



Drug Residues Analysis and Implications for Doping Controls

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Utmost sensitivity of doping control analytical assays

- is vital for detecting trace amounts of doping agents
- ensures best possible detection windows / retrospectivity
- allows for covering time periods between two doping controls

- Raises question(s) as to the origin of the observed drug (residue)
- Can result in adverse analytical findings where extensive result management is required



Illicit Drugs: Contaminants in the Environment and Utility in Forensic Epidemiology

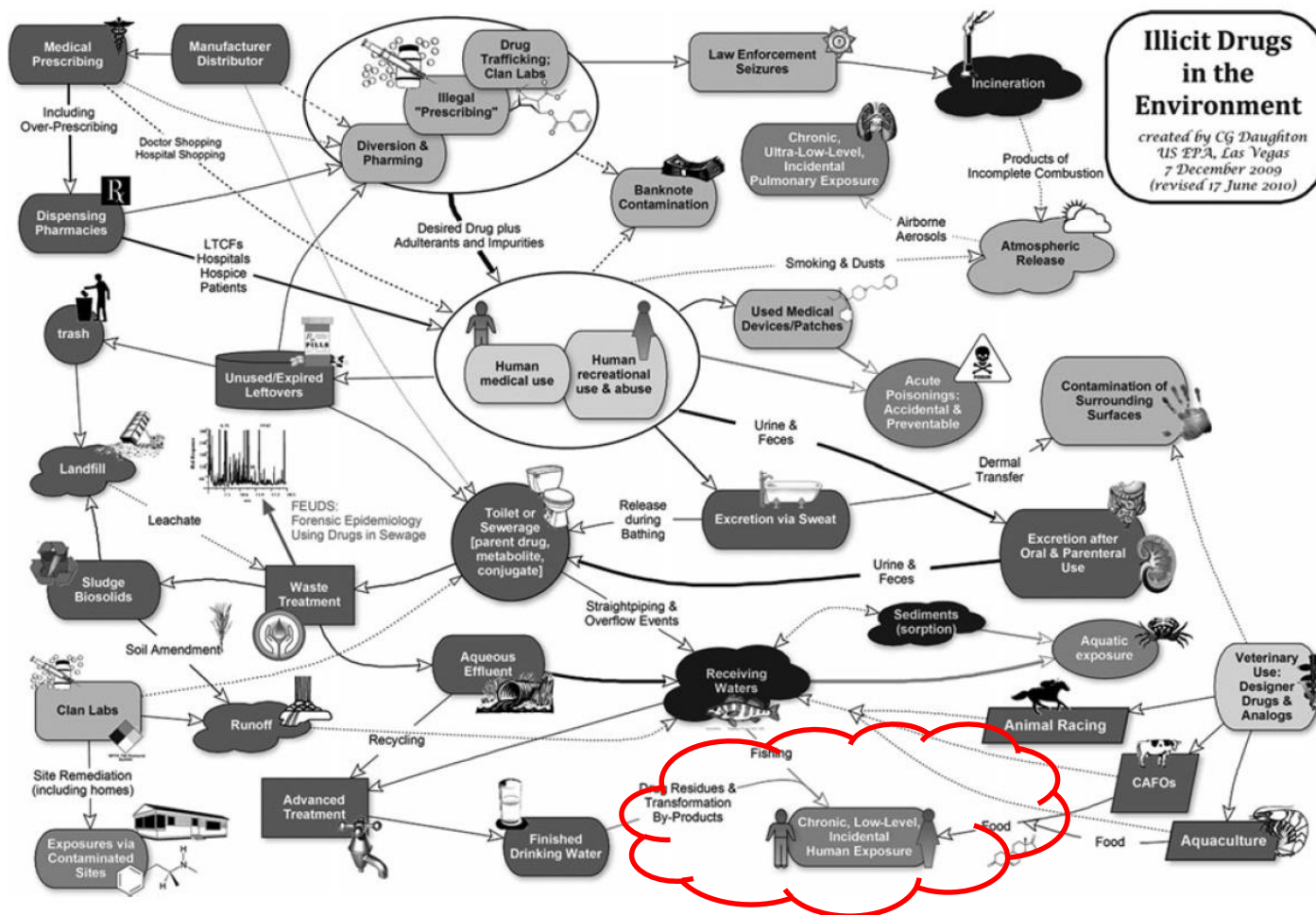




Table 4 Drugs of abuse targeted and identified in environmental compartments^a

	Wastewaters	Surface waters	Drinking water	Sewage sludge	Biosolids	Air	Banknotes	Wildlife tissue	Dermal transfer ^b
<i>Analgesics</i>									
6-AM (6-acetylmorphine; deacetylated heroin)	×√	×√	×				√		▲
6-AC (6-acetylcodeine)		×							
Codeine ^c	√√√	√√	××√				×		▲
Dihydrocodeine ^d	√	√							
Heroin (diacetylmorphine) ^c	××√	×	×			√	√√		▲
Hydrocodone ^c	√√	×√							
Morphine ^c	√√√	√	×	√√		×	√		▲
Morphine-3β-D-glucuronide	×√	×							
Norcodeine	√	√	××√						
Normorphine	√	×	×						
Fentanyl ^d (excreted mainly as norfentanyl)	××	××	×						‡
Norfentanyl	×								
Oxycodone ^c	√√								
Tramadol ^d		√						√	
<i>Methadone</i>									
Methadone ^c	√√√	√	√√	√√√		×			▲
EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine)	√√√	√√	√√						
<i>Stimulants</i>									
Amphetamine ^c	√√	×√	××√	√√		×√	√		▲
