Testing Androgenic Anabolic Steroids in Oral Fluid using Turbulent Flow Orbitrap Mass Spectrometry

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Outline

- Background in AAS testing OF
- Details of Method
- Details of Current Study
- Future Plans

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Developments with Western Slope Laboratory and beyond….

BACKGROUND IN ANABOLIC ANDROGENIC STEROID TESTING IN ORAL FLUID

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Foundation in Oral Fluid

- Western Slope Laboratory was founded on oral fluid technologies and have continued down that path
  - Test for hundreds of analytes in oral fluid
  - Same catalogue as urine analytes
  - Low limits of detection and quantification

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Oral Fluid-Matrix of the Future?

- Most toxicology laboratories now offer some testing with the oral fluid matrix
- BUT, there are not any commercial laboratories that offer oral fluid steroid testing….Western Slope Laboratory sought to develop the method

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DETAILS OF METHODOLOGY

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Goal of testing for steroids:

- For forensics: low limits of detection for as many compounds and their analogues
- For post mortem: large dynamic range for as many compounds and their analogues
- For clinical: low limits of detection for a specific group of compounds
- For doping: large dynamic range for a specific group of compounds

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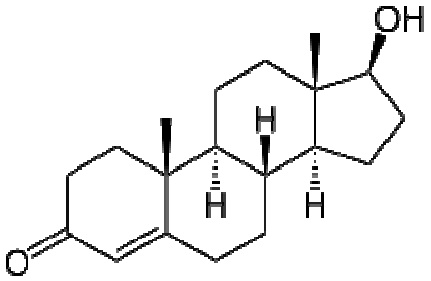
The Analytes



- Androgenic anabolic steroids are hormones that mimic testosterone
  - They are lipids, but much different structure than the classical lipid
  - they are non-polar

  ∴ Need solvent based sample preparation probably SPE and high organic in mobile phases

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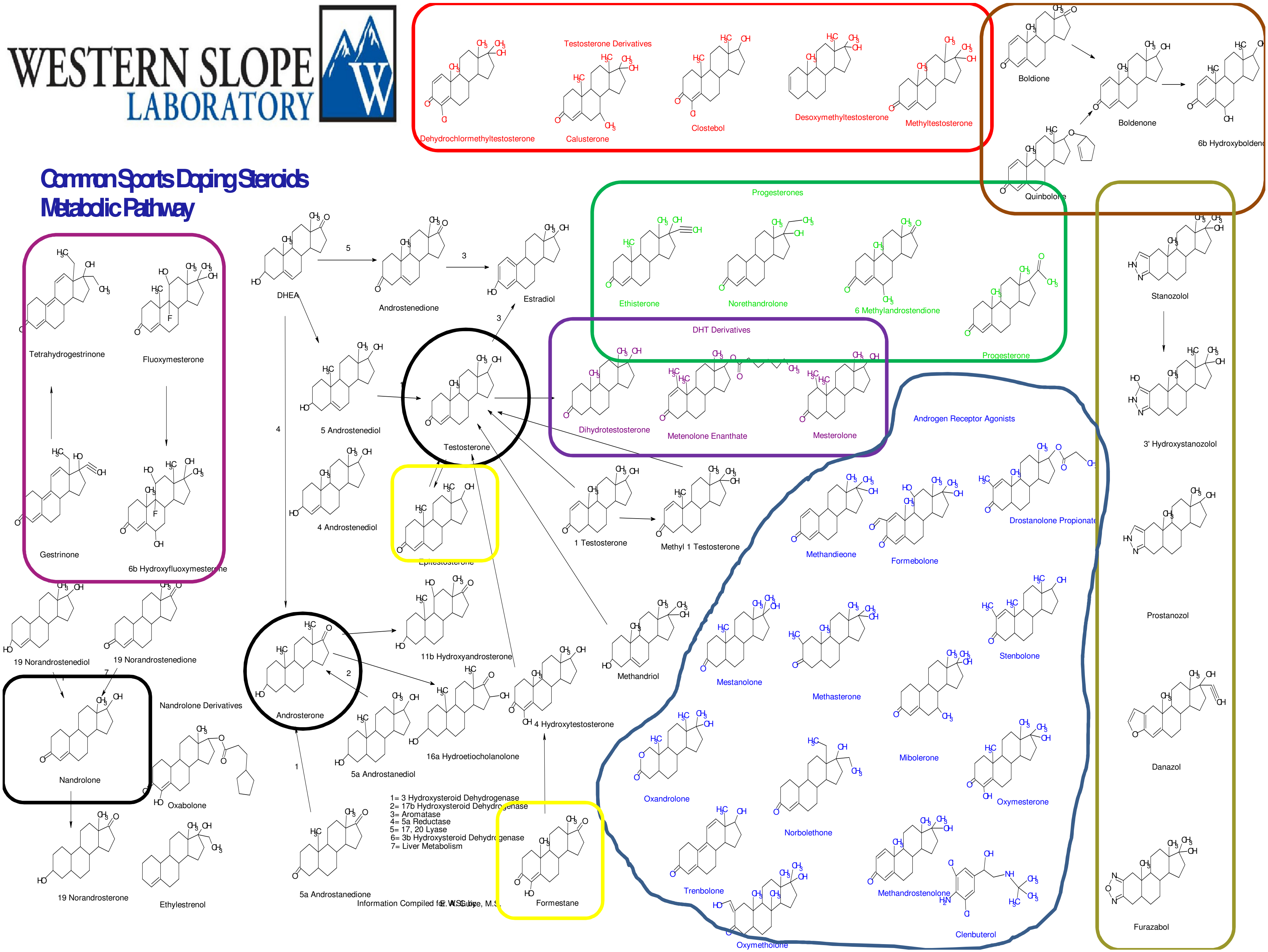
Common Sports Doping Steroids Metabolic Pathway



