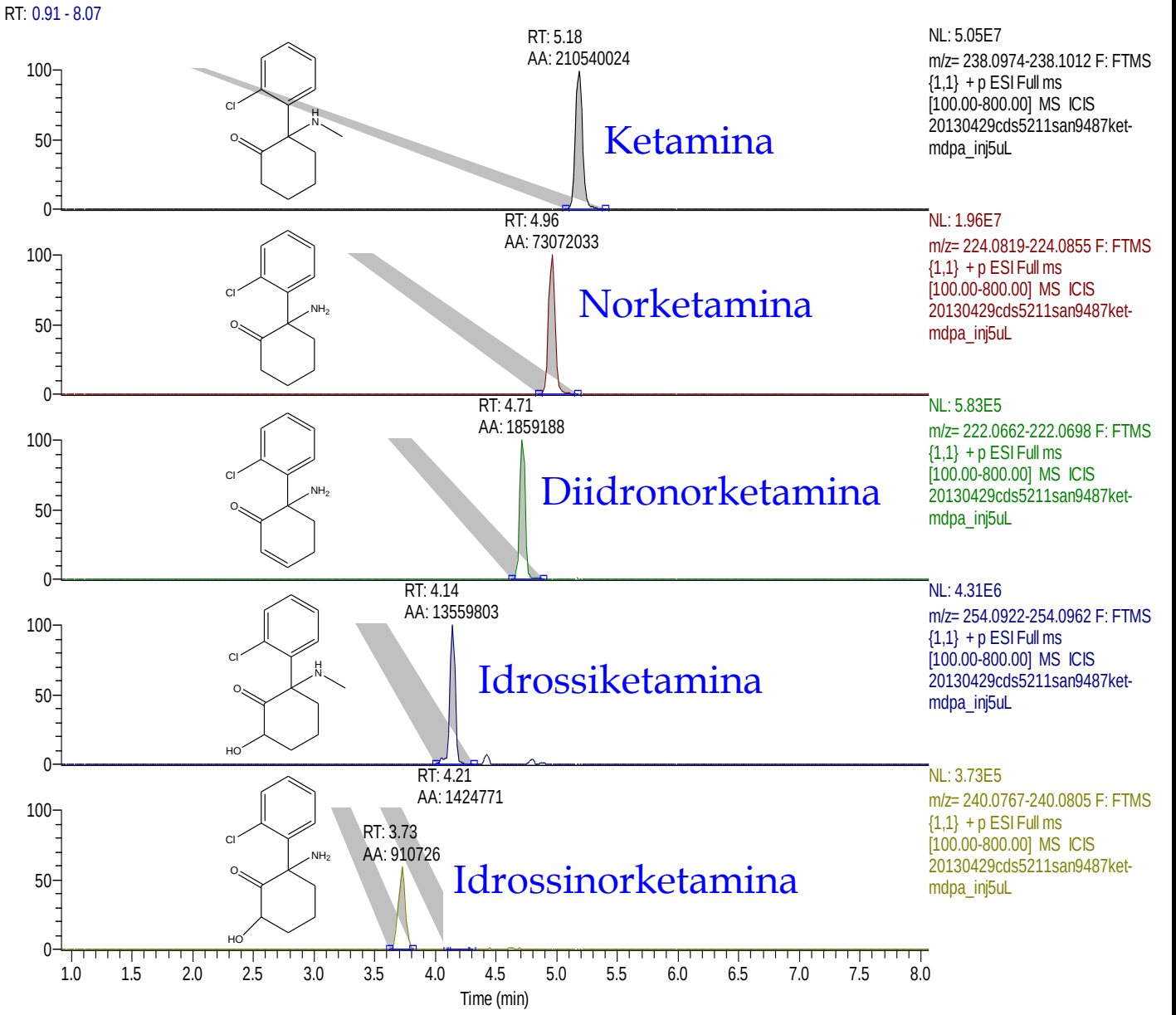
Sabina Strano Rossi,[a]* Luca Anzillotti,[a] Erika Castrignanò,[a] Giampietro Frison,[b] Flavio Zancanaro[b] and Marcello Chiarotti[a]

