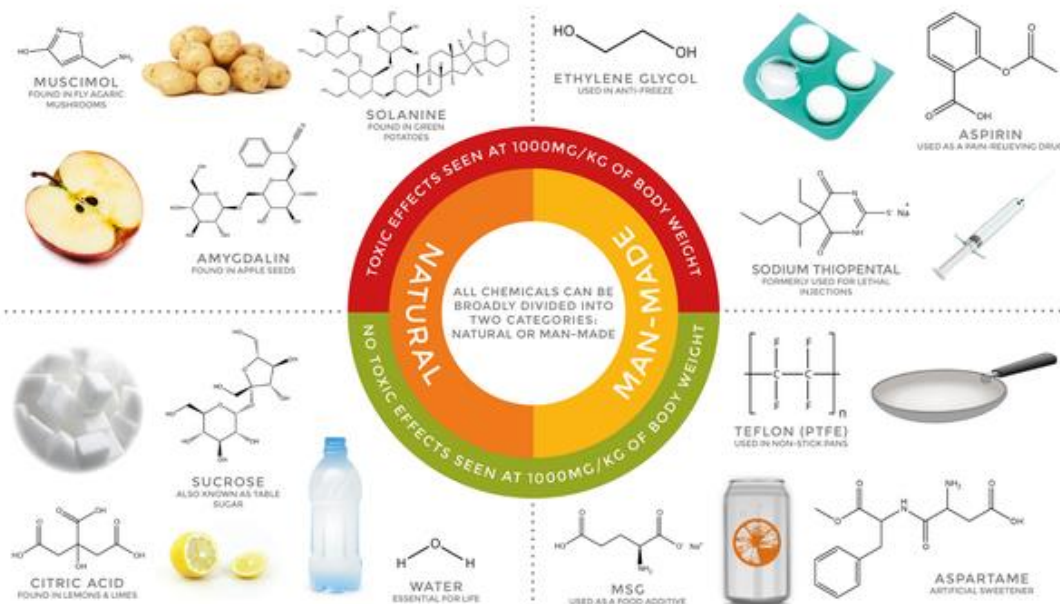


NATURAL & MAN-MADE CHEMICALS

A COMMON MISCONCEPTION IS THAT ALL MAN-MADE CHEMICALS ARE HARMFUL, AND ALL NATURAL CHEMICALS ARE GOOD FOR US. HOWEVER, MANY NATURAL CHEMICALS ARE JUST AS HARMFUL TO HUMAN HEALTH, IF NOT MORE SO, THAN MAN-MADE CHEMICALS.



"EVERYTHING IS POISON. THERE IS POISON IN EVERYTHING. ONLY THE DOSE MAKES A THING NOT A POISON."

PARACELSUS: 1493-1541, "THE FATHER OF TOXICOLOGY"

ANY SUBSTANCE, IF GIVEN IN LARGE ENOUGH AMOUNTS, CAN CAUSE DEATH. SOME ARE LETHAL AFTER ONLY A FEW NANOGRAMS, WHILST OTHERS REQUIRE KILOGRAMS TO ACHIEVE A LETHAL DOSE.

CHEMICAL TOXICITY IS A SLIDING SCALE, NOT BLACK AND WHITE - AND WHETHER A CHEMICAL IS NATURALLY OCCURRING OR MAN-MADE TELLS US NOTHING ABOUT ITS TOXICITY.

Mass Spectrometry and Workflow in Forensic Toxicology

Presentation Goal

- The goal of this presentation is to show how mass spectrometry can guide the workflow in a forensic toxicology laboratory.
 - Technology
 - Substances detected
 - Extraction procedures
 - Sample management
- Time line: 2004 – 2022.

Scope



- Approximately 4500 cases per year (*post-mortem* and *ante-mortem*).

