

INTEREST OF TOXICOLOGICAL ANALYSIS TO ASSESS THE CONSUMPTION OF NEW PSYCHOACTIVE SUBSTANCES AND CANNABIDIOL



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- **New psychoactive substances**
 - Introduction
 - Analysis
 - Case reports
- Cannabidiol
 - Introduction
 - In shops
 - In urine

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« New Psychoactive Substances »

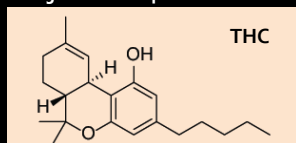
- Substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat
- The term “new” does not necessarily refer to new inventions — several NPS were first synthesized decades ago — but to substances that have recently become available on the market
- Designer drugs, research chemicals, legal highs, bath salts, spice,...



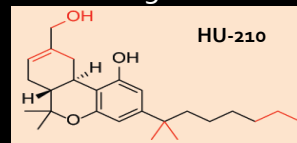
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Key points

- Objective: provide a legal alternative to illicit drugs



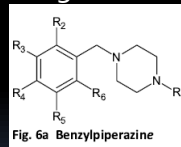
Psychoactive - controlled



Psychoactive - (Initially) not controlled

- BUT since September 2017 : generic legislation

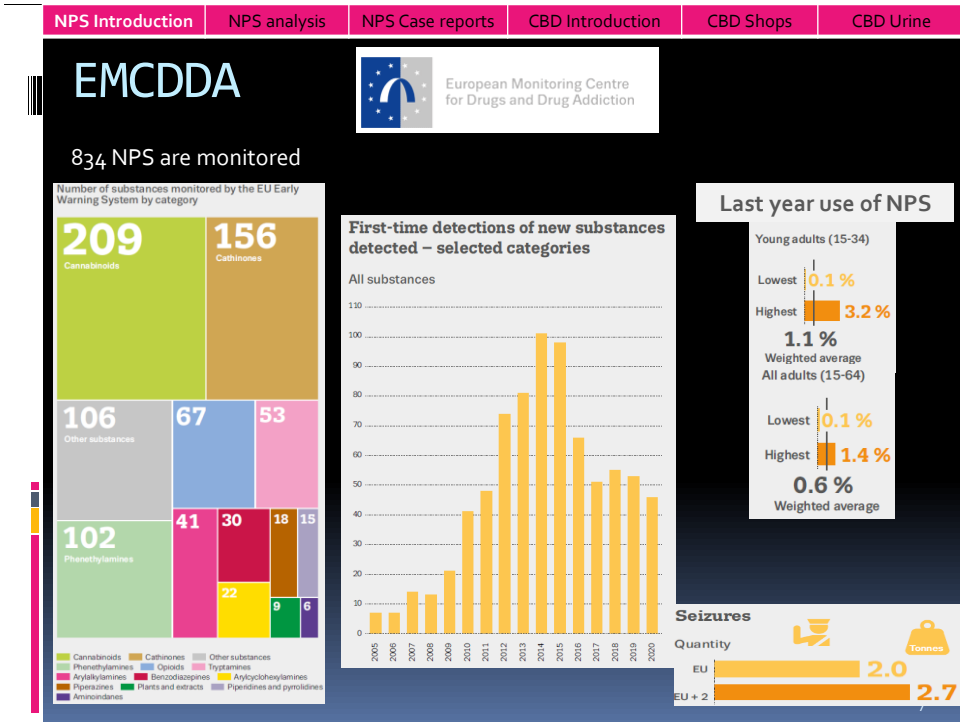
- Chemical basic structure
- Analogs using R-groups
- Decrease need for updates



R1 = H, CH₃, benzyl
 R2 = H, halogène, OCH₃
 R3 = H, CH₃, halogène, CF₃
 R4 = H, halogène, OCH₃
 R5 = H, OCH₃,
 R6 = H

- Internet is the main source of supply
- China remains the main supplier, followed by India
- Marginalised population (homeless, prisoners), high-risk drug users, psychonauts (young adults exploring new effects)

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- NPS Introduction** | **NPS analysis** | **NPS Case reports** | **CBD Introduction** | **CBD Shops** | **CBD Urine**
- **New psychoactive substances**
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Analytical challenge

- Number of substances
- Low concentration: even overdose (and death) cases may correspond to blood levels below 1 ng/mL
- Some NPS are largely metabolized

Gold standard: High Resolution Mass Spectrometry

- Targeted screening** : library
 - With retention time: 329 compounds
 - Without retention time :
 - Sciex: 2636 compounds
 - Designer drugs: 530 NPS



