UNIVERSITEIT GENT

FACULTEIT GENEESKUNDE EN GEZONDHEIDSWETENSCHAPPEN

Pharmacokinetics and Metabolism of designer drugs

P. Van Eenoo

What are designer drugs?

- Developed to circumvent:
  - Law
  - (WADA) Regulations
  - Detection

What are designer drugs?

- Examples:
  - Designer steroids
  - Designer stimulants
  - Designer cannabinoids
  - Designer peptides
  - Other...

1994 - 2004

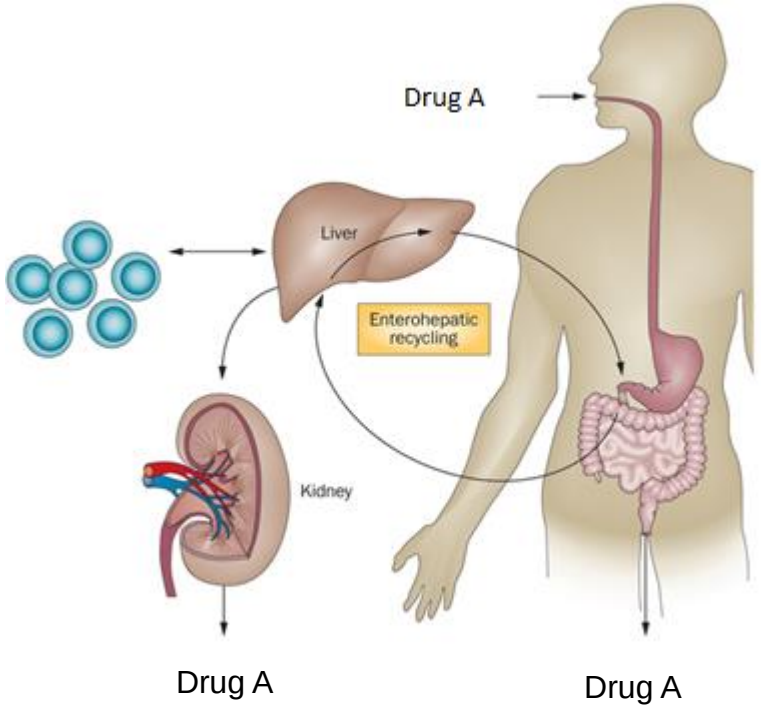
Drug Supplement Health and Education Act (DSHEA)

- in USA
- legal sales of anabolic steroids
  - DHEA (1994-…)
  - androstenedione, androstenediol (1996) — 1st wave: testosterone analogues
  - norandrostenedione, norandrostenediol (1998) — 2nd wave: nandrolone analogues
  - 1-androstenedione/1-androstenediol (2001) — 3rd wave: boldenone analogues
- Anabolic steroid control act 2005
  - nominative list
  - 6-oxo-androstenedione, 7-keto-DHEA, 4-OH-testosterone (2001-2008) — 4th wave

designer steroids on the internet

- madol
- methyldrostanolone
- prostanozol
- “halodrol”
- bromo-androstenedione
- 1,4,6-androstatrienedione
- epitiostane
- …