Zolpidem and zopiclone (Z-compounds) are non-benzodiazepine hypnotics of new generation that can be used in drug-facilitated sexual assault (DFSA). Their determination in biological fluids, mainly urine, is of primary importance; nevertheless, although they are excreted almost entirely as metabolites, available methods deal mainly with the determination of the unmetabolized drug. This paper describes a method for the determination in urine of Z-compounds and their metabolites by ultra-high-pressure liquid chromatography/tandem mass spectrometry (UHPLC-MS/MS) and UHPLC coupled with high resolution/high accuracy Orbitrap® mass spectrometry (UHPLC-HRMS). The metabolic profile was studied on real samples collected from subjects in therapy with zolpidem or zopiclone; the main urinary metabolites were identified and their MS behaviour studied by MS/MS and HRMS. Two carboxy- and three hydroxy- metabolites, that could be also detected by gas chromatography/mass spectrometry (GC-MS) as trimethylsylyl derivatives, have been identified for zolpidem. Also, at least one dihydroxilated metabolite was detected. As for zopiclone, the two main metabolites detected were N-demethyl and N-oxide zopiclone. For both substances, the unmetabolized compounds were excreted in low amounts in urine. In consideration of these data, a UHPLC-MS/MS method for the determination of Z-compounds and their main metabolites after isotopic dilution with deuterated analogues of zolpidem and zopiclone and direct injection of urine samples was set up. The proposed UHPLC-MS/MS method appears to be practically applicable for the analysis of urine samples in analytical and forensic toxicology cases, as well as in cases of suspected DFSA.
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Keywords: zolpidem; zopiclone; metabolites; LC-MS/MS; LC-HR-MS; GC-MS



Ketamina in camp. sangue conducente

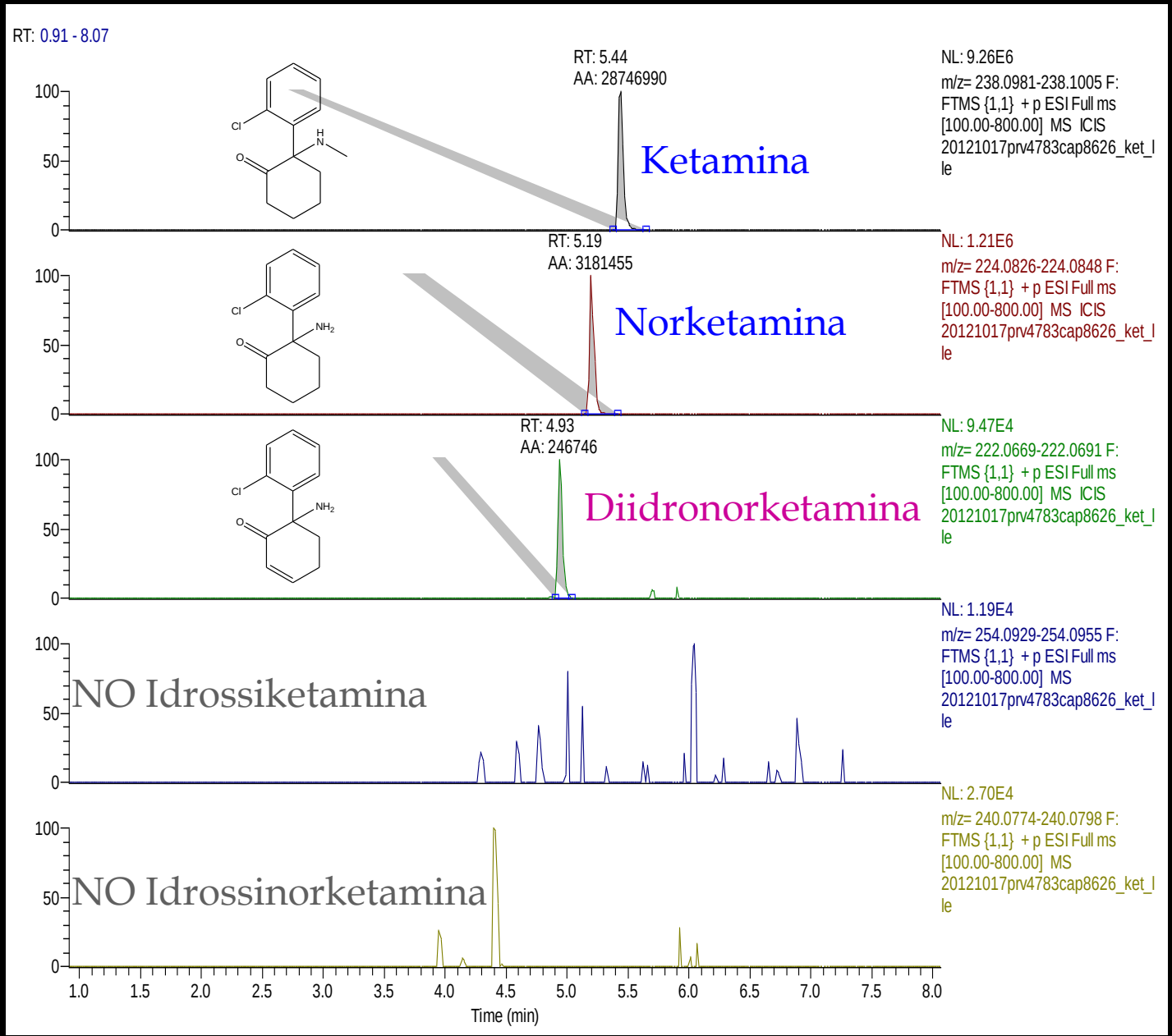


Ketamina: 230 ng/mL
Norketamina: 85 ng/mL

Identificazione altri metaboliti:

