



NEW PSYCHOACTIVE SUBSTANCES THE LABORATORY PERSPECTIVE

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Liege University Hospital

(Prof Charlier)

24/10/2023

Laboratory's missions



Industrial Toxicology

Clinical Toxicology

- Therapeutic Drug Monitoring
 - Antibiotics, immunosuppressants, anticonvulsants, ...
 - Screen for Intoxication
 - Alcohol
 - Psychoactive Medicines (antidepressants, anxiolytics, sleeping pills, opiates...)
 - Drugs of abuse (cannabis, cocaine, heroin, MDMA)
- Compare with reference range
- Explain symptoms



Forensic Toxicology

- Biological samples
 - Road safety (Crash/Control)
 - Cause of death
 - Crime (Victim/suspect)
 - Child Custody
 - ...
- Seized samples
 - Cannabis
 - Powders
 - Tablets
 - ...



Environmental Toxicology



How to perform an analysis

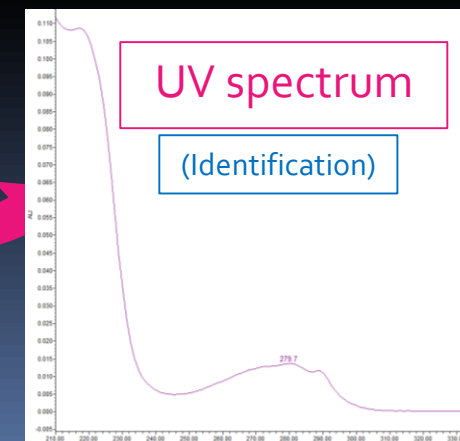
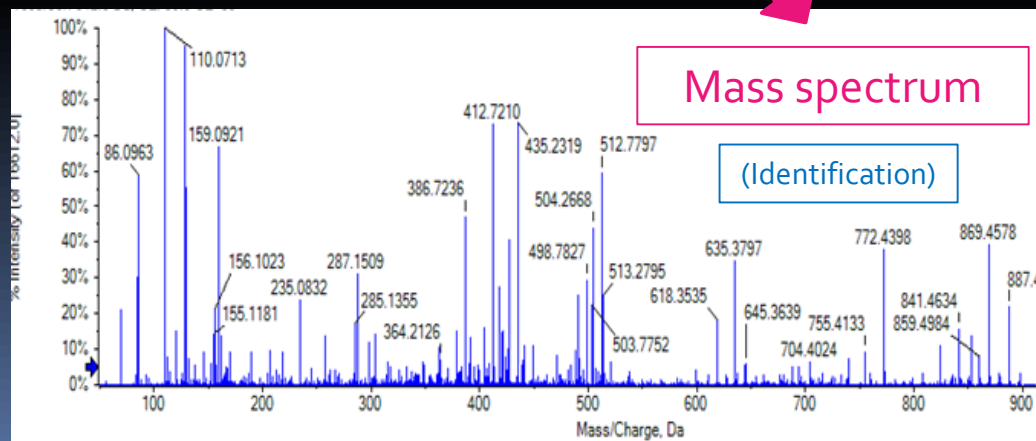
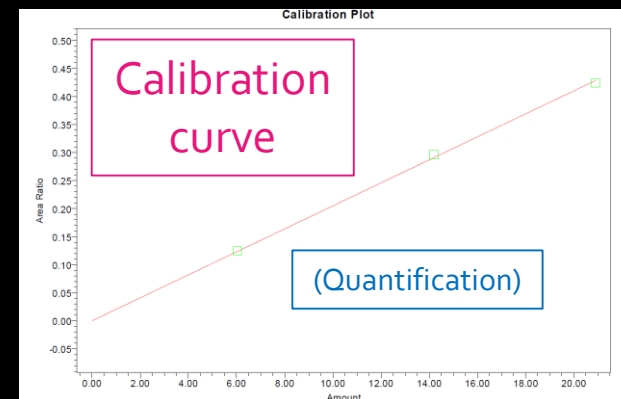
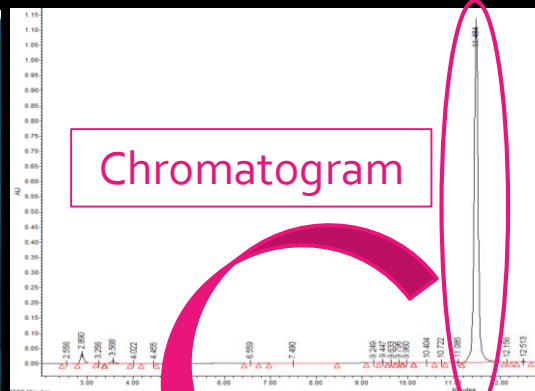