Issues with detection in biological fluids: Drug Metabolism



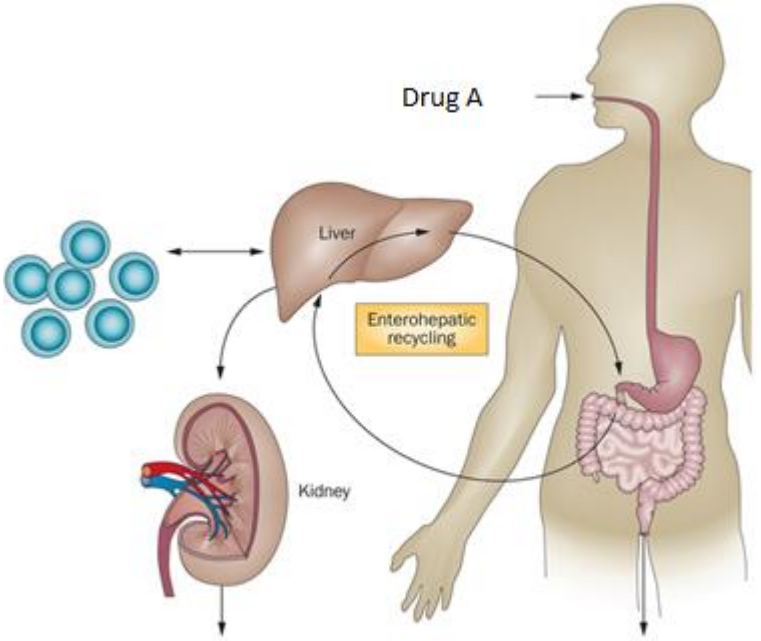
Ideal situation

No alterations

Low number of drugs

Clenbuterol

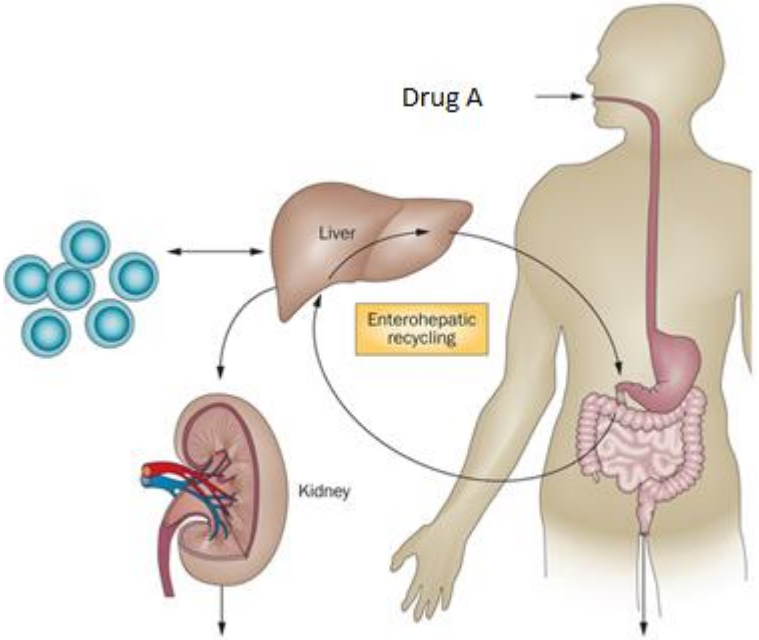
Issues with detection in biological fluids: Drug Metabolism



Phase I metabolism

Alterations to Basic structure
Introduction of reactive or polar groups

Issues with detection in biological fluids: Drug Metabolism



Drug A-Con
A1-Conj, A2-Conj,...

Drug A-Conj
A1-Conj, A2-Conj,...

Phase II metabolism

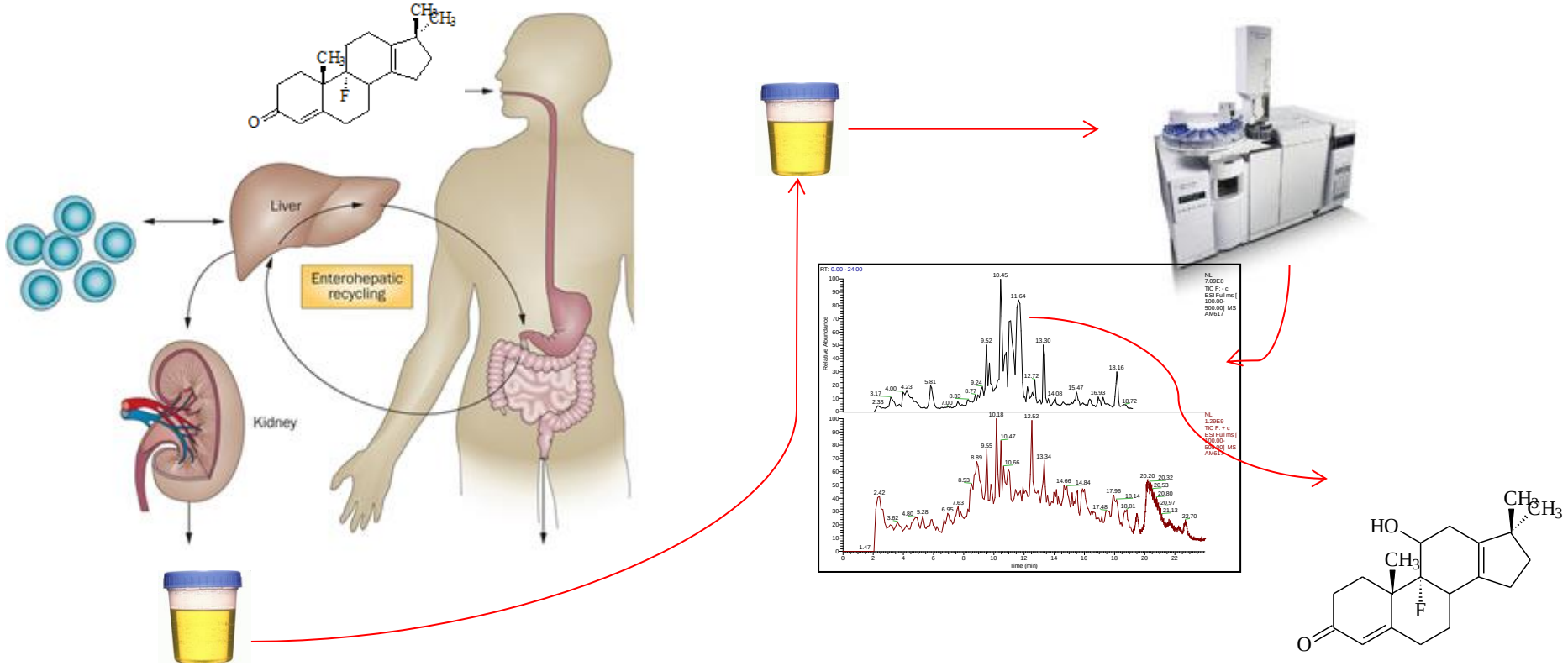
Conjugation to polar compounds
- Sulphate
- Glucuronide