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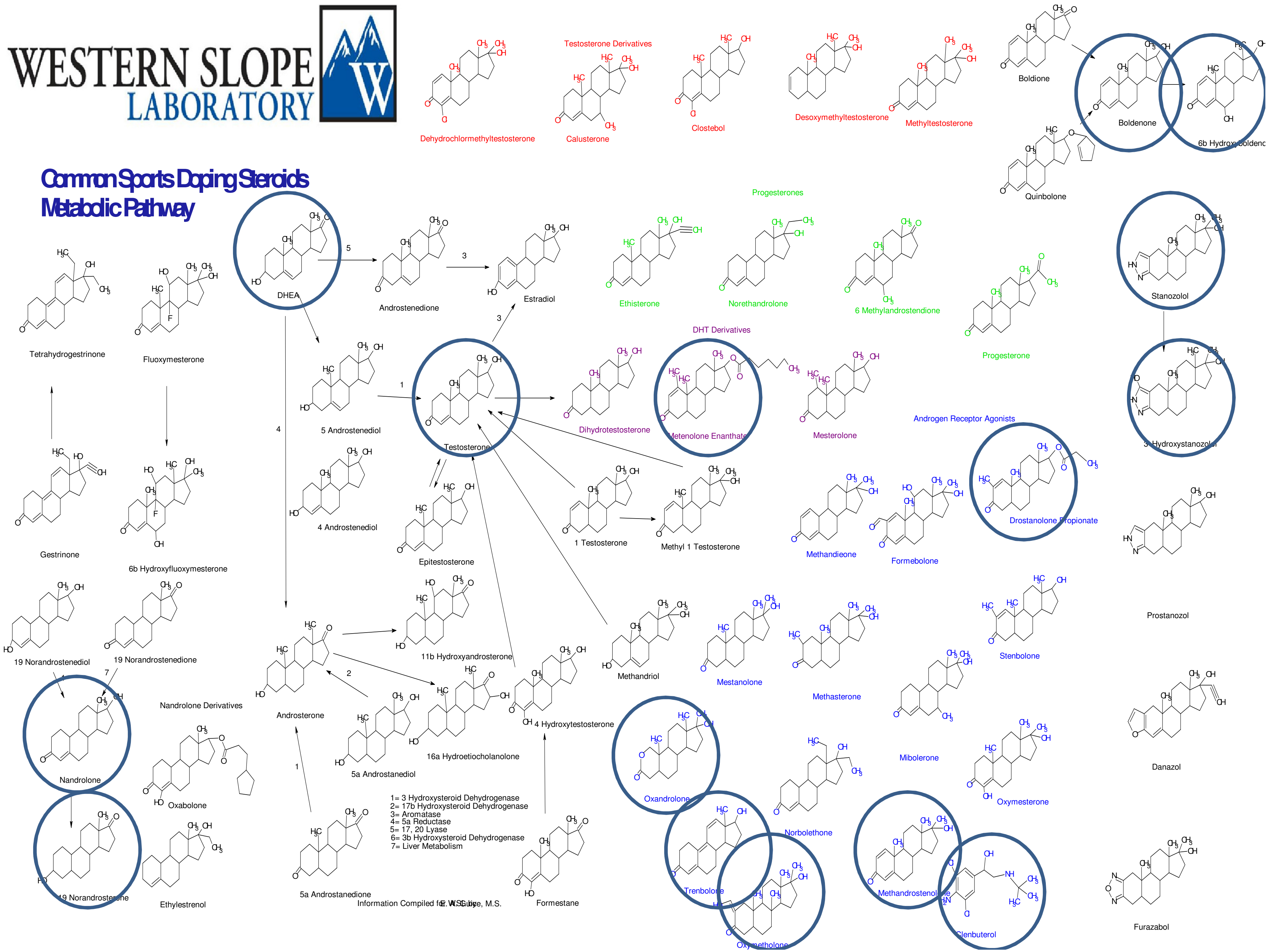