Before NPS

Reference standard



⇒ Targeted Chromatographic Method



NPS challenge

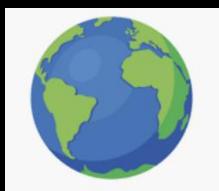


European Monitoring Centre
for Drugs and Drug Addiction

1. Number of substances – permanent increase



930 NPS



1184 NPS

245
SCRA



162
Cathinones



115
Autres substances

73
Opioides



57
Tryptamines



41
Arylalkylamines

27
Arylcyclohexylamines

106
Phénéthylamines



18
Pipérazines

33
Benzodiazépines



15

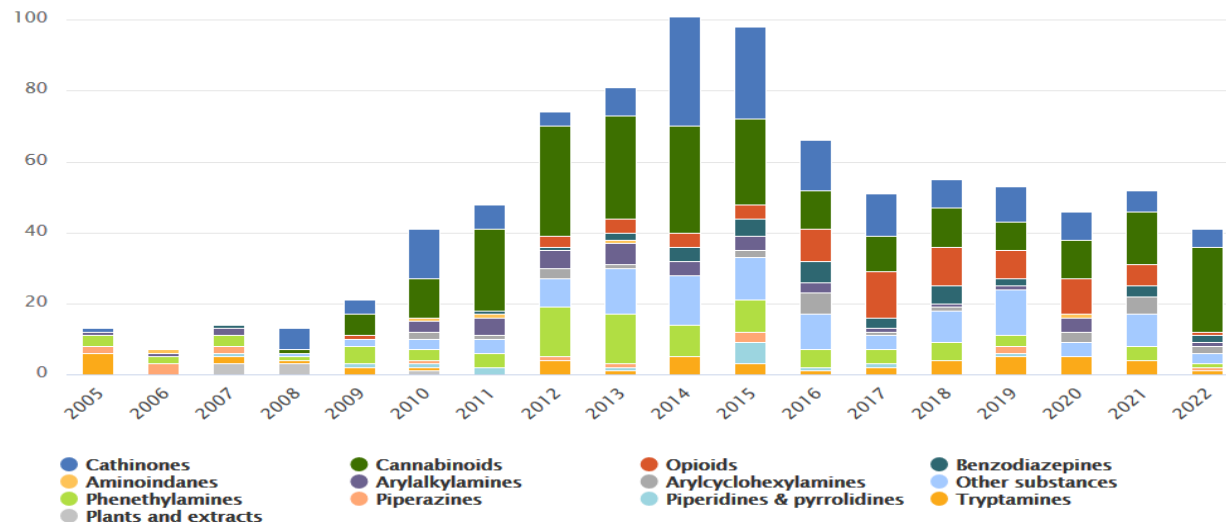
9

6

Pipéridines et pyrrolidines

Plants et extraits de plantes

Aminoindanes



A new drug appears
almost every week
on the market




NPS challenge

1. Cost +++

Availability?