Complications for detection

Detection

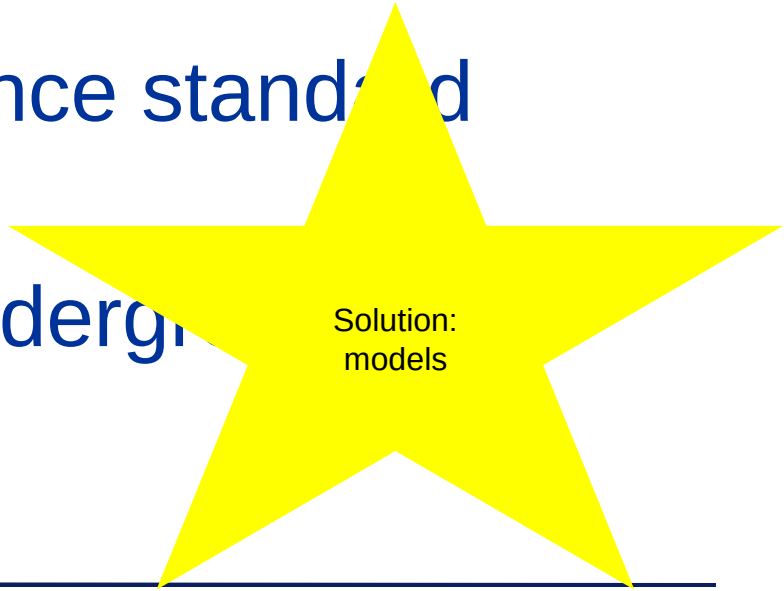
- Adequate detection methods should detect
  - Rapidly excreted metabolites
  - Long-term metabolites
- Identify
  - By direct comparison to a reference (standard, urine, etc)
  - unequivocally