- Untargeted screening** : ChempSpider®

High resolution mass spectrometry

5 identification criteria to fulfill

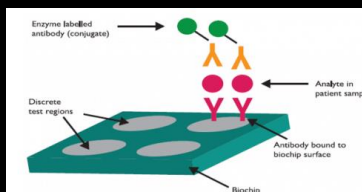
| | Mass Error Mass Error (ppm) | Retention Time % Error | Isotope Isotope Ratio % Difference | Library Hit Library Score | Formula Finder Formula Finder Score |
|---|--------------------------------|---------------------------|------------------------------------------|------------------------------|----------------------------------------|
| ✓ | < 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 |

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

ChempSpider suggests lot of candidates/peak

Immunoanalysis

- Evaluation of the Randox Evidence Investigator
 - Biochip Array Technology: analytes present in the sample bind to the antibody



| Category | Compounds |
|-----------------|--------------------------------------------------------|
| SCRA | JWH-018, UR-144, AB-PINACA, AB-CHMINACA |
| Fentanyl | Furanyl-, acetyl-, car-, suf-, oc-fentanyl |
| Cathinones | Mephedrone, α -PVP |
| Opioids | U-47700, AH-7921, MT-45, W-19, naloxone, buprenorphine |
| Benzodiazepines | Etizolam, clonazepam, |
| Piperazines | Phenylpiperazine, benzylpiperazine |
| Miscellaneous | Mescaline, salvinorine, mitragynine |

- Easy to use – small sample volume – quick

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Immunoanalysis - results

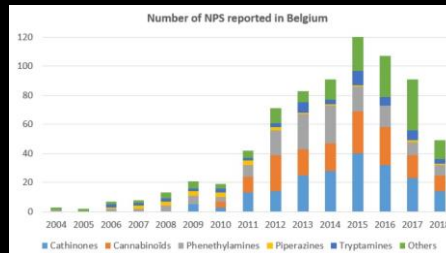
- 80 samples (urine and serum) - no real NPS consumer according to UHPLC
- Cross reactivity

| | Nb Samples + (Randox) | Nb Samples + (TOF) | Hypothetic explanation |
|--------------------------|-----------------------|--------------------|----------------------------------------------------------------------------|
| Clonazepam | 15 | 5 | Other benzodiazepines |
| Etizolam | 2 | 0 | Other benzodiazepines |
| Phenyl/benzyl-piperazine | 18 | 17 (medicine) | - Trazodone and mCPP - Other medicine containing a piperazine structure |
| Fentanyl derivatives | 10 | 4 | - Other opiates - Threshold too low |
| Naloxone | 3 | 1 | Threshold too low |
| JWH-018 | 6 | 0 | Overestimation of the result |
| α -PVP | 1 | 0 | No explanation |

- First intention before chromatographic confirmation

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In Belgium



- One team > lot of publication (fundamental research)
- Wastewater analysis; electronic music festivals
- Driving under the influence of drugs population (11% but near festivals/nightclubs)
- Few case reports

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Case Report

Clues



Drug addict death
(28-years old)

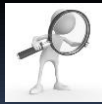


Unidentified powders
Material used to snort



Drugs: lorazepam,
prazepam,
zopiclone, amoxicilline,
bisoprolol

Medical Report



- Epistaxis
- No corporal lesion
- Blood and urine collection



- Toxicological cause of death highly suspected

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Material & Methods

General Unknown Screening

| | |
|-------------------------------------------------------------------------------------------------------------------------|--------|
| 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-amylic 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)



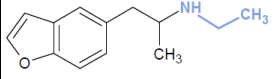
Eksigent LC 100 XL + TripleTOF 4600
Column: Kinetex 2.6 C18, 100 Å,
50 x 3.00 mm (Phenomenex)
Mobile Phase A: 10mM Ammonium
formate
Mobile Phase B: ACN/MeOH (50/50)

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

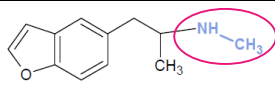

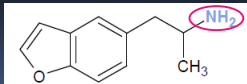
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NPS Introduction | NPS analysis | **NPS Case reports** | CBD Introduction | CBD Shops | CBD Urine

Results: NPS fatality

- Powders**
 -  5,6-MethyleneDioxy-2-AminoIndane
MDAI : 68 %
 -  EthylAminoPropylBenzofuran
EAPB : 87 %
 - MDMA-like stimulants**

- Biological samples**

| | Blood (mg/L) | Urine (mg/L) | Case 1 [1] | Case 2 [1] |
|------|--------------|--------------|------------|------------|
| 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 |

