

# ANALYTICAL CRITICAL ASPECTS FOR THE RAPID IDENTIFICATION OF NPS ON THE ROAD

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**ROME, 14-15 May 2014**



# DAMAGES DUE TO DRUGS AND ALCOHOL

- ❑ damage to the user's health
- ❑ damage to the society
- ❑ increase in medical expenses

responsible for many **car accidents!**



**CAR ACCIDENT IS THE FIRST CAUSE OF DEATH  
FOR YOUNG PEOPLE BETWEEN 18 AND 35 YEARS  
IN ITALY AND EUROPE**



# **“THE REPORT OF SIX YEARS OF PIRACY ROAD AND STREET KILLINGS (2008-2013)”**

According to ASAPS estimations,  
50% of the individuals having a car accidents  
were driving under the influence of alcohol or drugs



*ASAPS: Associazione Sostenitori ed Amici della Polizia Stradale*

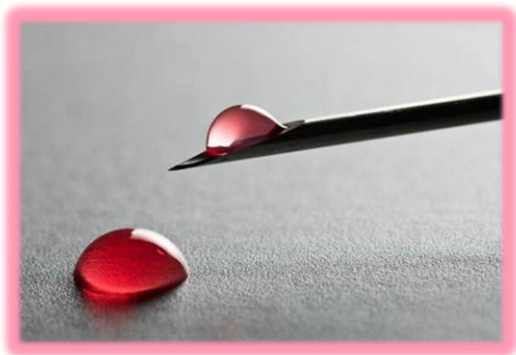
# HOW TO PREVENT AND FIGHT THIS PROBLEM?

- ❑ Educating young people on the toxic effects of drugs and alcohol
- ❑ Development of innovative methods for the analysis of drugs for on street controls



# DRIED BLOOD SPOTS (DBS)

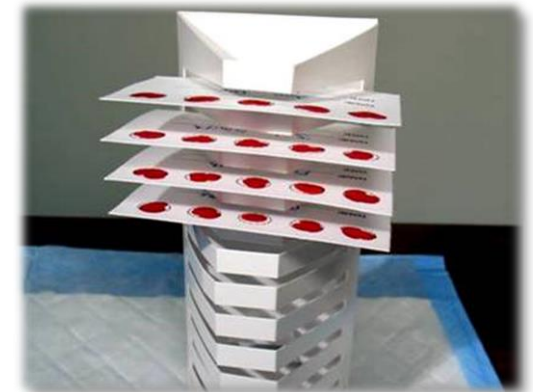
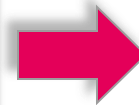
- A NEW MATRIX FOR THE TOXICOLOGICAL ANALYSIS IS IMPLEMENTED IN THE LABORATORY OF PHARMACO-TOXICOLOGICAL ANALYSIS OF THE UNIVERSITY OF BOLOGNA
- USEFUL TOOL TO ASSESS THE POSITIVITY TO DRUGS ON STREET



**BLOOD** is the biological sample of choice for evaluating the consumption of drugs of abuse when they are active in the body

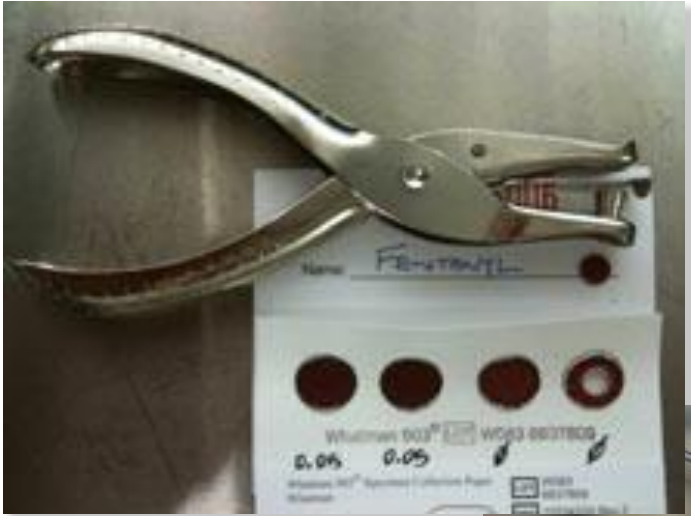
# DRIED BLOOD SPOT (DBS)

- ❑ Finger prick with disposable sterile lancets
- ❑ Deposition of blood drops on a special filter paper
- ❑ Card drying and storage



