

OVERCOMING BIOSAMPLING ISSUES IN SPORT DRUG TESTING: ANABOLIC STEROIDS IN DRIED URINES

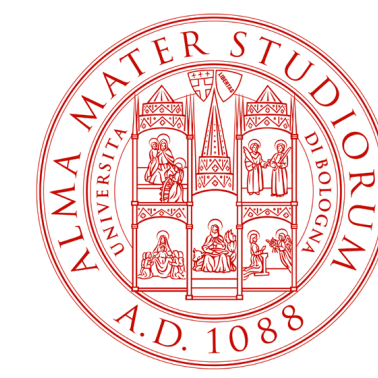
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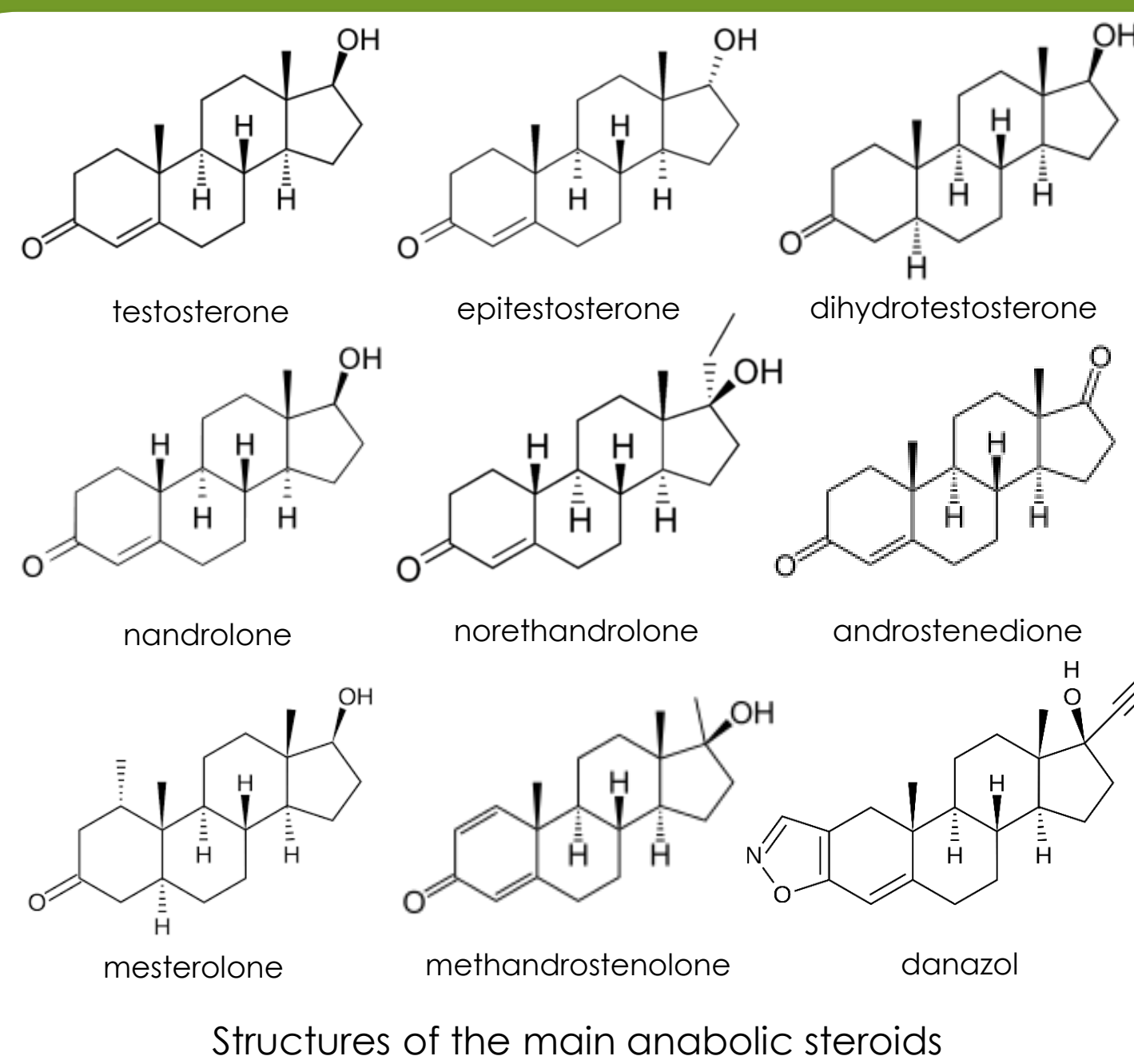


