



**Universiteit  
Leiden**  
The Netherlands

## **Recent Advances in LC-MS/MS Analysis of Ancient Hormones**

Schrader, S.A.; Brewster, K.; Hall, R.; Giera, M.; Sánchez-López, E.

### **Citation**

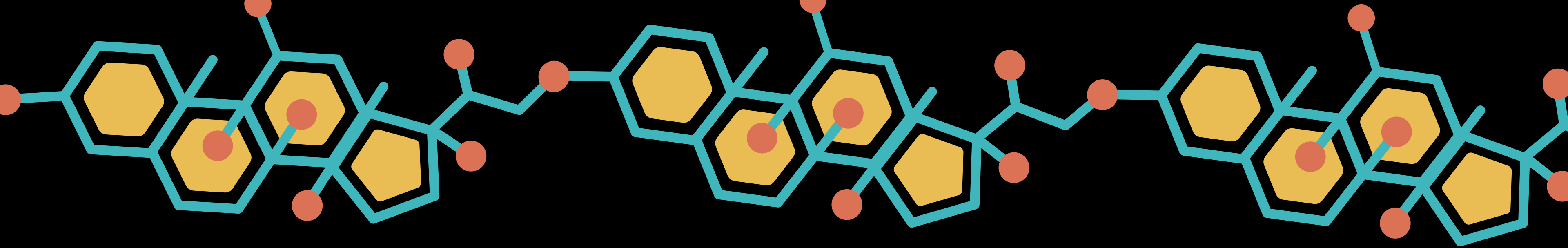
Schrader, S. A., Brewster, K., Hall, R., Giera, M., & Sánchez-López, E. (2024). Recent Advances in LC-MS/MS Analysis of Ancient Hormones. In . doi:10.5281/zenodo.14917697

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# RECENT ADVANCES IN LC-MS/MS ANALYSIS OF ANCIENT HORMONES

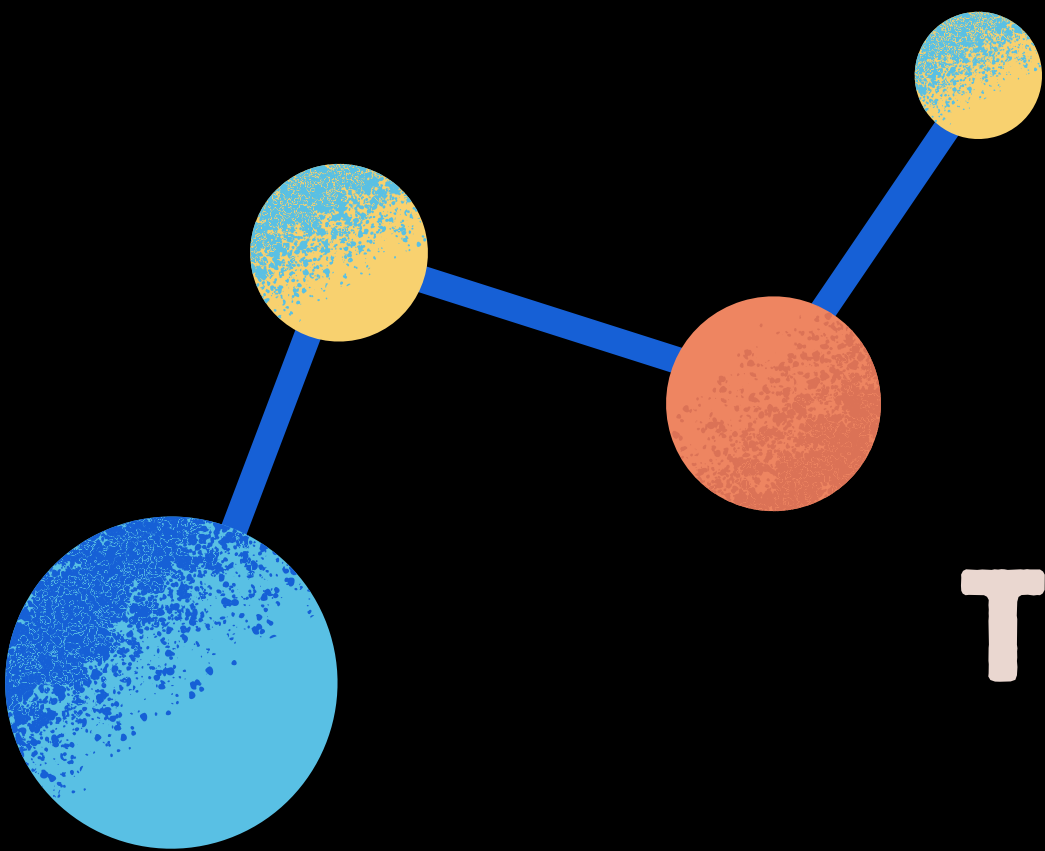
Sarah Schrader,<sup>1</sup> Kevin Brewster,<sup>2</sup> Rachael  
Hall,<sup>1</sup> Martin Giera,<sup>2</sup> Elena Sánchez-López<sup>2</sup>