Ephedrine ^d /pseudoephedrine ^c	√√√	√				×			
Methamphetamine ^c	√√√	√	××√		√	×√	√		▲
MDA ^c	√	×√	××√						
MDBD	×								



Table 4 (continued)

	Wastewaters	Surface waters	Drinking water	Sewage sludge	Biosolids	Air	Banknotes	Wildlife tissue	Dermal transfer ^b
MDEA ^d	×√	×							
MDMA ^c	√√	√	√			×√			▲
Methylphenidate ^c									‡
<i>Cocainics</i>									
Benzoylcegonine (BZE)	√√√	√	√			√	√		▲
Cocaethylene	×√	√				×			
Cocaine ^c	√√	√	×√			√√	√√√		▲
Ecgonine methyl ester (EME)	×√√								▲
Norbenzoylcegonine	√	√							
Norcocaine	×√	√							
<i>“Club” drugs (e.g., dissociative anesthetics)</i>									
Ketamine ^d	××√	×	×						
Norketamine	×								
PCP (phencyclidine) ^c	×√	×	×				√		
<i>Hallucinogens</i>									
LSD	××√	×	×			×			
Nor-LSD (<i>N</i> -desmethyl-LSD)	×√	××√				×			
<i>O</i> - <i>H</i> -LSD (2-oxo-3-hydroxy-LSD)	×√	××√				×			
<i>Cannabinoids</i>									
Cannabinol (CNB)						√√	×√		
Cannabidiol (CND)						×√	×√		
OH-THC (11-hydroxy- Δ^9 -tetrahydrocannabinol)	×√	××√				×			
nor-THC	√					×			
THC (Δ^9 -tetrahydrocannabinol) ^c	√√	√√	×			√√	×√		▲
THC-COOH (11-nor-carboxy- Δ^9 -tetrahydrocannabinol)	√√√	√√	×						



Old drugs – new challenges

Case Report Malaria Chemoprophylaxis

- 3 fencer return from Africa to Europe from competitions
- Two produce adverse analytical findings with chlorazanyl (diuretic)
- Chlorazanyl prohibited/tested since 1988 -> findings until 2015: **0**

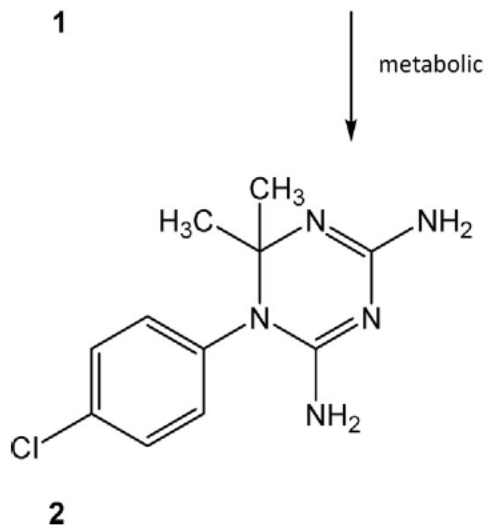
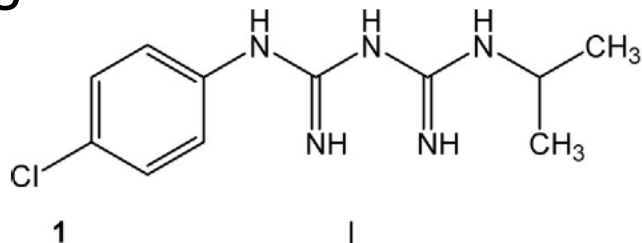