ALMA MATER STUDIORUM UNIVERSITA DI BOLOGNA

Commissione per la Vigilanza ed il controllo sul Doping E per la tutela della salute nelle attività sportive

Introduction

Anabolic steroids: the proper term for these compounds is anabolic-androgenic steroids. "Anabolic" refers to muscle building, and "androgenic" refers to increased male sex characteristics. Health care providers can prescribe steroids to treat hormonal issues, such as delayed puberty. Steroids can also treat diseases that cause muscle loss, such as cancer and AIDS. Moreover some athletes, and bodybuilders in particular, abuse these drugs to boost performance or improve their physical appearance. For these reason these compounds have been included in the list of prohibited substances in sports by the **World Anti-Doping Agency (WADA)**.



WADA Prohibited List Category S1. Anabolic agents: Prohibited substances at all times



From the International Standard for Testing and Investigations (ISTI) 2015:

"[...] Collection of blood samples in a manner that ensures:

- the Sample has not been manipulated, substituted, contaminated or otherwise tampered with in any way;
- the Sample is clearly and accurately identified;
- the Sample is securely sealed;
- the Sample is properly stored and dispatched in accordance with the relevant analytical guidelines"

From the International Standard for Laboratories (ISL) 2015:

"[...]"

- Analytical and Technical Processes
 - Receipt of Samples
 - Handling and retention of Samples
 - Sampling and preparation of aliquots for analysis
- Analytical Testing
- Results management"