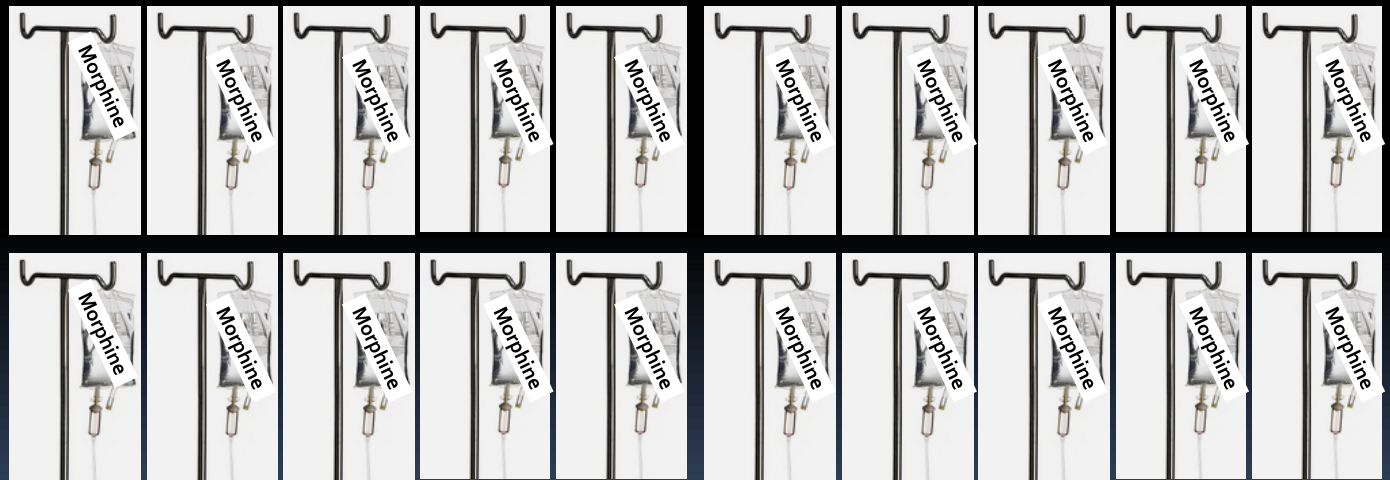
Delivery Time?



<p>Valeryl fentanyl (hydrochloride) [A neat solid]</p> <p>588,60 € / 5 mg</p> <p>Stock escompté ⓘ</p> <p>Code Article : CAY-18934-5MG Numéro CAS : 117332-91-9 Marque: Cayman Chemical</p> <p>ISO 17034</p> <p>+ Afficher plus</p> <p>1</p> <p>Ajouter au panier</p>	<p>Furanyl fentanyl (hydrochloride) [A neat solid]</p> <p>129,60 € / 1 mg</p> <p>Stock: veuillez faire une demande de renseignements ⓘ</p> <p>Code Article : CAY-18705-1MG Numéro CAS : 101365-56-4 Marque: Cayman Chemical</p> <p>ISO 17034</p> <p>+ Afficher plus</p> <p>1</p> <p>Ajouter au panier</p>	<p>Acetyl fentanyl (hydrochloride) [A neat solid]</p> <p>279,00 € / 5 mg</p> <p>Stock escompté ⓘ</p> <p>Code Article : CAY-ISO00128-5MG Numéro CAS : 117332-89-5 Marque: Cayman Chemical</p> <p>ISO 17034</p> <p>+ Afficher plus</p> <p>1</p> <p>Ajouter au panier</p>
<p>94 / 100 Bioz Stars</p> <p></p> <p>Butyryl fentanyl (hydrochloride) [A neat solid]</p> <p>174,60 € / 5 mg</p> <p>Stock escompté ⓘ</p> <p>Code Article : CAY-14728-5MG Numéro CAS : 1443-52-3 Marque: Cayman Chemical</p>	<p></p> <p>Cyclobutyl fentanyl (hydrochloride) [A cry...]</p> <p>566,10 € / 5 mg</p> <p>Stock escompté ⓘ</p> <p>Code Article : CAY-22389-5MG Marque: Cayman Chemical</p>	<p></p> <p>Methoxyacetyl fentanyl (hydrochloride) (CR...)</p> <p>199,80 € / 1 mg</p> <p>Stock escompté ⓘ</p> <p>Code Article : CAY-23540-1MG Numéro CAS : 101365-54-2 Marque: Cayman Chemical</p>