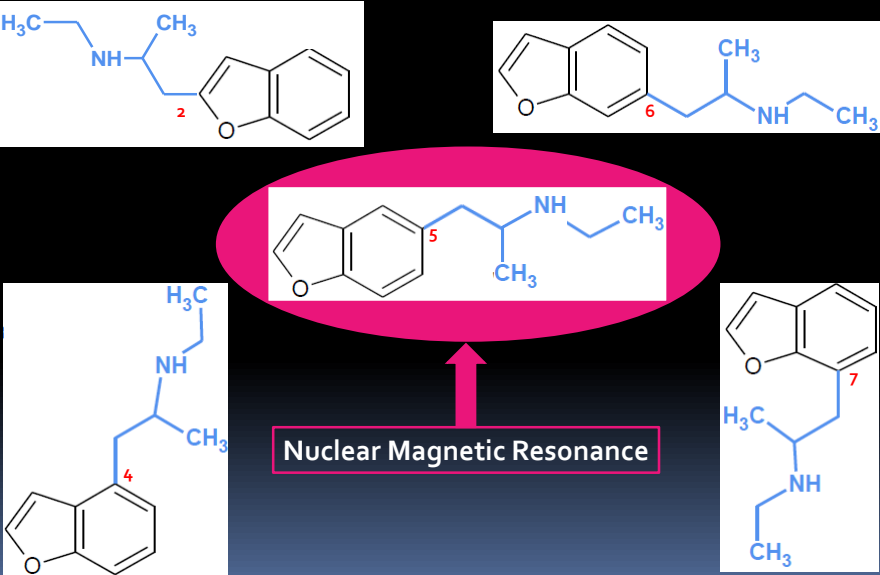
 -  MAPB
 -  MethylAminoPropylBenzofuran
 -  AminoPropylBenzofuran

[1] Corkery JM., et al., MDAI toxicity: a brief overview and update, Hum Psychopharmacol Clin Exp (2013)28; 345-355

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NPS Introduction | NPS analysis | **NPS Case reports** | CBD Introduction | CBD Shops | CBD Urine

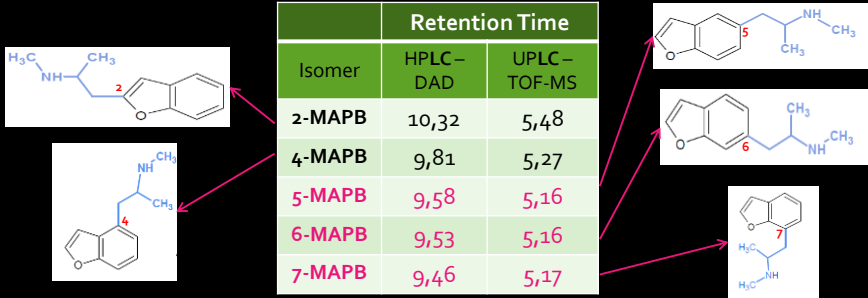
EAPB Isomeric distinction



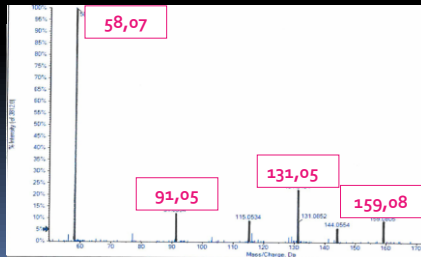
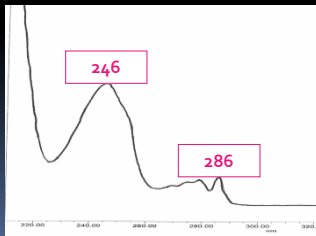
Nuclear Magnetic Resonance

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MAPB Isomeric distinction



| Isomer | Retention Time | |
|--------|----------------|---------------|
| | HPLC – DAD | UPLC – TOF-MS |
| 2-MAPB | 10,32 | 5,48 |
| 4-MAPB | 9,81 | 5,27 |
| 5-MAPB | 9,58 | 5,16 |
| 6-MAPB | 9,53 | 5,16 |
| 7-MAPB | 9,46 | 5,17 |

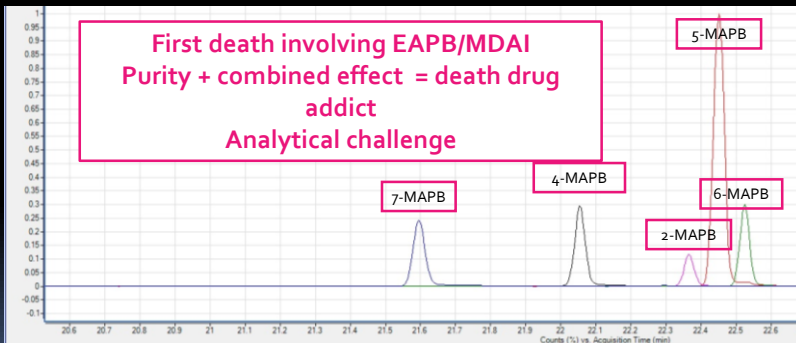


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MAPB Isomeric distinction

GC-MS-MS (EI) [2]:

- Heptafluorobutamide (HFBA) derivatization: 75°C – 30 min
- 30m x 0,25 mm HP-5MS; gradient