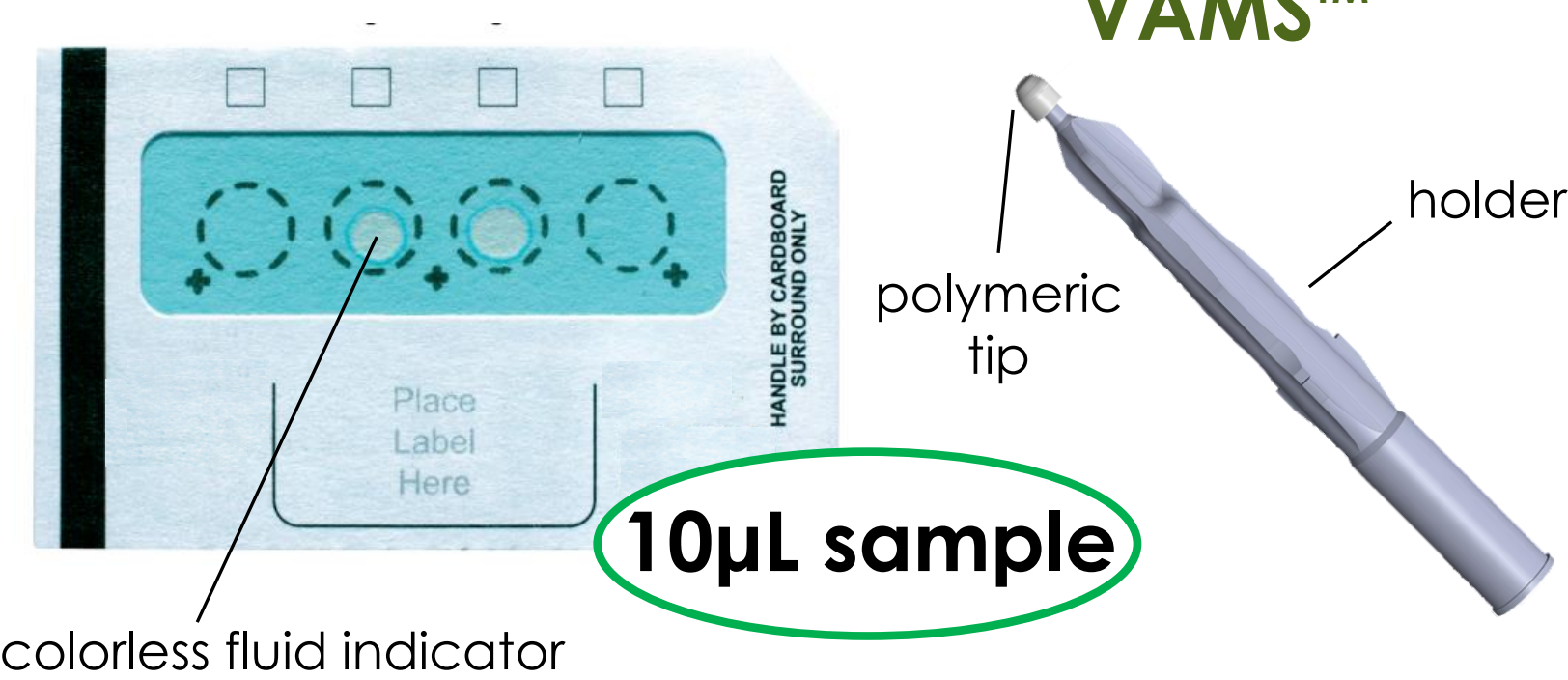
Question: How to

- Simplify procedures?
- Lower costs?
- Increase result reliability?
- Ease operator and athlete tasks?
- Shorten the time from sampling to results?
- Increase throughput?

Answer:

Dried urine microsampling approach

DRIED URINE SPOT VOLUMETRIC ABSORPTIVE MICRO SAMPLING



SAMPLING
↓
MICROSAMPLING



Features	CLASSIC IN-TUBE FLUID URINE	DRIED MATRIX SPOT	VOLUMETRIC ABSORPTIVE MICRO SAMPLING
Collection of a fixed volume of urine	✓	✓	✓
Easily stored and transported (without refrigerated needs)		✓	✓
Direct sampling approach with minimal training needs		✓	✓
No after-sampling processes (centrifugation, protein precipitation, L/L extraction, purification, spot excision)			✓
Sample stability towards enzymatic and bacterial activities		✓	✓
Cost and time saving		✓	✓
Easily automated		~	✓