Ante-mortem:

- Road side surveys testing
- Sexual aggressions
- Custody child in divorces
- Suspected poisoning

Post-mortem:

- Pathology cases
- Exhumations

Samples

Routine biological samples

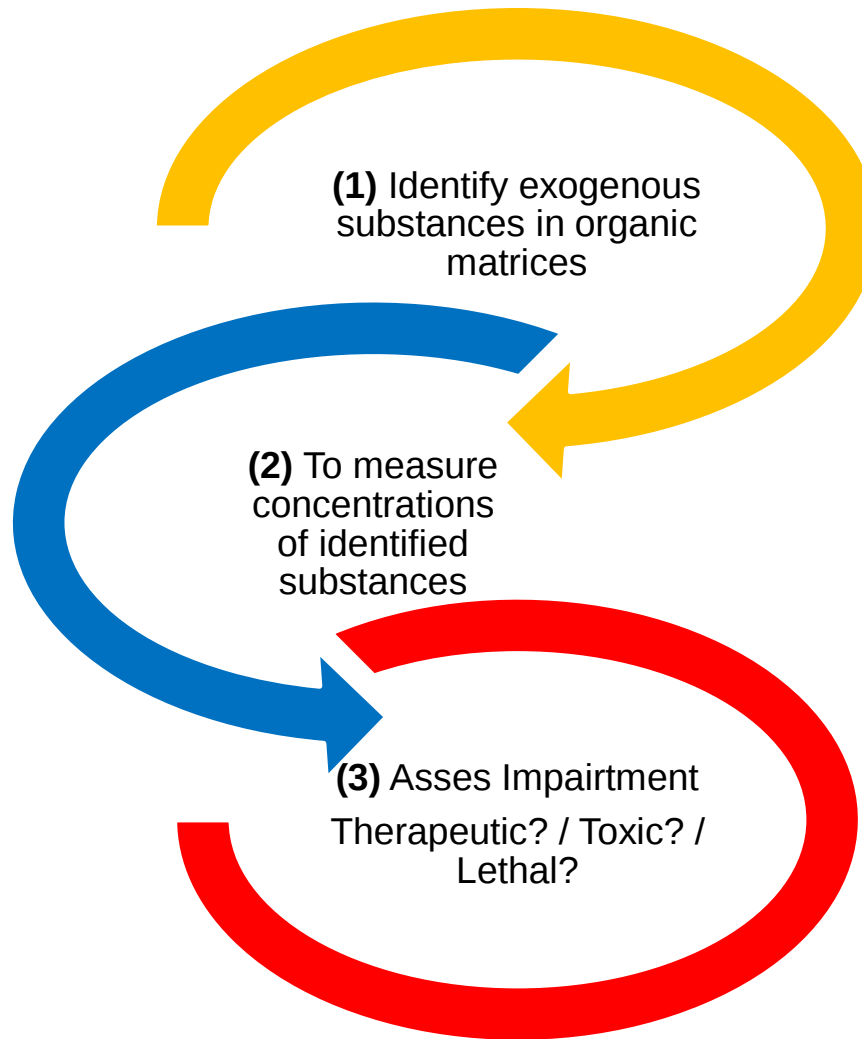
Heart blood	20-30 mL
Peripheral blood	10 mL
Urine	30 mL
Gastric content	50 g
Vitreous humor	~ 1 mL