NPS challenge

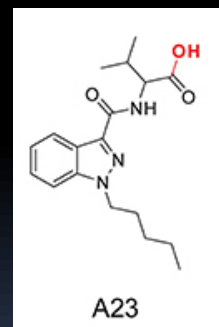
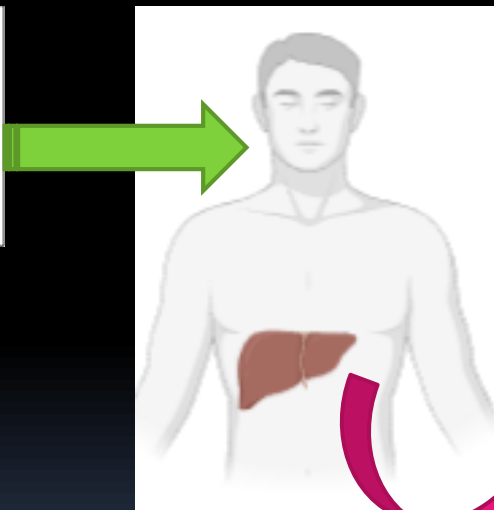
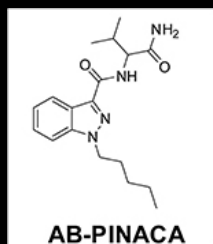
2. Low concentration: even overdose (and death) cases may correspond to blood levels below 1 ng/mL
- Carfentanil: 10.000 x more potent than morphine
 - 1 mL carfentanil \approx 10.000 mL morphine





→ Need for a sensitive method

NPS challenge

1. Number of substances
2. Low concentration
3. Some NPS are largely metabolized: in biological samples, we have to search for metabolites



Nouveau!	Nouveau!
	
AB-PINACA N-(5-Hydroxyphenyl) Metabolite	AB-PINACA N-(4-Hydroxyphenyl) Metabolite
1.729,80 € / 100 mg	4.324,50 € / 100 mg
En stock ①	En stock ①
Code Article : TRC-A109005-100MG	Code Article : TRC-A109020-100MG

Before

Reference Standard
⇒ Targeted Method

NPS

No Reference Standard
⇒ Untargeted Method with HR-MS

High resolution mass spectrometry

GOLD
STANDARD

5 identification
criteria to fulfill

→ SPECIFICITY +++

	Mass Error Mass Error (ppm)	Retention Time % Error	Isotope Isotope Ratio % Difference	Library Hit Library Score	Formula Finder Formula Finder Score
✓	< 5.0	< 5.1	< 10.0	> 70.0	> 70.0
▲	< 10.0	< 15.0	< 20.0	> 31.0	> 30.0
●	>= 10.0	>= 15.0	>= 20.0	<= 31.0	<= 30.0

- No need of reference standard to develop the method
(still necessary for confirmation & quantification)
- Can detect every compound in the sample
- Difficulty = data processing
 - Targeted screening: library, reference standard
 - Suspects screening: HighResNPS, *Chemspider*®
 - Untargeted screening: real unknowns



Complexity

High resolution mass spectrometry

Peak detection sensitivity ± exhaustive
Lots of peaks are found in one run

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High resolution mass spectrometry