- Valutazione comportamento cromatografico
- Misure di massa accurata delle specie ioniche MH^+
- Esame frammentazione ionica MS/MS
- Confronto pattern isotopici MH^+ sperimentali e calcolati

Ketamina in camp. capelli conducente



Ketamina: 3,1 ng/mg
Norketamina: 0,4 ng/mg

- Presenza Diidronorketamina!
- Analogo RT, stessi MS data (valore di massa accurata, pattern di frammentazione, cluster isotopico) del sangue!
- Concentrazione stimata: circa 1/100 e 1/10 di ketamina e norketamina
- Assenza di metaboliti idrossilati

Disabilità alla guida



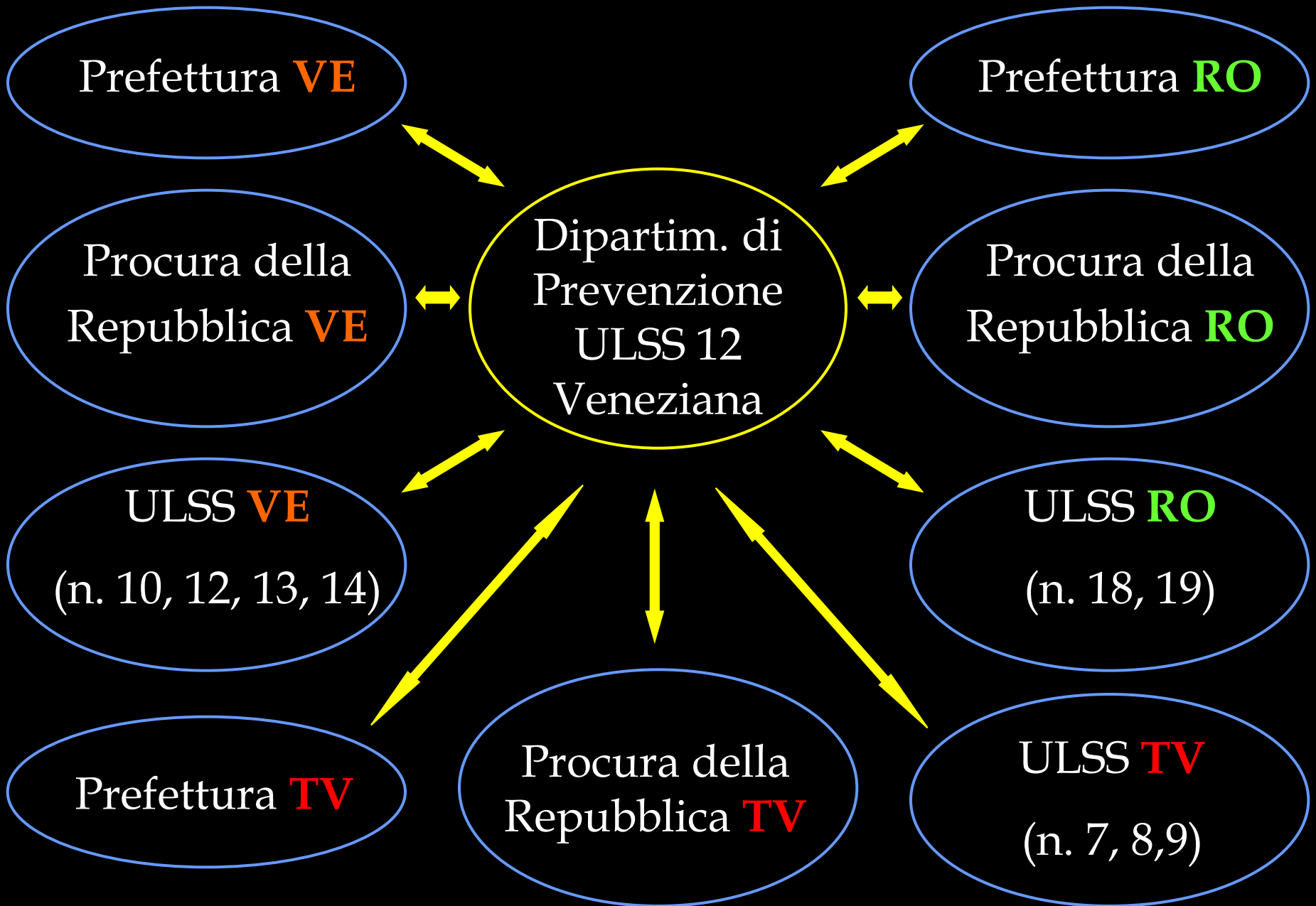


Conducenti di veicoli in stato di
intossicazione da alcol, farmaci
e sostanze psicoattive
(Artt. 186 e 187 Codice della Strada)