Alternative biological samples

Oral fluid	~ 0,5 mL
Hair	As available

Forensic toxicology main goals



Antonio Castañera & Susana Simões

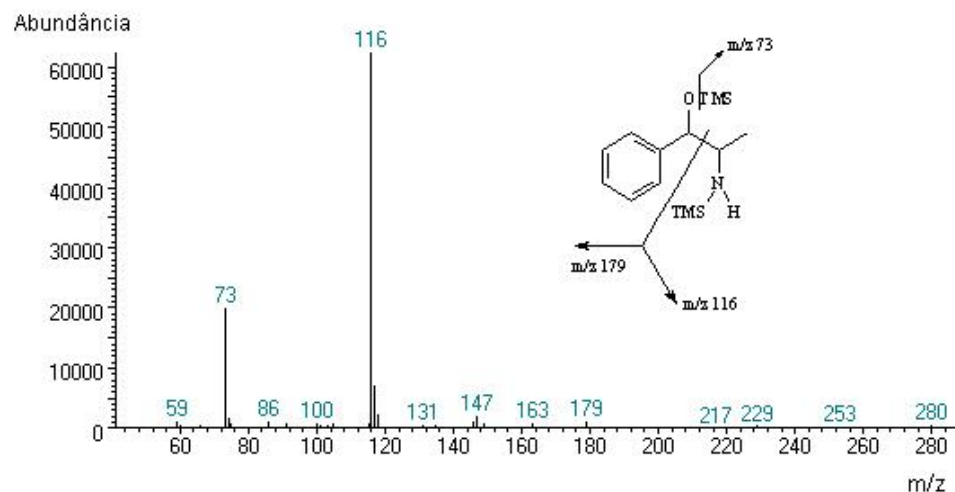
18-04-2022

Advanced Analytical Tools: Multiple applications for Mass Spectrometry

Identify substances in forensic toxicology

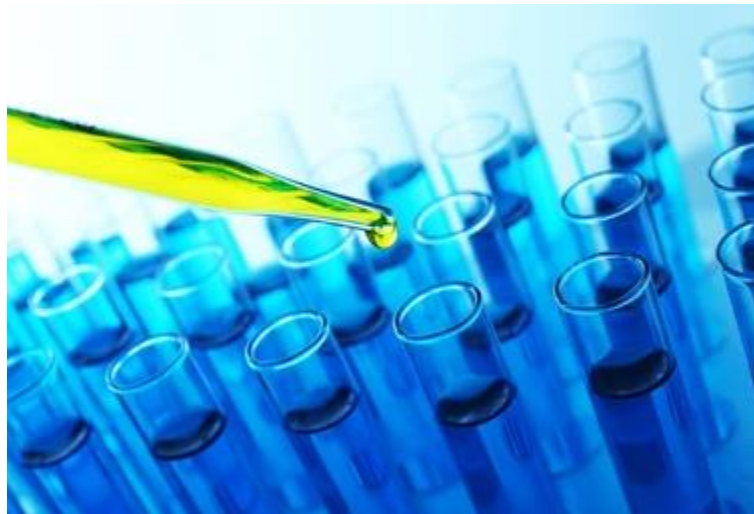


- Ethanol (GC/HS/FID)
- Drugs of abuse (mass spectrometry)
- Legal medicines (mass spectrometry)
- Pesticides (mass spectrometry)
- Carboxyhemoglobine (UV-Vis)



Identify substances in forensic toxicology

- Forensic toxicology is characterized by the analysis of very complex and unstable biological samples
- It is very important to pay attention to the metabolism of each substance and the detection time window.
- The potential number of substances analyzed can be hundreds or even thousands.



Antonio Castañera & Susana Simões

18-04-2022

Advanced Analytical Tools: Multiple applications for Mass Spectrometry

Identify substances in forensic toxicology

- Forensic toxicology is often related to overdose situations so it is expected to find large concentrations of substances in positives samples.
- In routine samples, only a small percentage of positive samples correspond to situations of overdose.
- It is even normal to find some drugs of abuse in low concentrations: 6-MAM, Δ 9-THC, NPS.



Antonio Castañera & Susana Simões

18-04-2022

Advanced Analytical Tools: Multiple applications for Mass Spectrometry

Analytical technique evolution (2004 – 2022)



GC/MS
Agilent 5973



LC/MS Waters
Quattro micro



UPLC/MS Waters
TQD



LC/MS Sciex
6500 QTrap



GC/LC TOF Impact
II Bruker

2004

- Complex extraction procedures:
 - SPE extractions
 - Derivatizations
- Sample volumes (0,5 – 1 mL).
- Acceptable sensibility.
- Several analytical methods.
- Not versatile equipment

2005

- Extraction procedures:
 - SPE extractions
- Sample volumes (0,2 – 0,5 mL).
- Sensitivity Increase (MS → MSMS)
- Versatile technology: Possibility of changing chromatography parameters (analytical columns and mobile phases)
- Ionizations sources (APCI, APPI, ESI)

2009

2021

- Faster and easier extraction procedures:
 - Protein precipitation
- Low sample volume (0,1 – 0,2 mL).
- High throughput
- Versatile technology: LC, GC, QQQ, Qtrap, Time of flight (TOF)
- Possibility of analyzing exact mass (TOF) → Unknown substances (e.g. NPS)

2022

2004



GC/MS
Agilent 5973

- Opiáceos: Morfina, Codeína, 6-MAM, Metadona, Papaverina
- Cocaína: Cocaína, benzoilecgonina, EME, Ketamina, Atropina
- Canabinóides: THC, 11-OH-THC; THC-COOH
- Anfetaminas: Anfetamina, MDA, MBDB
- Metanfetaminas: Metanfetamina, MDMA; MDE