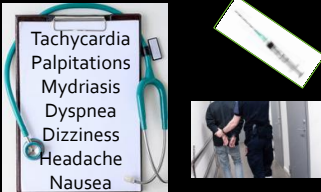
[2] Stanczuk A., et al., Identification of APB phenyl ring positional isomers in Internet purchased products, Durg Test Analysis(2013)5; 270-276

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Case reports (Emergency Room)

Case 2: 31-year-old drug addict

Day 0: injection of unknown compound



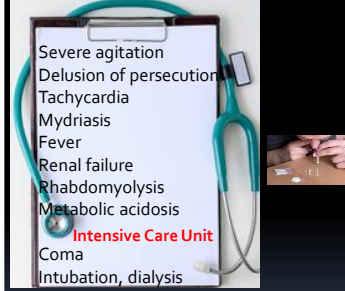
Left against medical advice

Day+1: aggressiveness, agitation
Day+1 month: sleeping disorders, hallucinations, anxiety

⇒ 21st detoxification cure

Case 3: 27-year-old man with no previous history

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

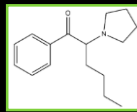


D+3: extubation, stop dialysis
D+5: symptoms resolution - discharge from ICU

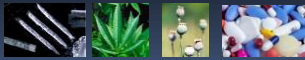
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Results: cathinones intoxication

Case 2: α -pyrrolidinohexiophenone (α -PHP)



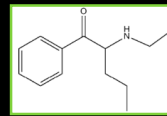
- ❖ 1st & 2nd admission only
- ❖ Still waiting for the reference standard → No quantification
- ❖ + cocaine, cannabis, opiates (heroin, methadone) benzodiazepines (clonazepam, diazepam, bromazepam)



→ Interpretation?

Stability in biological samples [5-6]
❖ 3rd amines > 2nd
❖ Depending on the t^o, some cathinones are highly unstable

Case 3: n-ethylpentedrone (α -ethylaminopentiophenone - α -EAPP)



Long T_{1/2}

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 [3]
 - 0.932 mg/L [4]

3. Fujita Y. et al. Acute Med Surg 2016; 3: 230-236 4. Pieprzyka E. et al. J Anal Toxicol 2021;00,1-7 2021;00,1-7 5. Glicksberg L. et al. J Anal Toxicol, 2017;41:711-719 6. Adamowicz P. et al., forensic Sci Int 295 (2019) 36-45

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Conclusion – NPS

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



Is there more cannabidiol (CBD) users in the Belgian population ?

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CBD: Commercial success



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Cannabis sativa L. = hemp

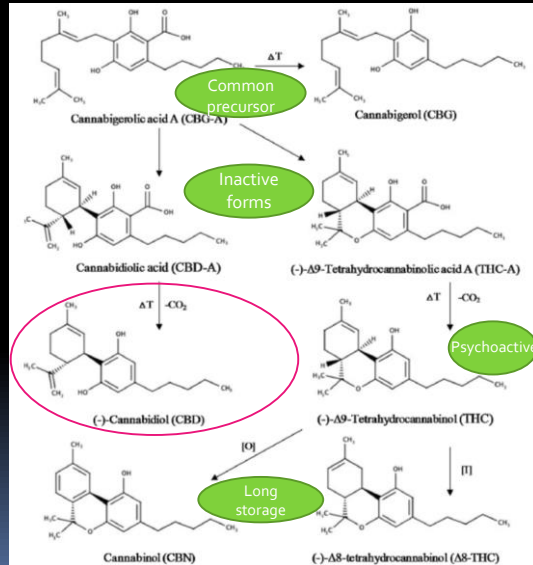
- Weed, marijuana = dried flowers (female plant)
- Haschich = resin coming from flowers and leaves
- Seeds, strong fiber...
- Glandular trichomes → aromatic compounds (terpenes), phytocannabinoids (main active components – 104 have been identified so far)



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104 Phytocannabinoids in Cannabis Sativa

- Various chemotypes depending on the ratio
 - 1: THC-A >> CBD-A
 - 2: THC-A ≈ CBD-A
 - 3: THC-A << CBD-A
 - 4: CBG-A = major
 - 5: no cannabinoid
- CBD
 - Flowers, resins
 - E-liquids
 - Oil, cream, tea, capsules
 - ...



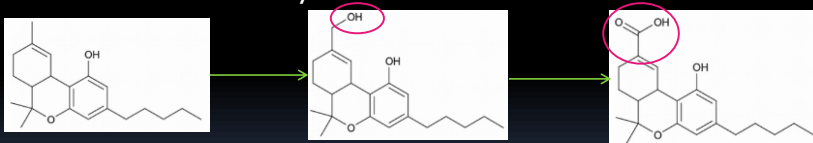
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Remarks

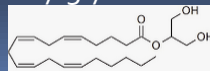
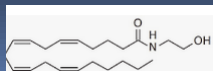
- **NOT** a synthetic cannabinoid
 - Dronabinol and nabilone
 - New Psychoactive Substance