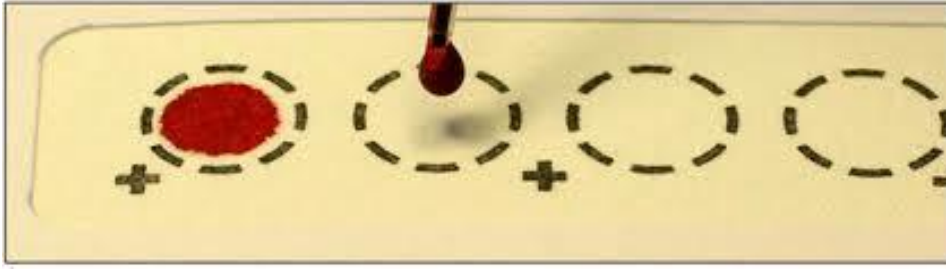


# ANALYSIS OF DRUGS OF ABUSE IN DBS





# DBS vs VENIPUNCTURE



- **Fast sampling**
- **Minimally invasive**
- Low biological hazard
- Easy to store
- Easy to transport
- **Compound stability**
- Analysis replication
- Low costs





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## Dried blood spots: Liquid chromatography–mass spectrometry analysis of $\Delta^9$ -tetrahydrocannabinol and its main metabolites

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HPLC–MS/MS

"On street" controls

### ABSTRACT

A sensitive and selective HPLC–MS/MS method has been developed for the first time for the analysis of  $\Delta^9$ -tetrahydrocannabinol (the most important active cannabinoid) and its hydroxylated and carboxylated metabolites in human Dried Blood Spots (DBSs). The simultaneous determination of  $\Delta^9$ -tetrahydrocannabinol and its two main metabolites allows assessing the time elapsed after the drug intake and distinguishing between acute or former consumption. This is an important information in specific contexts such as "on street" controls by police forces. DBSs have been chosen as the optimal biological matrix for this kind of testing, since they provide information on the actual state of intoxication, without storage and transportation problems usually associated with classical blood testing. The analysis is carried out on a C8 reversed phase column with a mobile phase composed of 0.1% formic acid in a water/methanol mixture and an electrospray ionisation (ESI) source, coupled to a triple quadrupole mass spectrometer. The method was validated according to international guidelines, with satisfactory results in terms of extraction yields, precision, stability and accuracy. Application to real DBS samples from Cannabis abusers gave reliable results, thus confirming the methodology suitability for roadside testing.

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# CAR ACCIDENT PREVENTION

Conference:

“DRUGS ON STREET: INNOVATIVE  
ANALYTICAL METHODS”



DEMONSTRATION

“DBS Testing”

**MOTORSHOW 2010 Bologna**



## STREET SAFETY

Conference:

“DRUGS ON STREET:  
TOXICITY/RISKS/TESTS”

## DRUG CONTROL



“DBS testing on volunteers”



**MOTORSHOW 2011 Bologna**



# WHAT CAN WE ANALYSE IN DBS?



**cocaine**

**cannabinoids**

## CLASSICAL DRUGS

**amphetamines**

**opiates**

**opioids**

**ketamine**

**technocannabis**

**GHB**

## NEW PSYCHOACTIVE SUBSTANCES (NPS)

**phenethylamines**

**cathinones**

**fentanyl**

# New Psychoactive Substances (NPS)



**Which type of drugs?** Synthetic drugs

(synthetic cannabinoids, synthetic cathinones, etc.)

**Which effects?** Serious toxic effects on the state of consciousness  
(a number of cases of acute intoxication are recorded)

**Which damages?** Damages to CNS, heart and lungs





**October 22<sup>nd</sup>, 2013**

**Horrific cost of taking legal highs: Mother releases shocking picture of her son, 20, on his deathbed after he suffered fatal heart attack from smoking herbal substance**



## ***Indictment of the «legal highs»***

*Jimmy was found dead near an envelope marked “UK Skunkworks”, a controversial brand that sells these new drugs.*

*He had a stroke and died a few hours after drug intake.*

A grieving mother today released this harrowing photograph of her dying son as a warning to others about deadly legal highs.

Keen sportsman Jimmy Guichard, 20, suffered a heart attack and severe brain damage within hours of taking one of the herbal substances.

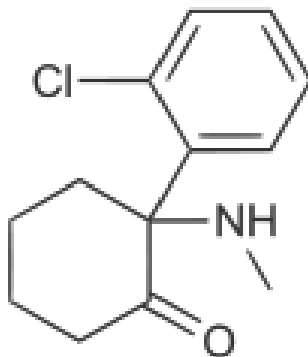
His mother Karen Audino said he was found unconscious next to an empty plastic bag from controversial legal high shop UK Skunkworks.

Just 24 hours later Karen held her 'fit and healthy' son in her arms after the heartbreaking decision to turn off his life-support machine.

