



## Testing therapeutics relevant for doping controls

Mario Thevis











## Sochi Olympic antidoping laboratory

Urine sample bottles  
were passed through  
a hole in the wall.

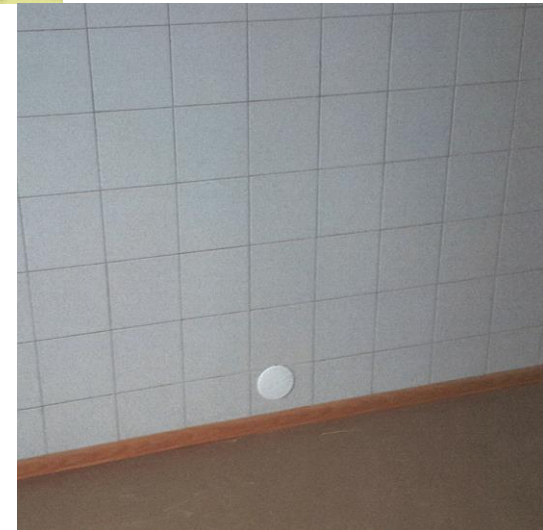
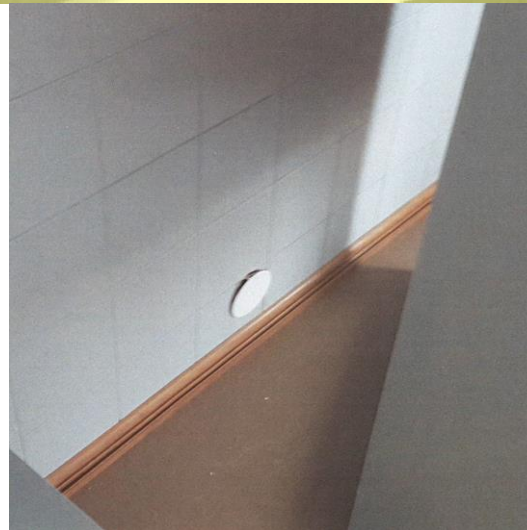
**The New York Times**

[www.nytimes.com](http://www.nytimes.com)

Storage  
space

Official urine  
sample room

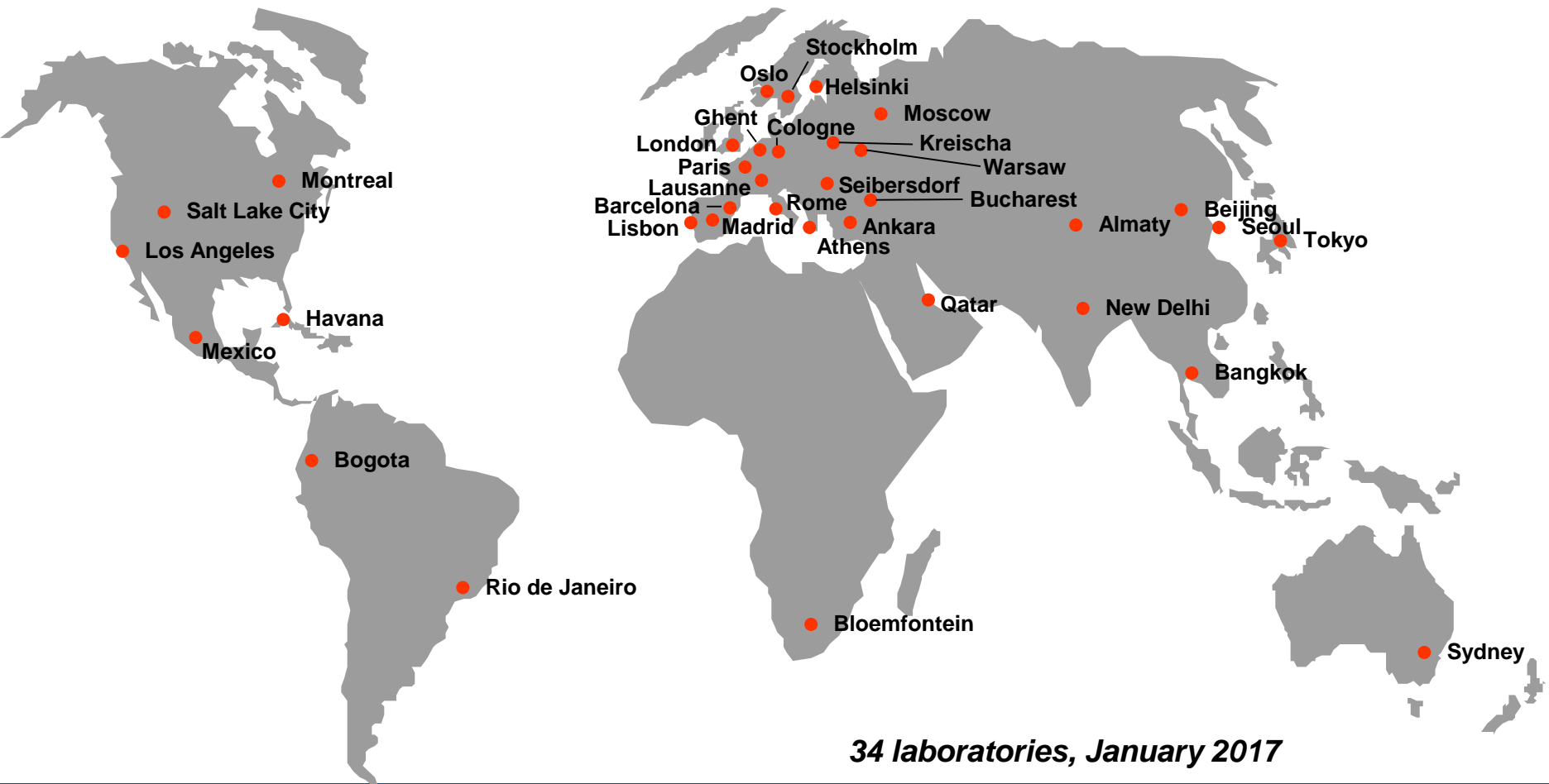
Hole







# Doping control laboratories with WADA/IOC accreditation





Deutsche  
Sporthochschule Köln  
German Sport University Cologne

Zentrum für Präventive Dopingforschung / Institut für Biochemie  
Center for Preventive Doping Research / Institute of Biochemistry

THE WORLD ANTI-DOPING CODE  
**INTERNATIONAL  
STANDARD**



# PROHIBITED LIST

JANUARY 2017



# SUBSTANCES & METHODS PROHIBITED AT ALL TIMES

(IN- AND OUT-OF-COMPETITION)

## PROHIBITED SUBSTANCES

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S0

### NON-APPROVED SUBSTANCES

Any pharmacological substance which is not addressed by any of the subsequent sections of the *List* and with no current approval by any governmental regulatory health authority for human therapeutic use (e.g. drugs under pre-clinical or clinical development or discontinued, designer drugs, substances approved only for veterinary use) is prohibited at all times.





# SUBSTANCES & METHODS PROHIBITED AT ALL TIMES

(IN- AND OUT-OF-COMPETITION)

## PROHIBITED SUBSTANCES

S1

### ANABOLIC AGENTS

Anabolic agents are prohibited.

---

#### 1. ANABOLIC ANDROGENIC STEROIDS (AAS)

---

#### 2. OTHER ANABOLIC AGENTS

#### Including, but not limited to:

Clenbuterol, selective androgen receptor modulators (SARMs, e.g. andarine and ostarine), tibolone, zeranol and zilpaterol.



# SUBSTANCES & METHODS PROHIBITED AT ALL TIMES

(IN- AND OUT-OF-COMPETITION)

## PROHIBITED SUBSTANCES

S2

### PEPTIDE HORMONES, GROWTH FACTORS, RELATED SUBSTANCES AND MIMETICS

The following substances, and other substances with similar chemical structure or similar biological effect(s), are prohibited:

#### 1. Erythropoietin-Receptor agonists:

- 1.1 Erythropoiesis-Stimulating Agents (ESAs) including e.g.
  - Darbepoietin (dEPO);
  - Erythropoietins (EPO);

EPO-Fc;

EPO-mimetic peptides (EMP), e.g. CNTO 530 and peginesatide;

GATA inhibitors, e.g. K-11706;

Methoxy polyethylene glycol-epoetin beta (CERA);

Transforming Growth Factor- $\beta$  (TGF- $\beta$ ) inhibitors, e.g. sotatercept, luspatercept;



# SUBSTANCES & METHODS PROHIBITED AT ALL TIMES

(IN- AND OUT-OF-COMPETITION)

## PROHIBITED SUBSTANCES

---

### S4 HORMONE AND METABOLIC MODULATORS

The following hormone and metabolic modulators are prohibited:

#### 5. Metabolic modulators:

- 5.1 Activators of the AMP-activated protein kinase (AMPK), e.g. AICAR; and Peroxisome Proliferator Activated Receptor  $\delta$  (PPAR $\delta$ ) agonists, e.g. GW 1516;
- 5.2 Insulins and insulin-mimetics;





# THE COLOR OF PEE



# UROSCOPY

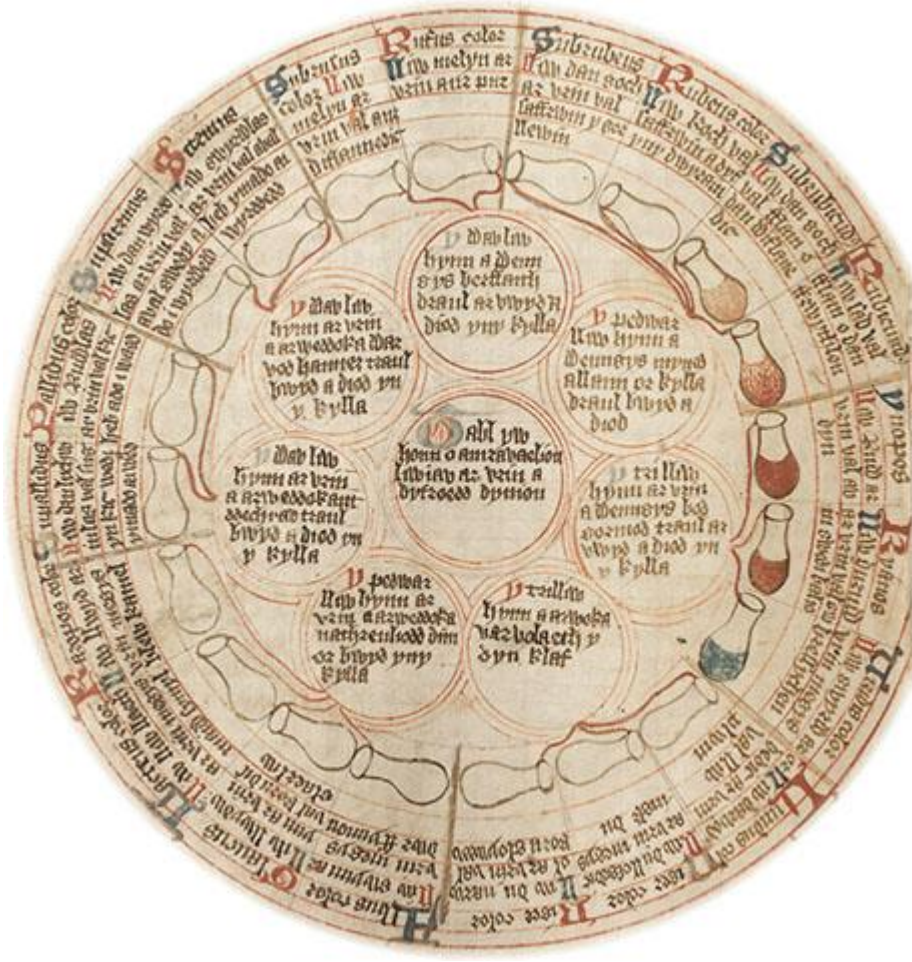
“black as (buck)horn”  
“spring water”







# UROMANCY



Divinization of uroscopy

*i.e.*

fortune telling and  
foreseeing the future by  
“reading” the bubbles in  
urine





# Result of Uromancy-Based Analysis: Athlete misused Xenon

## SUBSTANCES & METHODS PROHIBITED AT ALL TIMES

(IN- AND OUT-OF-COMPETITION)

### PROHIBITED SUBSTANCES

S2

#### PEPTIDE HORMONES, GROWTH FACTORS, RELATED SUBSTANCES AND MIMETICS

2. Hypoxia-inducible factor (HIF) stabilizers, e.g. cobalt and FG-4592; and HIF activators, e.g. argon, xenon;







## Characterizing best-possible target analytes





# The Telegraph

## IOC to act after new testing methods reveal hundreds of positive results

18.11.2013

Hundreds of urine samples have tested positive for heavy-duty anabolic steroids in recent months after they were analysed by scientists in laboratories in Cologne and Moscow using a new testing method, according to a report broadcast on German television on Monday night.

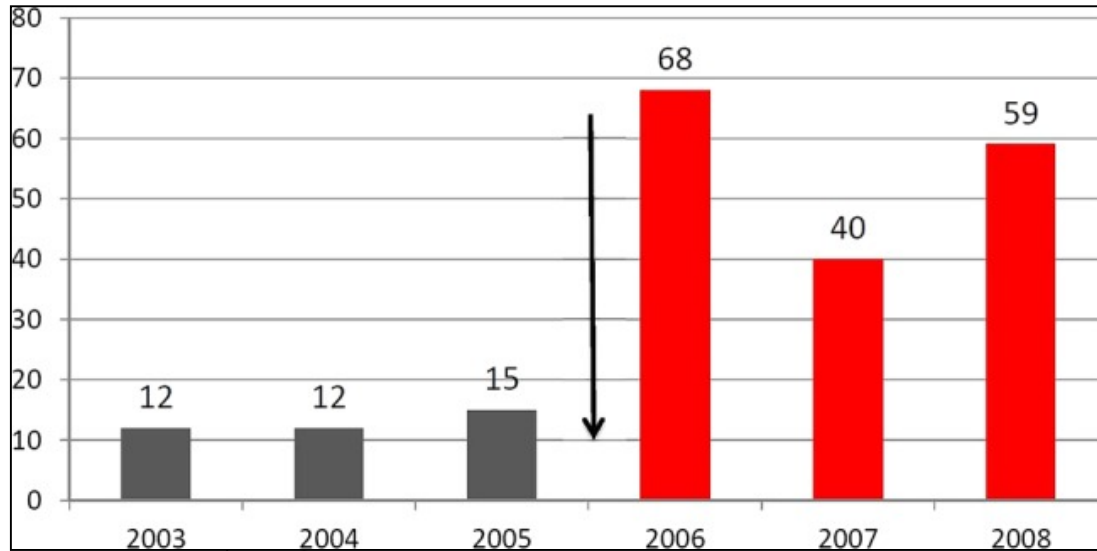
The substances detected were **Oral-Turinabol**, a steroid widely used in the state-run doping programme of the former East Germany, and **stanozolol**, the drug for which Canadian sprinter Ben Johnson tested positive at the 1988 Olympics in Seoul. Officials from the two laboratories told the TV programme Sport Inside that all of the samples would have produced negative results in 2012 due to the short detection window available at the time but a new testing procedure, known as the 'long-term metabolites method', meant steroids could now be detected more than six months after they were taken.