Drug of abuse analysis. Evolution 2004 – 2022

2022



UPLC/MS Waters
TQD



GC/LC TOF Impact
II Bruker

5-IT
PMA
6-APDB
PMMA
Ketamina
Methoxetime
5-MeO-DALT
4-chloro-2,5-DMA
25C-NBOMe
25B-NBOMe
25I-NBOMe
Methylone
3,4-DMMC
MDPV
AB-CHMINACA met.1MA
5-F PB 22-3 carboxindole
AM2201 6-hydroxyndole
AB-FUBINACA met3
JWH N-pentanoic acid
5-F-APINACA N-(4-hydroxybutyl)
4-MEC, JWH073 butanoic acid
JWH073 N-(4-hydroxybutyl)
JWH250 N-pentanoic acid
MDMB-CHMICA
MDMB-CHMICA O-desmethyl acid
MDA
Metanfetamina
Mephedrone
α-PVP

Pentedrone
JWH250 N-(5-hydroxypentyl)
JWH073 N-(3-hydroxybutyl)
AM2201 N-(4-hydroxypentyl)
JWH018 N-(hydroxypentyl)
JWH122 N(4-hydroxypentyl)
JWH210 N-pentanoic acid
JWH210 N-(4-hydroxypentyl)
4-Methylaminores
α-PVT
4-4-Dimethylaminorex
AH7921
MT-45
JWH018 N-pentanoic acid
Anfetamina
MDMA
JWH210 5-Hydroxyndole
THC-COOH
Morfina
Codeína
6-MAM
Benzoilecgonina
Tramadol
Cocaína
EDDP
Metadona
Fentanil
11-OH-THC
THC

Canabidiol
Canabinol
HMMA
Buprenorfina
M-CPP
Naltrexona
Naloxona
EME

New Psychoactive
Substances (NPS)

“Substâncias desconhecidas”



Aminoidanes – These substances, of which 5,6-methylenedioxy-2-aminoindane (MDAI) is an example, have been sold as NPS for their ability to produce the empathogenic and entactogenic effects of serotonin-releasing drugs, such as MDMA.



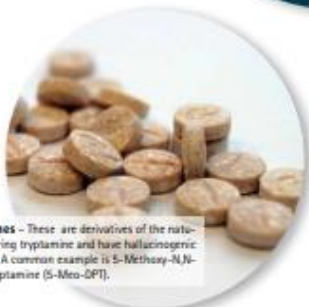
Synthetic cannabinoids – These are cannabinoid receptor agonists which produce effects similar to those of delta-9-tetrahydrocannabinol (THC), the principal psychoactive component in cannabis. Synthetic cannabinoids are often laced into herbal products and sold as Spice, K2, Kronic, etc.



Synthetic cathinones – These are analogues/derivatives of the internationally controlled substance cathinone, one of the active components of the khat plant. They generally have stimulant effects and include frequently reported NPS such as mephedrone and MDPV.



Tryptamines – These are derivatives of the naturally occurring tryptamine and have hallucinogenic properties. A common example is 5-Methoxy-N,N-diisopropyltryptamine (5-MeO-DIPT).



NPS

Categories of new psychoactive substances sold in the market

Ketamine and phencyclidine-type substances – Ketamine is a human and veterinary anesthetic which acts as a stimulant at low doses and a hallucinogen at high doses. It is one of the most widespread NPS in Asia. Phencyclidine-type substances are another group of NPS that has recently appeared in the market. Phencyclidine (PCP) and ketamine show structural similarity and are classified as arylcycloalkylamines. One of the most frequently reported substances in this group is 4-methoxyphencyclidine (4-MeO-PCP).



Plant-based substances – This group includes plants with psychoactive properties. The most frequently reported are:

- Kratom (*Mitragyna speciosa* Korth.), a plant indigenous to South-East Asia that contains the alkaloid mitragynine, a stimulant at low doses and sedative at high doses.
- *Salvia divinorum*, a plant indigenous to forest areas in Oaxaca, Mexico, which contains the active ingredient salvinorin A, a hallucinogenic substance.
- Khat (*Catha edulis*), a plant native to the horn of Africa and the Arabian peninsula. The leaves of the plant are chewed, resulting in the release of the stimulants cathinone and cathine.



Piperazines – These substances are frequently sold as 'ecstasy' due to their central nervous system stimulant properties. The most commonly reported members of this group are benzylpiperazine (BZP) and mCPP (1-[3-chlorophenyl] piperazine).



Phenethylamines – This group contains substances related to amphetamine and methamphetamine, and generally produces stimulant effects. However, modification of these compounds can lead to potent hallucinogens such as Bromo-Drongilly.