# KETAMINE



(*RS*)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone



Dissociative drug: “*club drug*”

**Low doses** → pleasant experience, called “wonder world”

**High doses** → distressing experience, called “K-Hole,” “out of body,” “near-death”

## Side effects (dose-dependent)

- attention and memory disorders
- hallucinations, synesthesia
- dizziness, abdominal pain
- tachycardia, hypertension
- pulmonary edema

*Overdose:  
respiratory depression, stroke*



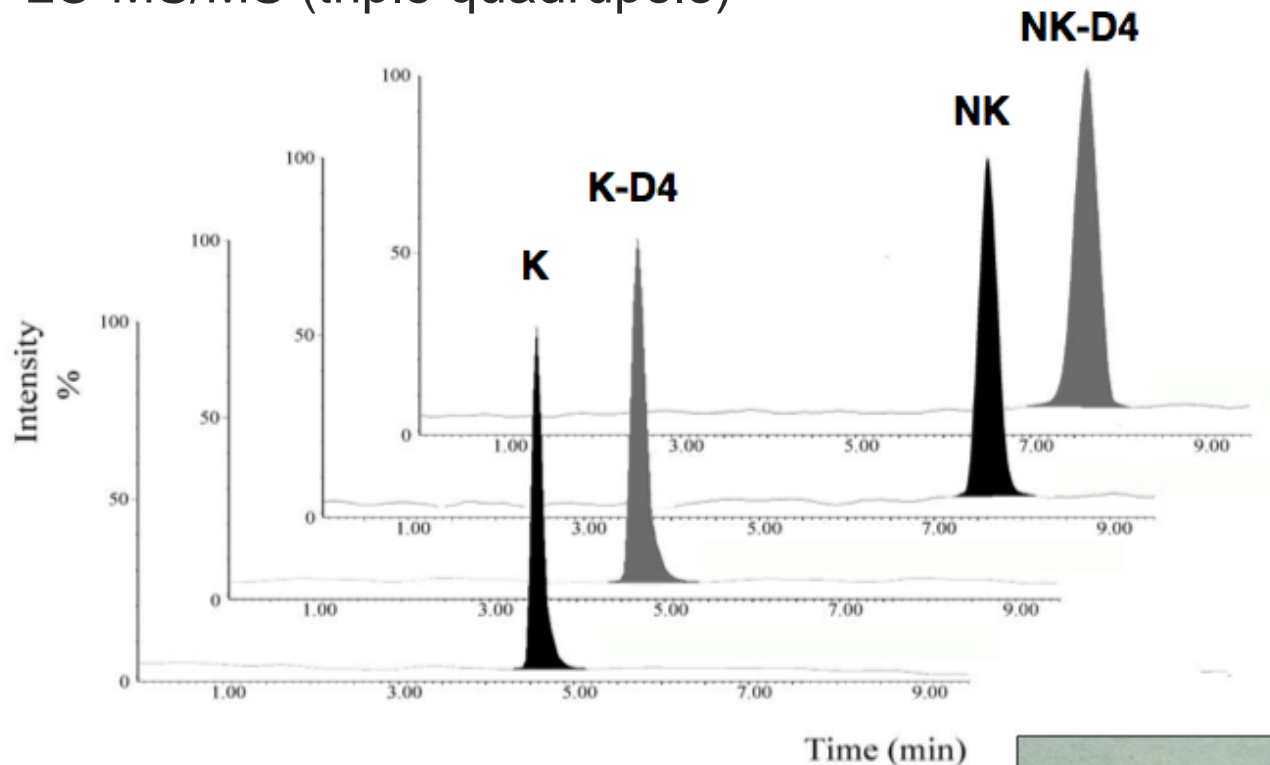


## CRITICAL ASPECTS IN THE ANALYSIS OF KETAMINE

- Short half-life ( $t_{1/2} = 1\text{-}3$  hours)
- Elimination within 24 hours
- False-positive results to screening tests (routine kits)
- Lack of sensitive analytical methods
  - false-negative results

# ANALYSIS OF KETAMINE AND NORKETAMINE IN DBS

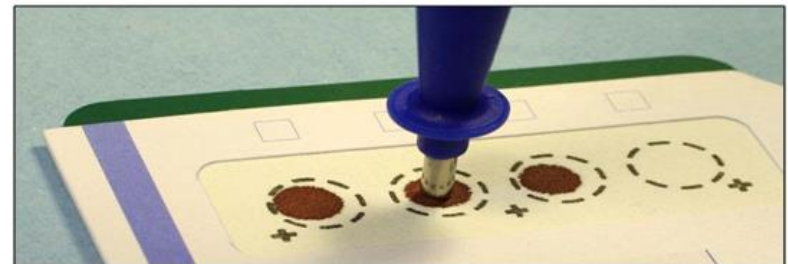
LC-MS/MS (triple quadrupole)