Traditional investigation of drug metabolism

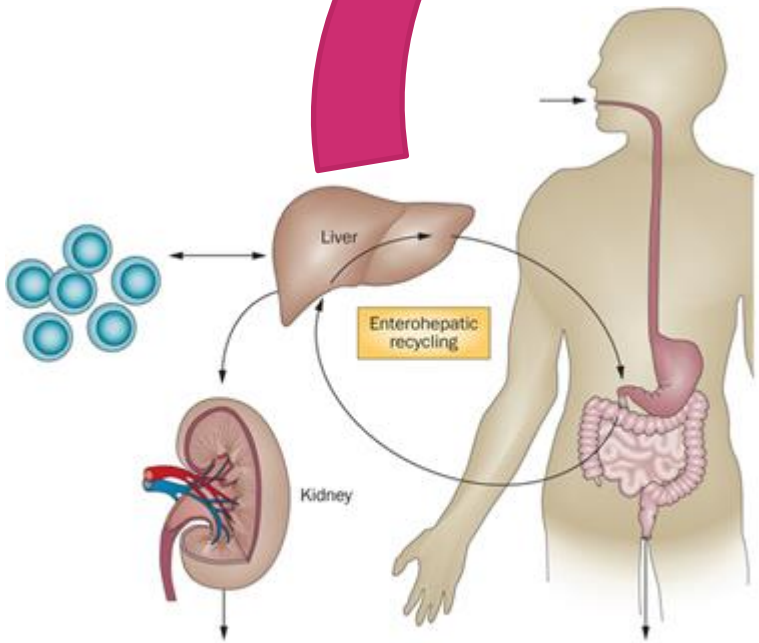


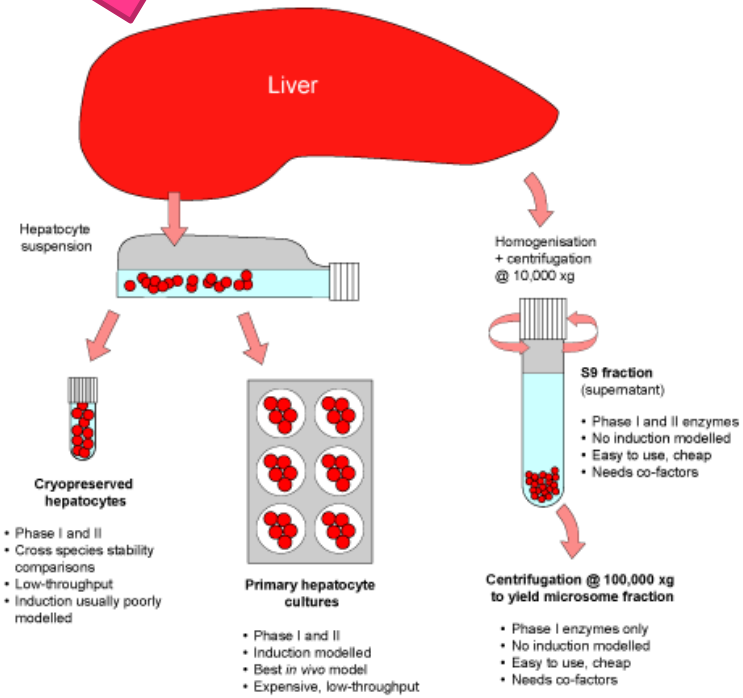
Problems with designer drugs

- No/limited Toxicological data available
- No/Limited data on quality
- No/Limited certified reference standard available
- No data or structure for underg preparations



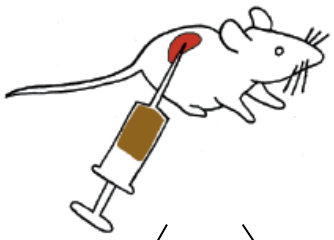
In-vitro models:

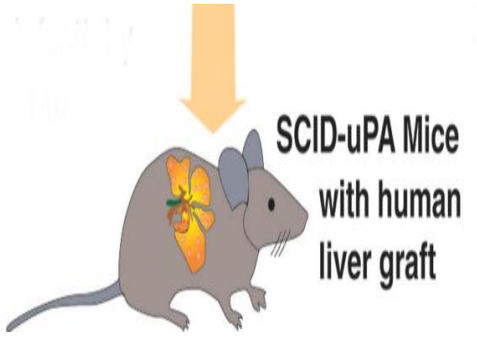




uPA$^{+/+}$SCID chimeric mouse

1. Human hepatocytes + 2. Transgene Mouse = **Chimeric Mouse**





uPA — Liver disease

SCID — No rejection

Knowledge: Synthetic Cannabinoids

- Pharmacokinetics: No/extremely limited scientific data available
- metabolism: limited scientific data available