- **NOT** a THC metabolite and does not convert to THC in the body



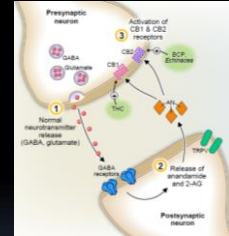
- **NOT** an endogenous cannabinoid
 - Anandamide and 2-arachidonoylglycerol



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CBD molecular targets

- **CB₁** (mainly in the CNS): antagonist? Inverse agonist? → could conteract some THC effects (anxiety, hunger, sedation, tachycardia)
- **CB₂** (mainly in the immune system): inverse agonist
- **Other receptors:**
 - 5HT_{1A} (5-HydroxyTryptamine)
 - PPAR-γ (Peroxisome Proliferator-Activated Receptor)
 - TRPA₁ (Transient Receptor Potential Ankyrin 1)
 - TRPV₁ & 2 (Transient Receptor Potential Vanilloide)
 - Adénosine A₂
 - GPR55 (Orphan G Protein Coupled Receptor)
 - ...
- **Others** : cytochromes P₄₅₀ 2D6, 3A₄,...



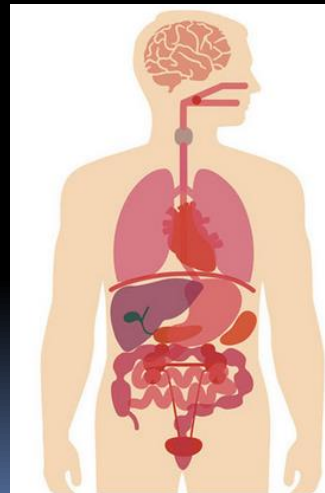
Very wide pharmacological activity

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Potential CBD effects

- Epilepsy
- Schizophrenia, depression, insomnia, anxiety, post traumatic stress, attention deficit hyperactivity disorder
- Nausea, vomiting
- Chronic pain
- Withdrawal (tobacco, cocaine, amphetamines, opiates)
- Tourette's syndrome
- Amyotrophic lateral sclerosis
- Fragile-X syndrome
- Parkinson's, Alzheimer's, Huntington's diseases
- Peripheral neuropathies
- Anticancer drug
- Obesity treatment
- Anti-inflammatory
- Rheumatic diseases, musculoskeletal diseases
- Refractory glaucoma
- Immunity stimulation

NOT PSYCHOACTIVE???



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Adverse effects

- CBD is generally well tolerated and shows a good safety profile but
 - Hepatic damages
 - Diarrhea, loss of appetite
 - Drug Interactions
 - Drowsiness, sedation, lethargy, fatigue
 - Irritability, agitation
 - Toxicity on male reproductive system (decrease in testicle size, sperm maturation, circulating testosterone)
- Unanswered questions:
 - Particular populations?
 - Long term toxicity?



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Medical applications



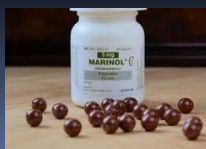
- **Sativex**: Spray: THC = CBD
- Multiple sclerosis: pain and loss of appetite



- Magistral preparation in oil: cost +++
- CBD Pharm®: OTC , pain, stress, sleep disorders,...



Seizures associated with Lennox-Gastaut & Dravet syndromes; tuberous sclerosis complex



Dronabinol & nabilone
Synthetic THC analogs
Nausea & vomiting in oncology



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Belgian legislation



- Drug type cannabis
 - Prohibited to import/export, transport, grow, produce, possess, sell, deliver, purchase
 - Detention for personal purpose (1 plant/3g without aggravating circumstances): simple report
 - Flower, resin, extract, tincture with THC + THC-A $\geq 0,2\%$
- Foodstuffs (prohibited) and cosmetics : other laws
- CBD: initially in a grey area > Since April 2019: same category as tobacco products
 - Duties on the selling price
 - Reduced profit margin for traders (/3 or 4)



Analysis of samples seized in CBD shops

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Forensic Science International 310 (2020) 110234

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forensi

Validation of an UHPLC/DAD method for the determination of cannabinoids in seized materials: Analysis of 213 samples sold in Belgian CBD shops


 CrossMark

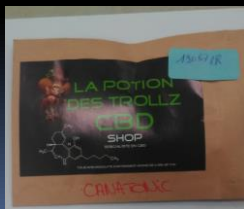


Marine Deville*, Nathalie Dubois, Raphaël Denooz, Corinne Charlier

Laboratory of Clinical, Forensic and Environmental Toxicology, Center for Interdisciplinary Research on Medicines (CIRM), University of Liege, Belgium

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Seized samples



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Sample preparation



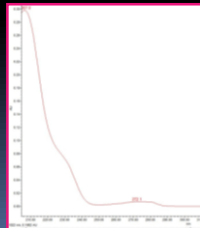
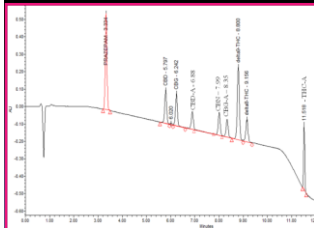
Chromatographic analysis