## ***DBS LEVELS:***

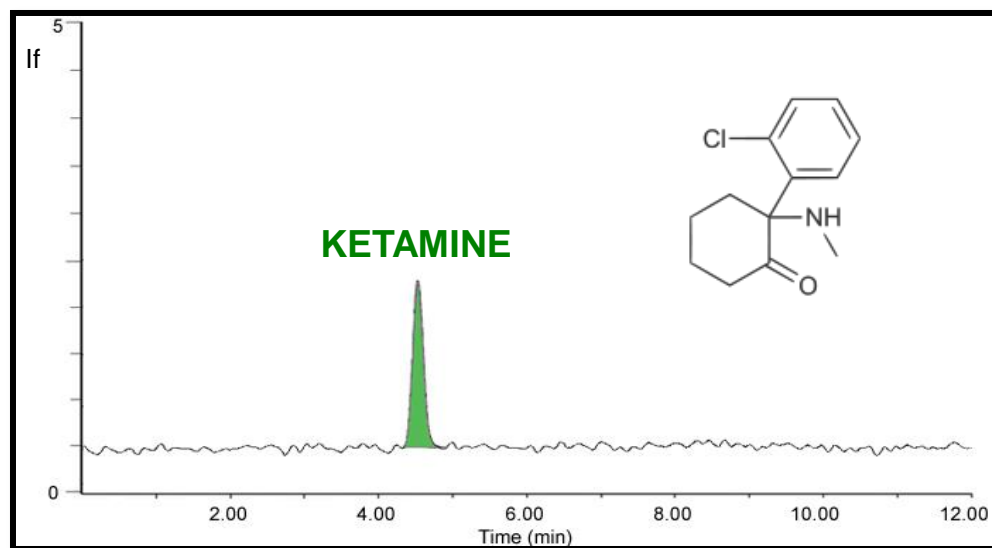
KETAMINE 22 ng/mL

NORKETAMINE 19 ng/mL



# ANALYSIS OF KETAMINE IN DBS

**ABUSER DBS ANALYSIS:**  
KETAMINE 23 ng/mL



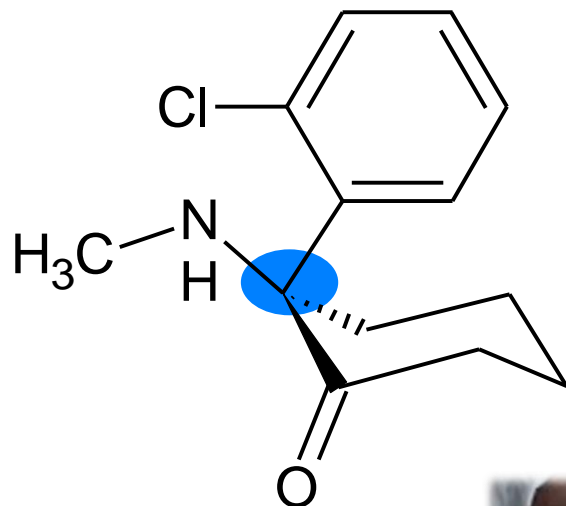
HPLC-F (native fluorescence)



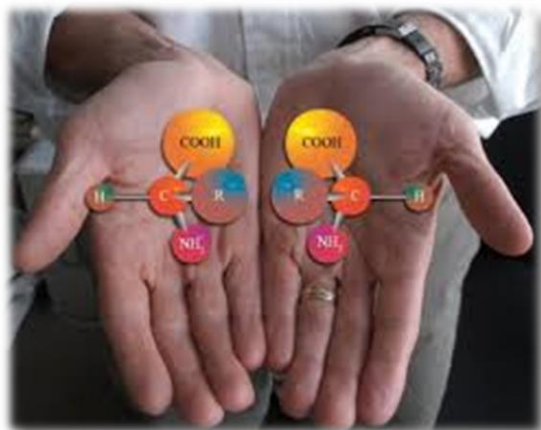
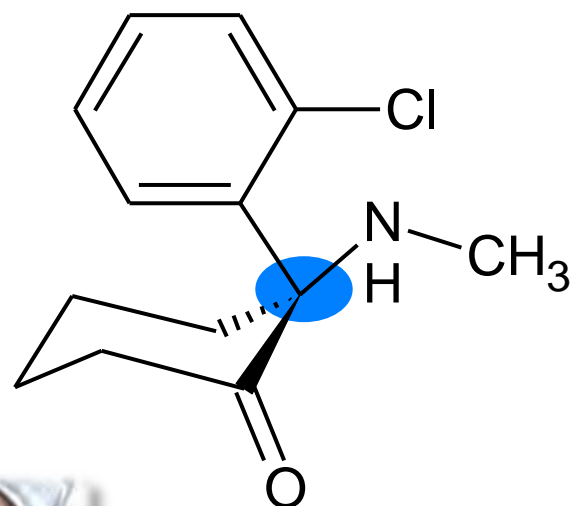