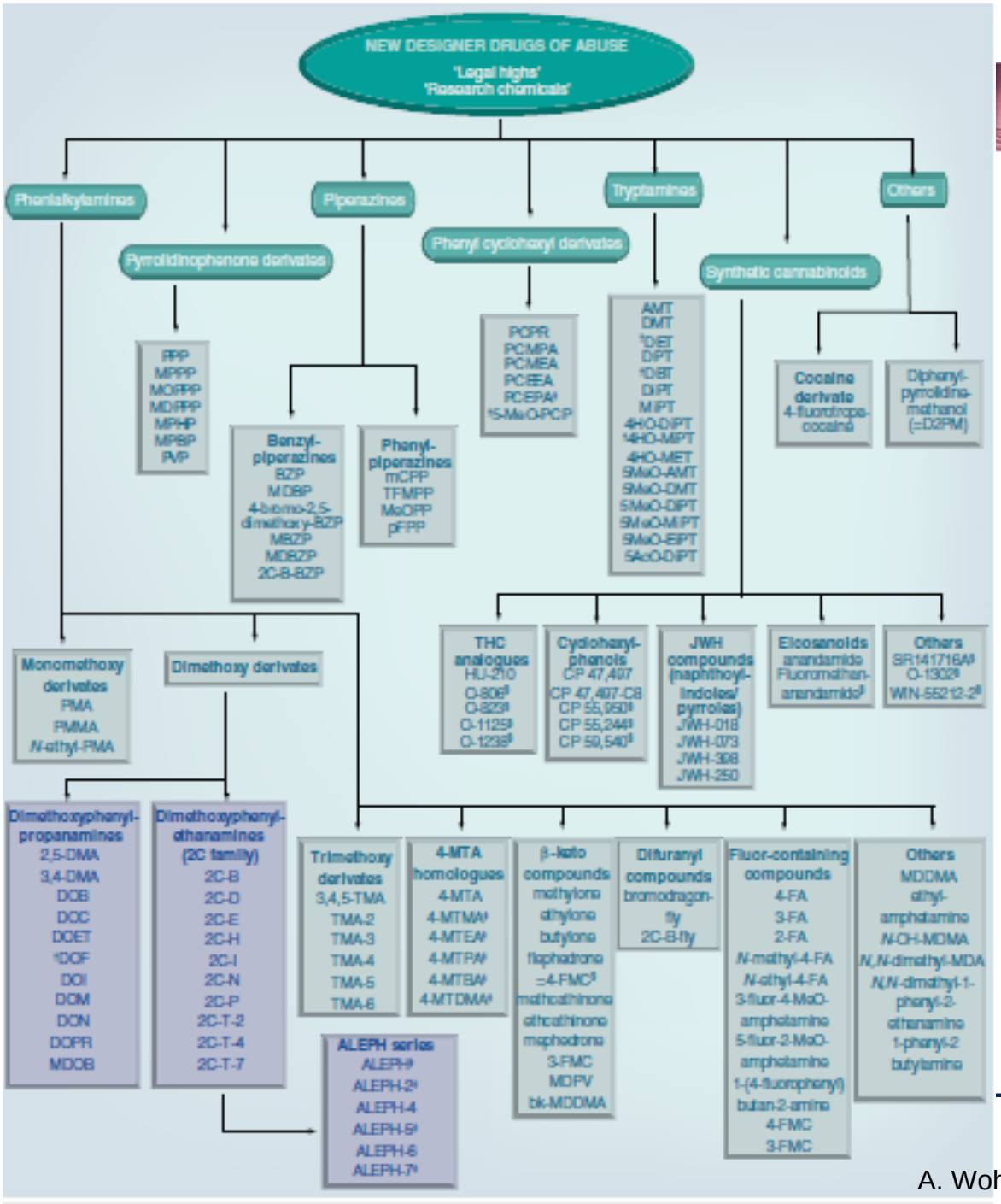
Cannabinoid	Reference
JWH-018	J. Teske, et al.. J. Chromatogr. B 2010, 878, 2659–63. A. Wintermeyer, et al. Anal Bioanal Chem. 2010, 398, 2141–53. Moran et al. Analytical chemistry. 2011, 83, 4228–36. S. Beuck et al. Anal Bioanal Chem. 2011, 401, 493–505. T. Sobolevsky et al. Forensic science international. 2010, 200,141–47
JWH-015	Q. Zhang, et al. Anal Bioanal Chem. 2006, 386, 1345–55.
AM-2201	T. Sobolevsky et al. Drug testing and analysis. 2012,doi:10.1002/dta.1418
UR-144	T. Sobolevskyet al. Drug testing and analysis. 2012,doi:10.1002/dta.1418
HU-210	U. Kim et al. J Pharm Biomed Anal. 2012, 64-65, 26–34.
JWH-122	N. De Brabanter et al.. Forensic Toxicology. 2013, 31, 212–22
JWH-073	Moran et al. Analytical chemistry. 2011, 83, 4228–36.
AM-694	A. Grigoryev et al. Drug testing and analysis. 2013, 5, 110–15.
JWH-200	N. De Brabanter et al. Rapid Commun Mass Spectrom. 2013, 27, 2115–2126