<sup>1</sup> Faculty of Archaeology, Leiden University

<sup>2</sup> Center for Proteomics and Metabolomics, Leiden University Medical Center

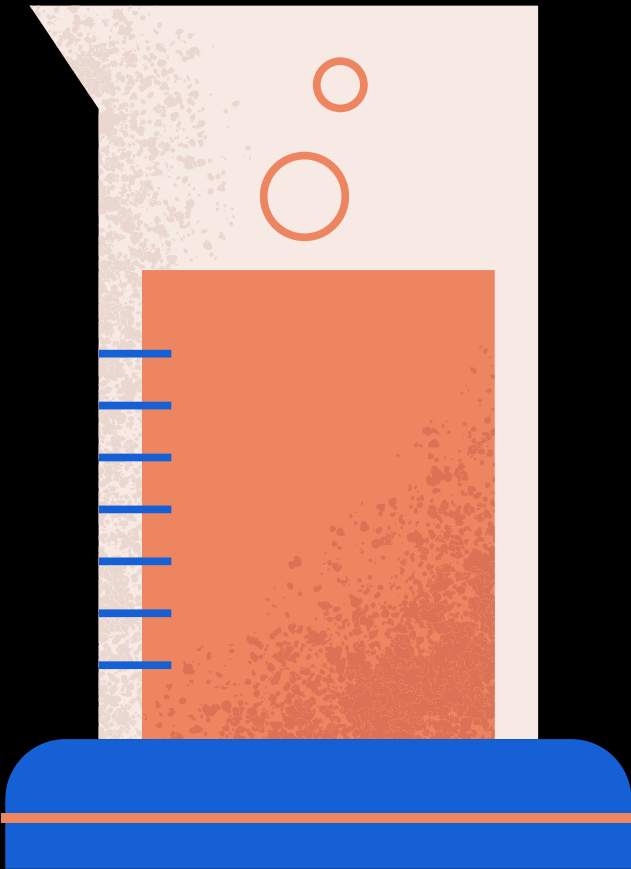






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03	Materials & Methods
04	Results
05	Summary
06	Next Steps



# PREVIOUS RESEARCH

- Hair
- Dentine

Contents lists available at [ScienceDirect](#)

 **Journal of Archaeological Science**  
journal homepage: <http://www.elsevier.com/locate/jas> 

Assessing individual systemic stress through cortisol analysis of archaeological hair

Emily Webb<sup>a,\*</sup>, Steven Thomson<sup>b</sup>, Andrew Nelson<sup>a</sup>, Christine White<sup>a</sup>, Gideon Koren<sup>b,c,d,h</sup>, Michael Rieder<sup>b,c,d,e,f,g</sup>, Stan Van Uum<sup>c,e</sup>

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**BRIEF COMMUNICATION**  **WILEY**

Desperately seeking stress: A pilot study of cortisol in archaeological tooth structures

Leslie Quade<sup>1</sup>  | Paul L. Chazot<sup>2</sup> | Rebecca Gowland<sup>1</sup> 

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Detection of cortisol, estradiol, and testosterone in archaeological human hair from the Dakhleh Oasis, Egypt

Elisha Tisdale<sup>a,\*</sup>, Lana Williams<sup>a</sup>, John J. Schultz<sup>a,b</sup>, Sandra M. Wheeler<sup>a</sup> 

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## **LC-MS/MS**

Higher sensitivity, with accuracy, reliability, and precision measurements

# **THE EVOLUTION OF STRESS**





# THE EVOLUTION OF STRESS



## **LC-MS/MS**

Higher sensitivity, with accuracy, reliability, and precision measurements

## **Multi-Tissue & Lifecourse Approach**

Bone, hair, dentine, fingernails



# THE EVOLUTION OF STRESS



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## **Inter-/Intra- Individual Variation**