# CHIRAL ANALYSIS OF KETAMINE IN DBS

**S-(+)-KETAMINE**



**R-(-)-KETAMINE**

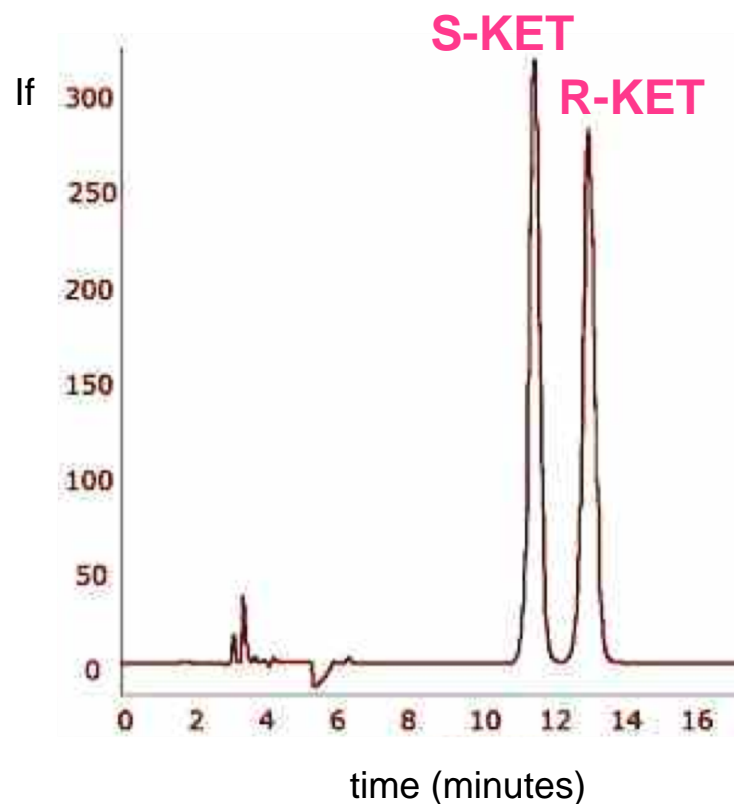
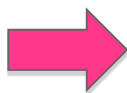


# DBS testing: application of chiral HPLC-F (native fluorescence)



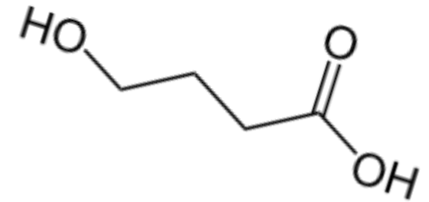
## ***KETAMINE ABUSER DBS:***

S-(+)-KETAMINE 14 ng/mL  
R-(-)-KETAMINE 11 ng/mL

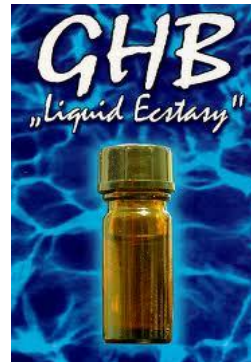


# GHB

gamma-hydroxybutyric acid



- *Liquid ecstasy*
- GHB is used for the treatment of alcoholism
- It is colorless, odorless and tasteless → it may be added to drinks without noting its presence (*date rape drug*)
- GHB also has an anabolic effect and is used by body builders



What You  
Need to Know  
About  
Drugs

GHB

## GHB effects

- anxiolytic
- euphorizing
- anabolic
- confusion, nausea, dizziness
- disorders of memory

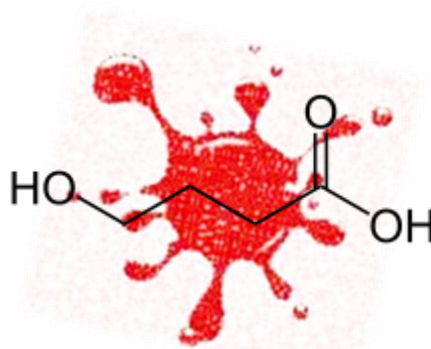
*Overdose: hallucinations, seizures, coma*



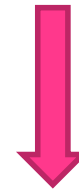
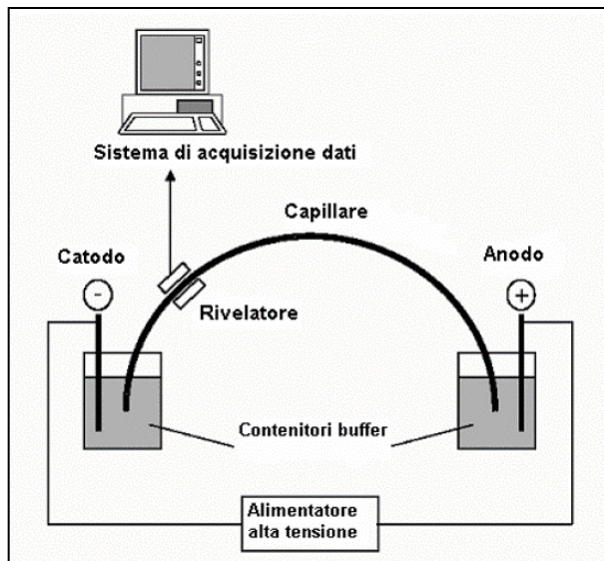
## CRITICAL ASPECTS IN THE ANALYSIS OF GHB