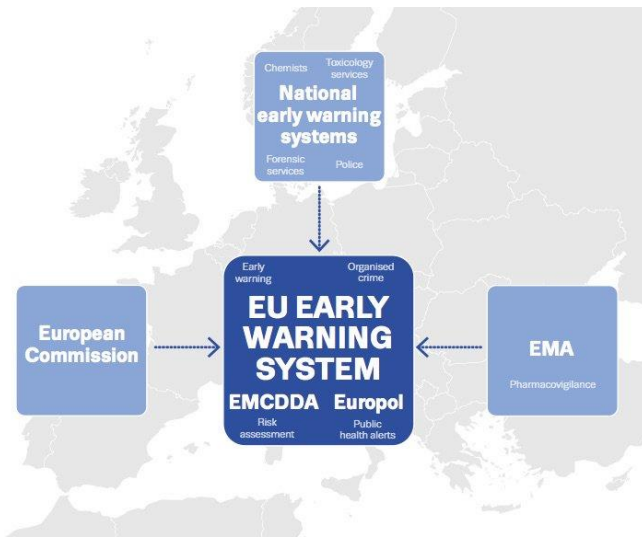


Other substances – NPS substances in this category are structurally diverse and do not fit into the categories mentioned above for example, 1,3-dimethyl-aminylamine (DMAA).



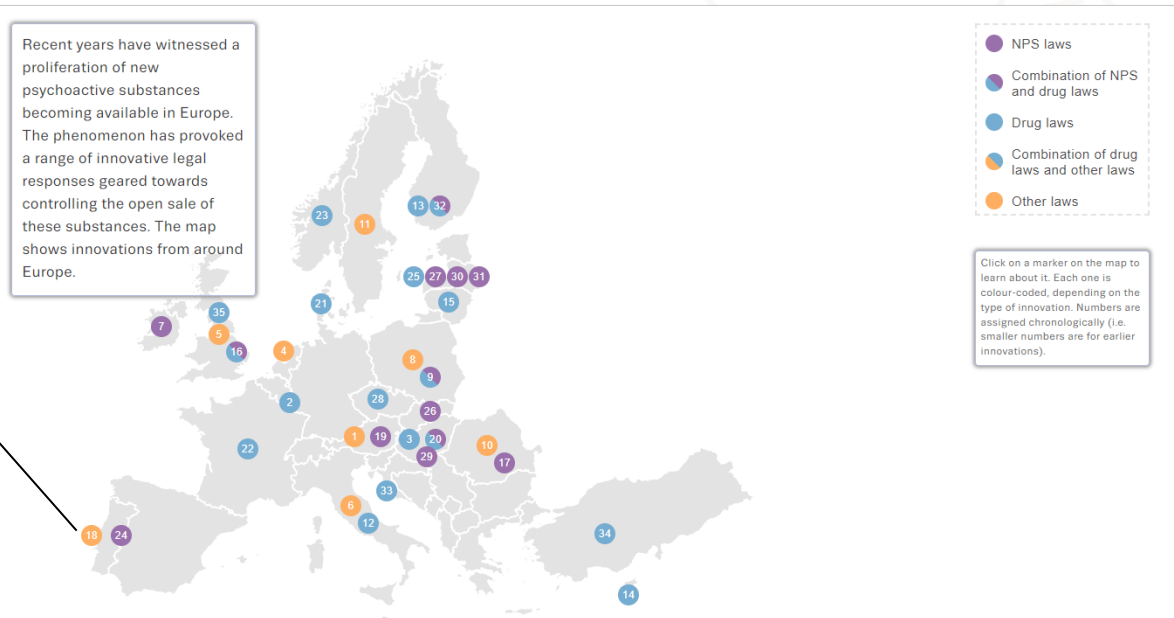
Make health your “new high” in life - not drugs.

Controlo Internacional de Substâncias (EU _ Early Warning System)



Interactive: legal innovations across Europe

December 2011, Portugal ✕
The Economy and Food Safety Authority (ASAE) confiscated over 65 000 packages of substances from fourteen 'smart' shops due to consumer health concerns.