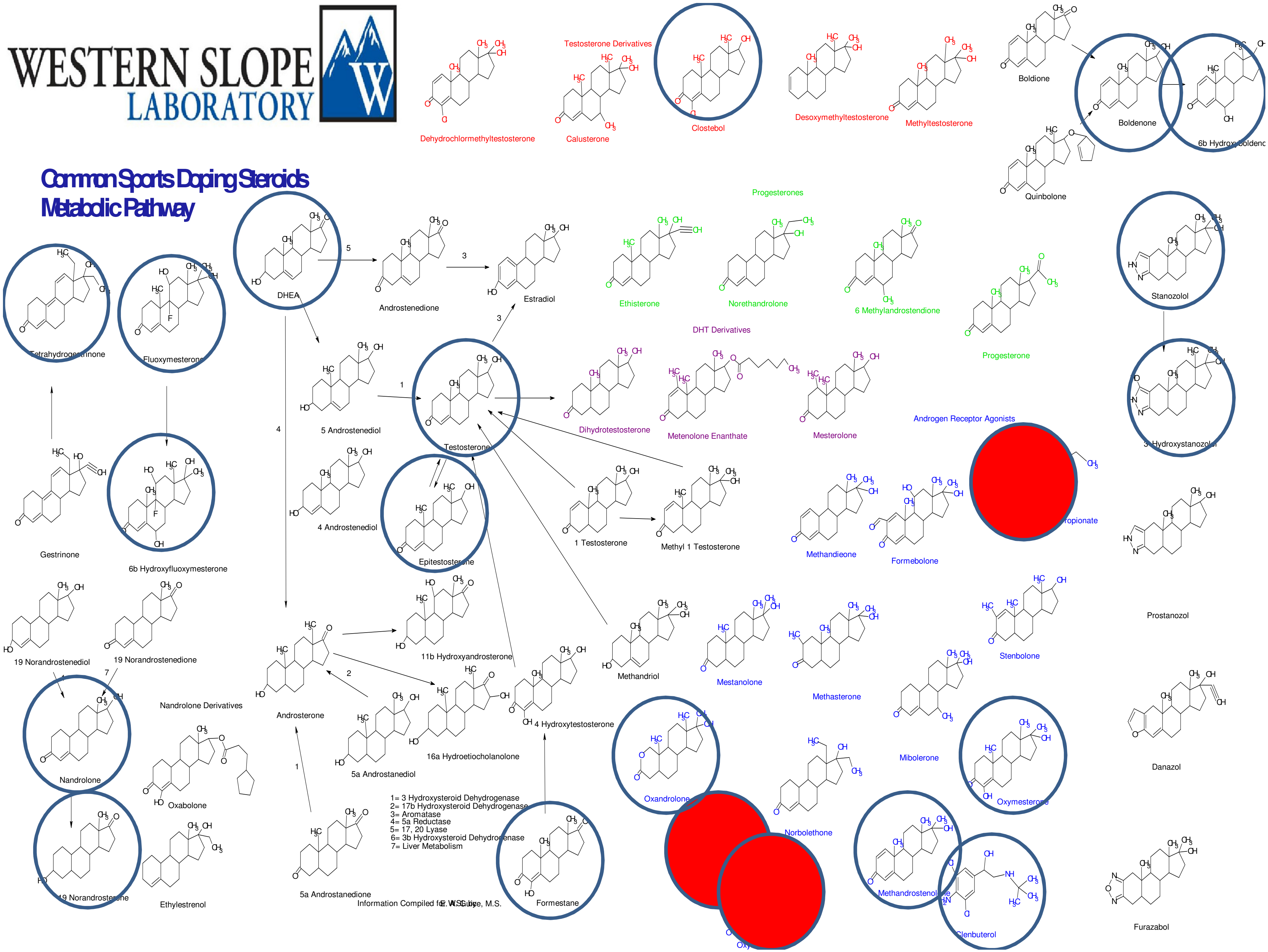
Common Sports Doping Steroids Metabolic Pathway