- Very short half-life ( $t_{1/2} = 0.5-2$  hours)
- Needs sample collection in the shortest possible time
- Lack of suitable chromophors → very low UV responses
- Endogenous GHB concentration

# ANALYSIS OF GHB IN DBS



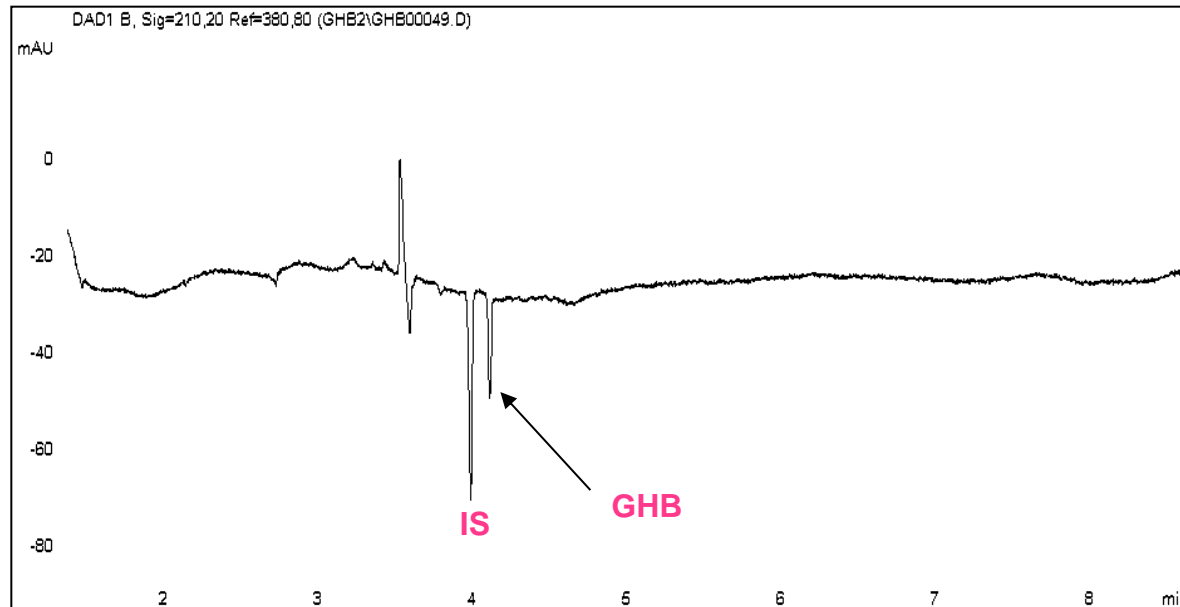
Development of a method based on capillary electrophoresis (CE) with indirect detection



No derivatization!



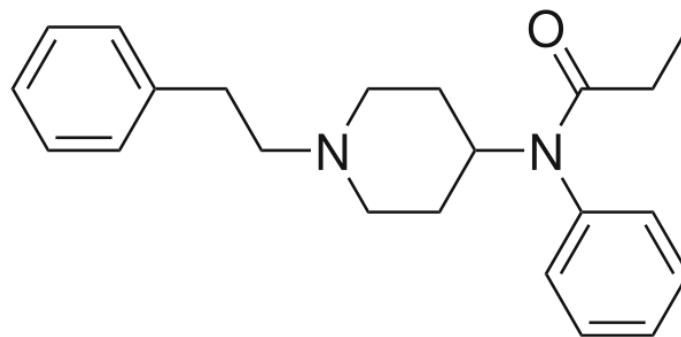
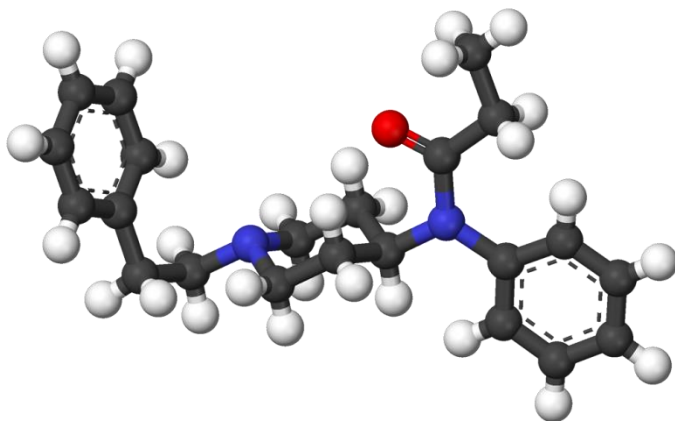
# DBS testing: method application



DBS OF GHB USER  
GHB = 14.0  $\mu\text{g/mL}$

# FENTANYL

*N*-(1-(2-phenylethyl)-4-piperidiny)-*N*-phenylpropanamide



- synthetic opioid analgesic
- powerful narcotic drug
- very lipophilic molecule
- 80 times more potent than morphine