Case Report Malaria Chemoprophylaxis

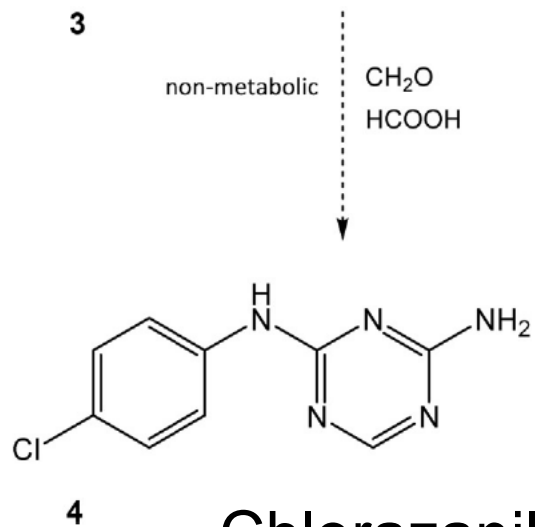
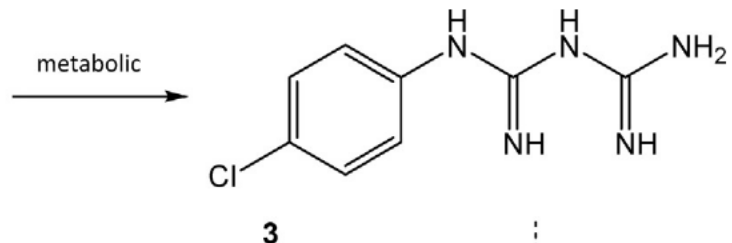
- These cases are the worldwide first two findings with the obsolete diuretic
- The laboratory's sensitivity increased over the past years to an LOD of 0.1 ng/mL
- Structural relationship to proguanil (anti-malaria drug) triggered in-depth investigation