Arne Ljungqvist, the chairman of the International Olympic Committee's medical commission, said he was surprised by the high number of positive cases and would be recommending that urine samples frozen from previous Olympic Games should be retested.

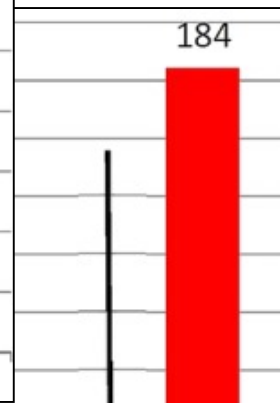
"This case is a good example of the necessity of performing retests on Olympic doping samples," said Ljungqvist. "I would certainly conduct retests here. We have the mandate for that, after all."



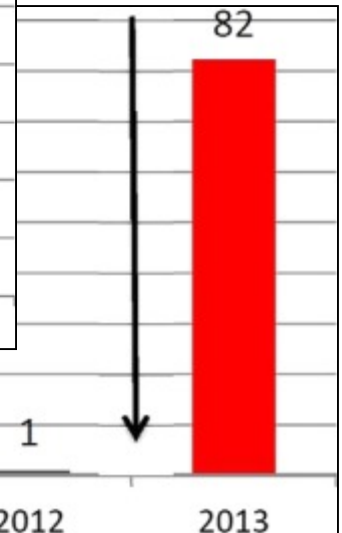
## Value of implementing long-term metabolites...



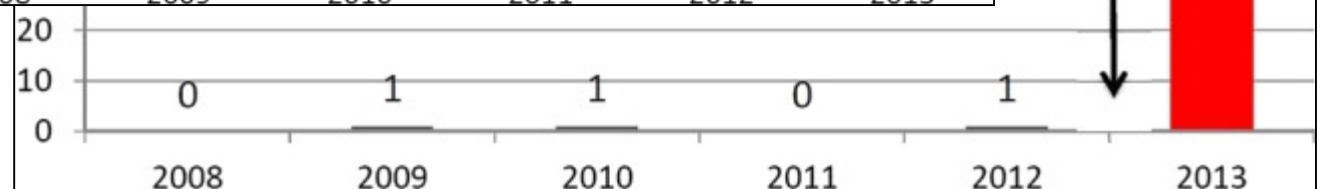
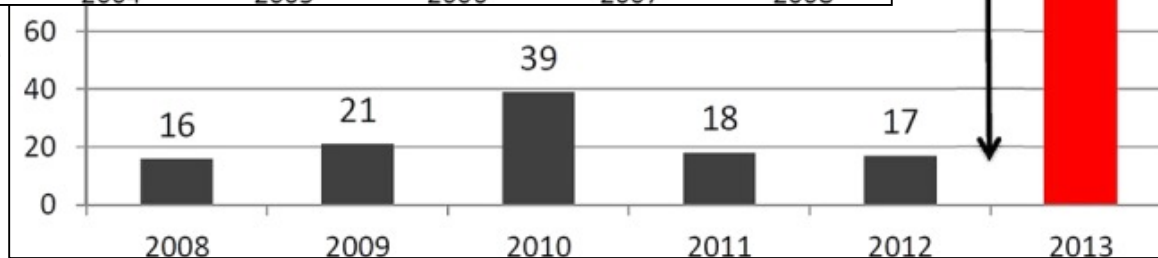
Stanozolol



Oral-Turinabol



Metandienone





# Stanozolol glucuronides as screening target analytes

Journal of Chromatography A, 1292 (2013) 195–200



Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: [www.elsevier.com/locate/chroma](http://www.elsevier.com/locate/chroma)



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

E. Tudela<sup>\*</sup>, K. Deventer, P. Van Eenoo

*DocLab, Ghent University (UGent), Department of Clinical Chemistry, Microbiology and Immunology, Technologiepark 30, B-9052 Zwijnaarde, Belgium*

## Research article

Drug Testing  
and Analysis

Received: 18 June 2013

Revised: 26 June 2013

Accepted: 26 June 2013

Published online in Wiley Online Library: 19 July 2013

([www.drugtestinganalysis.com](http://www.drugtestinganalysis.com)) DOI 10.1002/dta.1516

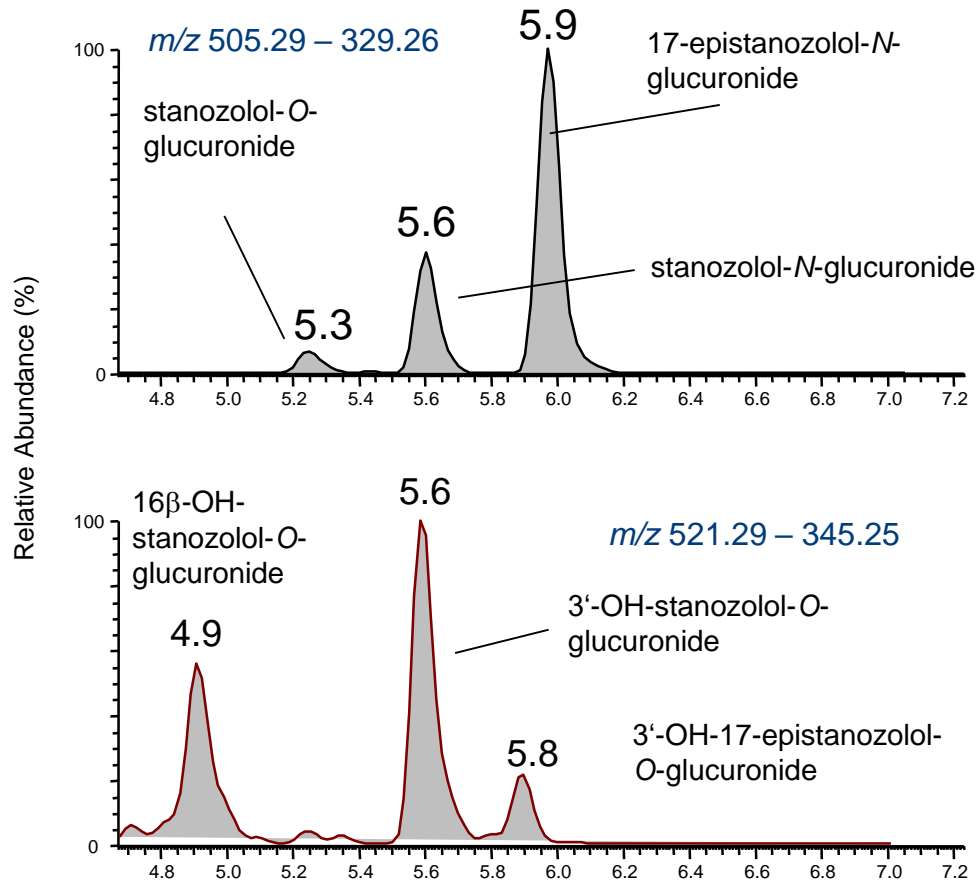
**Expanding analytical possibilities concerning the detection of stanozolol misuse by means of high resolution/high accuracy mass spectrometric detection of stanozolol glucuronides in human sports drug testing**

Wilhelm Schänzer,<sup>a</sup> Sven Guddat,<sup>a</sup> Andreas Thomas,<sup>a</sup> Georg Opfermann,<sup>a</sup> Hans Geyer<sup>a</sup> and Mario Thevis<sup>a,b,\*</sup>

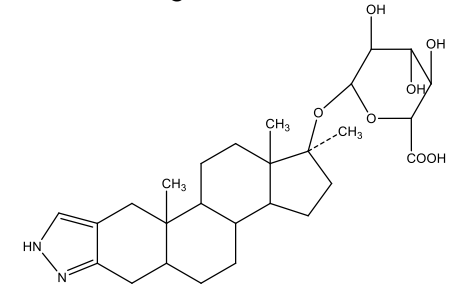
....were recognized to be important in doping controls



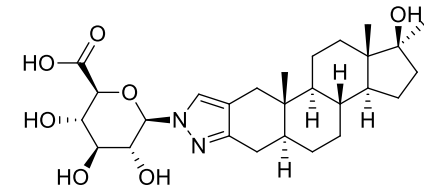
## excretion study urine sample



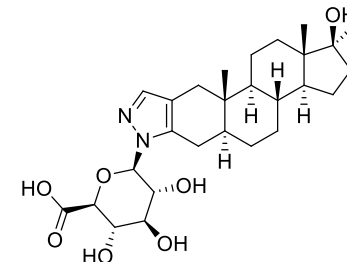
stanozolol-O-glucuronide



stanozolol-2`N-glucuronide

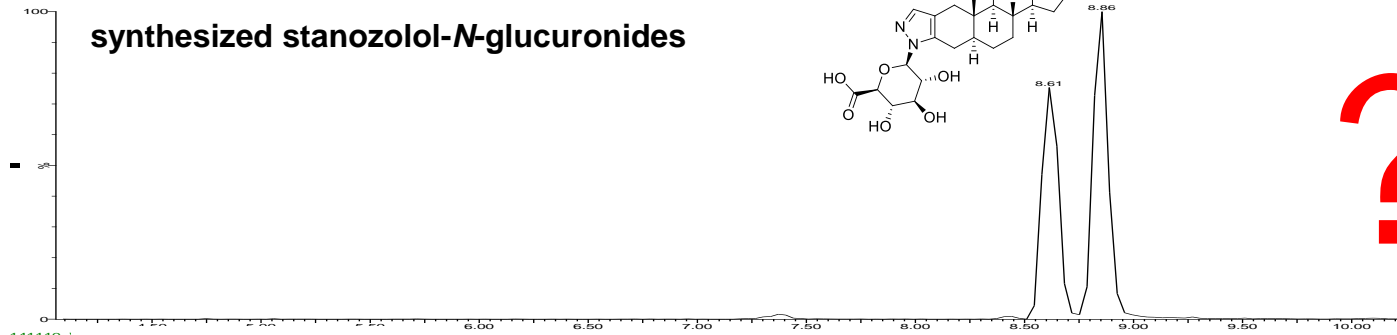
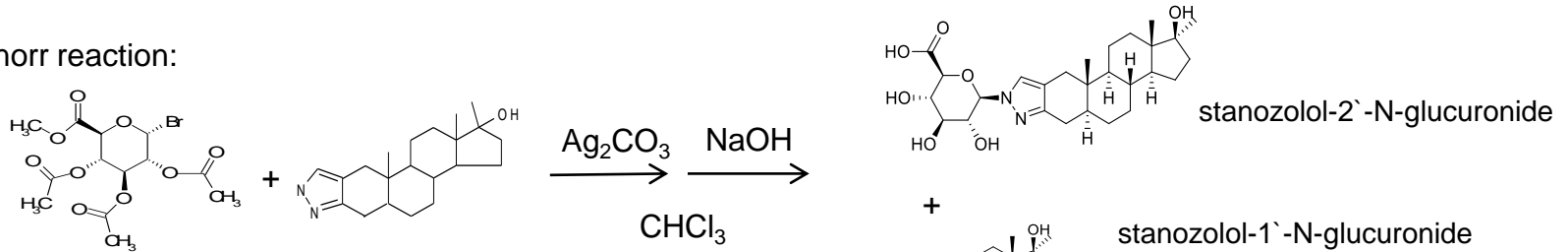


stanozolol-1`N-glucuronide



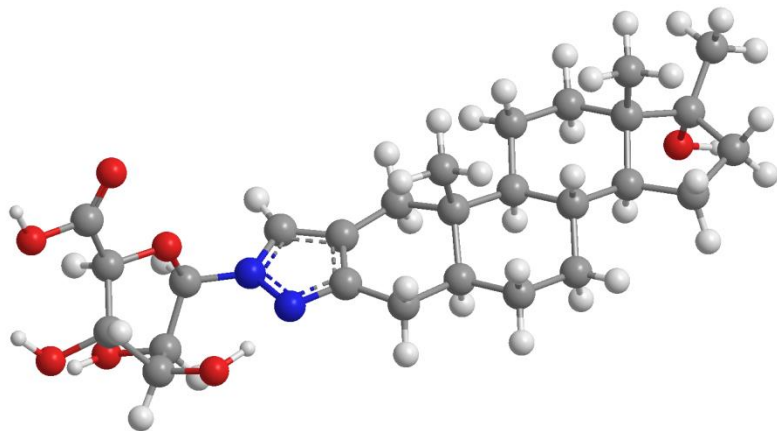
## Extracted ion chromatograms acquired at $m/z$ 505.3

Koenigs-Knorr reaction:





## Computing the collision cross section (CCS):



stanozolol-2'-N-glucuronide

1. molecular structure was created with ChemBio3D Ultra (CambridgeSoft, version 12.0)
2. optimization with ORCA according to density functional theory (DFT) using the data set B3LYP/6-31G
3. CCS values of the ORCA-optimized 3D coordinate data set were calculated with the open source software MOBCAL



## Collision Cross Section [ $\text{\AA}^2$ ]

	LC-retention time [min]	drift time [ms]	drift time [bins]	computed (N <sub>2</sub> )	measured (poly-Ala calibration)	measured (AAS-gluc. calibration)	$\Delta$ (poly-Ala - AAS-gluc.)	proton affinity [kJ/mol]	[M+H] <sup>+</sup> [m/z]
<b>19-nortestosterone glucuronide</b>	<b>7.95</b>	<b>3.57</b>	<b>66.05</b>	<b>181.18</b>	<b>143.93</b>	-	-	<b>955.8</b>	<b>451.2326</b>
<b>boldenone glucuronide</b>	<b>8.01</b>	<b>3.62</b>	<b>67.05</b>	<b>184.48</b>	<b>145.21</b>	-	-	<b>961.4</b>	<b>463.2326</b>
<b>testosterone glucuronide</b>	<b>8.48</b>	<b>3.67</b>	<b>67.92</b>	<b>184.99</b>	<b>146.63</b>	-	-	<b>939.8</b>	<b>465.2483</b>
<b>methyltestosterone glucuronide</b>	<b>8.74</b>	<b>3.79</b>	<b>70.12</b>	<b>188.39</b>	<b>149.79</b>	-	-	<b>960.7</b>	<b>479.2639</b>
<b>3'hydroxystanozolol glucuronide</b>	<b>9.03</b>	<b>4.18</b>	<b>77.34</b>	<b>208.39</b>	<b>159.88</b>	-	-	<b>862.7 – 1074.3</b>	<b>521.2857</b>
<b>stanozolol-1'N- glucuronide*</b>	8.62	3.93	72.78	204.1	153.42	195.83	42.41	878.4 – 1073.0	505.2908
<b>stanozolol-2'N- glucuronide*</b>	8.85	4.08	75.63	205.1	157.50	201.91	44.41	817.4 – 1043.6	505.2908
<b>stanozolol-O- glucuronide</b>	7.40	4.23	78.26	212.4	161.30	208.00	46.7	801.9 – 990.8	505.2908
<b>17-epistanozolol-1'N- glucuronide</b>	9.90	3.89	72.04	203.1	152.44	194.20	41.76	841.3 – 1046.9	505.2908