Chemspider suggests lot of candidates/peak

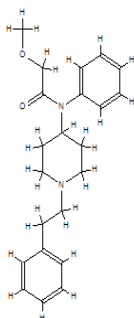
ChemSpider results for: C22H28N2O2

41-80 of 7868

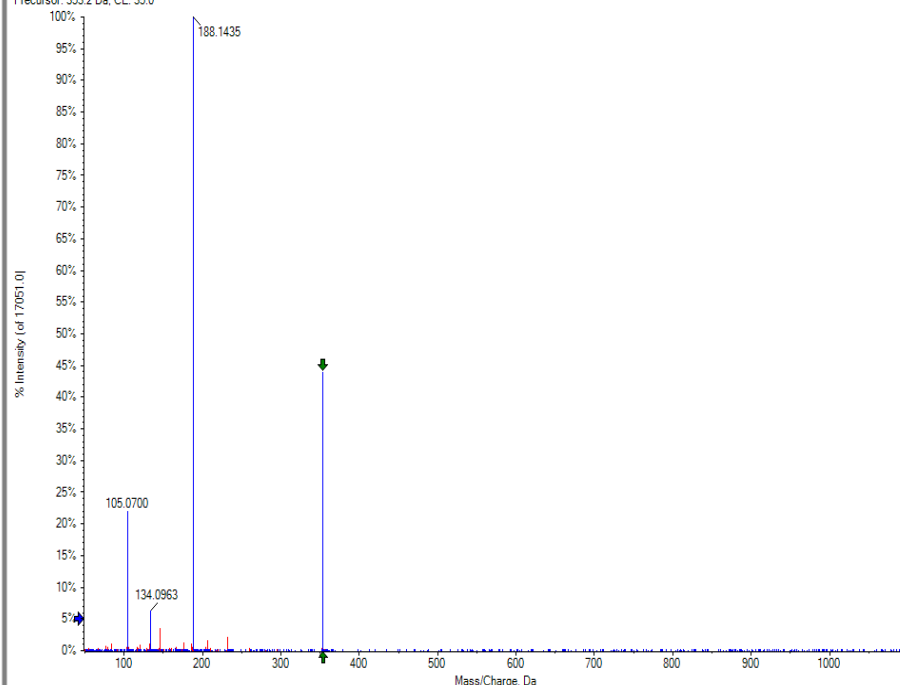
CSID	Common Name	Molecular Weight
1255880	N-(1-Benzyl-4-piperidinyl)-2-(4-ethylphenoxy)acetamide	352.4699
1275139	4-(3-Methylphenoxy)-N-[4-(1-piperidinyl)phenyl]butanamide	352.4699
1661565	3,3'-[1,3-Butadiene-1,4-diylbis(1,1-cyclohexanediylloxy)]dipropenenitrile	352.4699
1277252	(2E)-N-[4-(Diethylamino)phenyl]-3-(4-propoxyphenyl)acrylamide	352.4699
2228585	4-(Pentanoylamino)-N-(4-phenyl-2-butanyl)benzamide	352.46992
2204110	N,N'-1,4-Phenylenebis(cyclo[2.2.1]heptane-2-carboxamide	352.46992
1114035	4-tert-Butyl-N-[4-(isobutylcarbamoyl)phenyl]benzamide	352.4699
1061659	Cyclohex-2-enone, 2-butyl-3-[2-(2,5-dimethyl-1H-indol-3-yl)ethylamino]-	352.4699
1042606	3-[(2-Ethylbutanoyl)amino]-N-(2-isopropylphenyl)benzamide	352.4699
1047376	[4-(2-Ethoxyphenyl)-1-piperazinyl](4-propylphenyl)methanone	352.4699
3840322	Cyclohex-2-enone, 3-[2-(2-methyl-1H-indol-3-yl)ethylamino]-2-pentanoyl-	352.4699
3429649	2-(3,4-Dimethoxyphenyl)-1-heptyl-1H-benzimidazole	352.46992
2943918	2-(3,4-Dimethylphenoxy)-N-[4-(1-piperidinylmethyl)phenyl]acetamide	352.46992
20318630	1-[1-[3-(4-Ethylphenoxy)propyl]-1H-benzimidazol-2-yl]-1-butanol	352.4699
20318656	1-[1-[4-(4-Methylphenoxy)butyl]-1H-benzimidazol-2-yl]-1-butanol	352.4699
12007203	N-[4-(Dimethylamino)benzyl]-2-methyl-N-(tetrahydro-2-furanyl)methylbenzamide	352.4699
21279742	1-[1-[3-(2,4-Dimethylphenoxy)propyl]-1H-benzimidazol-2-yl]-1-butanol	352.4699
2871957	1-(2-Ethoxyethyl)-2-[4-(2-methyl-2-propanyl)phenoxy]methyl-1H-benzimidazole	352.46992
838859	Methoxyacetylentanyll	352.4699
2041279	Benzyl methyl[1-phenyl-3-[1-pyrrolidinyl)propyl]carbamate	352.46992
2488711	N-Benzyl-1-(2-methoxybenzyl)-N-methyl-3-piperidinecarboxamide	352.4699
1274846	4-(4-Methylphenoxy)-N-[4-(1-piperidinyl)phenyl]butanamide	352.4699
1261164	[4-(2-Ethoxyphenyl)-1-piperazinyl](4-isopropylphenyl)methanone	352.4699
1620022	N,N'-1,4-Butanediylbis(3,4-dimethylbenzamide)	352.4699
1649502	N-(4-Butylphenyl)-4-(pentanoylamino)benzamide	352.4699