Proguanil



Cycloguanil

4-chlorophenyl-biguanide



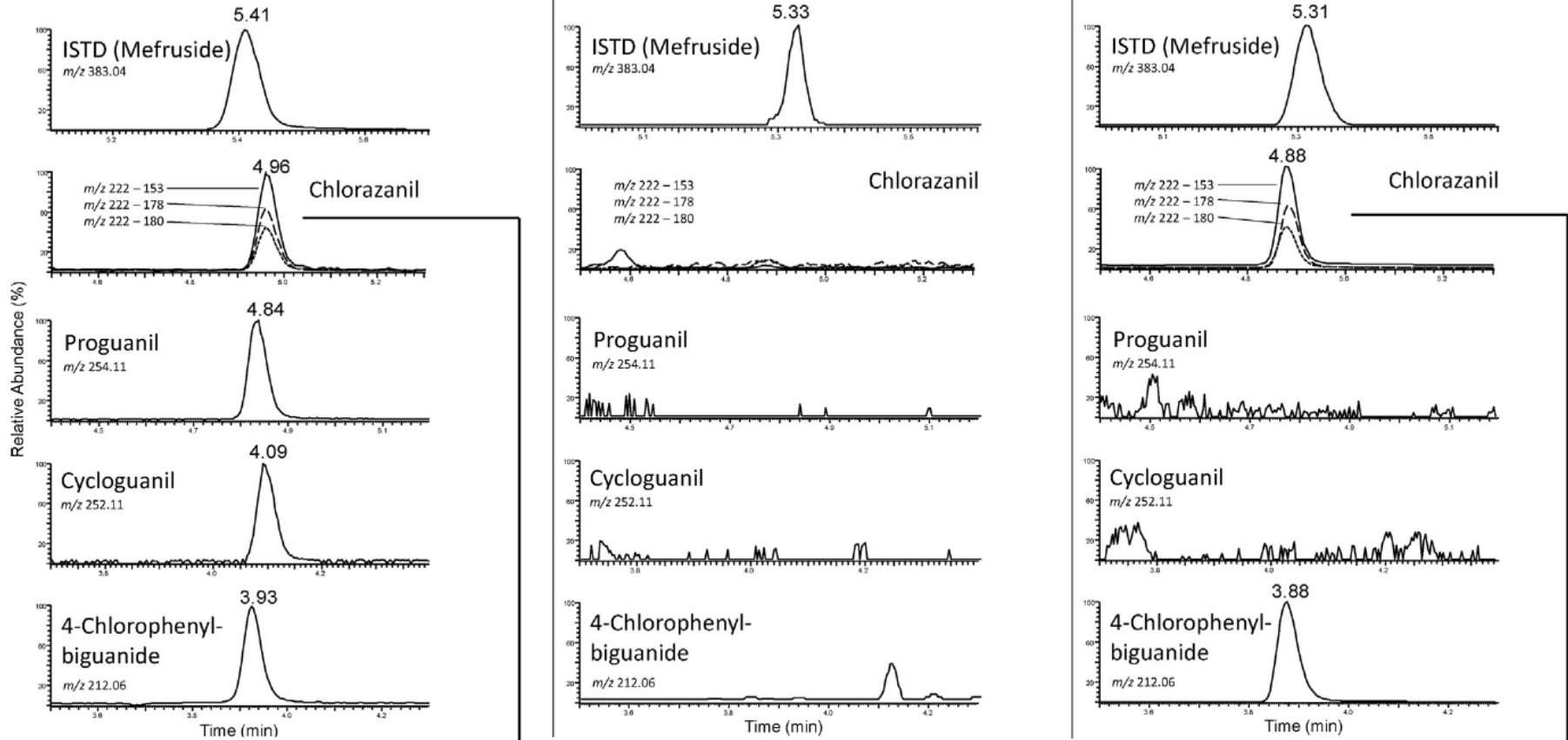
Chlorazanal



Reference compounds

blank urine

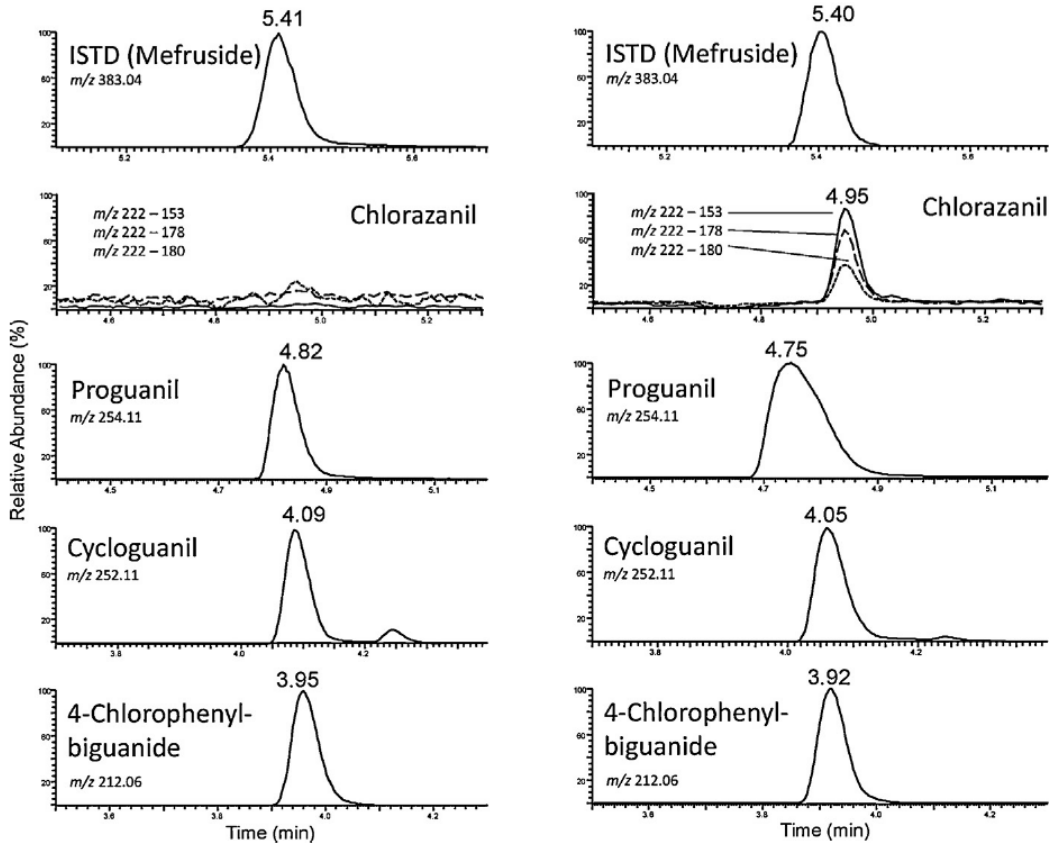
incubation mixture (urine)





Patient's urine sample (no chlorazanyl)

Patient's urine sample (with chlorazanyl)





Case Report Malaria Chemoprophylaxis

- Findings plausibly explained by anti-malarial chemoprophylaxis
- Arguably supported by dietary habits and increasing sensitivity of doping control analytical assays
 - → Athletes not sanctioned!



Permitted drugs – new challenges

Case Report contaminated NSAID

- Handball player tested positive for hydrochlorothiazide, urinary concentration ca. 5 ng/mL
- Administration of non-steroidal anti-inflammatory drug declared, remaining pills of used batch provided to RMA