Assessing relationship between tissues AND individuals



# THE EVOLUTION OF STRESS



## **LC-MS/MS**

Higher sensitivity, with accuracy, reliability, and precision measurements

## **Multi-Tissue & Lifecourse Approach**

Bone, hair, dentine, fingernails

## **Inter-/Intra- Individual Variation**

Assessing relationship between tissues AND individuals

## **Population Variation**

Studying how steroids differ between populations





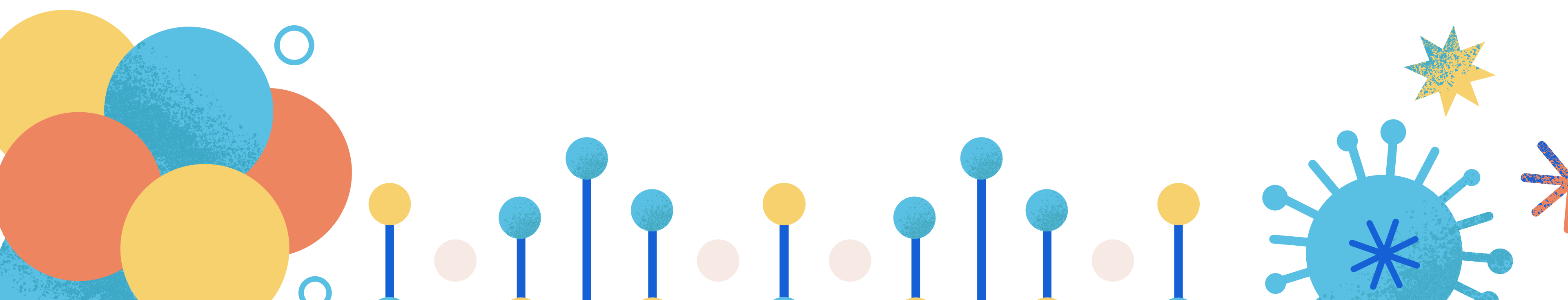
# MATERIALS, METHODS & ETHICS

## Arnhem

Post-medieval (1626-1850 CE) Dutch collection  
Permission for destructive analysis was provided  
by the local community and ethical clearance  
was granted from Leiden University.

## Sex/Age

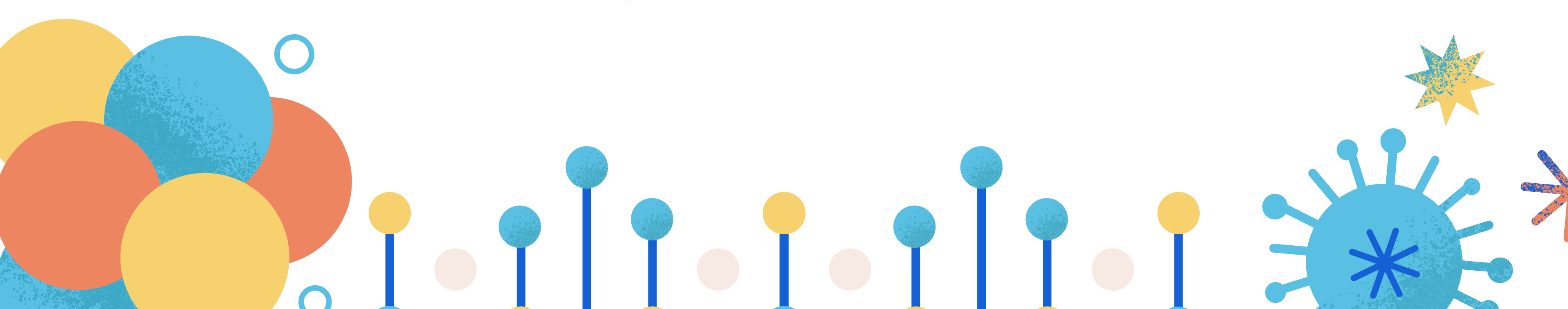
Standard pelvic and cranial traits were  
used for the estimation of sex and age.