☐ Display all Carbon Atoms

Options...



Spectrum from 191210_SCRmL_QCE-LGC-Quartz round79 ech6 2mL.wiff (sample 1) - 191210...E-LGC-Quartz round79 ech6 2mL, Experiment 16, +TOF MS² (50 - 1100) from 6.574 min
Precursor: 353.2 Da, CE: 35.0



Fragments Peaks

Mass/Charge	Intensity (%)	Assigned	Error (Da)
50.0163	0.15	<input type="checkbox"/>	
51.0285	0.15	<input type="checkbox"/>	
55.0540	0.46	<input type="checkbox"/>	
56.0476	0.15	<input type="checkbox"/>	
64.0938	0.15	<input type="checkbox"/>	
67.0546	0.31	<input type="checkbox"/>	
69.0690	0.31	<input type="checkbox"/>	
77.0389	0.77	<input type="checkbox"/>	
79.0549	0.62	<input type="checkbox"/>	

Matches: 17 of 92 peaks, 85.0% of total intensity

Sample Preparation

■ Biological Samples

Blood/Urine (treated or not by glucuronidase)	1 mL
Internal Standard (prazepam)	100 µL
Na ₂ CO ₃ 1M	500 µL
Diethyl Ether/ Dichloromethane/Hexane/n-amyl alcohol (50/30/20/0.5:v/v) → Agitation → Centrifugation → Evaporation	5 mL
Mobile Phase	100 µL



HPLC-DAD (*Waters*)

Alliance 2695 + PDA 2996
Column: Symmetry C8, 5 µm,
250 X 4,6 mm (*Waters*)
Mobile Phase A : 43,5 mM
Phosphate buffer pH 3,8
Mobile Phase B: Acetonitrile



UPLC-TOF-MS (*Sciex*)

Eksigent LC 100 XL + TripleTOF 4600
Column: Kinetex 2.6 C18, 100 Å,
50 x 3.00 mm (*Phenomenex*)
Mobile Phase A : 10mM Ammonium
formate
Mobile Phase B: ACN/MeOH (50/50)



■ Powders: dilution → HPLC-DAD & UPLC-TOF-MS

Detection rate

- Epidemiological studies - CHU Liège

- Emergency Room Patients (n=80)
- Psychiatric Patients (n=59)
- Drivers in car accident/control (n=273)

NO NPS FOUND

- Main explanation

- Analytical difficulty
- Low consumption
- Selection bias? Problematic consumption...