LC-MS/MS

Stationary phase
• RP C18, 100x3.0 mm, 5µm

Mobile phase
• ACN/MeOH/H₂O + 0.25% Formic acid
• gradient elution

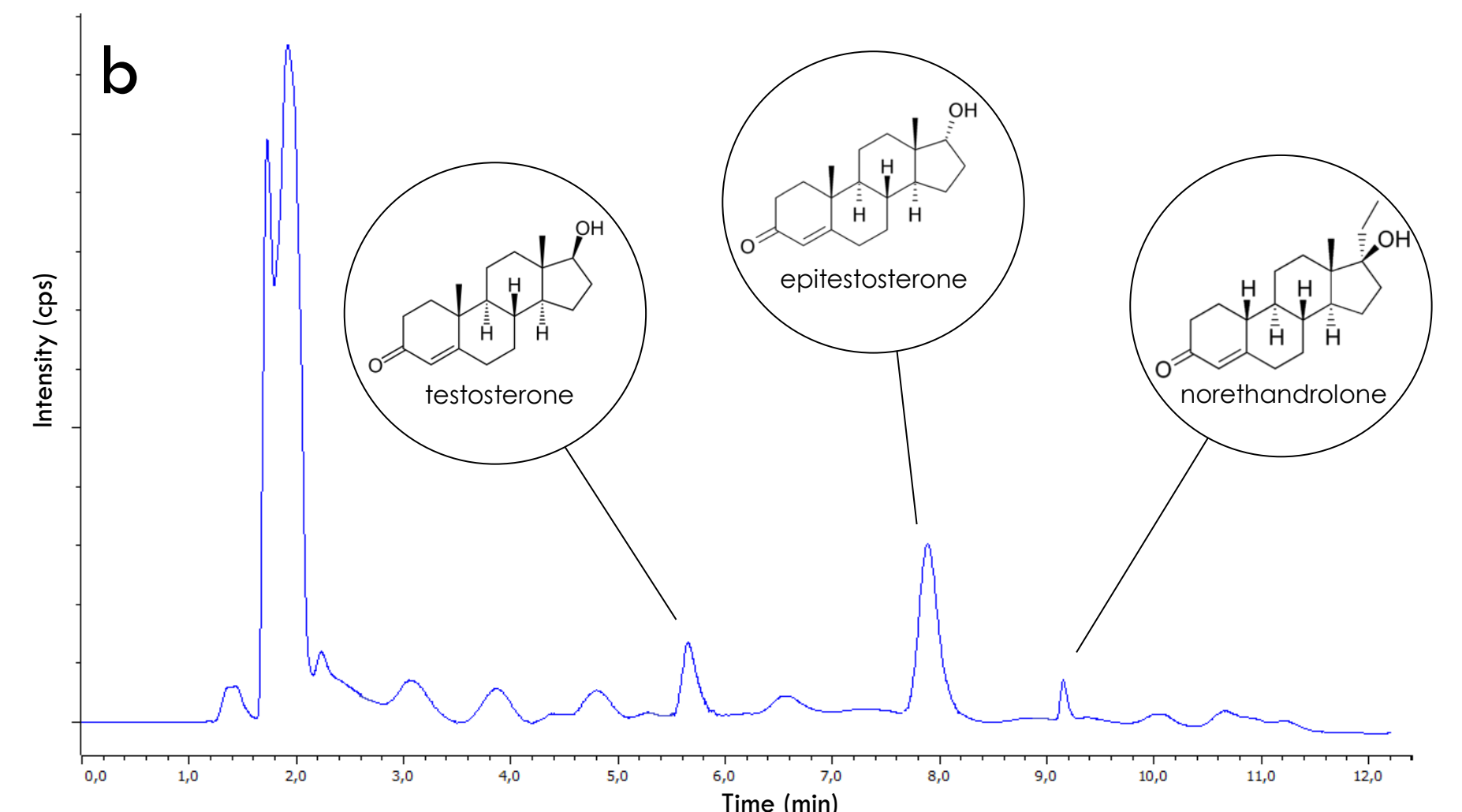