Common Sports Doping Steroids Metabolic Pathway



Why Liquid Chromatography…

- Derivatization process can be quite lengthy and often times irreproducible. In addition, this process does not generally yield high conversion of the steroid to the derivatized product

∴ Liquid can reduce time and sample manipulation, while still allowing for accurate analysis

∴ We can take advantage of online sample extraction systems i.e. turbulent flow

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Why Turbulent Flow Online Extraction?

- Reduced ion suppression
- Eliminate the need for extensive sample clean-up:
  - Saving time, money, and people time
  - Very versatile
    - Urine
    - Saliva
    - Plasma

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Instrument requirements with steroids

- Resolution of structurally similar compounds, either by chromatography, mass, fragmentation, or combination
- Sensitivity to detect as low as femtogram amounts on column; quantify picogram per milliliter solutions
- Dynamic range required for industry
- Ability to interface with online sample extraction

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LC/MS vs LC/MS/MS vs LC/HR-TOF or MS

SIM vs QQQ

- Single Ion Monitoring would require the separation of isobars through chromatography
- QQQ would require the use of Ion Ratios to determine of specific compounds (isobars)

TOF vs Orbitrap

- Both can use CID to provide fragment information
- Also use the full scan spectrum for retention time verification
- Biggest difference here is dynamic range and spatial/temporal association of the chromatographic data

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Which instrument meets the needs?

	SIM (LC/MS)	QQQ (LC/MS/MS)	Q-TOF (LC/HR-TOF)	Orbitrap (LC/HR-MS)
Resolution of structurally similar compounds	✓	✓	✓	✓
Sensitivity for low limits of detection		✓	✓	✓
Dynamic range requirements met	✓		✓	✓
Able to do online extraction			Maybe	✓

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The Column: The Other “Instrument”

- With a turbulent flow-liquid chromatography system set-up, there are two columns selections that have a bearing on the results of the method:
  - The turbulent flow column
  - The analytical column
- Column selection is affected by the pH and the additives used.