Knowledge: Synthetic Cannabinoids

- Metabolism
  - Extensive Phase 1 metabolism
  - Phase 2 metabolism
- Low concentrations in urine

Knowledge: Designer stimulants

- Metabolism
  - Often excreted (to some extent) as such
  - Synthetic cathinones often metabolized via
    - carbonyl reduction into respective substituted ephedrines
    - *Followed by N*-dealkylation into norephedrines
  - Relatively high concentrations in urine



FACULTEIT GENEESKUNDE EN GEZONDHEIDSWETENSCHAPPEN

A. Wohlfarth, W. Weinmann Bioanalysis 2 (2010) 965–979

Alternatives to urine: 1. Saliva

- Advantages
  - Parent compound = often target
  - Non-invasive - privacy
- Disadvantages
  - Limited volume
  - Detection time*
  - Limited knowledge so far except for DoA

* shorter; but better correlation to activity for some drugs

Alternatives to urine: 1. Saliva

Table II. Pseudoephedrine Concentrations in OF Samples*

Time after Drug Intake (h)	Subject 1 ng/mL	Subject 2 ng/mL	Subject 3 ng/mL	Subject 4 ng/mL	Subject 5 ng/mL	Subject 6 ng/mL	Subject 7 ng/mL	Subject 8 ng/mL
2	**80**	**171**	40	**73**	**196**	**219**	**400**	**215**
4	48	107	11	31	131	–	99	170
6	19	69	15	36	50	85	64	132
8	18	57	20	43	42	38	86	80
12	0	–	**66**	9	20	18	30	44
24	0	23	0	0	0	13	0	0

* Taken from eight subjects after intake of 60 mg of pseudoephedrine. Peak concentrations are indicated in boldface.

Table III. Pseudoephedrine and Cathine Concentrations in Urine Samples*

	Subject 1		Subject 2		Subject 3		Subject 4		Subject 5		Subject 6		Subject 7		Subject 8	
Time (h)	PSE µg/mL	Cat µg/mL	PSE µg/mL	Cat µg/mL	PSEL µg/mL	Cat µg/mL	PSE µg/mL	Cat µg/mL	PSE µg/mL	Cat µg/mL	PSE µg/mL	Cat µg/mL	PSE µg/mL	Cat µg/mL	PSE µg/mL	Cat µg/mL
2	19	2.0	–	–	12	1.2	69	–	–	–	62	1.2	4	0.7	25	1.0
4	15	4.5	**150**	2.1	24	1.6	28	1.4	84	1.4	43	1.4	**128**	3.4	**145**	2.0
8	5	2.3	–	–	20	1.9	28	1.8	48	1.8	12	1.2	**160**	**6.0**	37	1.3
12	10	2.8	–	–	8	1.6	51	1.9	83	1.9	**107**	2.3	86	4.6	55	1.9
16	17	1.9	73	2.7	19	2.5	–	–	11	1.2	36	1.7	81	4.4	32	1.6
24	19	1.6	23	1.7	4	1.4	15	1.2	18	1.4	3	1.0	53	3.1	37	1.7
28	0	0.8	2	1.0	1	1.8	0	1.4	6	1.1	5	1.1	9	1.5	10	1.1

* Taken from eight subjects after intake of 60 mg of pseudoephedrine. Values exceeding 100 and 5 µg/mL for pseudoephedrine and cathine, respectively, are indicated in boldface.

Ca. 1000-fold lower concentrations
Detection time much shorter

S . Strani-Rossi et al. J. Anal. Toxicol. 34 (2010) 210-215

Rome lab

Alternatives to urine: 1. Saliva

Table 1
Cut off levels for analysis in whole blood, oral fluid and urine.