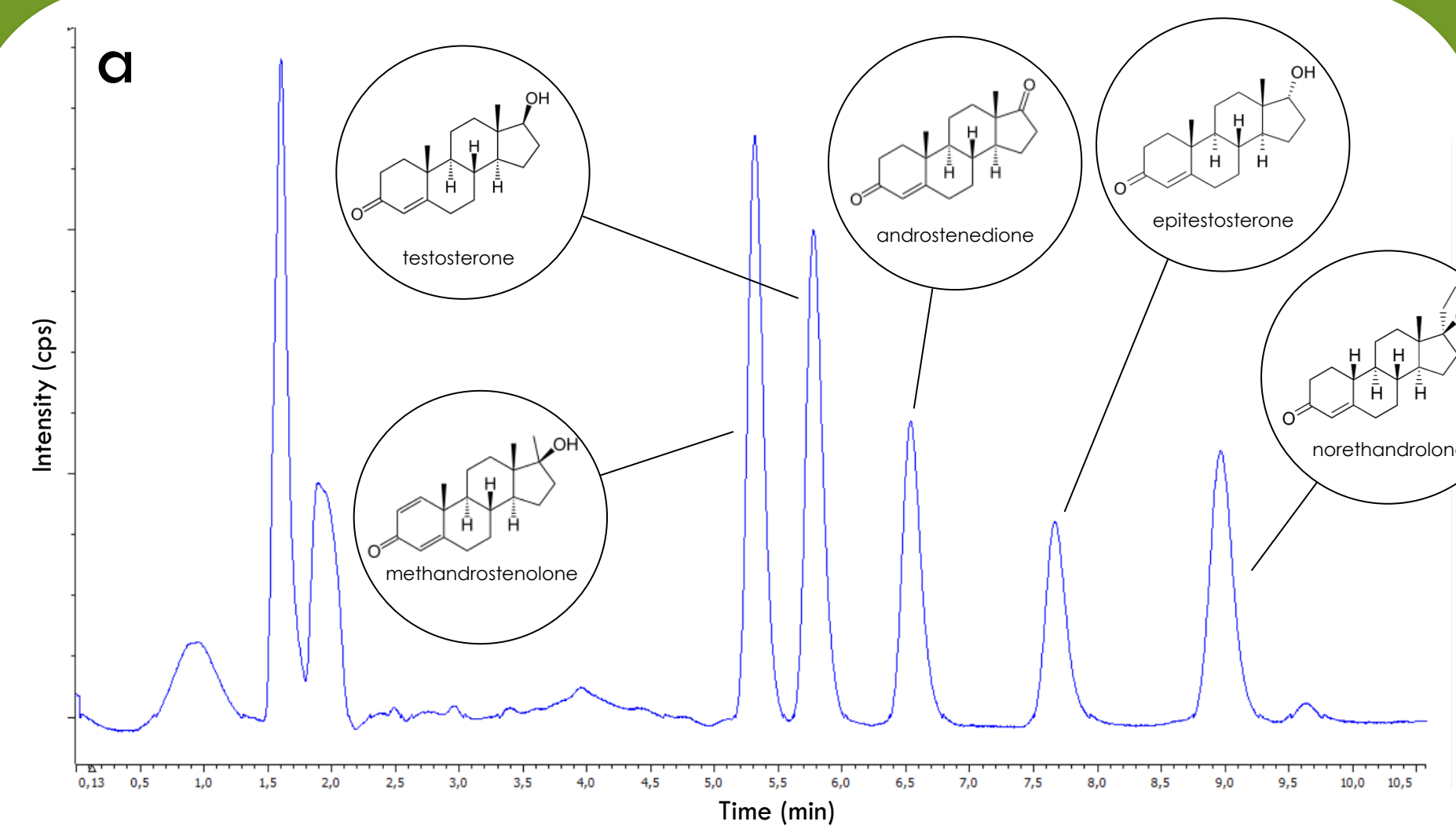
Mass Spectrometry
• Triple quadrupole
• ESI+, MRM
• Qualitative + quantitative m/z transitions
• deuterated ISS