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The Turbulent Flow Column

- There are several chemistries that can be employed including:
  - C8, C18, Fluoro, Phenyl, and C2
- There are 0.5 and 1.0 mm diameter columns available as well; the lengths are all standard

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The Analytical Column

- As usual with any chromatographic method, you have to select the appropriate analytical column chemistry, length, diameter, bead size, and porousity
- Cation/Anion exchange can also be utilized depending on the chemistry selected

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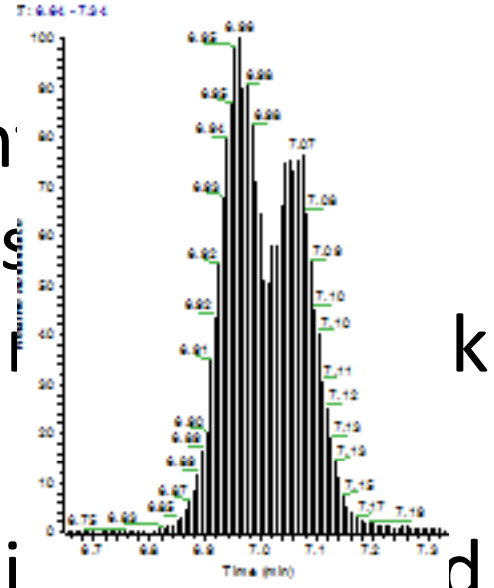
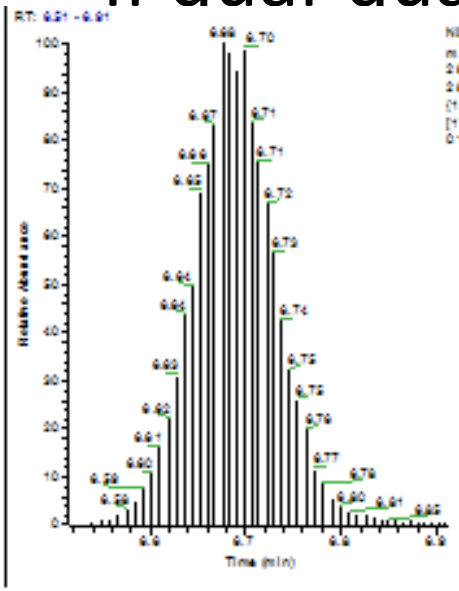
Scans across the curve

- In order to quantify the amoun
  steroid(s) present in any given s
  appropriate number of scans u k
  is required.
- If qual-quan peaks are used, it i d
  have the appropriate amount of





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Why human growth hormone

DETAILS OF CURRENT STUDY

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Where you are vs. Where you want to go

- We are able to detect endogenous and exogenous compounds in urine and oral fluid using the aforementioned method
- But one major group of compounds needed to be considered: Hormones and Growth Factors
  - Insulin-like Growth Factor-1 (Deer Antler Spray)
  - Somatotrophin (GH Male/Vermont Maxi)