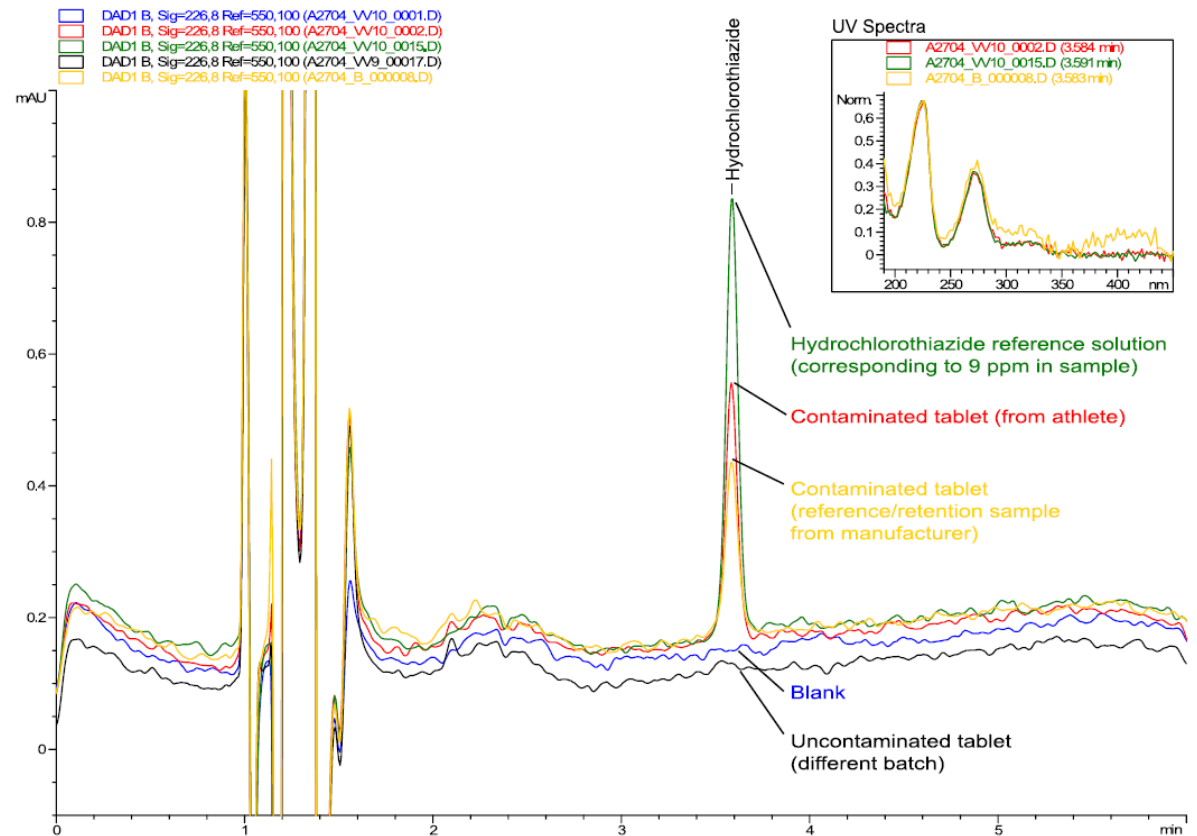
Permitted drugs – new challenges

Case Report contaminated NSAID

- Athlete's NSAID blister was analyzed for the presence of hydrochlorothiazide
- Manufacturer's retention sample was analyzed for the presence of hydrochlorothiazide