	Whole blood (ng/mL)	Oral fluid (ng/mL)	DRUID cut off oral fluid	Urine quantification (ng/mL)	Urine screening (ng/mL)
6-MAM	9.8	1	0.8	33	20
7-Aminoflunitrazepam	NA	0.2	0.1	28	NA
7-Aminoclonazepam	NA	0.6	0.7	29	NA
7-Aminonitrazepam	NA	0.5	0.6	25	NA
Alprazolam	9.3	0.5	0.5	31	NA
Amphetamine	41	5	24	135	300
Benzodiazepines	NA	NA	NA	NA	200
Benzoylecgonine	58	10	7	58	30
Buprenorphine/ Buprenorphine gluc[a]	0.9	2.3	NA	193	5
Cannabis	NA	NA	NA	NA	20
Clonazepam	9.5	0.6	0.5	NA	NA
Codeine	9.0	2	8	60	NA
Cocaine	61	1	1.8	61	NA
Diazepam	57	0.4	0.4	NA	NA
Flunitrazepam	1.6	0.2	0.3	NA	NA
Lorazepam	9.6	0.6	NA	32	NA
MDA	54	6	NA	1434	NA
MDEA	104	6	NA	207	NA
MDMA	58	26	NA	77	NA
Methadone	62	10	7.7	62	300
EDDP	NA	NA	NA	111	NA
Methamphetamine	45	5	15	149	NA
Morphine	8.6	4	7	29	NA
N-Desmethyldiazepam	54	0.4	0.7	135	NA
Nitrazepam	14	0.6	0.4	NA	NA
Opiates	NA	NA	NA	NA	300
Oxazepam	287	0.6	5	143	NA
THC/THC-COOH[a]	0.63	0.6	0.6	10	NA
Zolpidem[b]	15	1	NA	6	NA
Zopiclone[b]	19	1.3	1	4	NA

NA: not analysed.
[a] THC is analysed in oral fluid and blood. THC-COOH in urine. Buprenorphine is measured in oral fluid and whole blood and buprenorphine glucuronide in urine.
[b] Analysed only in urine if detected in oral fluid. Cut off levels in oral fluid is reported in oral fluid without buffer, e.g. corrected with a factor of 2 compared to measured values.

- Lower cut-off levels
- Mainly drugs of abuse
- Sometimes different targets needed

V. Vindenes et al. Forens. Sci. Int 219 (2012) 165-171

Alternatives to urine: 2. Blood

- Advantages
  - Lower concentrations
  - Dose-effect correlation for stimulants, narcotics...
  - Shorter detection time for some drugs
- Disadvantages
  - Invasive – difficult to collect
  - Limited volume
  - Detection time
  - Relatively limited knowledge so far

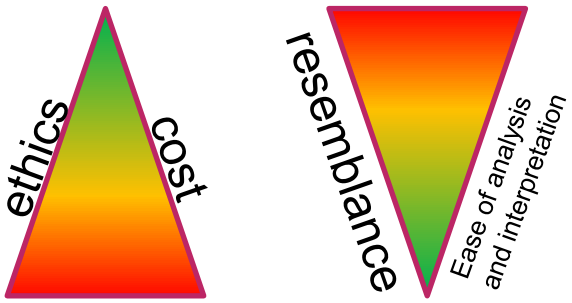
Detection of new drugs in Practice

- Option 1:
  - Knowledge on the drugs existence
  - Possession of the drug
- How
  - Active monitoring of the (internet) market
  - Cooperation with law enforcement/customs
  - Other (e.g. Balco)

Detection of new drugs in Practice: Option 1

- Include parent in existing methods
  - Fairly easy and fast
  - Uncertainty on effectivity

- Metabolism studies
  - In-vitro studies
  - In-vivo models
  - Administration studies



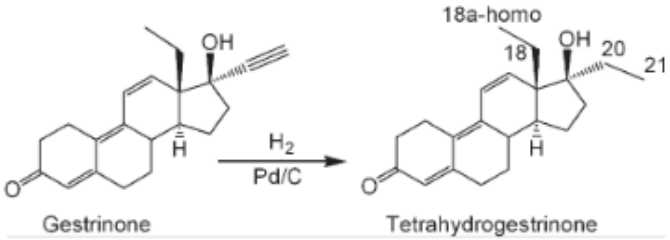
Detection of new drugs in Practice: Option 1

- Example: THG
  - Syringe sent to the lab (anonymous)
  - Lab investigates
    - 'Classic' GC-MS analysis after TMS derivatisation
      - Multiple peaks with resemblance to steroid
      - Doesn't match any known steroid
    - LC-MS analysis
      - 1 peak – steroid resemblance
    - Puzzle!
      - Tentative structure: resembles gestrinone... THG?