Sensitivity
LOD = 0.2 ÷ 0.3 ng/mL
LOQ = 0.7 ÷ 0.8 ng/mL

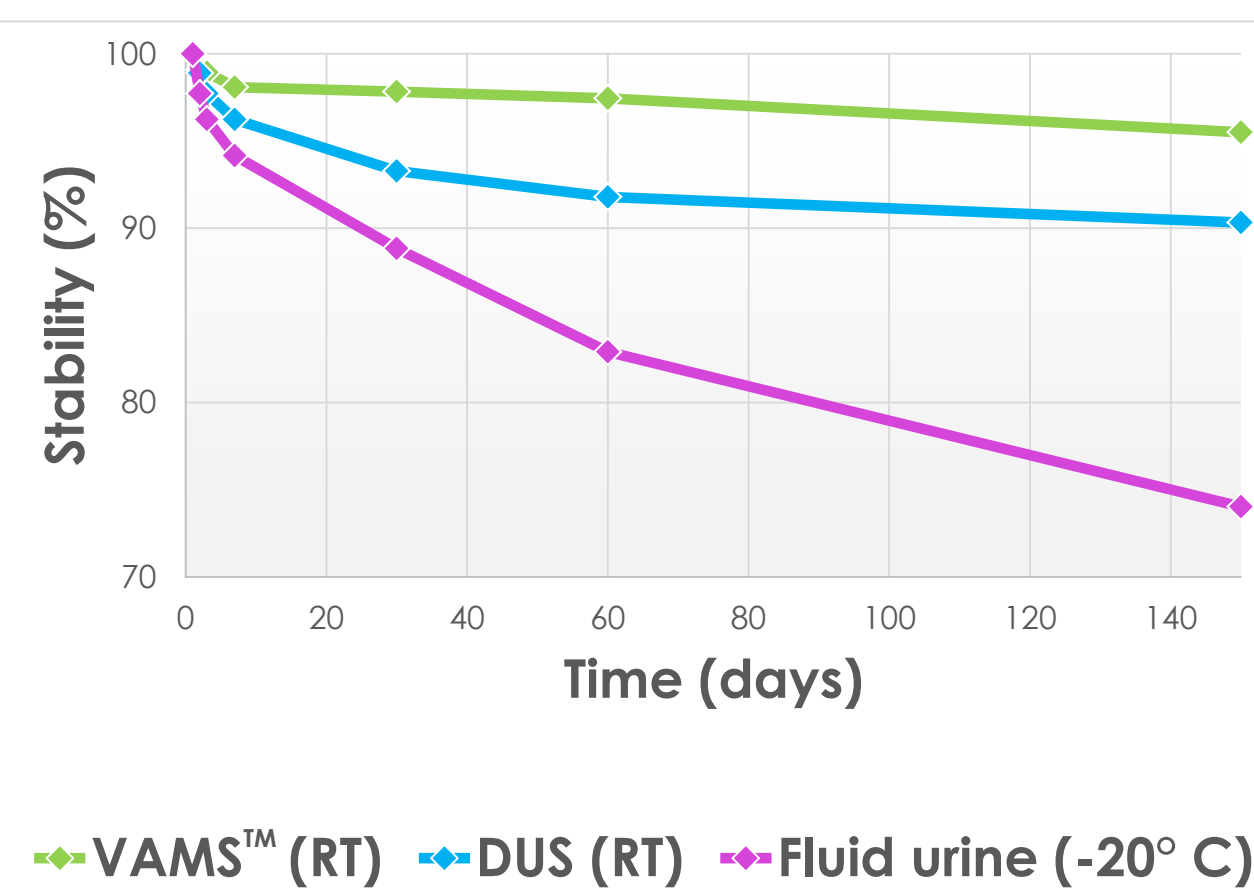
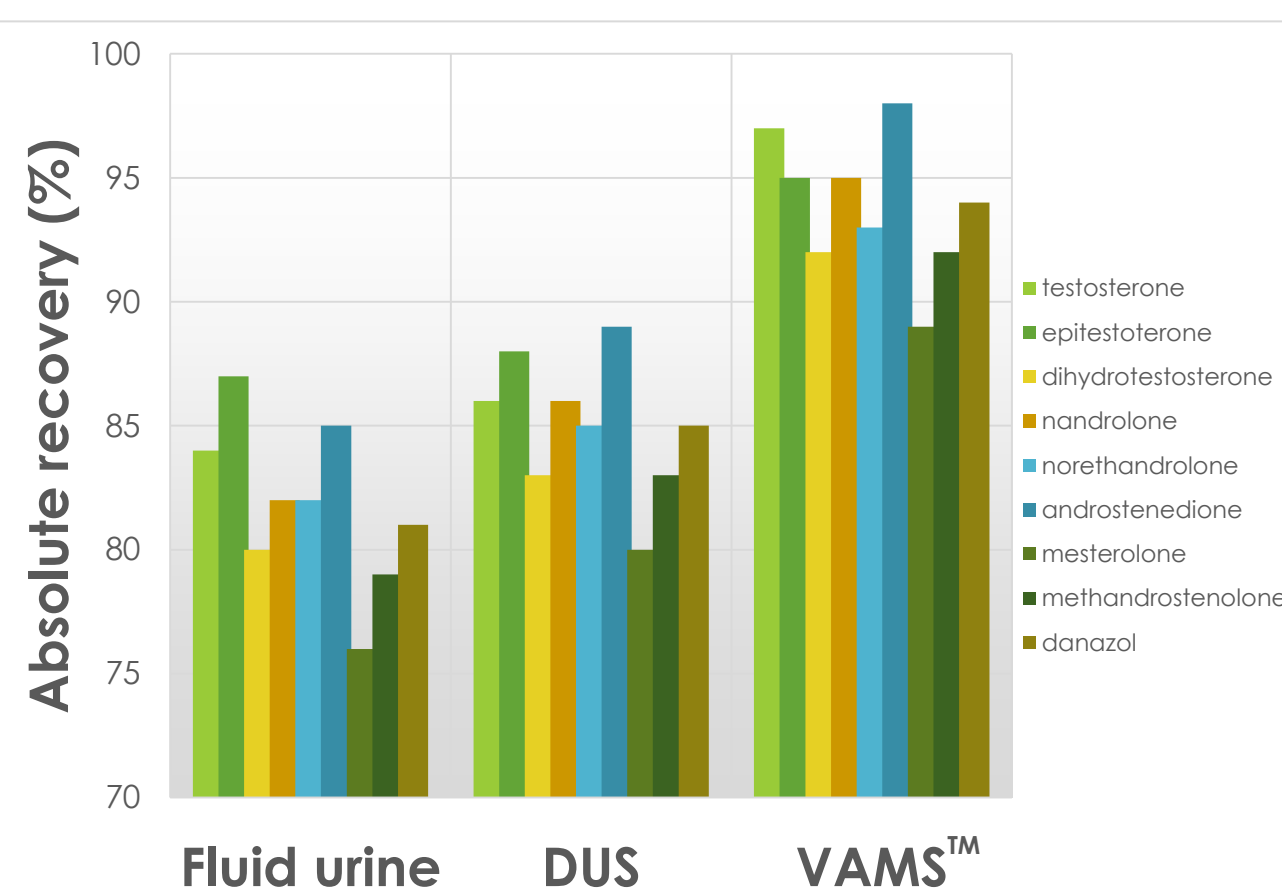
Linearity
0.8 – 250 ng/mL
r² > 0.9995