Permitted drugs – new challenges

LC-UV analysis

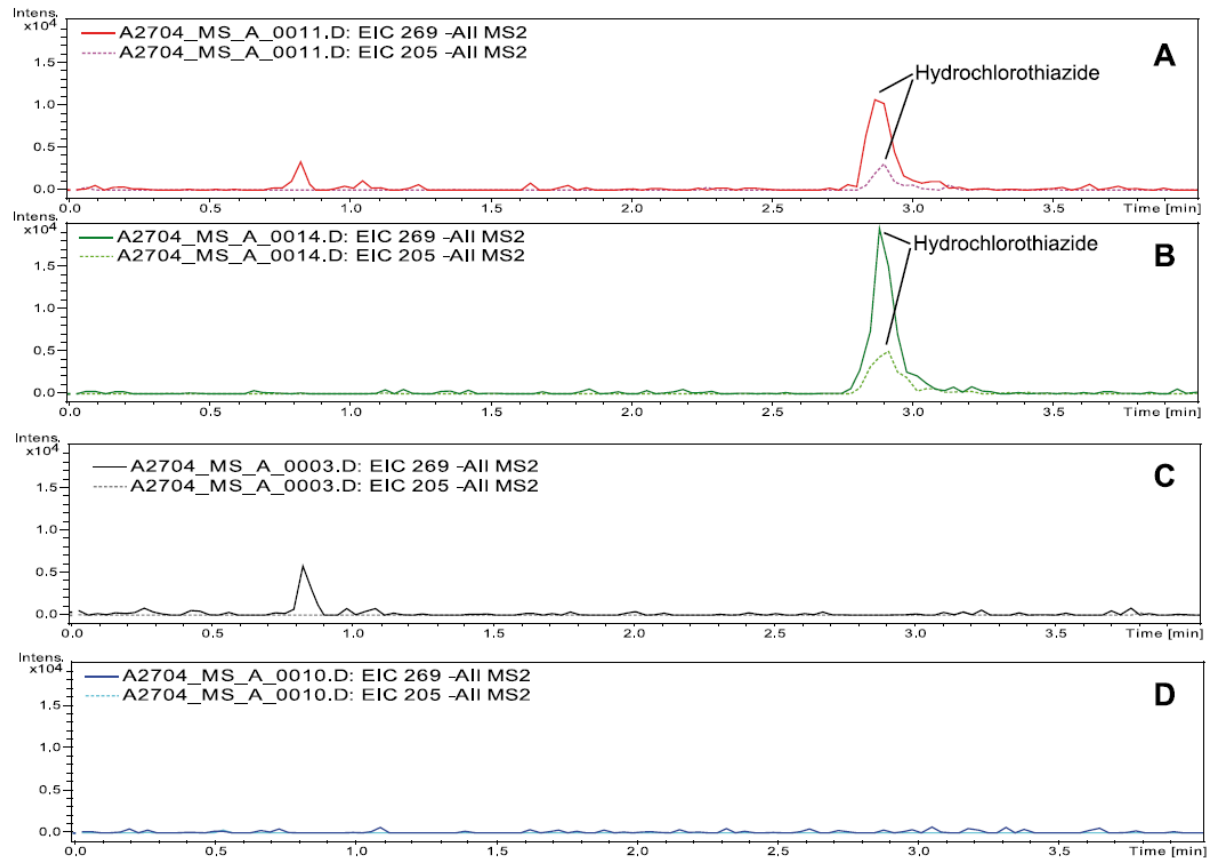


Permitted drugs – new challenges

LC-MS/MS analysis

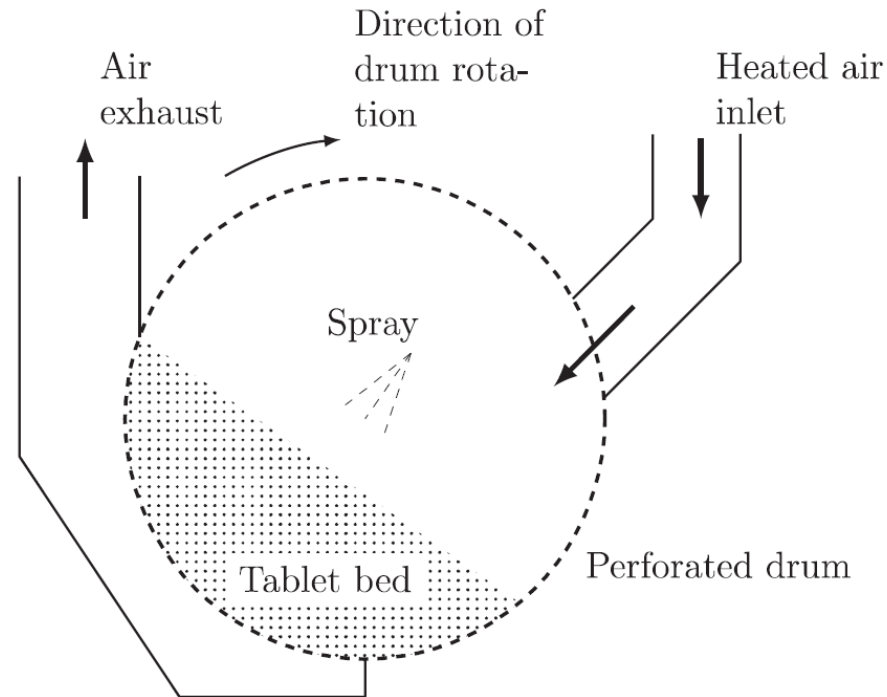


In-depth analysis proved a
contaminated coating
with ca. 2 µg/tablet





Coating process



- Sugar coating
 - Seal coating
 - Subcoating
 - Syrup coating
 - Polishing / finishing (coloring)
- Film coating
- Enteric coating
- Press coating



Coating process objectives

- Masking taste, odor or color of the drug
- Provide physical and chemical protection of the drug
- Control of drug release from tablet
- etc.