\* confirmed by NMR





## Re-analyses of samples from Beijing 2008 and London 2012

Status: February 2017

Number of re-analyses:	1550
Findings of prohibited substances:	101
Predominantly affected countries:	Russia, Kazakhstan, Belarus
Predominantly affected sports :	athletics, weightlifting, wrestling

→ More than 50 medalists tested positive



# Re-analyses of samples from Beijing 2008 and London 2012

Status: February 2017

INTERNATIONAL  
OLYMPIC  
COMMITTEE

Latest findings:

Three Chinese female gold medalists in weightlifting used GHRP-2  
(Pralmorelin)





# Challenge of GHRPs identified in 2007 – test methods available only since 2010

## Research Article

Drug Testing  
and Analysis

Received: 16 June 2010

Revised: 9 July 2010

Accepted: 9 July 2010

Published online in Wiley Online Library: 1 September 2010

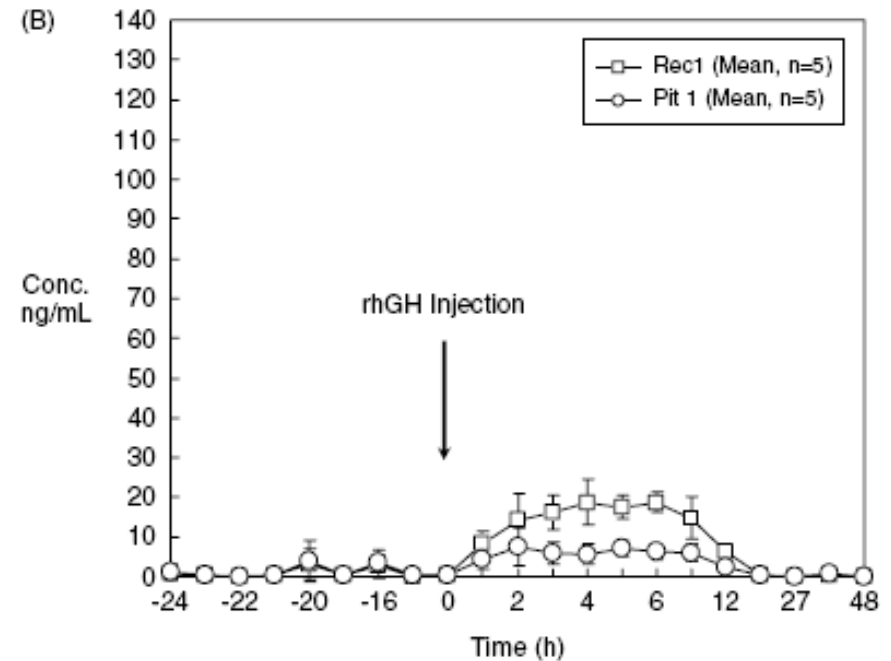
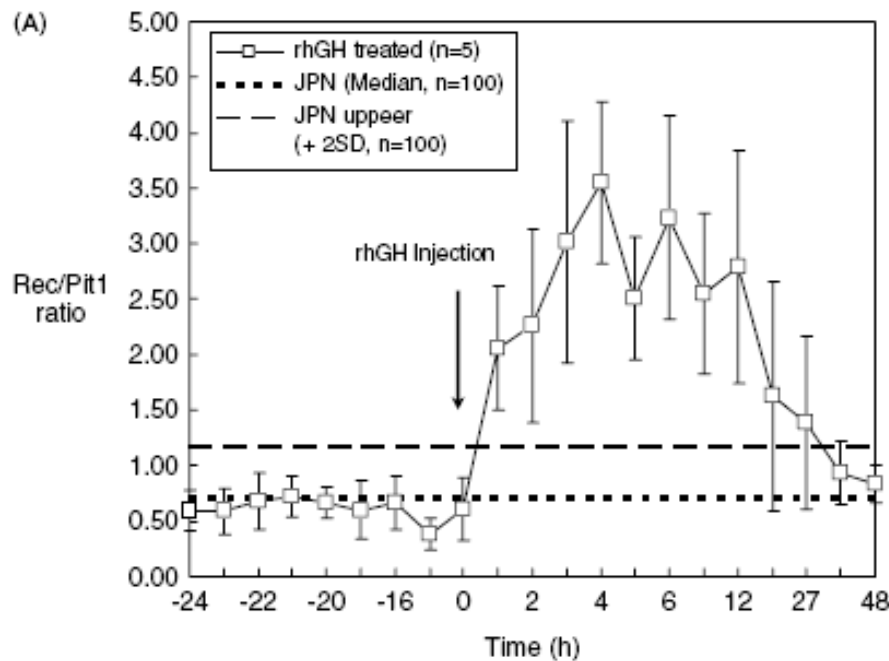
(www.drugtestinganalysis.com) DOI 10.1002/dta.166

## **Influence of intravenous administration of growth hormone releasing peptide-2 (GHRP-2) on detection of growth hormone doping: growth hormone isoform profiles in Japanese male subjects**

**Masato Okano,\* Yasunori Nishitani, Mitsuhiko Sato, Ayako Ikekita  
and Shinji Kageyama**



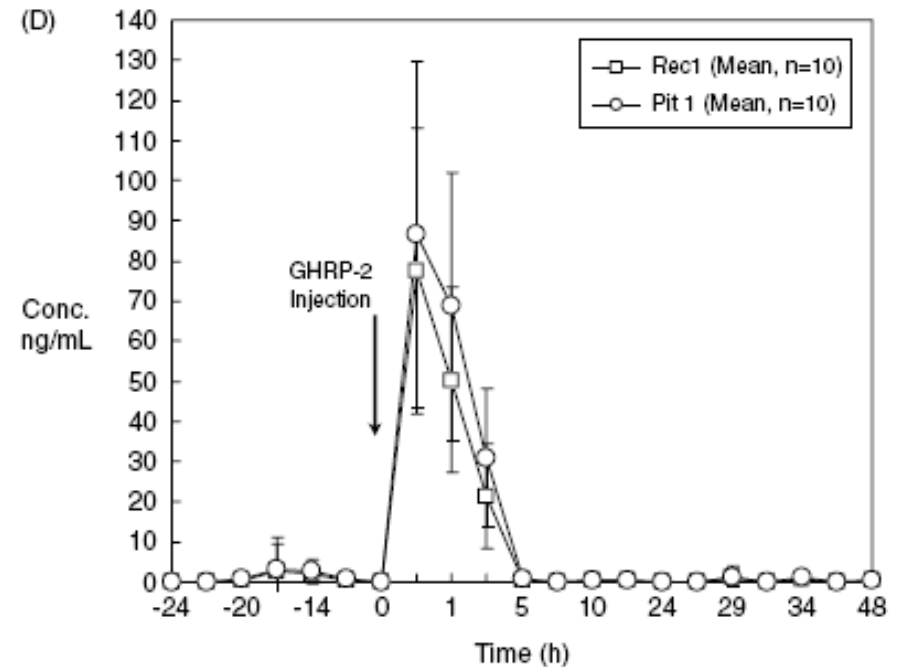
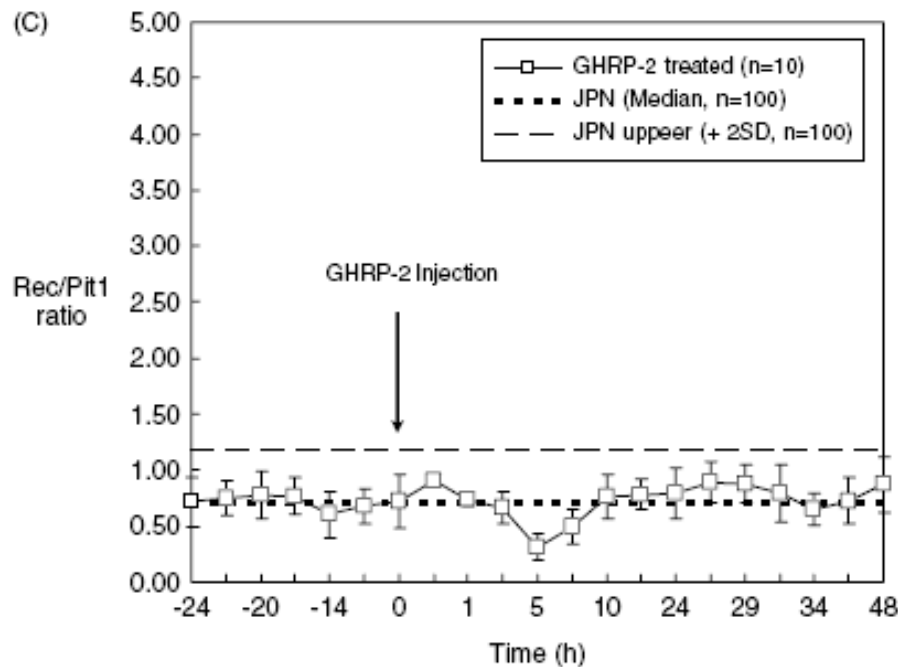
## Challenge of GHRPs identified in 2007 – test methods available only since 2010







## Challenge of GHRPs identified in 2007 – test methods available only since 2010





## Challenge of GHRPs identified in 2007 – test methods available only since 2010

Compound	Sequence
GHRP-1	Ala-His-D-βNal-Ala-Trp-D-Phe-Lys-NH <sub>2</sub>
GHRP-2	D-Ala-D-βNal-Ala-Trp-D-Phe-Lys-NH <sub>2</sub>
GHRP-4	D-Trp-Ala-Trp-D-Phe-NH <sub>2</sub>
GHRP-5	Tyr-D-Trp-Ala-Trp-D-Phe-NH <sub>2</sub>
GHRP-6	His-D-Trp-Ala-Trp-D-Phe-Lys-NH <sub>2</sub>
Hexarelin	His-D-Mrp-Ala-Trp-D-Phe-Lys-NH <sub>2</sub>
Ipamorelin	Aib-His-D-2-Nal-D-Phe-Lys-NH <sub>2</sub>
Alexamorelin	Ala-His-D-Mrp-Ala-Trp-D-Phe-Lys-NH <sub>2</sub>

Non-standard abbreviations: Aib = aminoisobutyric acid; Nal = naphthylalanine; Mrp 2-methyltryptophane.



# SUBSTANCES & METHODS PROHIBITED AT ALL TIMES

(IN- AND OUT-OF-COMPETITION)

## PROHIBITED SUBSTANCES

S1

### ANABOLIC AGENTS

Anabolic agents are prohibited.

---

#### 1. ANABOLIC ANDROGENIC STEROIDS (AAS)

---

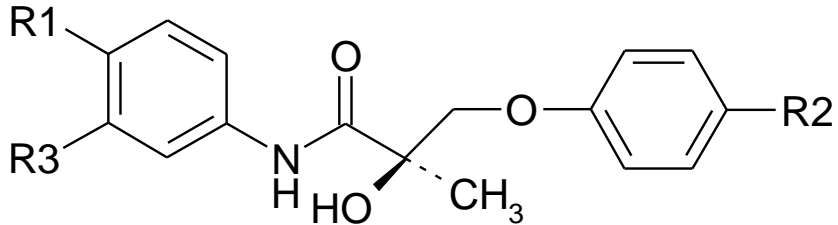
#### 2. OTHER ANABOLIC AGENTS

**Including, but not limited to:**

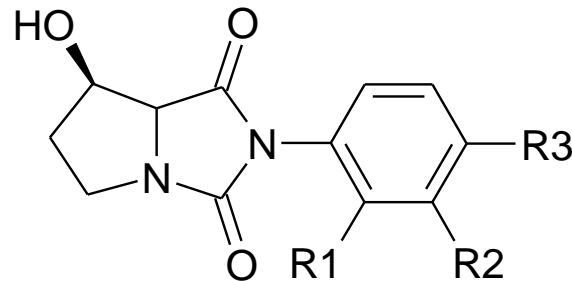
Clenbuterol, selective androgen receptor modulators (SARMs, e.g. andarine and ostarine), tibolone, zeranol and zilpaterol.