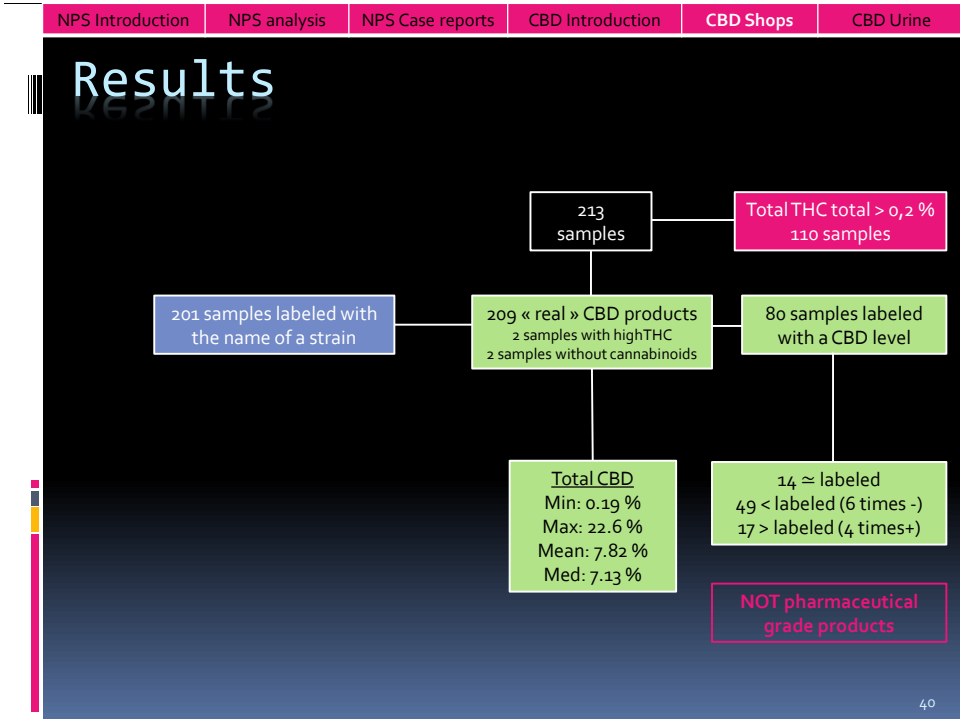
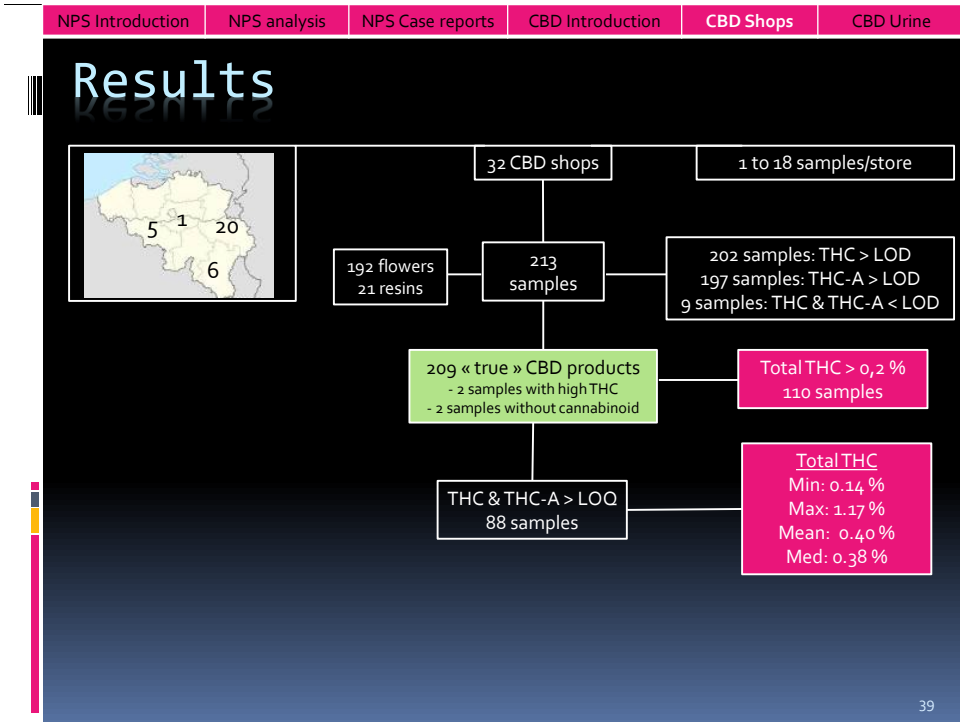
Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