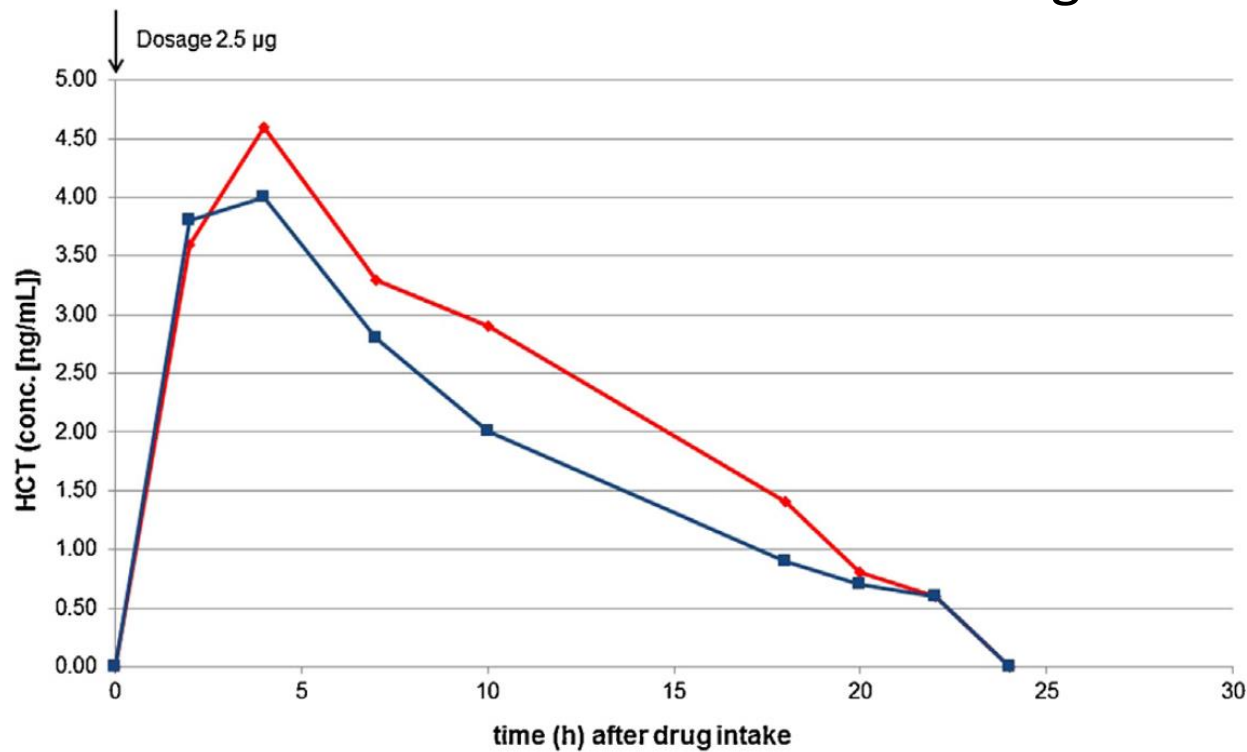
Probing for plausibility of finding

- The athlete declared the use of the NSAID with 1 tablet every 6 h on competition day (3 tablets in total)
- Urine sample was collected ca. 3 h after last tablet ingestion
- Placebo tablets enriched with 2.5 µg of hydrochlorothiazide were produced
- Elimination studies with a single dose and multiple doses were conducted
- Urine samples were analyzed according to established doping control methods



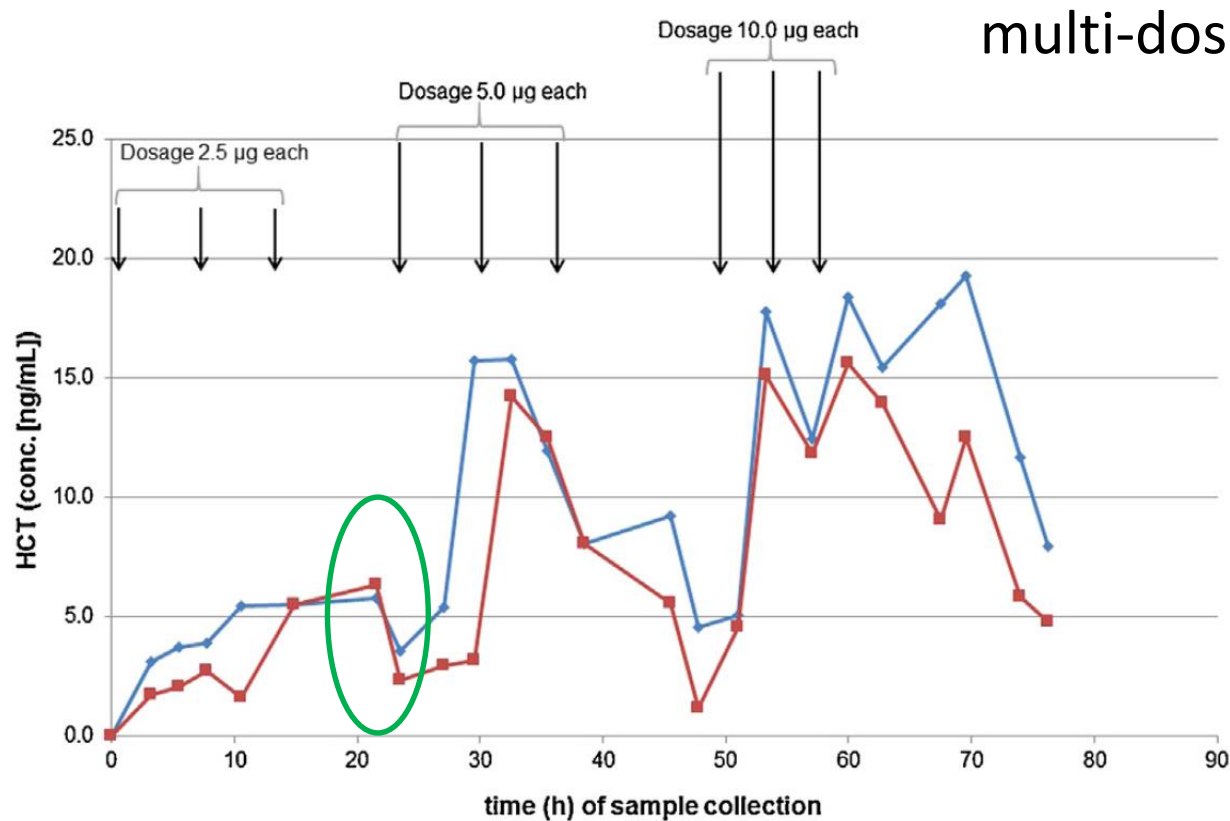
Probing for plausibility of finding

single-dose elimination study





Probing for plausibility of finding





Detection of stanozolol in the urine of athletes at a pg level: The possibility of passive exposure

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It is, therefore, possible that the detection of 3'-hydroxystanozolol in the urine of athletes at a pg level could correspond to an inadvertent doping case without the intentional use of stanozolol by the athlete and through an administration route, such as meat consumption for everyday dietary needs, that could not have been prevented by the athlete. In such a case, any advantage over other co-athletes while competing and any intention to enhance performance by steroids also becomes questionable.