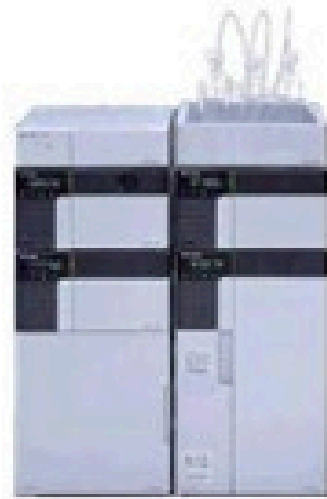


# MATERIALS, METHODS & ETHICS

## Steroid Extraction

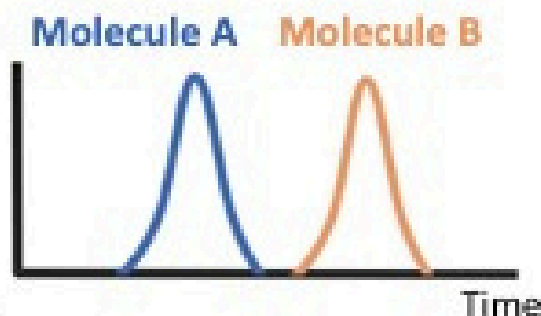
1. HCl: 200mg, demineralize in 100mL 0.01 N HCl (Longin, 1971), 1mL MeOH (Van Uum et al., 2008)
  2. EDTA: 200mg, demineralize in 10% EDTA (Tuross, 2012), 1mL MeOH
  3. Ground Bone: 200 mg, 1mL MeOH
  4. MeOH: 1mL MeOH (Charapata et al., 2018)
- 

## Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS analysis)



**Liquid chromatography (LC) from Shimadzu**

Molecules are separated based on chemical properties (polarity) in a chromatographic column



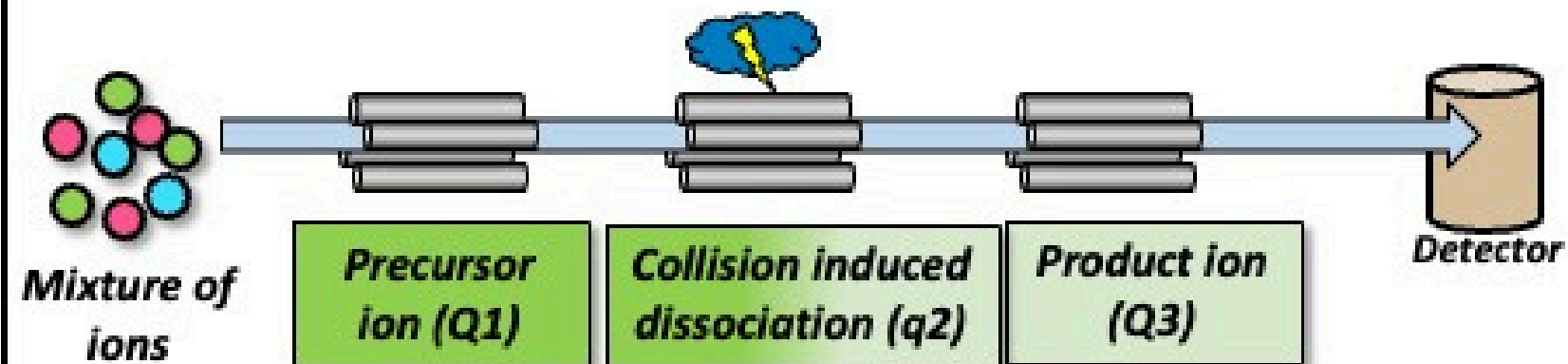
+



**Mass spectrometer (MS) with a Triple Quadrupole (QqQ) analyzer (QTrap 6600+ from Sciex)**

Molecules coming from LC (liquid phase) are **ionized (gas phase)** in the MS. A combination of **three quadrupoles** leads to:


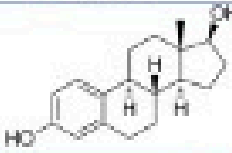

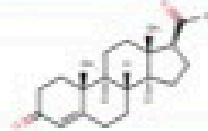



- **Isolation** of each molecule based on its mass-to-charge ratio ( $m/z$ ) (in Q1)
- **Fragmentation** (in q2), and
- **Isolation of a specific fragment** coming from the parent ion (Q3)