Protoc. d'intesa accert. Artt. 186, 187 CdS



Forze di P.G. coinvolte (ottobre 2013)

- Compagnie/Tenenze/Stazioni Carabinieri Leg. Veneto
- Sezioni/Distaccamenti Polizia Stradale Compart. Veneto
- Commissariati P.S./Squadre Vol. Questure
- Strutture di Polizia Locale/Municipale
- Guardia di Finanza

Strutture di P.S. coinvolte (ottobre 2013)

ULSS 10, 12, 13, 14

Mestre
Venezia
Chioggia
San Donà di Piave
Portogruaro
Jesolo
Bibione
Caorle
Dolo
Mirano

ULSS 7, 8, 9

Treviso
Oderzo
Conegliano
Vittorio Veneto
Castelfranco V.to
Montebelluna



ULSS 18, 19

Rovigo
Adria
Porto Viro
Trecenta

Procedure Operative Protocollo

Polizia Giudiziaria

Richiesta di accertamenti, ex Artt. 186 – 187,
al personale sanitario di PS

Pronto Soccorso ULSS

Prelievo campioni biologici (sangue e urina) e invio
(< 72 h) al LIATF a T controllata, con catena di custodia

LIATF - Dip. Prev. ULSS 12 VE

Ricezione campioni biologici, analisi, refertazione,
conservazione aliquote controanalisi per 12 mesi

Medicina Legale - Dip. Prev. ULSS 12 VE

Giudizio tossicologico-forense risultati analitici

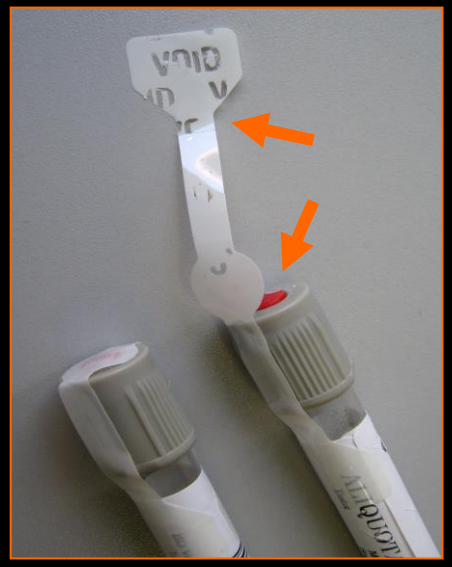
Invio refertazione
integrata a P.G.
richiedente

Gestione campioni biologici











- ✓ **Caratt. tossicol.-forensi**
  - Dispositivi dedicati sigillati
  - Provette con NaF e K-Ossalato
  - Ogive e sigilli antieffrazione
  - Aliquote analisi, controanalisi
  - Verbale di raccolta campioni
  - Catena di custodia campioni

Risultati attività (giugno 2010 – ottobre 2013)

Richieste accertamenti	ca. 4200
Richieste ex Art. 186	13,0 %
Richieste ex Art. 187	7,6 %
Richieste ex Art. 186 + 187	79,4 %

Campioni biologici	ca. 7500
Sangue	ca. 4000
Urina	ca. 3500

Camp. sangue POS Totale	32%
Camp. sangue POS Alcol	22%
Camp. sangue POS Sostanze	15%
Camp. sangue POS Alcol + Sost.	5%

Risultati attività (giugno 2010 – ottobre 2013)

Conducenti POSITIVI su sangue per Droghe

Classe	Cannabis	Cocaina	Oppiacei	Metadone	Amfetamine
%	54%	37%	9% *	7%	3,5%

*= Esclusi casi con terapia a base di Morfina o Codeina

Conducenti POSITIVI su sangue per Alcol

Alcol (g/L)	0,1 – 0,5	0,5 – 0,8	0,8 – 1,5	> 1,5
Incidenti stradali	17,9%	10,3%	28,2%	43,6%

Il gruppo della Tossicologia Forense al LIATF



Grazie per l'attenzione!