Precision
RSD interday < 9.7%
RSD intraday < 5.8%

Absolute recovery
DUS > 80% VAMS™ > 89%



LC-MS/MS chromatograms in total ion current (TIC) mode of a) A DUS sample spiked with 10 ng/mL of some representative anabolic steroids and b) a VAMS™ sample from a steroid user



Absolute recovery (a) and long-term stability (b) assays performed on fluid urine, DUS and VAMS™ samples spiked with anabolic steroids at a concentration of 10 ng/mL

Conclusion

Simple but reliable protocols have been developed and validated for the collection of dried urine microvolumes, unlikely to be tampered but transportable and storable at room temperature, aimed at performing screening tests and targeted analysis according to the World Anti-Doping Code guidelines. These protocols would substantially reduce overall analysis costs, allowing their application not only to elite athletes, but also to amateurs in local laboratories.

Acknowledgements

This research has been funded by and carried out in collaboration with Commission for the Monitoring and Control of Doping and the Protection of Health in Sporting Activities, Italian Ministry of Health, project "Innovative sampling and analysis strategies for an effective doping control" (CUP J52114001600005), Principal Investigator: Dr. Laura Mercolini, Laboratory of Pharmacotoxicological Analysis, Alma Mater Studiorum - University of Bologna. laura.mercolini@unibo.it