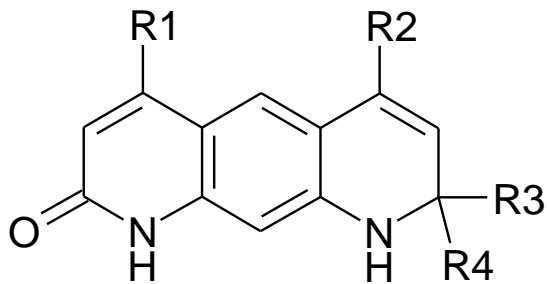
# New drugs – new challenges



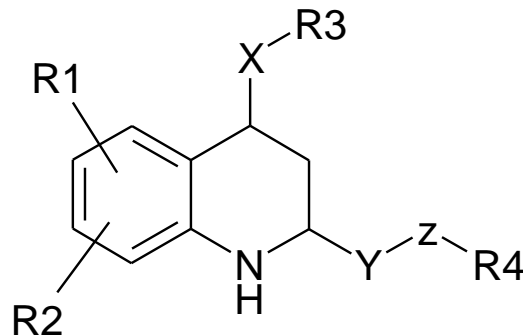
**arypropionamide**



**bicyclic hydantoin**



**quinolines**

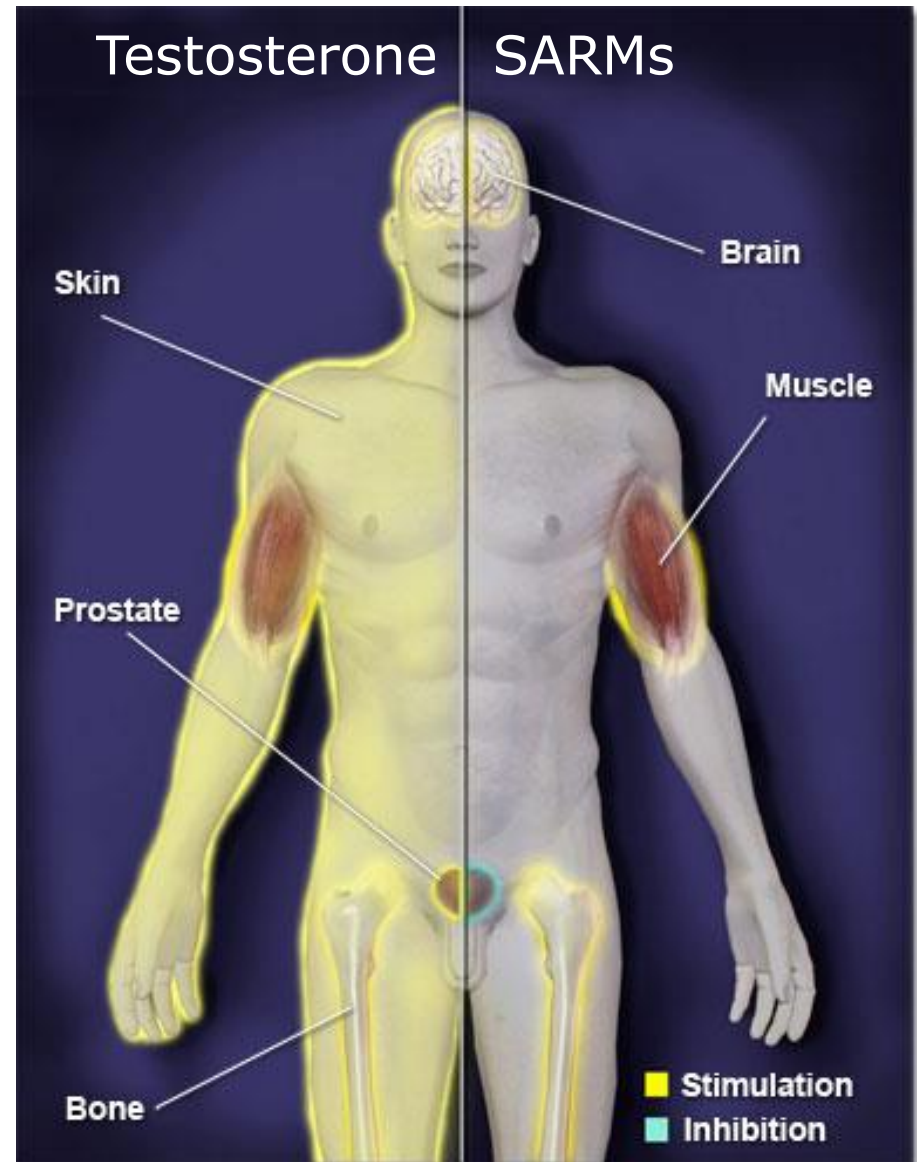


**tetrahydroquinolines**





# ...SARMs benefits





# ...SARMs benefits

**Potentially useful for prevention or treatment of**

**-muscle wasting**

**-osteoporosis**

**-frailty**

**-male contraception**

Basically without androgenic side effects – high potential for misuse in sports



# SARMS S-4

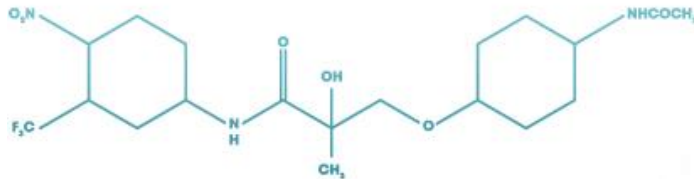
*S-3-(4-acetylamino-phenoxy)-2-hydroxy-2-methyl-N-(4-nitro-3-trifluoromethyl-phenyl)-propionamide*

Welcome!

We are the leading provider of **SARMS S-4** in the United States.  
At [www.XXXXXXXXXX.com](http://www.XXXXXXXXXX.com) we work very closely with our customers  
to provide the very best products available on the market.

Our business is built on a philosophy of strong customer focus,  
regular communication with a high degree of quality and value.

**NOT FOR HUMAN CONSUMPTION**



CONTACT

PURCHASE





# SARMS S-4

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The materials for sale are intended for laboratory and research use only. They are not for use as food additives, drugs, cosmetic, household chemicals, or other inappropriate applications. YOU MUST BE A MINIMUM OF 18 YEARS OF AGE TO ORDER. The listing of a material on this website does not constitute a license to, or a recommendation for its use in infringement of any patent. All of the products will be handled only by qualified and trained individuals. In purchasing these products, the customer acknowledges that there are hazards associated with their use.

**I AGREE**



# SARMS S-4

S-3-(4-acetylamino-phenoxy)-2-hydroxy-2-methyl-N-(4-nitro-3-trifluoromethyl-phenyl)-propionamide

Description	Unit Price	Quantity	Amount
30 mL Sample	\$100.00	<input type="text" value="1"/>	\$100.00
			Item total: \$100.00
			Shipping and handling: \$15.00
			<b>Total: \$115.00 USD</b>
<input type="button" value="Update Totals"/>			

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The image shows a hand holding a brown glass dropper bottle in front of a computer monitor. The monitor displays a website for SARMS S-4. The website content includes:

# SARMS S-4

*S-3-(4-acetylamino-phenoxy)-2-hydroxy-2-methyl-N-(4-nitro-3-trifluoromethyl-phenyl)-propionamide*

We are the leading provider of **SARMS S-4** in the United States.

Our business is built on a philosophy of strong customer focus, regular communication with a high degree of quality and value.

**NOT FOR HUMAN CONSUMPTION**

CC(C)(O)C(=O)Nc1ccc(C(F)(F)F)cc1OCCOc2ccc(O)cc2

**PURCHASE** **CONTACT**

The bottle label on the monitor reads: **SARMS**, **100MG/ML 30**, and **Not for Human Consumption**. The hand is holding a similar bottle in the foreground.



Name: Mario Thevis  
 Am Sportpark Muenzgerstr 6  
 Mönchengladbach - Donike Institut

Description (1)	Qty. (2)	Net Weight (3)		Value (U.S. \$) (5)
		lbs.	oz.	
Tea Tree Extract <del>Free</del> face Mouswizer				\$10

Certificate Number(s) (14):  
 Invoice Number (15):  
 Date and Sender's Signature (16): *CT* 9/14/09  
 USA, October 2007 ISBN 7530-01-000-9834  
 Do not duplicate this form without USPS® approval.

NOTE: Item is subject to return charges at sender's expense.  
 Redirect to Address Below.

1 - Manifest

USPS  
 SEP 14 2009  
 DOWNTOWN  
 ATLANTA, GA 30603







# First Adverse Analytical Finding 2010

Lausanne / 13<sup>th</sup> IAAF WIC, Doha, Qatar



SPORT

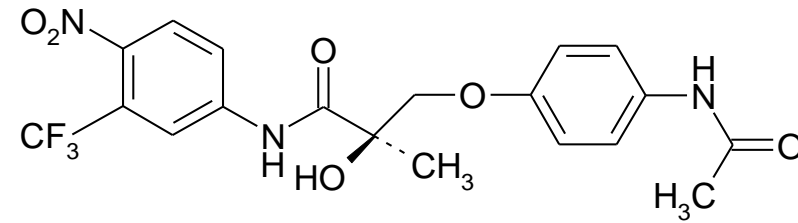
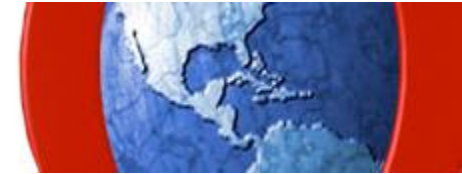
## JAAA awaits result of Wilkins drug hearing

BY DANIA BOGLE Observer staff reporter  
Wednesday, July 14, 2010



THE Jamaica Amateur Athletic Association (JAAA) is expecting a result soon from the disciplinary hearings being held into the positive drugs test result returned by quarter-miler Bobby-Gaye Wilkins, Dr Warren Blake told the Observer.

Wilkins tested positive for the Selective Androgen Receptor Modulator (SARM) Andarine, which is listed by the World Anti-Doping Agency (WADA) as an anabolic agent, at the 13th IAAF World Indoor Championships (WIC) in Doha, Qatar in March.





Anti-doping

# Two American triathletes test positive for Ostarine

Two American pro triathletes have tested positive for the banned substance Ostarine. Beth Gerdes tested positive after winning Ironman Australia in 2016, while Lauren Barnett's positive test occurred in July, 2016.

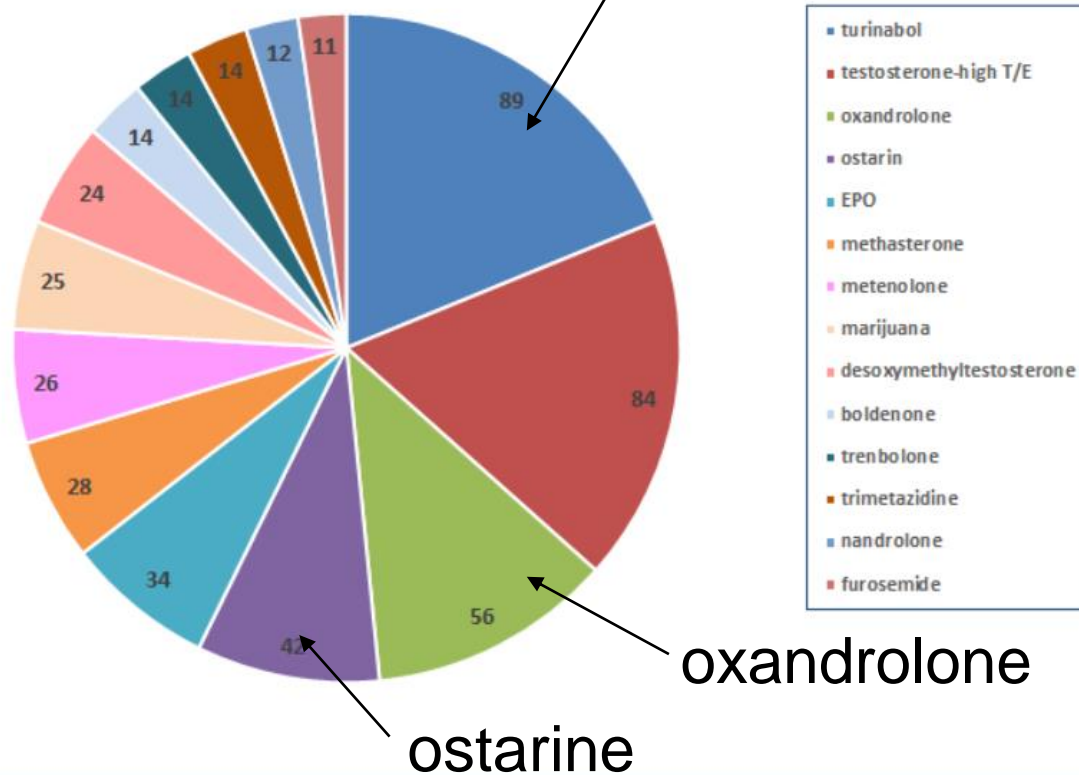
[FABIAN FIEDLER](#) | [FEBRUARY 4, 2017](#) | [NEWS](#)





# The Independent Person Report pt. 2: 2011-2015 -> 42 SARM-findings (Ostarine) but none reported / sanctioned

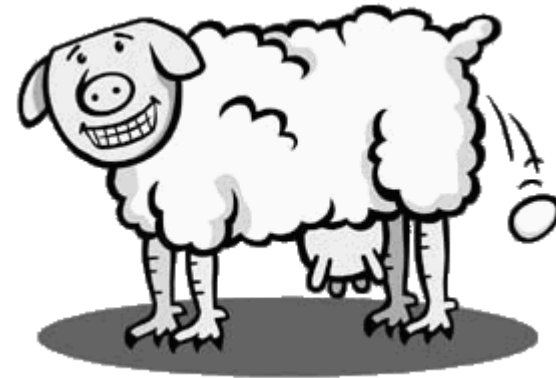
dehydrochloromethyltestosterone







## Facilitating flexible multi-analyte test methods





# Value of flexible and fast analytical approaches...

WORLD ANTI-DOPING CODE  
INTERNATIONAL  
STANDARD



## PROHIBITED LIST

JANUARY 2016

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- 1.1 Erythropoiesis-Stimulating Agents (ESAs) including e.g. Darbepoietin (dEPO); Erythropoietins (EPO); EPO-Fc; EPO-mimetic peptides (EMP), e.g. CNTO 530 and peginesatide; Methoxy polyethylene glycol-epoetin beta (CERA).

#### 1.2 Non-erythropoietic EPO-Receptor agonists, e.g.

- ARA-290;
- Asialo EPO;
- Carbamylated EPO.

#### 2. Hypoxia-inducible factor (HIF) stabilizers, e.g. cobalt and FG-4592; and HIF activators, e.g. argon, xenon.

#### 3. Chorionic Gonadotrophin (CG) and Luteinizing Hormone (LH) and their releasing factors, e.g. buserelin, gonadorelin and triptorelin, in males.

#### 4. Corticotrophins and their releasing factors, e.g. corticorelin.

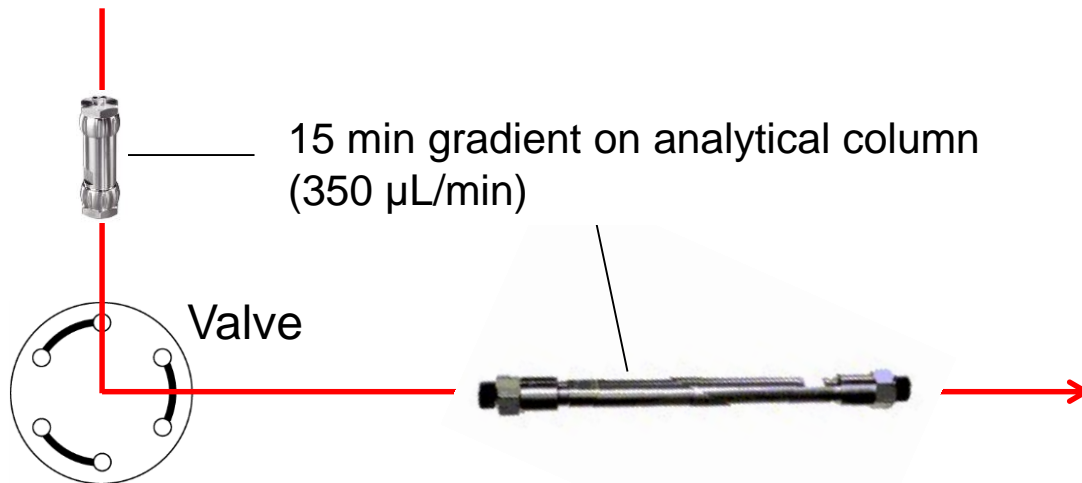
5. Growth Hormone (GH) and its releasing factors including Growth Hormone Releasing Hormone (GHRH) and its analogues, e.g. CJC-1295, sermorelin and tesamorelin; Growth Hormone Secretagogues (GHS), e.g. ghrelin and ghrelin mimetics, e.g. anamorelin and ipamorelin, and GH-Releasing Peptides (GHRPs), e.g. alexamorelin, GHRP-6, hexarelin and pralmorelin (GHRP-2).

Additional prohibited growth factors:

- Fibroblast Growth Factors (FGFs);
- Hepatocyte Growth Factor (HGF);
- Insulin-like Growth Factor-1 (IGF-1) and its analogues;
- Mechano Growth Factors (MGFs);
- Platelet-Derived Growth Factor (PDGF);
- Vascular-Endothelial Growth Factor (VEGF) and any other growth factor affecting muscle, tendon or ligament protein synthesis/degradation, vascularisation, energy utilization, regenerative capacity or fibre type switching.