Case reports: Emergency Room

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Special Issue

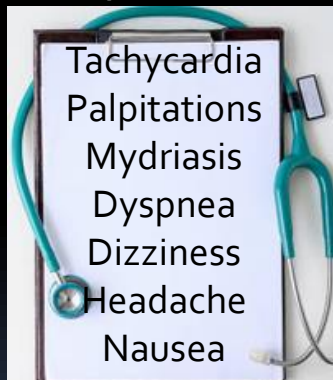
OXFORD

Synthetic Cathinones in Belgium: Two Case Reports with Different Outcomes Observed in the Emergency Room

M. Deville^{1,*}, R. Fedorowicz², F. Grandjean³, M. Simon² and C. Charlier¹

Case 1: 31-year-old drug addict

Day 0: injection of unknown compound



Left against medical advice

Day+1: aggressiveness, agitation

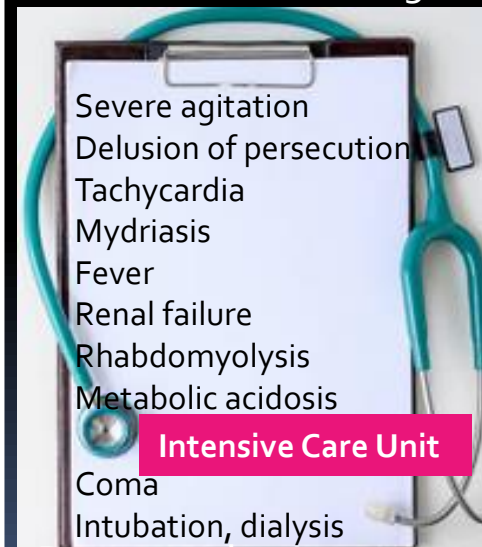
Day+1 month: sleeping disorders, hallucinations, anxiety

⇒ 21st detoxification cure



Case 2: 27-year-old man with no previous history

Day 0: wandered in the night
Confessed the snorting of NEP



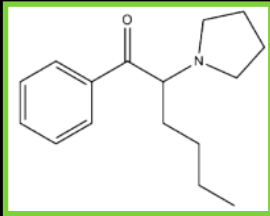
D+3: extubation, stop dialysis

D+5: symptoms resolution - discharge from ICU

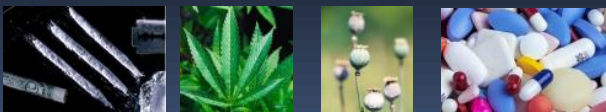
Case reports: Emergency Room

Case 1:

**α -pyrrolidinohexiophenone
(α -PHP)**



- ❖ 1st & 2nd admission only
- ❖ No reference standard → No quantification
- ❖ + cocaïne, cannabis, opiates (heroin, methadone) benzodiazepines (clonazepam, diazepam, bromazepam)

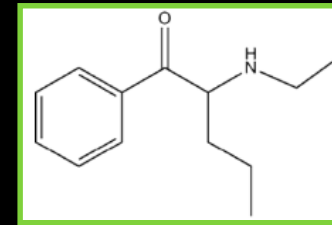


→ Interpretation?