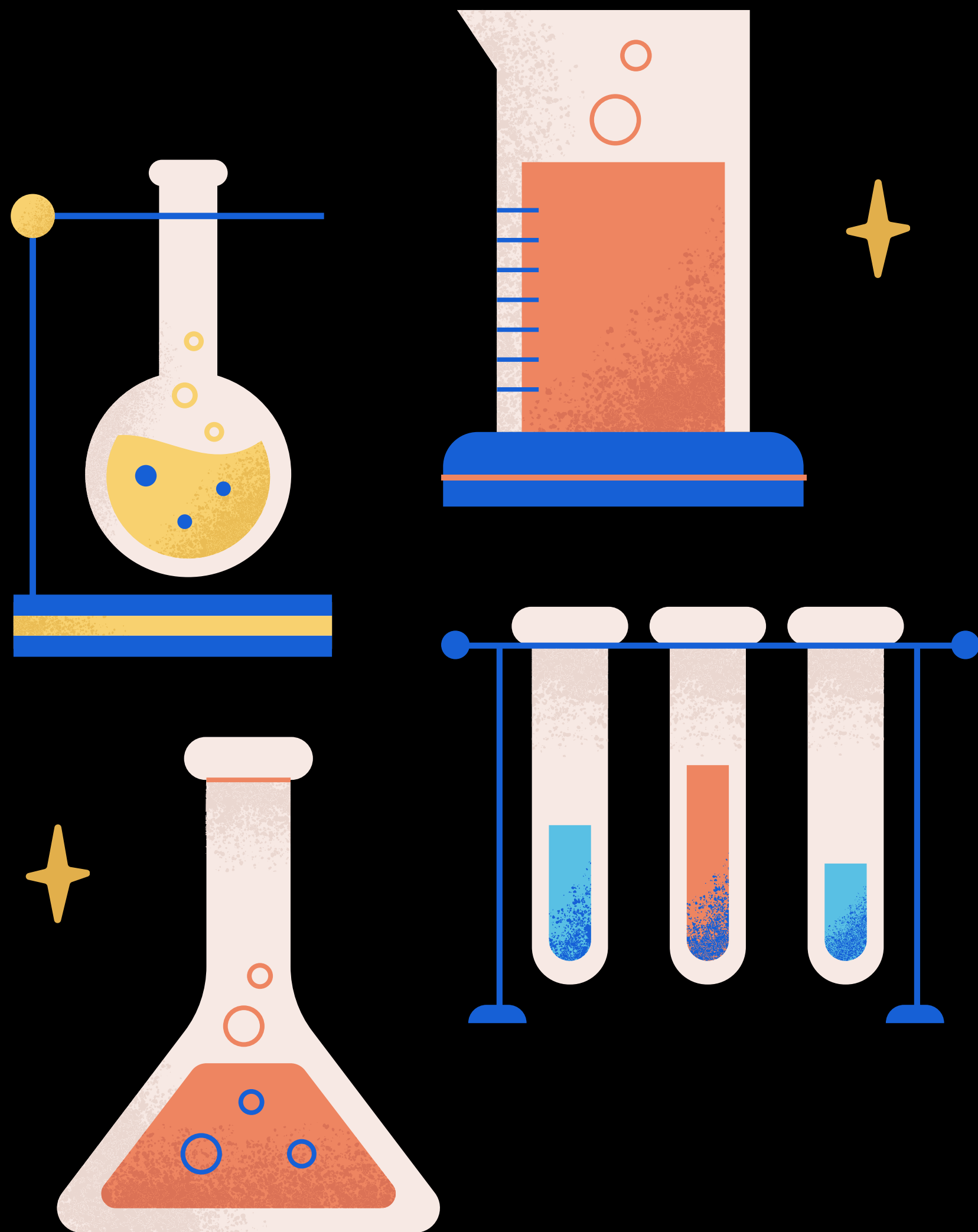
This combination offers **great sensitivity and selectivity** with **absolute quantification**

Q1 → Q3 transitions are monitored for each steroid of interest.