Catlin et al. Rapid Commun. Mass spectrom. 18 (2004) 1245-1249

Detection of new drugs in Practice: Option 1

- Example: THG
  - Synthesis



  - Verify with data from Syringe
  - Baboon excretion study
  - Reanalyze anti-doping urine samples
  - Prove anabolic properties

Catlin et al. Rapid Commun. Mass spectrom. 18 (2004) 1245-1249

Detection of new drugs in Practice: Option 1

- Example: Many more steroids since...

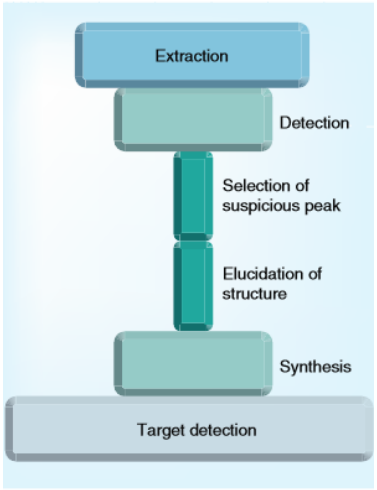


Figure 2. Analytical steps in the detection of unknown androgenic anabolic steroids. The width of the box illustrates the difficulty of the step.

- Underground
  - Madol, norbolethone
- Internet

Pozo et al. Bioanalysis 5 (2013) 2661-2677

Barcelona & Ghent

Detection of new drugs in Practice

- Option 2:
  - No knowledge on the drugs existence
  - Detection of a ‘strange peak’ in a real sample

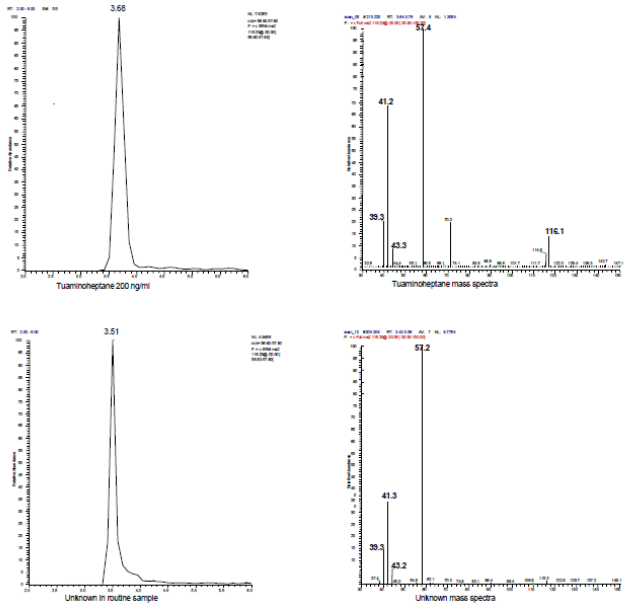
Detection of new drugs in Practice: Option 2

- Run library search
  - GC-MS (mainly in full scan) against several libraries
- Combine technologies
  - LC-HRMS (bruto formula)
  - LC-Prep (correlate LC with GC)
  - GC-MS(/MS)
  - LC-MS/MS

} Study fragmentation pattern – compare to other ‘known’ substances

Detection of new drugs in Practice: Option 2

- Real case: Methylhexaneamine



Routine detection of stimulants

Detection of a substance with:
- Similar RT as tuaminoheptane
- Similar MS as tuaminoheptane

HRMS-data:
- Merck Index
- Reference standard

Perrenoud et al. J. Chromatogr. B 877 (2009) 3767–3770

Lausanne lab

Detection of new drugs in Practice: Option 2

- Real case: Methylhexaneamine

Relatively easy case...

- PC detected; Standard available; high concentration; easy structure...

- More complex?

Metabolite; Low concentration; Little resemblance to known substance; No reference standard available- custom synthesis

Perrenoud et al. J. Chromatogr. B 877 (2009) 3767–3770

Lausanne lab

Appeal for concerted action: Option 1 = easiest

Law enforcement/Customs

- share information with WADA/WAADS/Labs (and vice-versa)
- share seized materials
- assist laboratories

Pharmaceutical Industry

- cooperate with WADA/WAADS/laboratories
- set up a system to detect misuse of patents
- share substances/information

Scientific community: laboratories/WADA/WAADS

- communicate results and findings