Stanozolol glucuronides as screening target analytes



Sensitive detection of 3'-hydroxy-stanozolol glucuronide by liquid chromatography–tandem mass spectrometry

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Research article

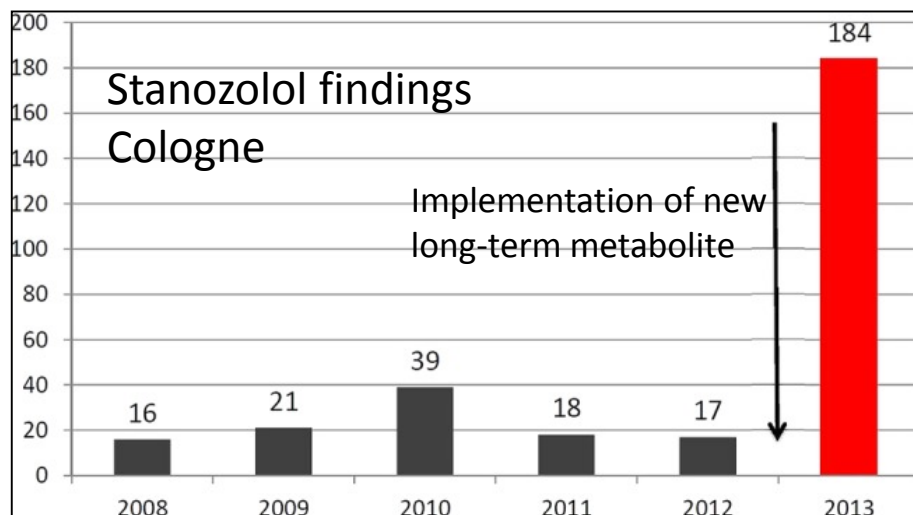
Drug Testing
and Analysis

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(www.drugtestinganalysis.com) DOI 10.1002/dta.1516

Expanding analytical possibilities concerning the detection of stanozolol misuse by means of high resolution/high accuracy mass spectrometric detection of stanozolol glucuronides in human sports drug testing

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Stanozolol in food ... little (if any) evidence today

- Numerous findings (injection sites) in the late 1990s in Belgium / The Netherlands (Le Bizec, Sterk, *et al*)
- Continuously decreasing number of findings ever since
- Vigilance however warranted



Stanozolol in food ... little (if any) evidence today

Table 6 Determination of stanozolol in environmental and domestic water samples

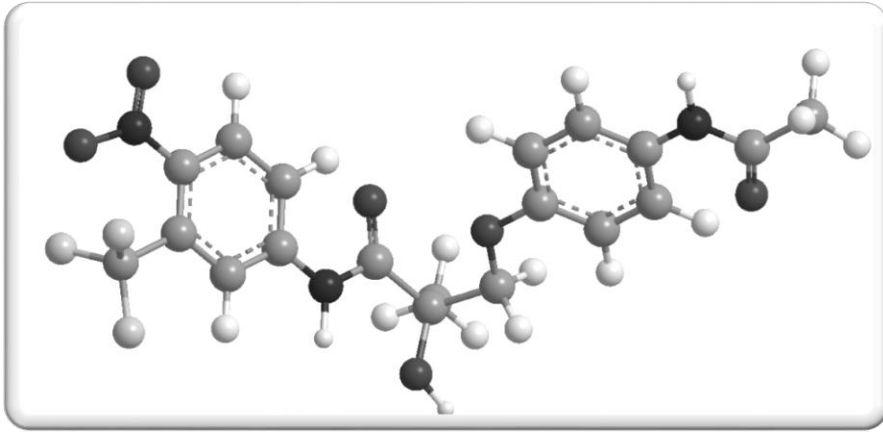
Environmental water sample (N = 3)	Average concentration pg/mL					
	31 st December 2009	18 th April 2010	21 th July 2010	01 st September 2010	24 th October 2010	05 th November 2010
River Danube	1.82 ± 0.19	0.71 ± 0.06	0.54 ± 0.03	ND	ND	ND
Budapest Tap	1.19 ± 0.03	0.31 (BLQ)	ND	ND	ND	ND
Lake Balaton	-	ND	-	-	-	-
Spring 'Rózsika'	-	ND	-	-	-	-

BLQ means below limit of quantification

ND means not detectable



Thank you



Antidoping Switzerland

World Anti-Doping Agency (WADA)

Federal Ministry of the Interior (D)



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