≠ Route of administration

≠ Clinical consequences

Case 2: **n-ethylpentedrone (α -ethylaminopentiophenone - α -EAPP)**

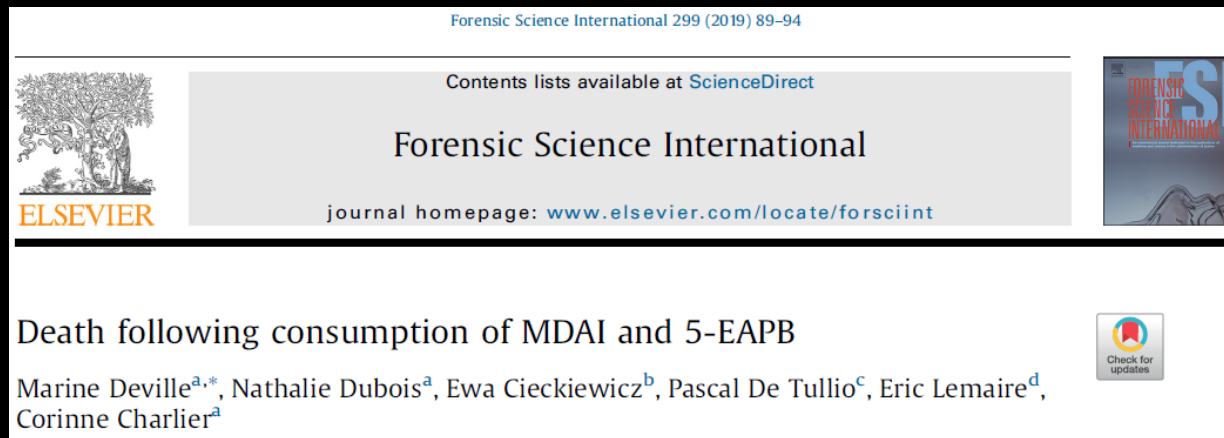


2.23 mg/L
Admission

2.804 mg/L
+17 hours

- ❖ Nordazepam (1.30 mg/L) and oxazepam (0.094 mg/L)
- ❖ Subcontracting: time to transfer the samples
- ❖ **Lethal** concentrations
 - 3.10 mg/L [1]
 - 0.932 mg/L [2]

Case report: forensic toxicology



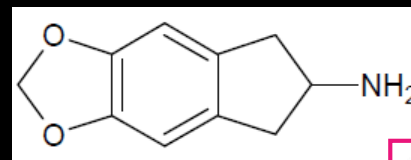
- Drug addict death (28-years old)
- Found at the scene:
 - Unidentified powders
 - Material used to snort (cards, homemade straw)
 - Medicines (lorazepam, prazepam, zopiclone, amoxicilline, bisoprolol)
- External examination: epistaxis without corporal lesion
- Toxicological cause of death highly suspected: NPS screening

Case report: forensic toxicology

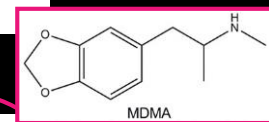
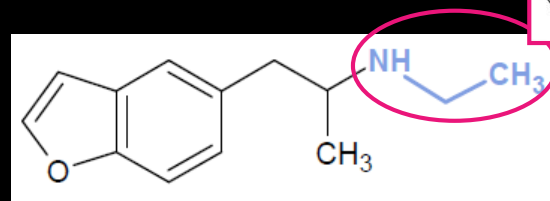
■ Powders



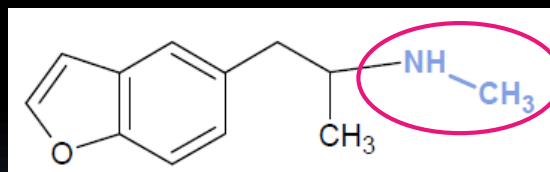
5,6-MethyleneDioxy-2-AminoIndane
MDAI : 68 %



EthylAminoPropylBenzofurane
EAPB : 87 %

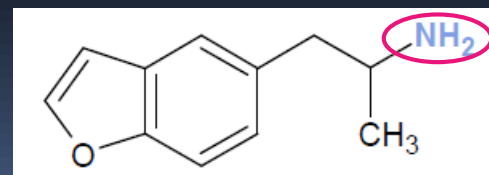


Isomer distinction with RMN



MAPB

MethylAminoPropylBenzofurane



APB

AminoPropylBenzofurane

■ Biological samples

	Blood (mg/L)	Urine (mg/L)	Blood Cas 1 [5]	Blood Cas 2 [5]
MDAI	2,09	69,4	26,3	3,3
EAPB	6,45	14,8	-	-
MAPB	0,089	1,00	-	-
APB	0,546	4,88	-	0,34

Conclusion

- NPS consumption is low compared to classical drugs of abuse
- Various routes of administration and clinical consequences
- Need for a sensitive and specific technique