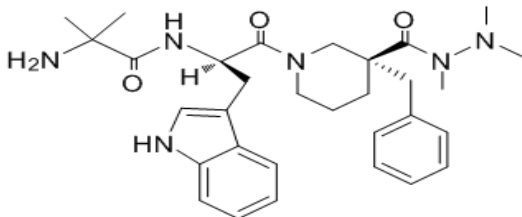
## Liquid chromatography – mass spectrometry



# Target analytes

peptide	amino acid sequence	category	WADA List
Desmopressin			
LH-RH			
Buserelin			
Triptorelin			
Leuprolide			
GHRP-1			
GHRP-1 metab.			
GHRP-2			
GHRP-2 metab.			
GHRP-3			
GHRP-4			
GHRP-5			
GHRP-6			
Alexamorelin			
Alexamorelin metab.			
Ipamorelin			
Hexarelin			
<b>Anamorelin</b>			
TB-500			
ARA-290			
AOD-9604			

Anamorelin (RC-1291, ONO-7643, ST-1291):



Synthetic, non-peptidic, orally active ghrelin receptor agonist, sum formula:  $C_{31}H_{42}N_6O_3$  (MW: 546.7)

Nal: Naphthylalanine  
Aib: Amioisobutyric acid  
Pyr: Pyroglutamic acid

Mpa: Mercaptopropionic acid

Mrp: 2-methyltryptophane



Monday, March 19, 2012

## Colombian doctor Beltrán Niño arrested with AICAR and TB-500 doping products

by Shane Stokes at 8:48 AM EST ☆☆☆☆

Categories: [Pro Cycling](#), [Doping](#)

### *Previously worked with several cycling teams*

Reports that the doping substances AICAR and TB-500 are being used by some in the peloton have gained weight with the news that a doctor linked to cycling was arrested earlier this month with both substances in his possession.

According to El Pais, Alberto Beltrán Niño was arrested on March 7th in Barajas airport in Madrid, from where he was due to fly to Colombia. The two substances were seized, as were his laptop plus flash drives.

These will presumably be screened in order to try to determine who he was supplying products to. Beltrán has been in the Soto del Real prison since March 8th.







# TB-500

DRUG FREE PERFORMANCE

HOME

WHAT IS TB500

BENEFITS OF TB500

BUY NOW

FAQ

TECHNICAL DATA

CONTACT US



TB-500 offers many benefits to the equine world in performance racing.



DB Genetics LLC is proud to introduce the latest, most potent Drug Free performance substance to the animal racing world\*... **TB-500 Synthetic Peptide**. This exclusive formula is a patented and customised Synthetic Peptide of the active region of *ThymosinBeta 4* (Tβ4).

Tβ4, a unique polypeptide of 43 amino acids was originally isolated in 1981 from the thymus gland and is a potent mediator of cell migration and differentiation. Tβ4 is different from other repair factors, such as growth factors, in that it promotes endothelial and keratinocyte migration which enables it to travel long distances through tissues.

**TB-500 Synthetic Peptide** is an exclusive sequence peptide containing a unique amino acid chain that provides many **benefits** in achieving peak performance.

**This potent, drug free product is the competitive edge all owners and trainers have been waiting for – Don't Miss Out**

## WARNING

Medivet International is the sole exclusive manufacturer of TB 500; this product is exclusive to Medivet and its approved distribution network internationally. Cheap non approved imitations have been circulating the World Wide Web, if you believe you have been subjected to the purchase of an imitation product or would like verification of the authenticity of your local distributor please contact us.

**Buy Now**

\* These statements have not been evaluated by the Food and Drug Administration.  
This product is not intended to diagnose, treat, cure or prevent any disease.

## what is tb500?

A special sequence peptide promoting muscle growth & wound healing.

[read more >](#)

## the benefits?

TB-500 offers many benefits in performance racing.

[read more >](#)

## want a discount?

Contact Us TODAY for Bulk-Order Discounts – Don't Miss Out!

[read more >](#)

**Contact Us TODAY for Bulk-Order Discounts 'Don't Miss Out'**



# MediVet America

---

## Product Fact Sheet

# TB-500

### Is TB500 Safe?

As TB-500 is a synthetic version of the naturally occurring peptide found in all animal cells, it does not pose as a foreign substance to the animal's body. When used within the recommendations of this fact sheet, TB-500 is completely safe.

### Side Effects?

TB500 is a naturally occurring peptide promoting the repair and regeneration of skin and blood vessels. It is completely Drug and Chemical free. There are no side effects with the use of this product. It does not swab.

### Benefits of TB500

In performance animals, as in all animals, the benefits include faster and better wound repair, and faster recovery from injury. Trials to date indicate faster recovery time (wear and tear) from training; increased muscle girth; increased muscular endurance; increased muscular strength; increased energy levels.

### Is It Legal for Performance Animals?

TB500 is 100% drug and chemical free. If used according to the Directions For Use, it will not compromise any performance animal in any event or race in which it competes. IT DOES NOT SWAB.



## Test purchase from black market 2012

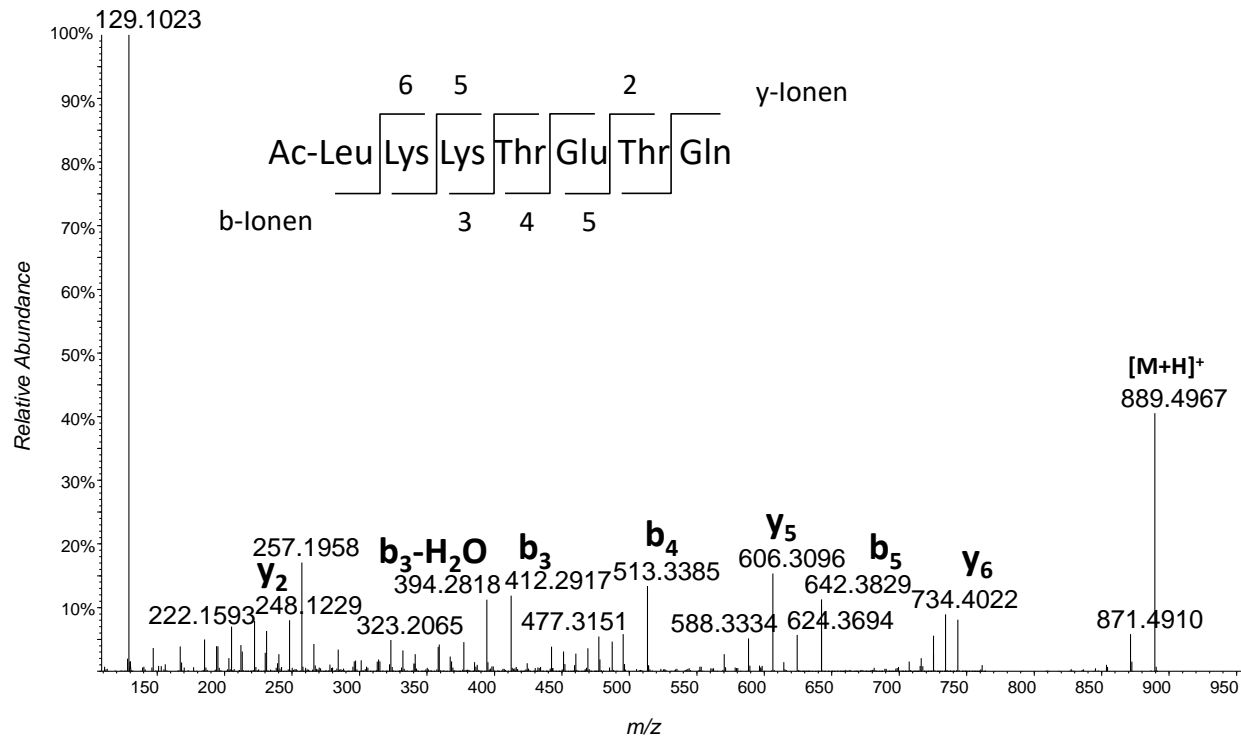
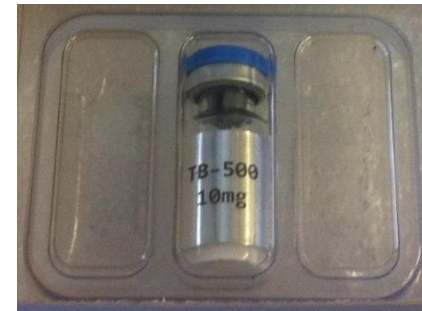






## Test purchase from black market 2012



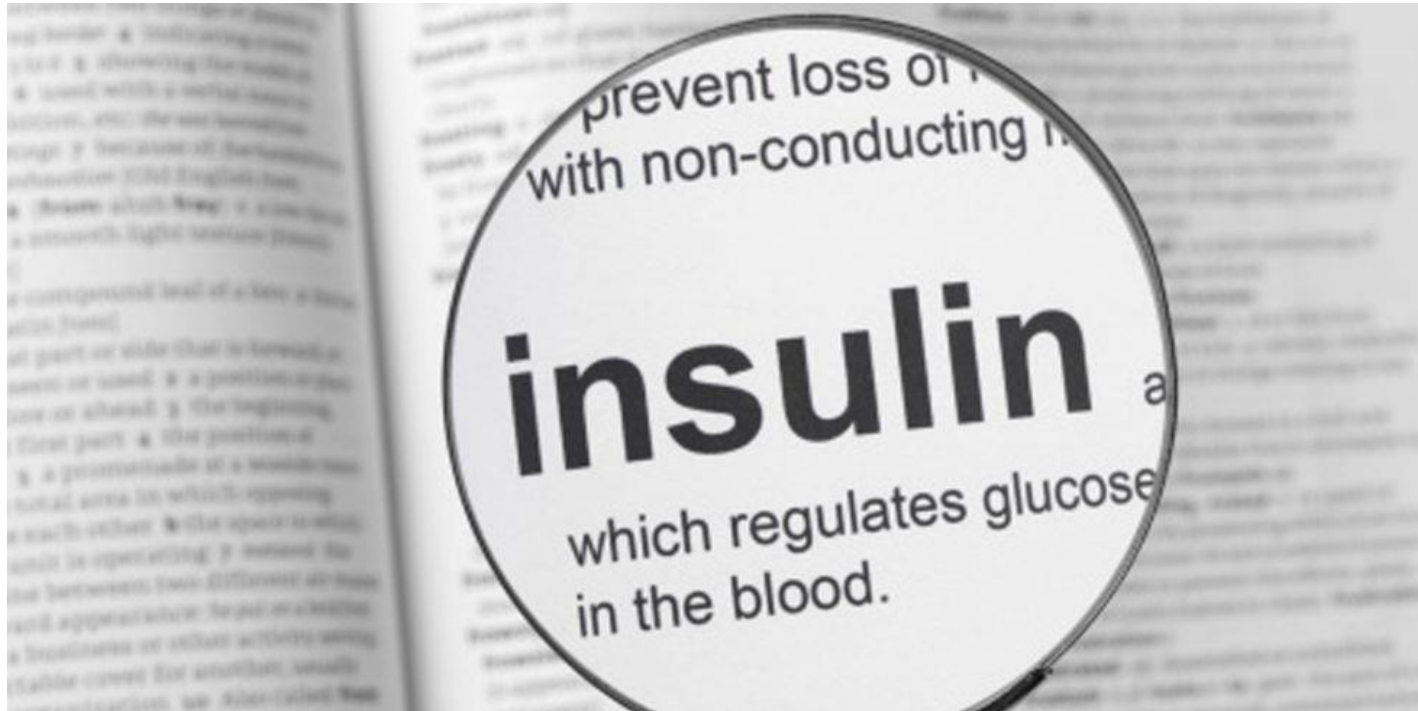






## Accelerating multi-analyte test methods







# SUBSTANCES & METHODS PROHIBITED AT ALL TIMES

(IN- AND OUT-OF-COMPETITION)

## PROHIBITED SUBSTANCES

---

### S4 HORMONE AND METABOLIC MODULATORS

The following hormone and metabolic modulators  
are prohibited:

#### 5. Metabolic modulators:

5.1 Activators of the AMP-activated protein kinase (AMPK), e.g. AICAR; and Peroxisome Proliferator Activated Receptor  $\delta$  (PPAR $\delta$ ) agonists, e.g. GW 1516;

5.2 Insulins and insulin-mimetics;

Why would that be of importance for doping controls / forensics?

Insulin –  
Abuse in sport and otherwise



Former ProTour cyclist:

Insulin was the only banned substance he administered every day within his doping program!

Immediately after competition (and respective doping control sampling) or training



# Why would that be of importance for doping controls / forensics?

Insulin –  
Abuse in sport and otherwise

## Review

Drug Testing  
and Analysis

Received: 25 March 2009

Revised: 29 April 2009

Accepted: 6 May 2009

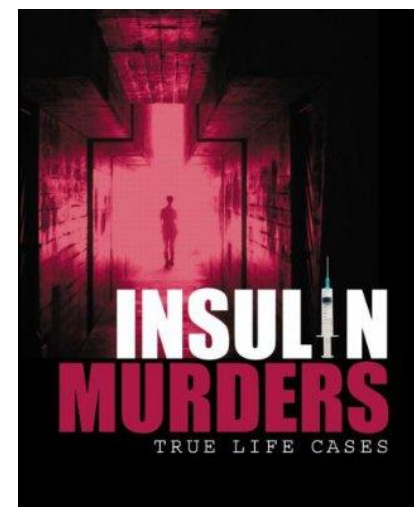
Published online in Wiley InterScience:

(www.drugtestinganalysis.com) DOI 10.1002/dta.38

## Murder by insulin: suspected, purported and proven – a review

Vincent Marks\*

Murder by insulin – whether attempted, suspected or proven – is rare. Only 66 cases worldwide. A conviction was secured in 31 cases and additional weapon was employed in 11. Differentiation Munchausen syndrome by proxy in the young and from ‘mercy killing’ in the elderly was not a were close relatives and most victims were alive when discovered and responded to treatment. H to homicidal insulin use in living subjects and requires the demonstration of a plasma insulin cor than 1000 pmol/L and undetectable plasma C-peptide concentration to establish the diagnosis. § are valueless in victims found dead. The presence near the body of insulin vials, syringes or needle perpetrator or their ready access to insulin may be the only clue. The demonstration of insulin in t by immunohistopathology or by measuring it in an extract clinches the diagnosis. Immunoassa detect and measure insulin and C-peptide are subject to random errors and cannot be relied up including separation by gel filtration or HPLC are undertaken prior to analysis. They do not detec generation of synthetic insulin analogues. Mass spectrometry will be required to do this and to va upon which convictions have always had to rely in the past. Copyright © 2009 John Wiley & Sons



## BRITISH MEDICAL JOURNAL

LONDON SATURDAY AUGUST 23 1958

### INVESTIGATIONS IN A CASE OF MURDER BY INSULIN POISONING

BY

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Beckett Hospital, Barnsley, Yorks, and Home Office  
Forensic Science Laboratory, Harrogate, Yorks