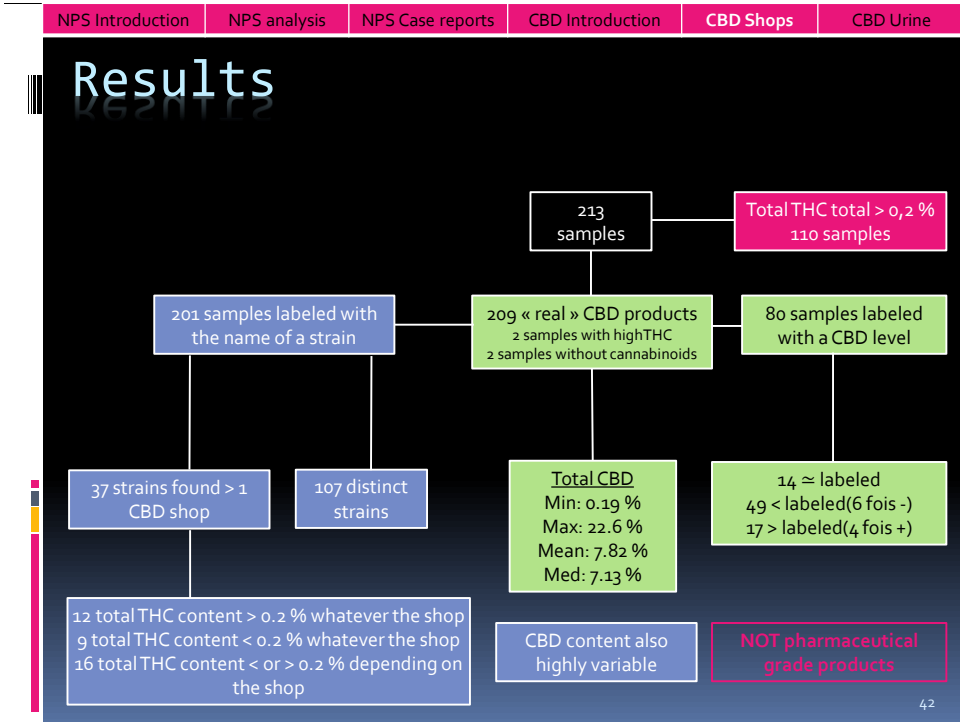
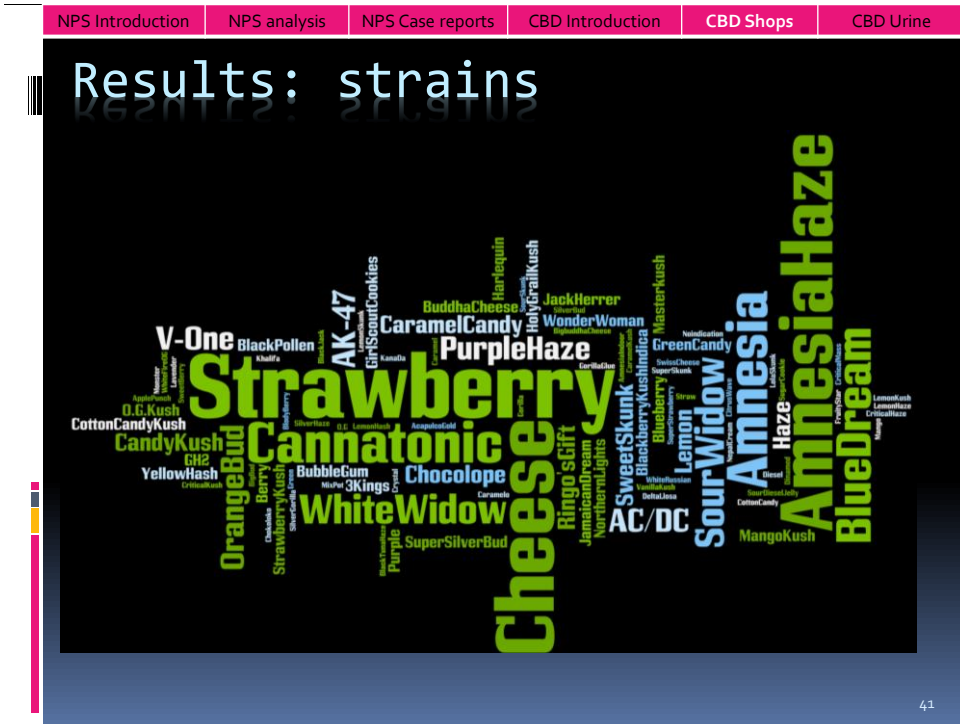
UPLC-DAD
 Column: Acquity HSS C18 SB
 1.8 µm (2.1 x 100 mm)
 Injection Volume: 10 µl
 Temperature: 45°
 Mobile Phase A: methanol
 Mobile Phase B: water + 0,1% formic acid
 Flow rate: 0,35 ml/min

| Time (min) | A (%) | B (%) |
|------------|-------|-------|
| 0 | 65 | 35 |
| 1 | 65 | 35 |
| 9 | 78 | 22 |
| 10 | 95 | 5 |
| 12 | 95 | 5 |
| 12,1 | 65 | 35 |
| 15,5 | 65 | 35 |