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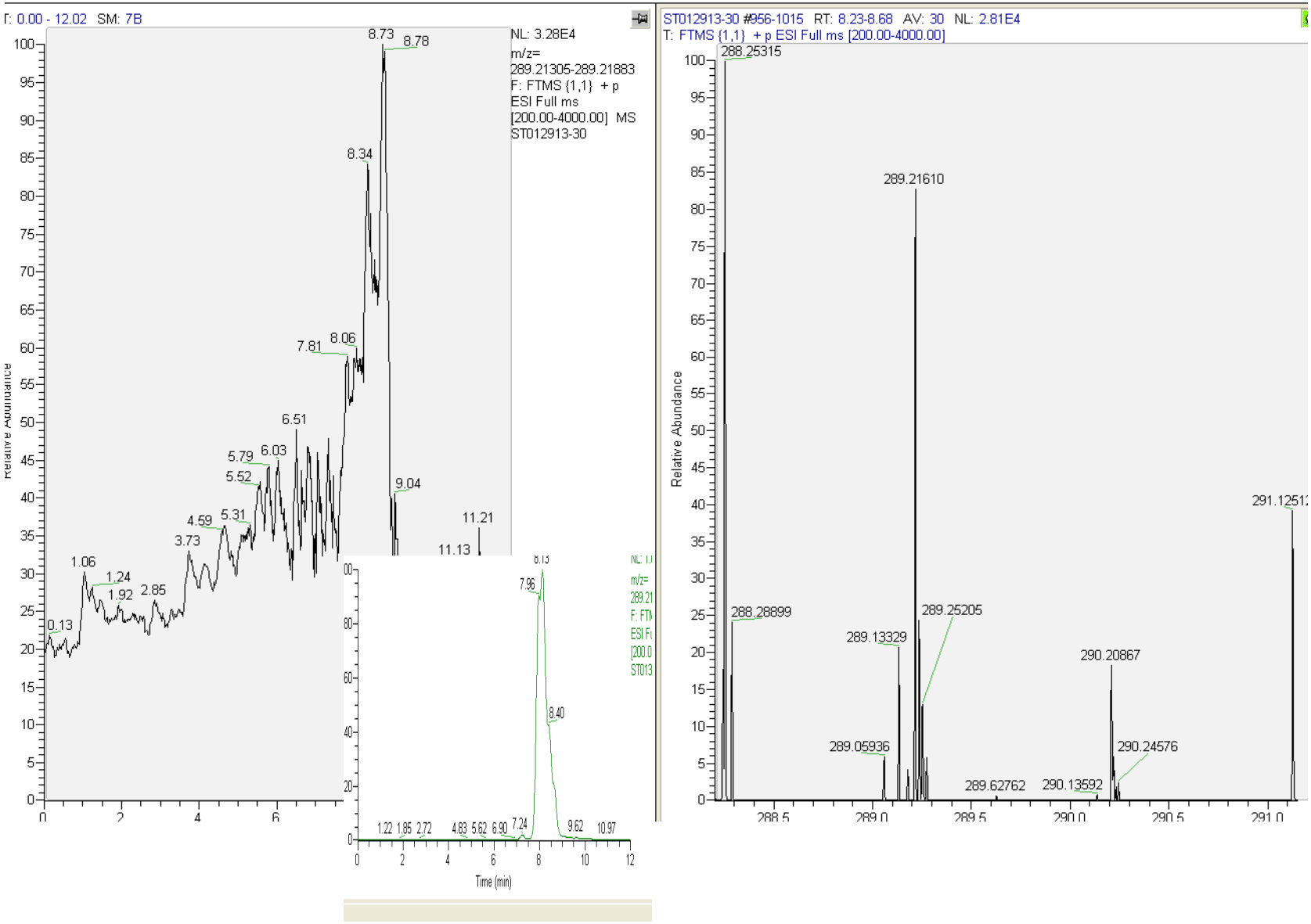