AND

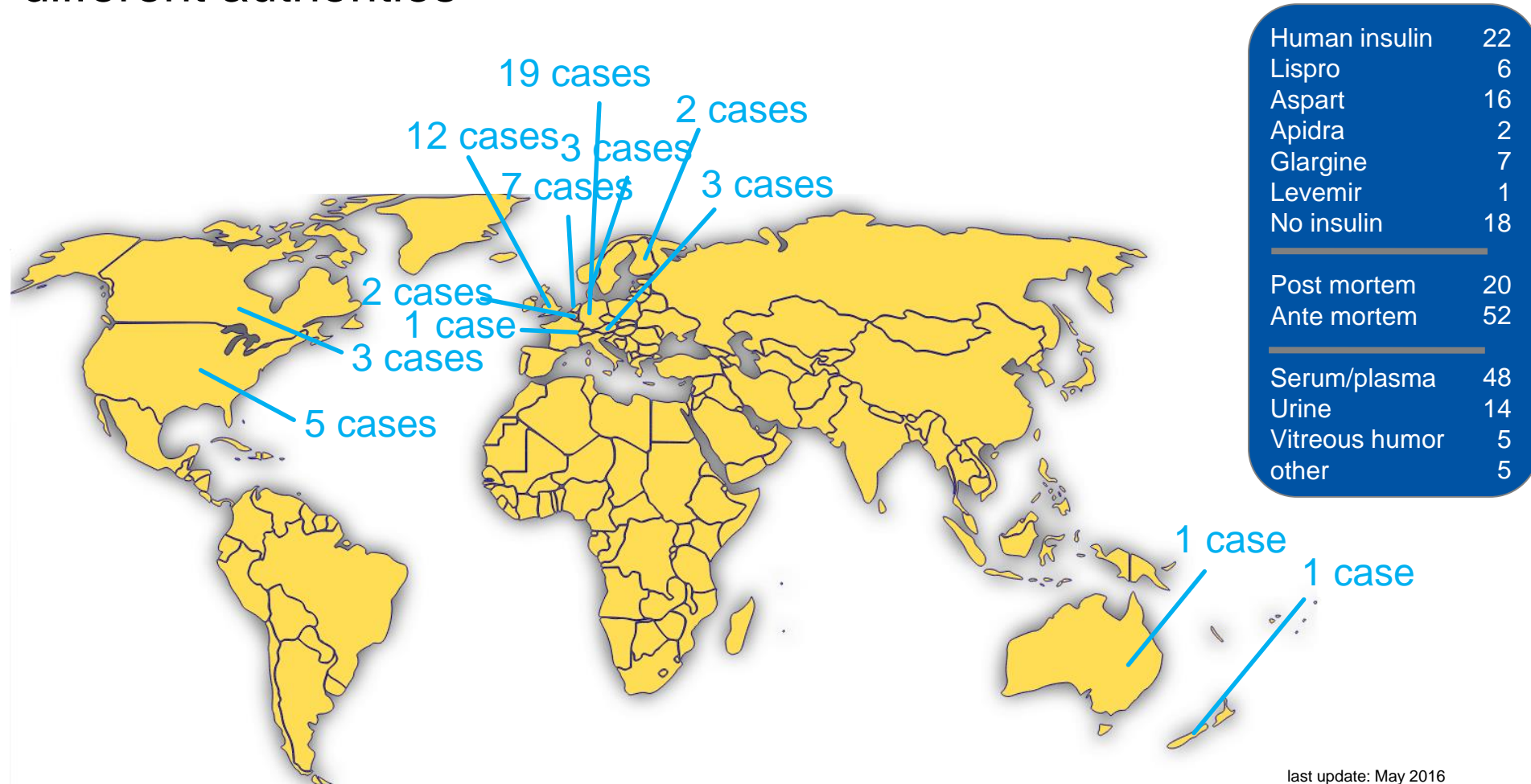
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Insulin –  
Abuse in sport and otherwise

# Investigations in cooperation with different authorities





# Selected Detection Assays: *Synthetic insulins*

Insulin –  
Abuse in sport and otherwise

Human Insulin:	GIVEQCCTSI <b>C</b> SLYQLENYCN - FVNQHLCGSHLVEALYLVCGERGFFYTP <b>K</b> T
Lispro:	GIVEQCCTSI <b>C</b> SLYQLENYCN - FVNQHLCGSHLVEALYLVCGERGFFY <b>T</b> <b>K</b> P <b>T</b>
Glargine Met.:	GIVEQCCTSI <b>C</b> SLYQLENY <b>C</b> <b>G</b> - FVNQHLCGSHLVEALYLVCGERGFFYTP <b>K</b>
Bovine Insulin:	GIVEQ <b>C</b> <b>C</b> <b>A</b> <b>S</b> <b>V</b> <b>C</b> SLYQLENYCN - FVNQHLCGSHLVEALYLVCGERGFFYTP <b>K</b> <b>A</b>
Porcine Insulin:	GIVEQCCTSI <b>C</b> SLYQLENYCN - FVNQHLCGSHLVEALYLVCGERGFFYTP <b>K</b> <b>A</b>
Glulisine:	GIVEQCCTSI <b>C</b> SLYQLENYCN - F <b>V</b> <b>K</b> QHLCGSHLVEALYLVCGERGFFYTP <b>E</b> <b>T</b>
Aspart:	GIVEQCCTSI <b>C</b> SLYQLENYCN - FVNQHLCGSHLVEALYLVCGERGFFY <b>T</b> <b>D</b> <b>K</b> <b>T</b>
Detemir:	GIVEQCCTSI <b>C</b> SLYQLENYCN – FVNQHLCGSHLVEALYLVCGERGFFYTP <b>K</b> <sub>-myrist.</sub>



Insulin –  
Abuse in sport and otherwise

1<sup>st</sup> SPE  
(5 mL of urine)

elution of analytes  
into Eppendorf tube

immunoaffinity  
purification using  
magnetic beads

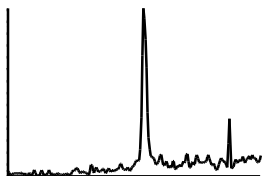
primary monoclonal  
antibody, secondary  
antibody-coated magnetic  
beads



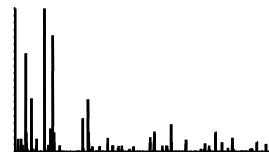
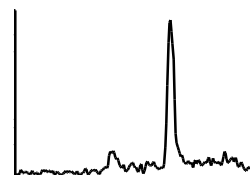
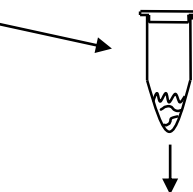
- Elution with 50  $\mu$ L

-cleavage into A- and B-chain

-LC-ESI-MS/MS  
analysis of intact  
insulins



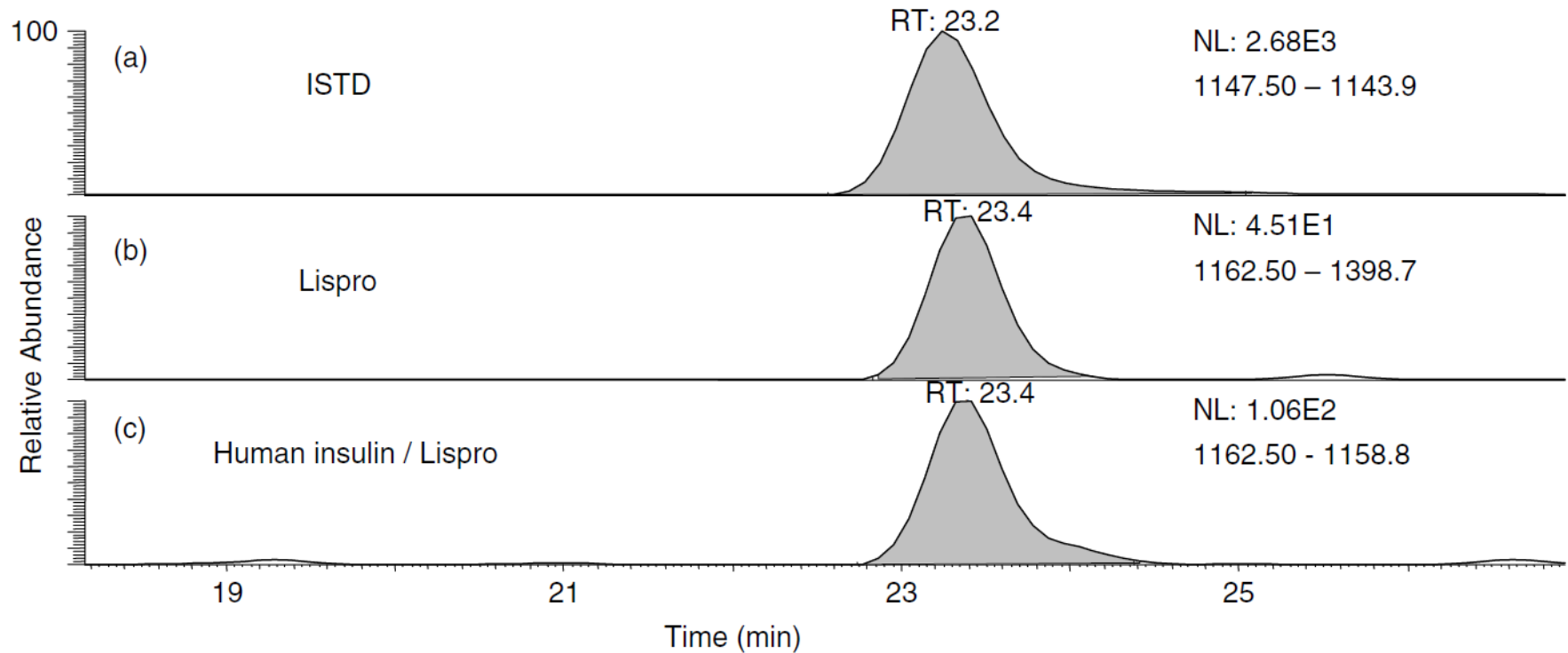
-LC-ESI-MS/MS  
analysis of B-  
chain



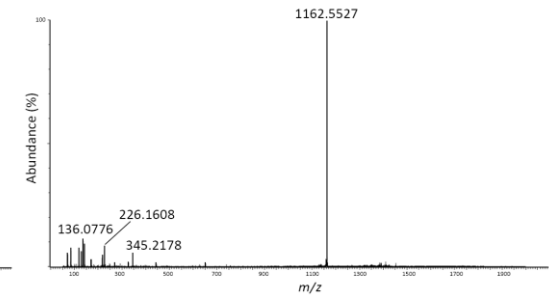
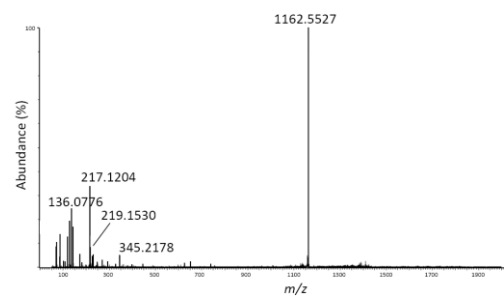
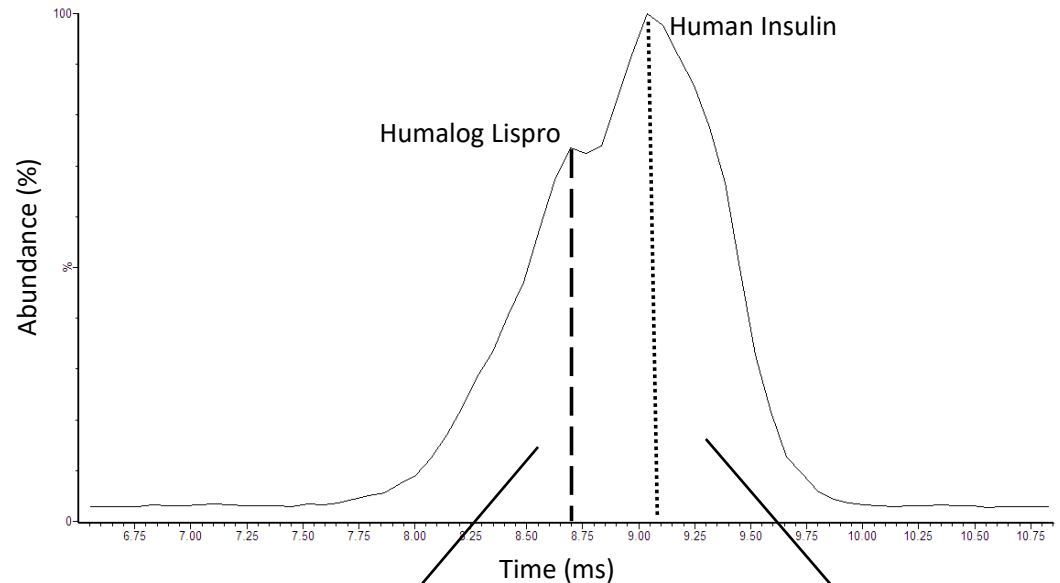
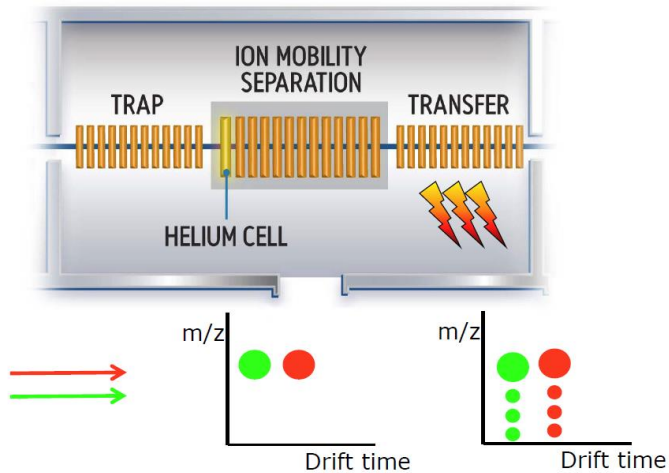