- Validation:**
- Specificity
 - Calibration Curve
 - Precision, trueness
 - LOD, LOQ
 - Carry-over
 - Extraction yield
 - Drying duration, stability during evaporation
- BELAC**
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« Doctor, I don't
smoke weed, I
swear! »

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Sample preparation

| Extraction | |
|---------------------------------------------------------|---------|
| Urine | 1000 µL |
| Internal Standard 1 mg/L | 100 µL |
| Vortex mix | |
| Phosphate buffer 0.1M pH 6.8 | 2 mL |
| Vortex mix | |
| β-glucuronidase | 40 µL |
| Vortex mix | |
| Incubate one night 40°C | |
| KOH 12 M | 50 µL |
| Vortex mix | |
| Incubate ± 30 min 60°C | |
| HCl 1 M | 400 µL |
| Vortex mix | |
| Acetic acid 10 % | 200 µL |
| Vortex mix → pH 5 (4.90-5.10) | |
| Hexane/Ethyl acetate (g/1 : v/v) | 5 mL |
| Agitation ± 10 minutes | |
| Centrifugation ± 10 minutes | |
| Take the organic layer | |
| Evaporate to dryness under a nitrogen stream (max 40°C) | |
| Methanol/H ₂ O (80/20 : v/v) | 100 µL |
| Vortex mix → Vial UPLC | |



**Rapid drug
screen do not
react with CBD**



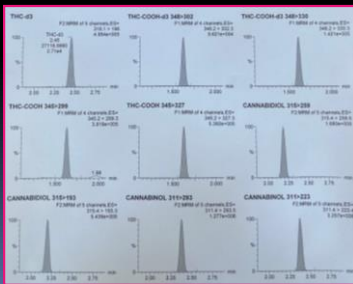
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Chromatographic analysis



UPLC-MSMS
 Column: Acquity BEH C18 1.7 μm (2.1 x 50 mm)
 Injection volume: 10 μl
 Temperature: 40°
 Mobile phase A: Ammonium bicarbonate (10 mM pH10)
 Mobile phase B: Methanol
 Flow rate: 0,45 ml/min

| Temps (min) | A (%) | B (%) |
|-------------|-------|-------|
| 0 | 50 | 50 |
| 0,3 | 50 | 50 |
| 2 | 5 | 95 |
| 2,9 | 5 | 95 |
| 3 | 50 | 50 |

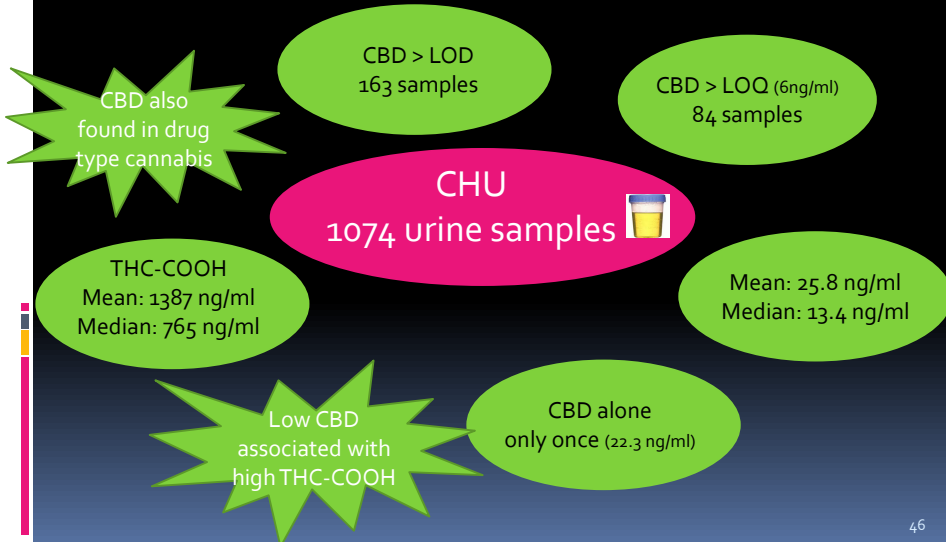


- Validation:
- Specificity
 - Calibration curve
 - Precision, trueness
 - LOD, LOQ
 - Matrix effect
 - Extraction yield



Frequently asked question

CBD or drug-type cannabis consumption?



Conclusions

- Variability of CBD and THC levels in products seized in CBD shops
 - Only pharmaceutical specialties guarantee optimal effectiveness and safety
- Anticonvulsivant effect is the only one which is proved
- Chromatographic analysis allows the distinction between a CBD and a drug type consumption