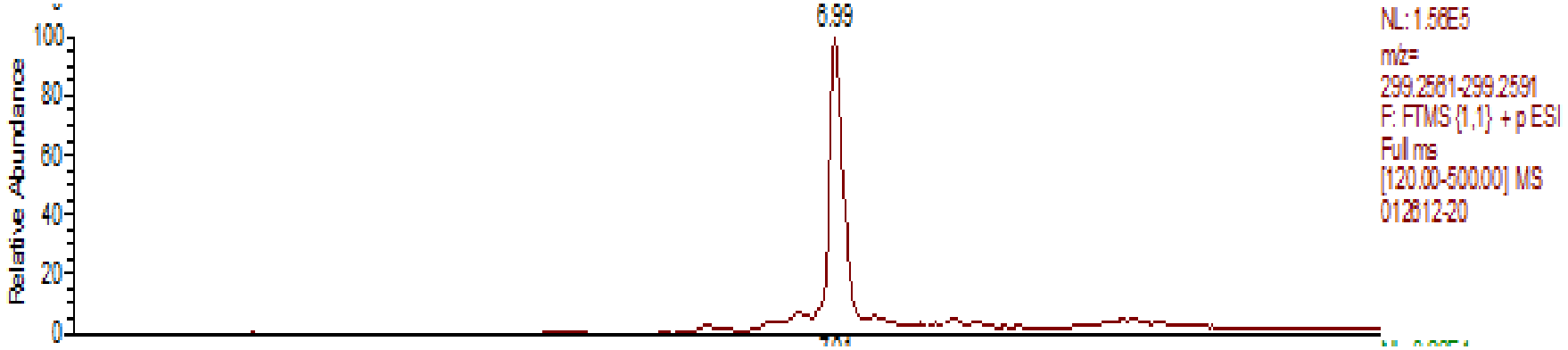
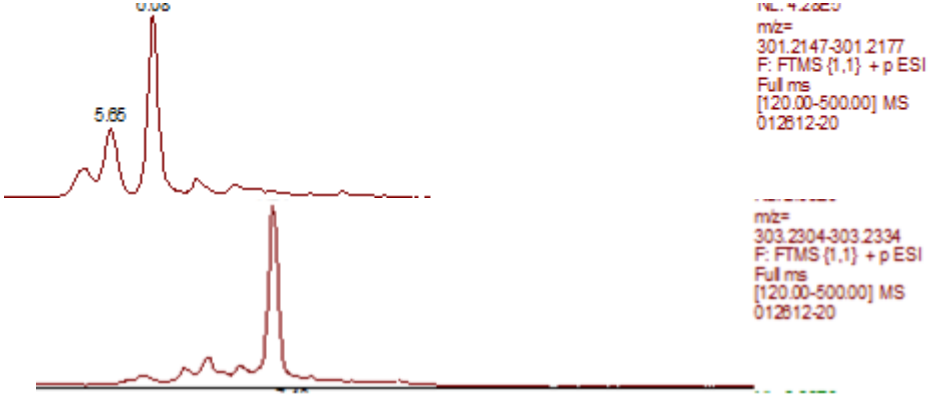
Targeted OF Sample