# Fentanyl effects

- analgesic
- anesthetic
- drowsiness
- nausea, dizziness
- peripheral edema
- addiction
- tolerance
- withdrawal symptoms
- severe drug interactions



*Overdose:  
respiratory depression, stroke*



## CRITICAL ASPECTS IN THE ANALYSIS OF FENTANYL

- Very low hematic levels
- Not detectable in screening tests for biological samples  
→ false-negative results

# ANALYSIS OF FENTANYL IN DBS

HPLC-F (native fluorescence)



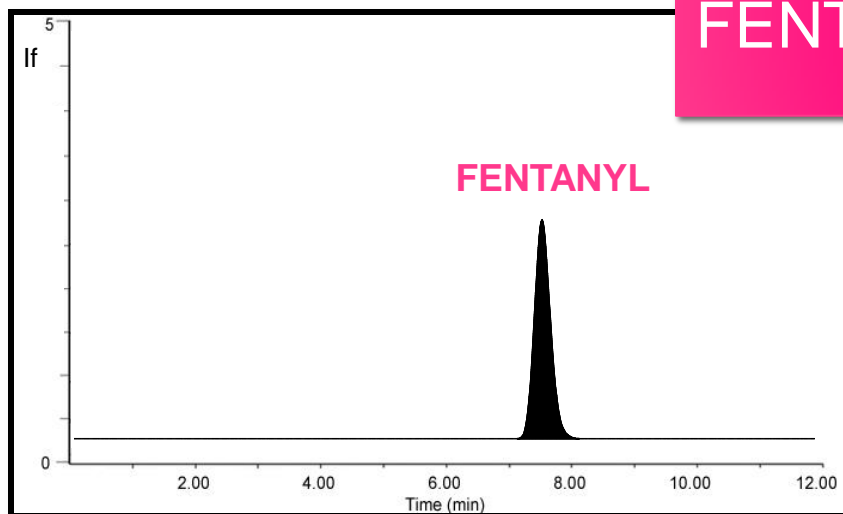
DBS clean-up: microextraction by packed sorbent (MEPS)