Insulin –  
Abuse in sport and otherwise

# Selected Detection Assays: *Synthetic insulins*



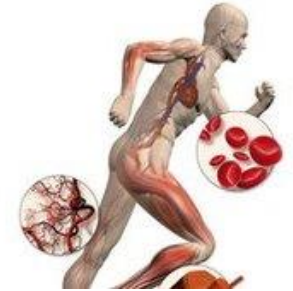
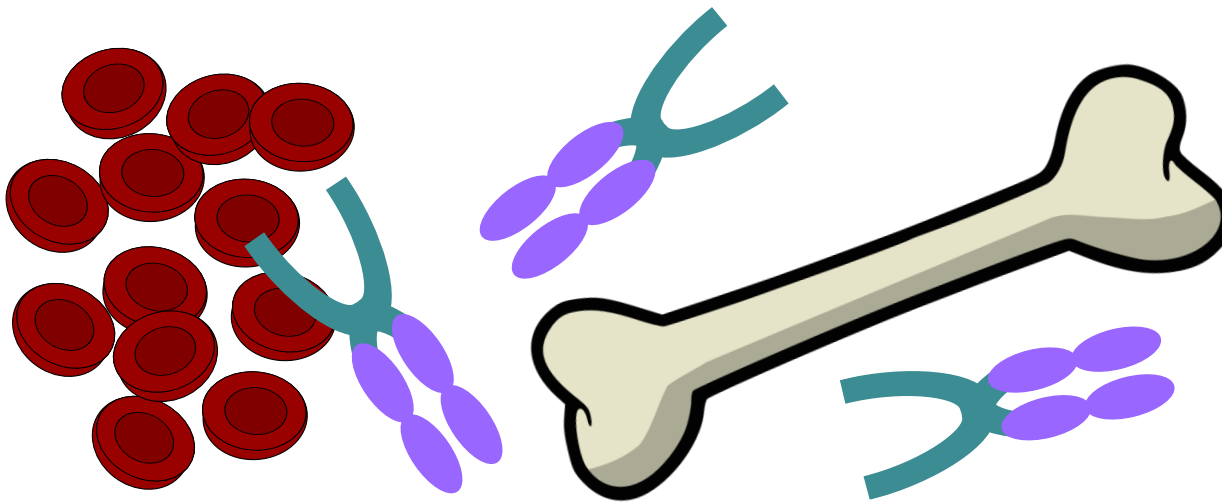
# Selected Detection Assays: *Synthetic insulins – alternative/faster options?*





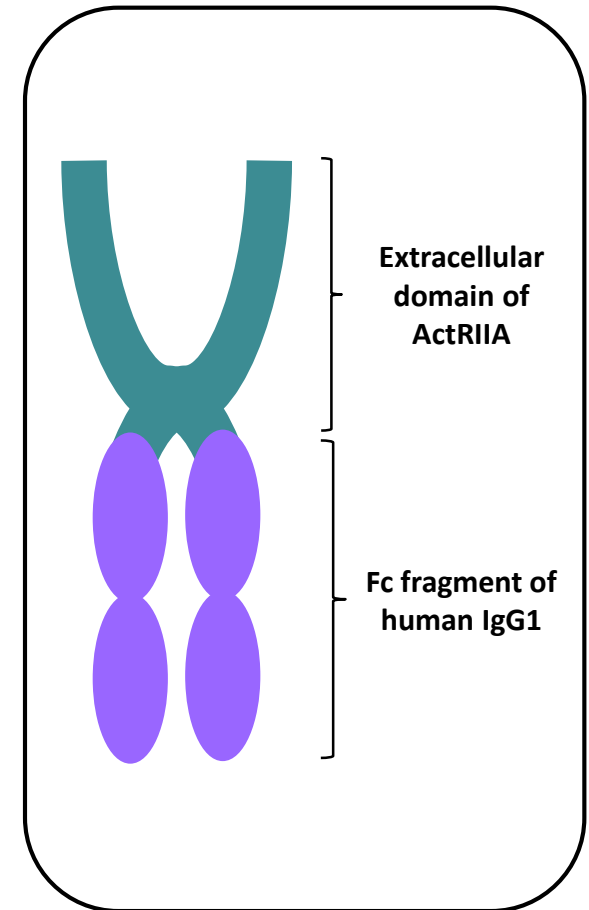
## Therapeutic ligand traps / decoy receptors

- ActRIIA-Fc (Sotatercept)



## Sotatercept/ACE-011:




- Soluble fusion protein consisting of the extracellular domain of activin receptor type IIA and the Fc fragment of human IgG1 (ActRIIA-Fc)
- **Clinical indications:**
  - Treatment of osteoporosis
  - Treatment of anemia in rare blood diseases where erythropoiesis-stimulating agents (ESAs) are either not approved or not well suited (e.g.  $\beta$ -Thalassemia, Diamond-Blackfan)





## Method development: *In-silico* enzymatic digestion of Sotatercept/ACE-011

ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS  
 IEIVKQGCWL DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPPEM  
 EVTQPTSNPV TPKPPTGGGT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI  
 SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV  
 SVLTVLHODW LNGKEYKCKV SNKALPVPKIE KTISKAKGQP REPQVYTLPP  
 SR**EEM**TKNOV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS  
 FFLYSKLTVD KSRWQOGNVF SCSVMHEALH NHYTQKSLSL SPGK

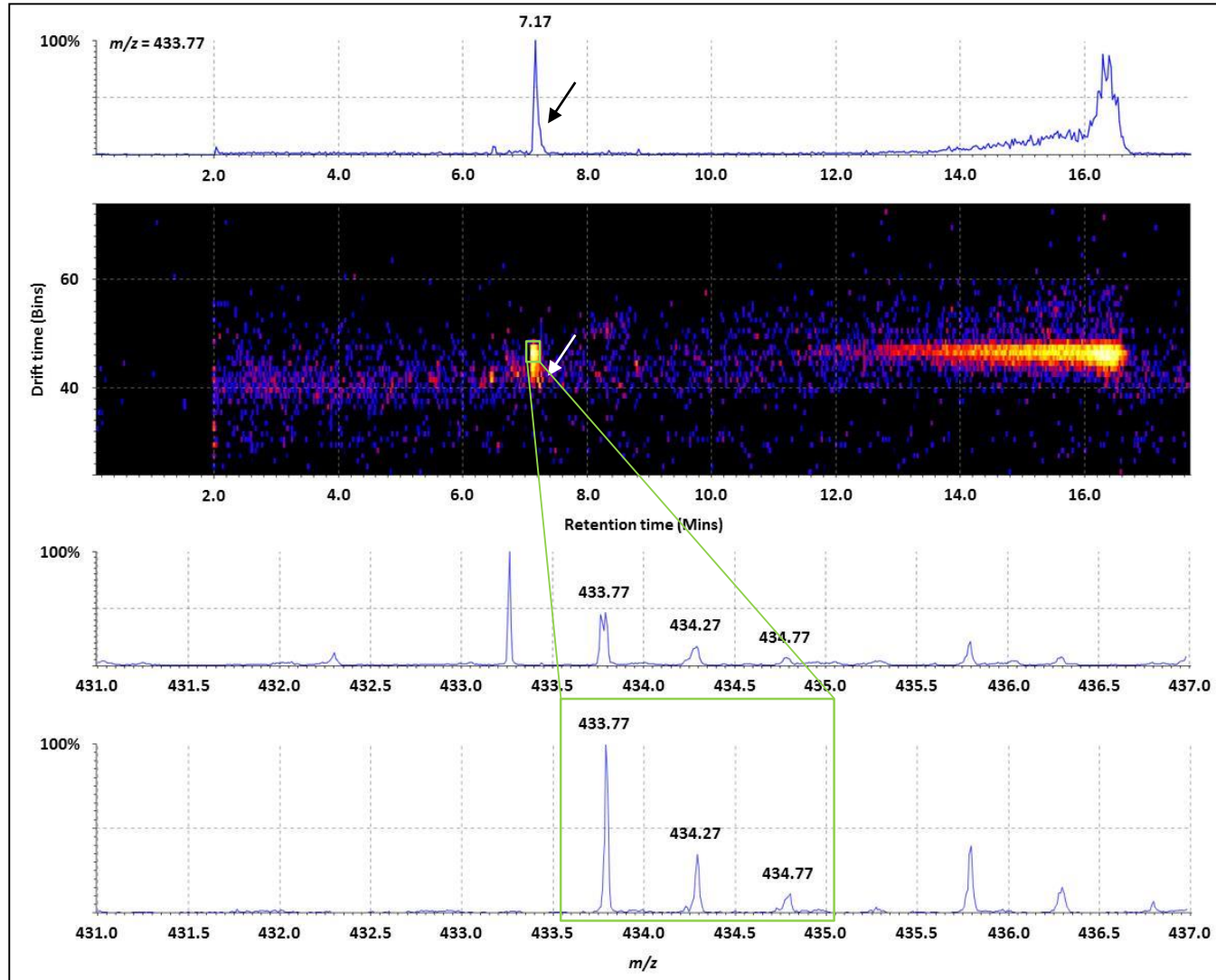
 = ActRIIA  
 = Linker  
 = IgG1 Fc

### *Proteolytic digestion: Trypsin*

- Tryptic peptides: ActRIIA T<sub>1</sub>-T<sub>13</sub>  
 Linker T<sub>14</sub>  
 Fc T<sub>15</sub>-T<sub>36</sub>
- Potential diagnostic peptides:  
 T<sub>2</sub>, T<sub>8</sub>, T<sub>10</sub>, T<sub>11</sub>, T<sub>13</sub>, T<sub>24</sub>



Waters SYNAPT G2S: Ion mobility  
0.05 µg/mL Sotatercept





## Case Report Malaria Chemoprophylaxis

- 3 fencer return from Africa to Europe from competitions
- Two produce adverse analytical findings with chlorazanyl (diuretic)





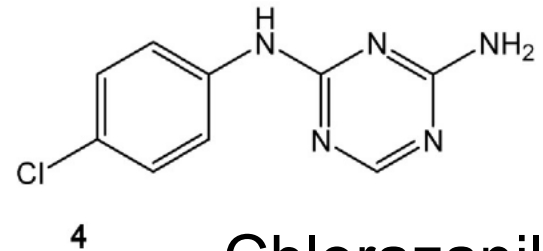
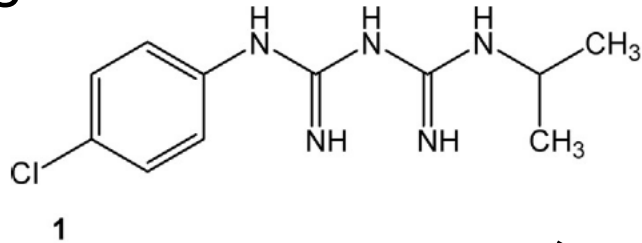
# Case Report Malaria Chemoprophylaxis

- These cases are the worldwide first two findings with the obsolete diuretic (since 1988)
- Structural relationship to proguanil (anti-malaria drug) triggered in-depth investigation





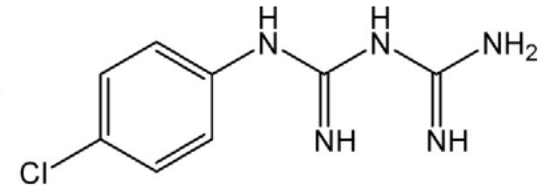
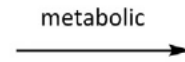
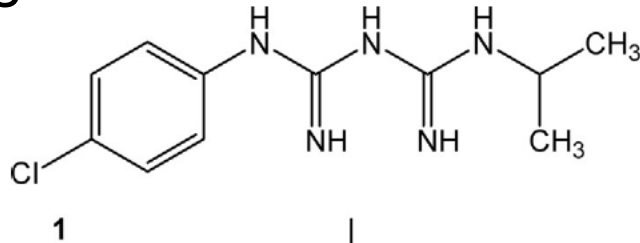
# Proguanil



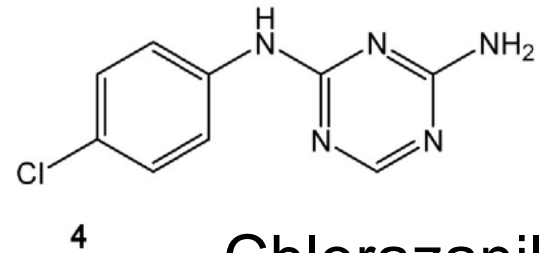
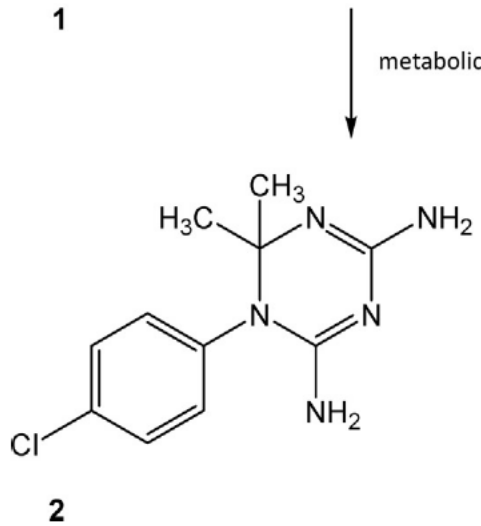
# Chlorazanyl

## 4-chlorophenyl-biguanide

### Proguanil



metabolic



### Cycloguanil

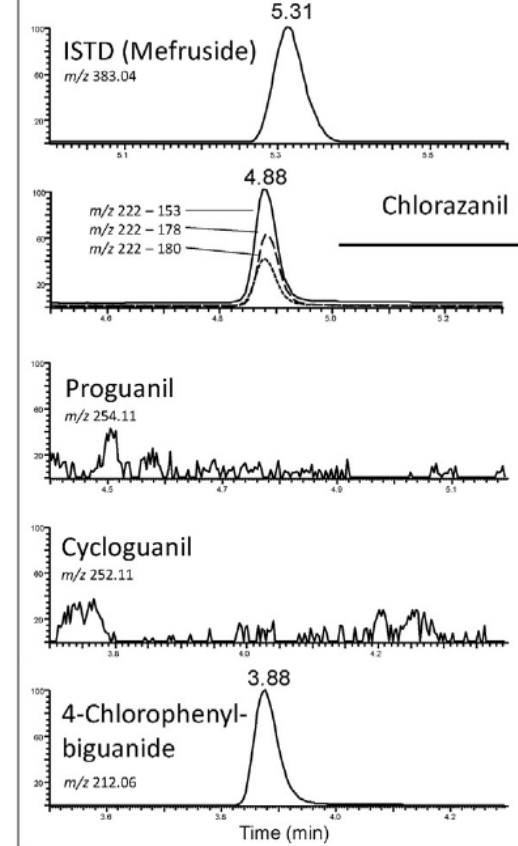
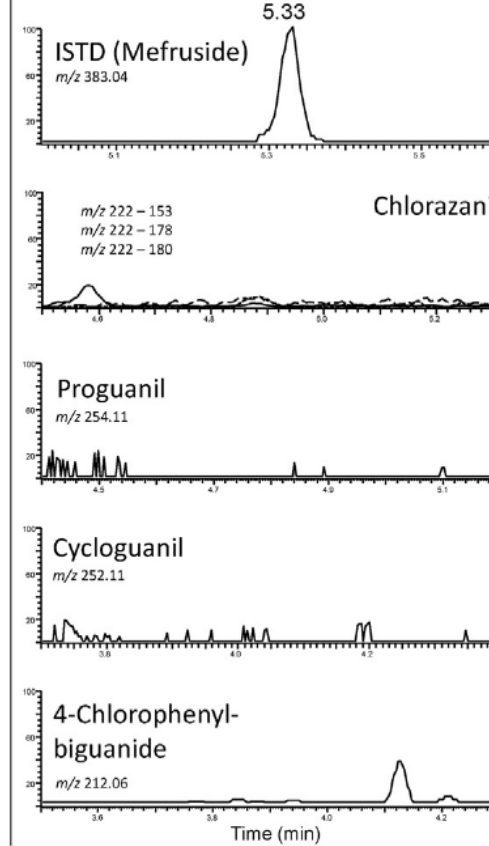
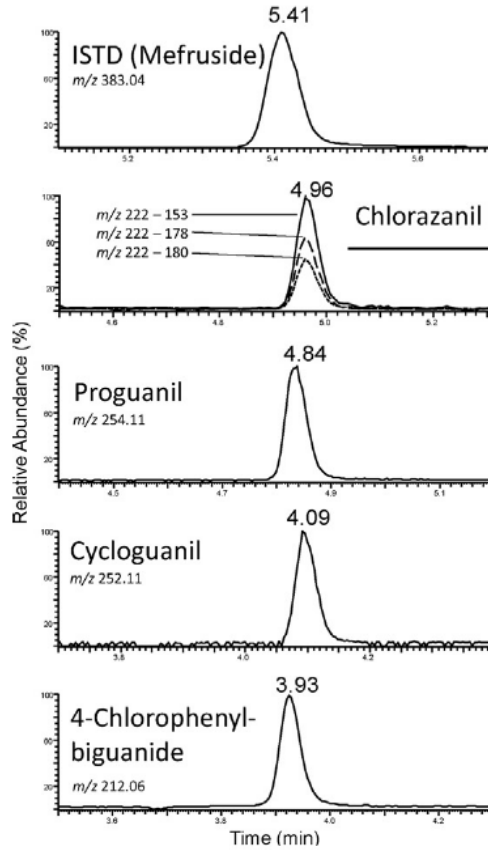
### Chlorazanyl