Untargeted OF Sample

- $C_{20}H_{26}O_2$ formula was given was a calculated mass of 298.193268 m/z of 299.25757
- Match to Norethindrone was given
- Donor questioned and admitted taking compound as birth control





The compound and its major metabolites were found in the sample.

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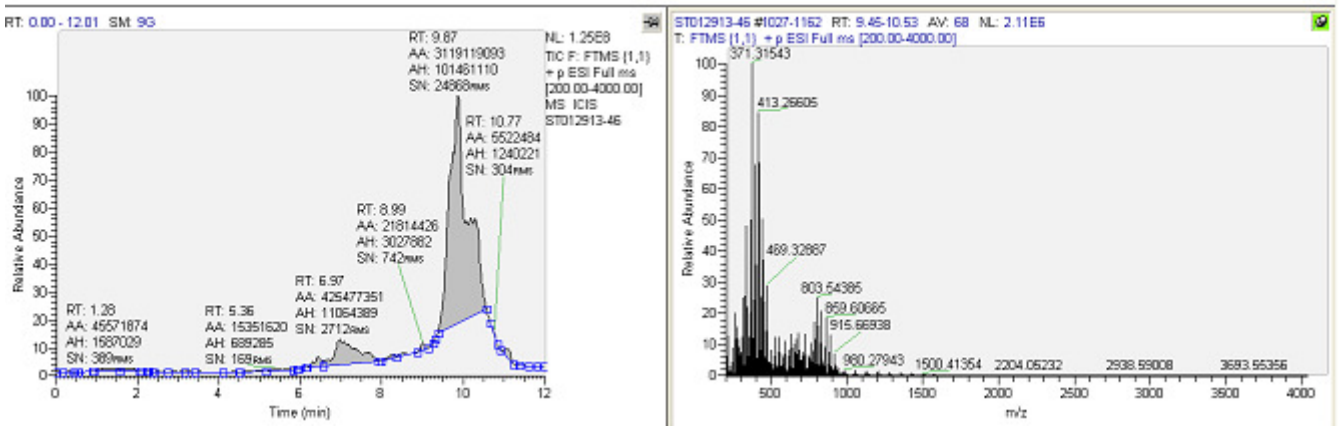
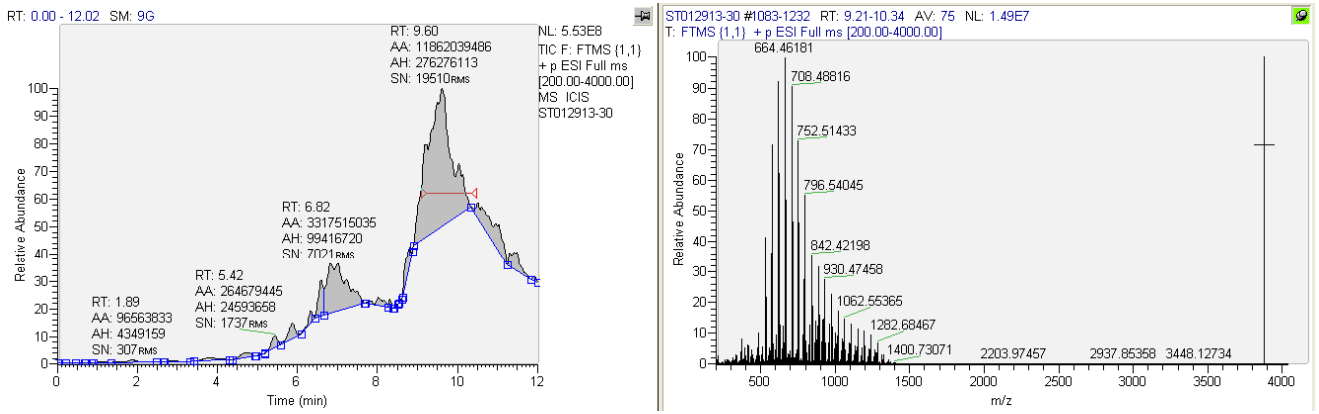
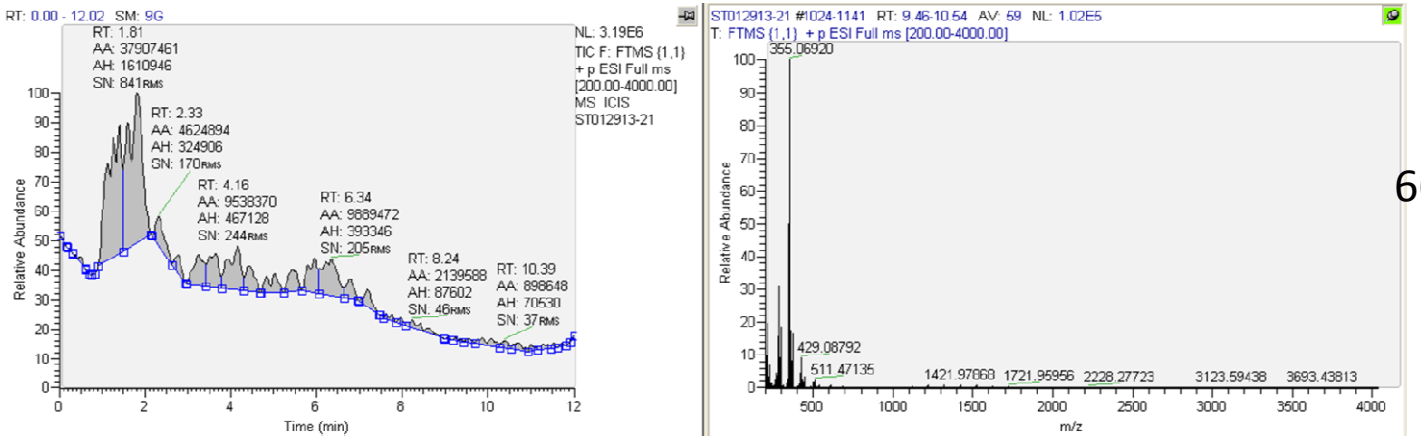


Standard



1 yr Female Donor



66 yr Female Donor

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FUTURE PLANS

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Future Plans

- Conduct a clinical trial:
  - Goal is to include a number of children/adolescents
  - Also want to recruit persons currently taking hGH recreationally (and other AASs)
- Try to detect the parent (22kDa) hGH compound
- Look to detect the difference between endogenous and exogenous hGH (preferably by size)

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  - Susan DiPietro
  - Subodh Nimkar

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Thank you for your attention

References

- Guice (2012) SOFT Workshop #4 *Method Development for Testing Androgenic Anabolic Steroids in Oral Fluid and Urine using Turbulent Flow Orbitrap MS*
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