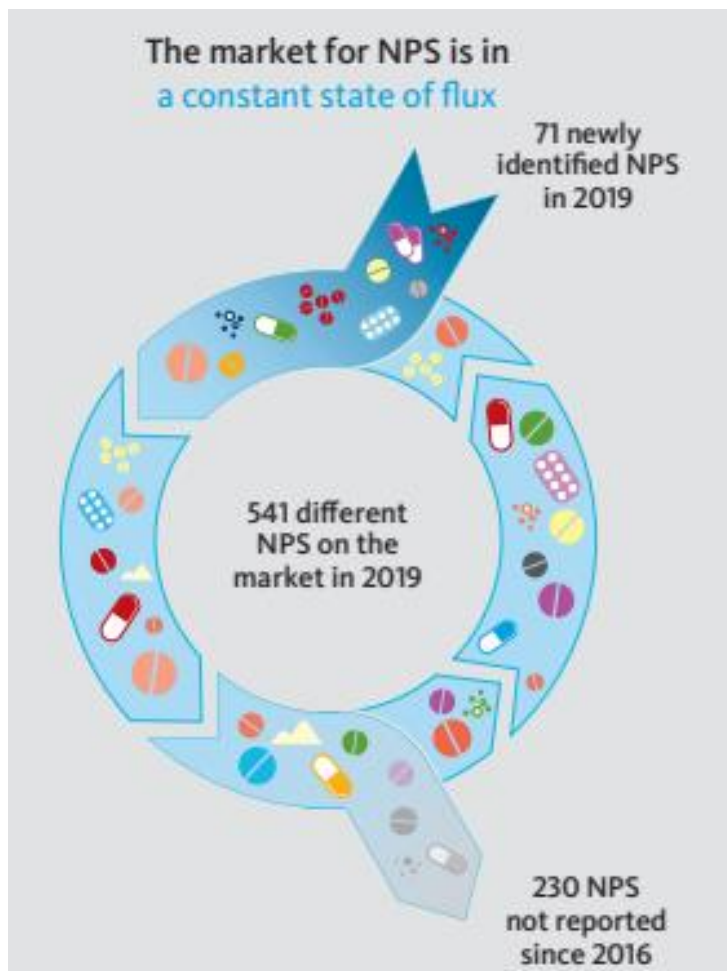
Group

Main effects

Ketamine and phencyclidine der.	-> Ketamine is an anesthetic with clinical and veterinary applications in low doses, acting as a stimulant, in high concentrations with hallucinogenic properties.
Synthetic cathinones	-> Analogues and derivatives of the controlled substance cathinone (main psychoactive component of the "khat" plant).
Synthetic cannabinoid	-> Receptor agonists of cannabinoids with properties similar to D9-THC, frequently mixed with products of vegetable origin and sold under the commercial name of "K2, spice, chronic".
Aminoindanes	-> Produce empathogenic and entatogenic effects similar to those caused by drugs that act on the neurotransmitter serotonin (e.g. MDMA).
Tryptamines	-> Hallucinogenic properties.
Plants base products	-> Plant extracts with psychoactive properties: Kratom (stimulant in low properties and sedative in high concentrations; Salvia divinorum (hallucinogenic properties); Khat (stimulant properties).
Piperazines	-> Frequently sold as "Ecstasy" because of its stimulating properties of the central nervous system.
Phenethylamines	-> Substances related to amphetamines and methamphetamines, modifications in molecular structure can cause powerful hallucinogenic effects (e.g. Bromo-Dragonfly)
Other substances	-> Diverse group of NPS that doesn't fit into any of the above categories.



. The market for New Psychoactive Drugs.....



What to do with reference standards....



2004



GC/MS
Agilent 5973

- Sample volume: 1 mL
- SPE extraction
- Run time: app. 35 min
- Target substances: App. 50

Legal medicines analysis. Evolution 2004 – 2022



2021



LC/MS Sciex
6500 QTrap

- Sample volume: 0,2 mL
- Protein precipitation
- Run time: 15 min
- Target Substances: Aproximadamente 200

Workflow....

- I. Screening. Imunoassays
- II. Confirmation procedures (GC/MS; LC/MSMS)
- III. Quantification procedures (GC/MS; LC/MSMS)



- I. Screening. (LC/MS/MS)
- II. Confirmation and quantification procedures (LC/MS/MS)

	2004	2022
Analytical procedures	Many	Few
Sample volume	2-10 mL	1-2 mL
Target compounds	App 70	App 300
Unknown compounds	No	Yes

Advantages

- Easier and cheaper analytical procedures.
- High throughput
- High sensitivity analytical methods
- Target analysis and detection of unknown compounds

Disadvantages

- Expensive technology.
- Expensive maintenance contracts
- One analytical equipment and several technicians are not always a good idea



Thank you for your attention !