Reference compounds

blank urine

incubation mixture (urine)



## 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!





## Review

## Drug Testing and Analysis

Received: 16 May 2013

Revised: 26 July 2013

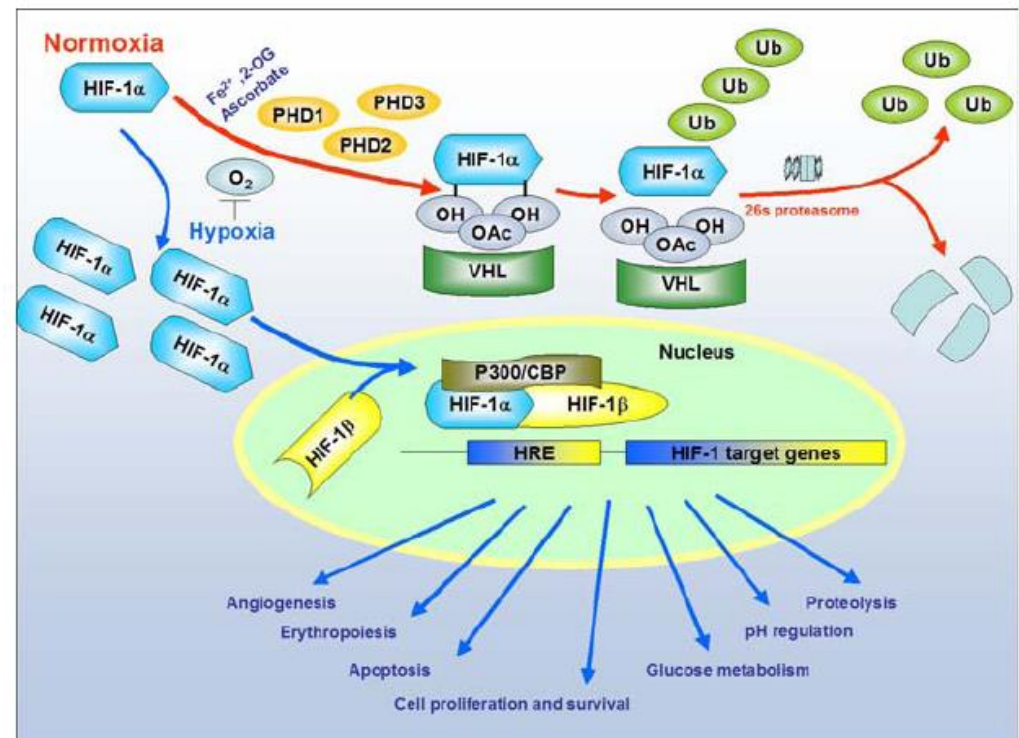
Accepted: 27 July 2013

Published online in Wiley Online Library: 30 August 2013

(www.drugtestinganalysis.com) DOI 10.1002/dta.1528

# Intolerability of cobalt salt as erythropoietic agent

Bastian Ebert and Wolfgang Jelkmann\*





Review

Drug Testing  
and Analysis

Received: 16 May 2013

Revised: 26 July 2013

Accepted: 27 July 2013

Published online in Wiley Online Library: 30 August 2013

(www.drugtestinganalysis.com) DOI 10.1002/dta.1528

## Intolerability of cobalt salt as erythropoietic agent

Bastian Ebert and Wolfgang Jelkmann\*

of the Epo gene (*EPO*).<sup>[5]</sup> With respect to doping practices, of particular interest are cobalt (II) ions ( $\text{Co}^{2+}$ ). The mechanism of the action of  $\text{Co}^{2+}$  is completely separate from that of the organic cobalt-containing vitamin, cobalamin.<sup>[6]</sup>  $\text{Co}^{2+}$  activates the hypoxia-inducible transcription factors (HIFs) that increase *EPO* expression.<sup>[7]</sup> By this way,  $\text{Co}^{2+}$  stimulates Epo production, as first observed in experimental animals in the late 1950s.<sup>[8]</sup>  $\text{Co}^{2+}$  is the reference substance for the *in vivo* calibration of rhEpo drug substance; 5  $\mu\text{mol Co}^{2+}$  elicits the same erythropoiesis-stimulating activity as one international unit (IU) of rhEpo. Weisbecker first noted that the oral administration of  $\text{CoCl}_2$  increases reticulocytes, red blood cells (RBCs) and [Hb] in healthy men. In another investigation of healthy humans, Davies and Fields<sup>[11]</sup> showed that the daily intake of 150 mg  $\text{CoCl}_2$  increases RBC numbers by about 1 Mio. per  $\mu\text{l}$  within 7 to 22 days, with the values returning to normal within 9 to 15 days after cessation of  $\text{Co}^{2+}$  administration.<sup>[9]</sup> In the late 1940s, the late 1950s, cobalt chloride ( $\text{CoCl}_2$ ) was applied to treat anaemic patients.<sup>[10,12]</sup> The medicine was usually given as tablets, in divided doses at meal times.

**$\text{Co}^{2+}$  activates the hypoxia-inducible transcription factors (HIFs) that increase *EPO* expression**

**From the late 1940s to the late 1970s, cobalt chloride ( $\text{CoCl}_2$ ) was applied to treat anaemic patients. The medicine was usually given as tablets, ...**



Cart Total \$0.00

\$19.95

Quantity \*

[Add to Cart](#)



### COBALT 8oz

## Cobalt Dietary Supplement / Cobalt Mineral Concentrate

#### Product Information

\*96 Day Supply\*

An advanced aqueous form of Cobalt Ions (IsoIonic™)\*\* in clear solution for quicker absorption than tablets or capsules which must first dissolve in the digestive system before being absorbed. Patented, clinically tested professional grade CHD-Fulvic Acid has been added to further increase absorption and enhance overall well-being as fulvic acid has natural anti-viral, anti-bacterial, anti-inflammatory, and detoxification properties.

Concentration: 200 PPM or mg/L

ITEM# 156

## Supplement Facts

Serving Size : 2.5ml (1/2 cap)

Servings Per Container : 96

	Amount Per Serving	%DV
Cobalt	500 mcg	*
Fulvic Acid	2 mg	*

\* Daily Value not established.

**Ingredients:** Double Reverse Osmosis Water  
Ionic Cobalt from ionized Cobalt Chloride,  
Natamycin, Nisin (100% natural, protects  
freshness).



**Table 1** Product description and analytical results.

product #	advertised effect	product formulation (suggested route of administration)	determined content relevant for doping controls ( $\geq 0.1$ mg/mL)	declared on label	remark
1	erythropoiesis	aqueous solution ( <i>i. v.</i> )	cobalt (0.1 mg/mL) nickel (7.5 mg/mL)	no no	cyanocobalamin (ca. 2.0 mg/mL) detected, accounting for ca. 90 $\mu$ g/mL cobalt
2	erythropoiesis	aqueous solution ( <i>i. v.</i> )	cobalt (4.8 mg/mL)	no	cyanocobalamin (ca. 1.7 mg/mL) detected, accounting for ca. 75 $\mu$ g /mL cobalt
3	increased oxygen supply	aqueous solution ( <i>i. v.</i> )	–		
4	counteracts fatigue	aqueous solution (injection)	–		
5	anti-inflammatory properties	gel ( <i>i.m.</i> or <i>i. v.</i> )	–		
6	–	aqueous solution	cobalt (3.4 mg/mL)	no	
7	–	aqueous solution	–		
8	erythropoiesis	aqueous suspension ( <i>i. v.</i> )	cobalt (1.9 mg/mL)	no	cyanocobalamin (ca. 2.6 mg/mL) detected, accounting for ca. 110 $\mu$ g/mL cobalt
9	erythropoiesis	aqueous suspension ( <i>i. v.</i> )	cobalt (2.2 mg/mL)	no	
10	erythropoiesis	aqueous solution ( <i>i. v.</i> )	cobalt (3.3 mg/mL)	no	cyanocobalamin (ca. 3.0 mg/mL) detected, accounting for ca. 270 $\mu$ g/mL cobalt
16	supports erythropoiesis	aqueous suspension ( <i>i.m.</i> or <i>i. v.</i> )	cobalt (0.2 mg/mL)	yes	accounting for ca. 5 $\mu$ g/mL cobalt label declares cobalt gluconate (0.7 mg/ mL) accounting for ca. 90 $\mu$ g/mL cobalt label declares cyanocobalamin (0.15 mg/ mL) accounting for ca. 7 $\mu$ g/mL cobalt cyanocobalamin (ca. 0.3 mg/mL) detected, accounting for ca. 15 $\mu$ g /mL cobalt
17	erythropoiesis	aqueous solution ( <i>i. v.</i> )	cobalt (0.1 mg/mL)	no	cyanocobalamin (ca. 4.0 mg/mL) detected, accounting for ca. 175 $\mu$ g/mL cobalt
18	erythropoiesis	aqueous solution ( <i>i. v.</i> )	cobalt (0.1 mg/mL)	no	cyanocobalamin (ca. 3.3 mg/mL) detected, accounting for ca. 140 $\mu$ g/mL cobalt
19	–	aqueous solution, residue in confiscated syringe	cobalt (5.5 mg/mL)	n/a	cyanocobalamin (ca. 5.3 mg/mL) detected, accounting for ca. 230 $\mu$ g/mL cobalt

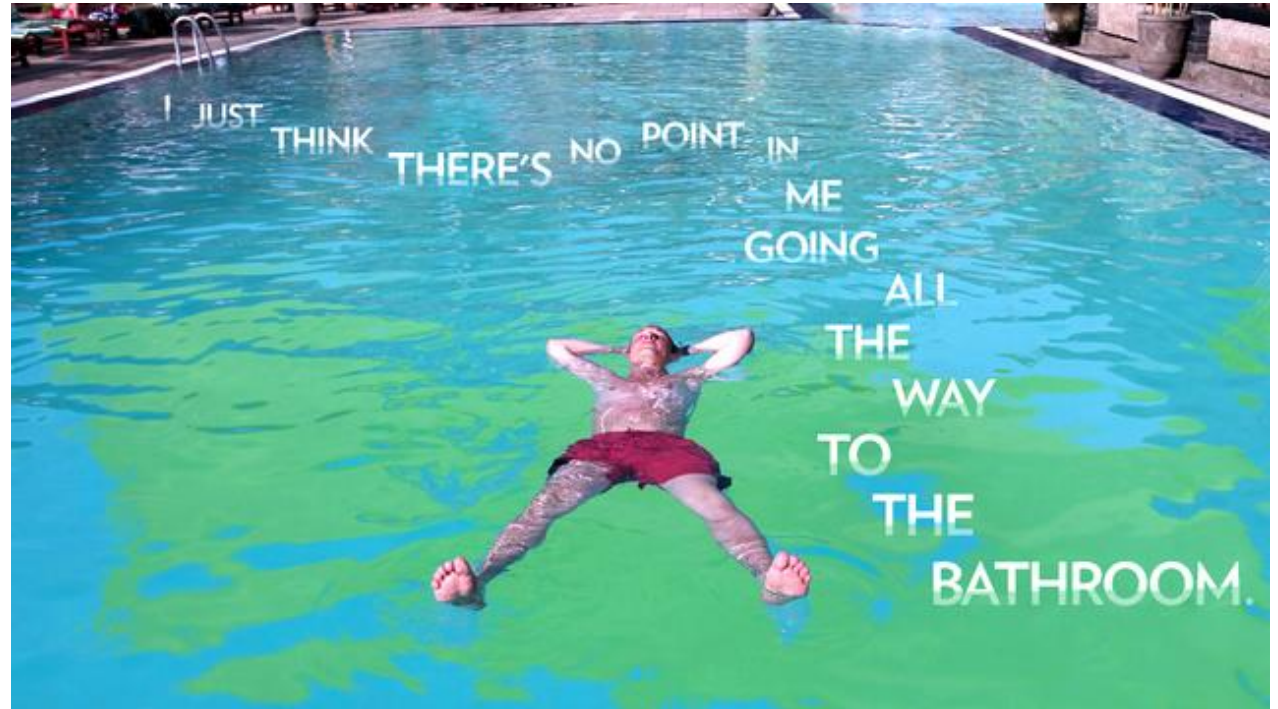




## Keeping in mind the urine specimen...

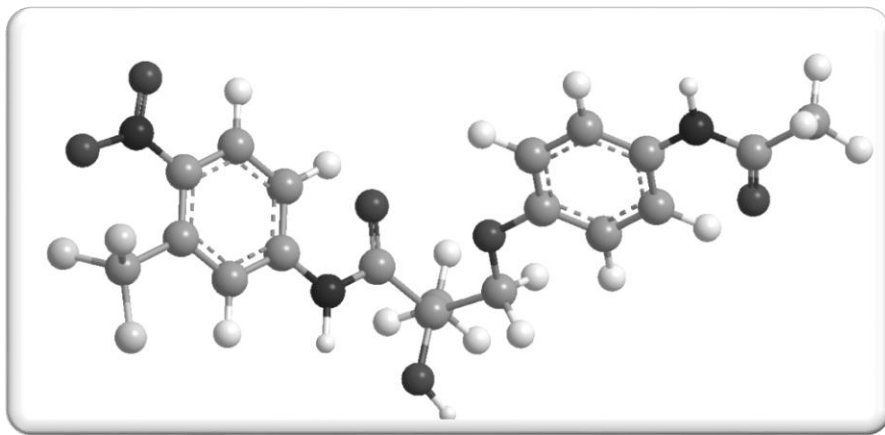








# Thank you



**Antidoping Switzerland**

**World Anti-Doping Agency (WADA)**

**Federal Ministry of the Interior (D)**





**Deutsche  
Sporthochschule Köln**  
German Sport University Cologne

**Institut für Biochemie**  
Institute of Biochemistry