# DBS testing: method application

## HPLC-F CHROMATOGRAM

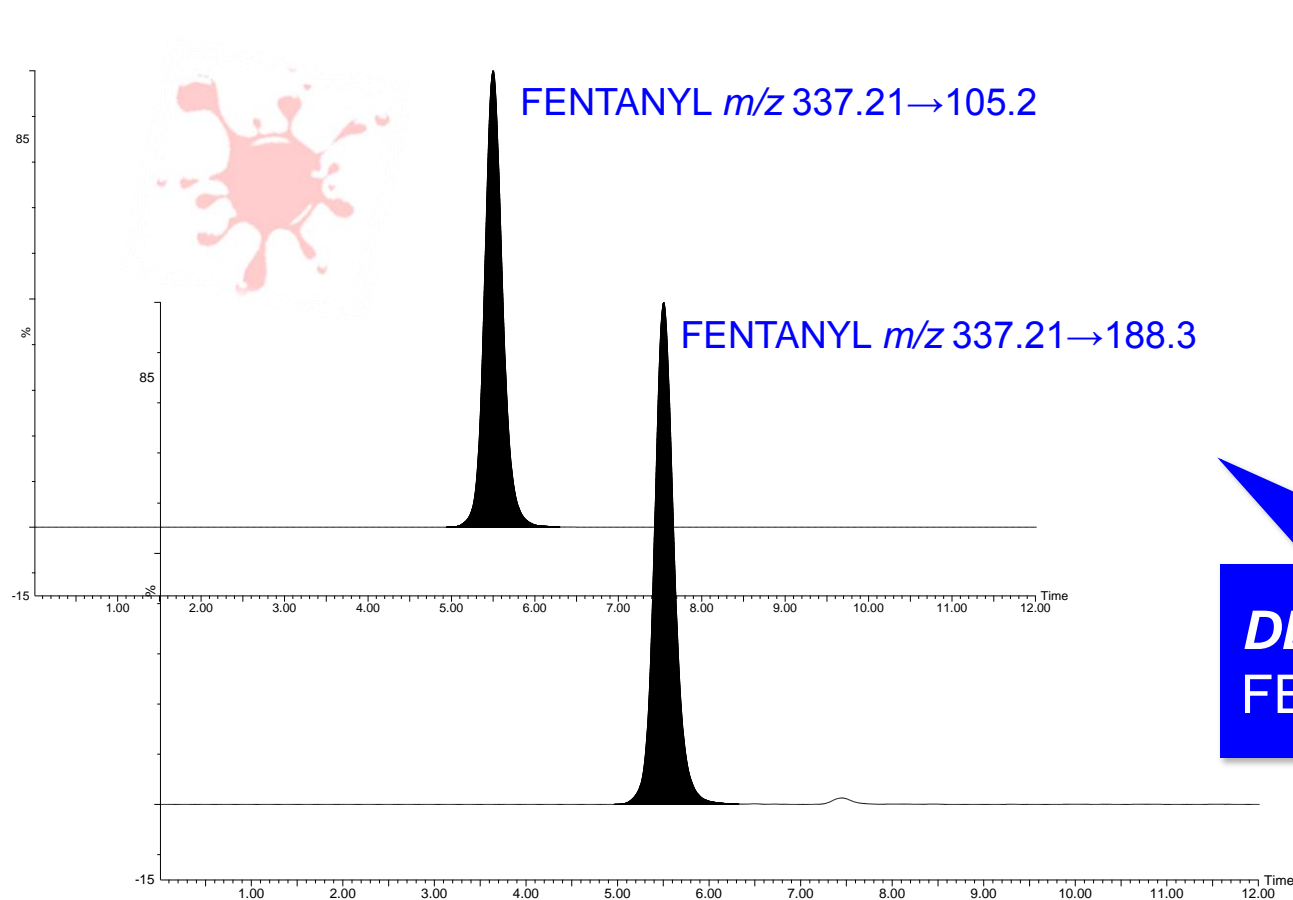


**DBS ANALYSIS:**  
FENTANYL 26 ng/mL



# DBS testing: method application

ESI+ LC-MS/MS CHROMATOGRAM



**DBS ANALYSIS:**  
FENTANYL 27 ng/mL



## ***WORK IN PROGRESS...***

- *Qualitative and quantitative analysis of synthetic cannabinoids (JWH) and synthetic cathinones (4-MEC) in seized materials by mass spectrometry*
- *Fast identification and quantitation of synthetic JWH and cathinones by LC-MS/MS in DRIED BLOOD SPOT (DBS) for “on the road” controls*



# CONCLUSION

**NOVEL TOOLS FOR RELIABLE ANALYSES  
OF “ON STREET” NPS ARE URGENTLY NEEDED**

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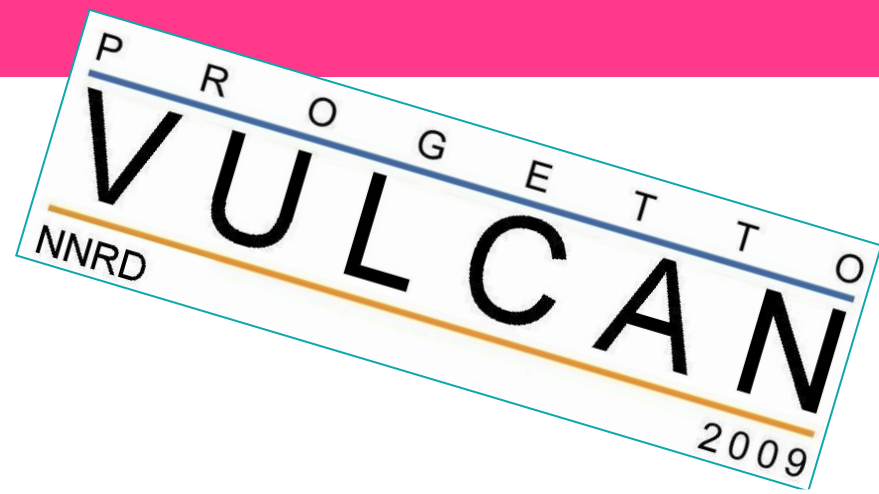
**THIS IS THE CHALLENGE FOR THE LABORATORY  
OF PHARMACO-TOXICOLOGICAL ANALYSIS  
IN THE FIGHT AGAINST DRUG ABUSE**



# LABORATORY OF PHARMACO-TOXICOLOGICAL ANALYSIS ALMA MATER STUDIORUM – UNIVERSITY OF BOLOGNA

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<b>Agnese Samorì</b>	Grant holder in Analytical Chemistry
<b>Nadia Ghedini</b>	Professor of Medicinal Chemistry
<b>Anna Ferranti</b>	Professor of Medicinal Chemistry
<b>Francesca Bugamelli</b>	Laboratory Technician





## RESEARCH PROJECT *VulCan*

**"Assessment of the condition of vulnerability to *Cannabis*  
and to the development of addiction: neuroendocrine correlations  
and quali-quantitative aspects"**

*Years 2009 - 2014*

*Selected and funded by the Department of Antidrug Policy  
Presidency of the Council of Ministers*



PRESIDENZA DEL CONSIGLIO DEI MINISTRI  
Dipartimento Politiche Antidroga



SISTEMA NAZIONALE DI ALLERTA PRECOCE  
NATIONAL EARLY WARNING SYSTEM - N.E.W.S.

# ***THANKS!***



**ROME, 14-15 May 2014**