A mixture of **isotopically labeled compounds** (cortisol-d<sub>4</sub>, testosterone-d<sub>3</sub> and progesterone-d<sub>9</sub>) are used to account for losses during sample preparation and to ensure identification of the steroids of interest

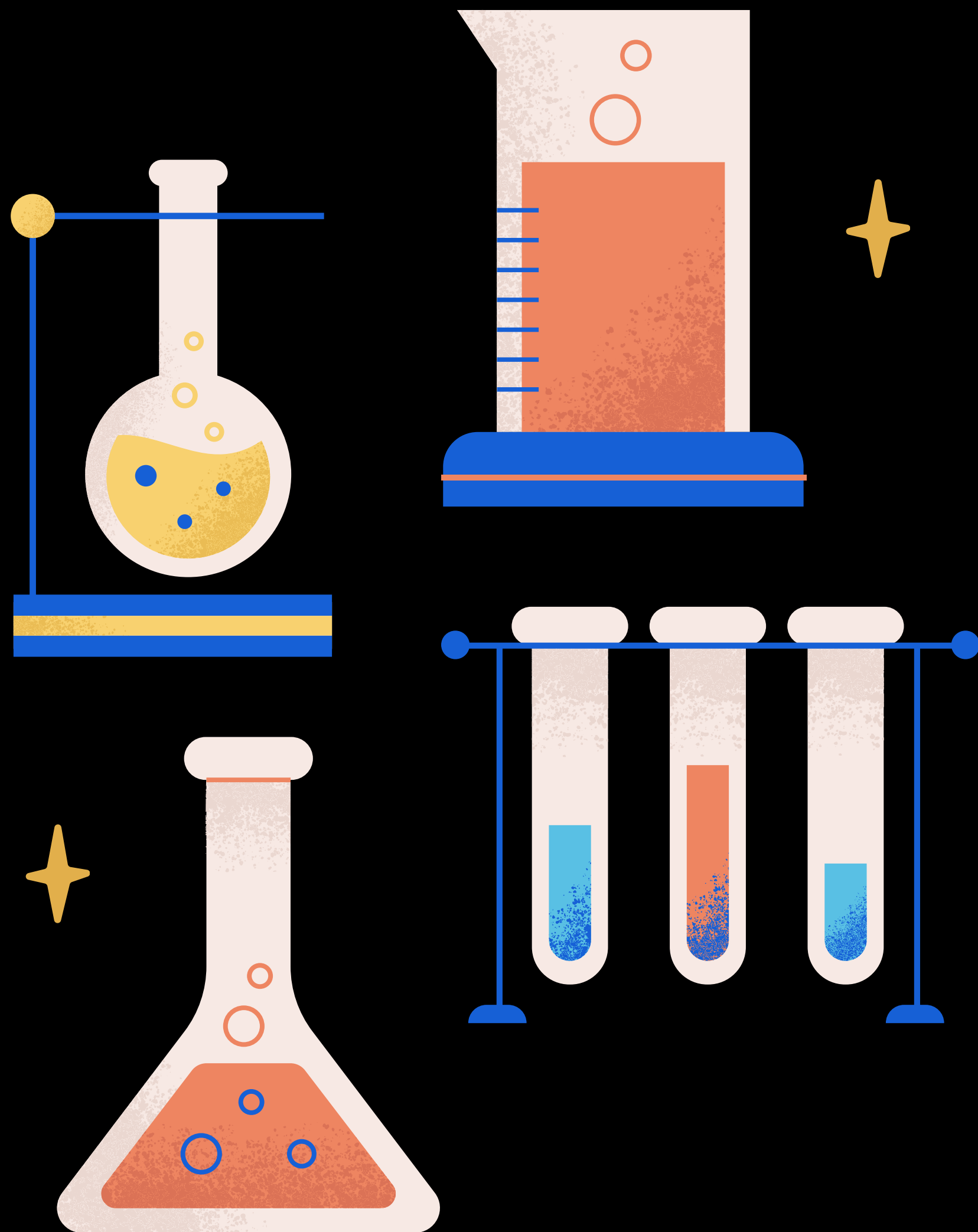
Steroid	Structure	Nominal mass (Da)	Protonated ions ( <i>m/z</i> )	Q1 → Q3 transitions
Cortisol		362	363.2	363.3 → 121.1
β-estradiol		272	273.1	237.1 → 107.0
Testosterone		288	289.2	289.1 → 97.0
Progesterone		314	315.2	315.1 → 109.0
Cortisol-d <sub>4</sub>		366	367.2	367.1 → 121.1
Testosterone-d <sub>3</sub>		291	292.2	292.1 → 97.0
Progesterone-d <sub>9</sub>		323	324.2	324.1 → 100.0

Method adapted from previous publication: Koorneef et al. 2022 Front. Endocrinol. 13:960279



# RESULTS

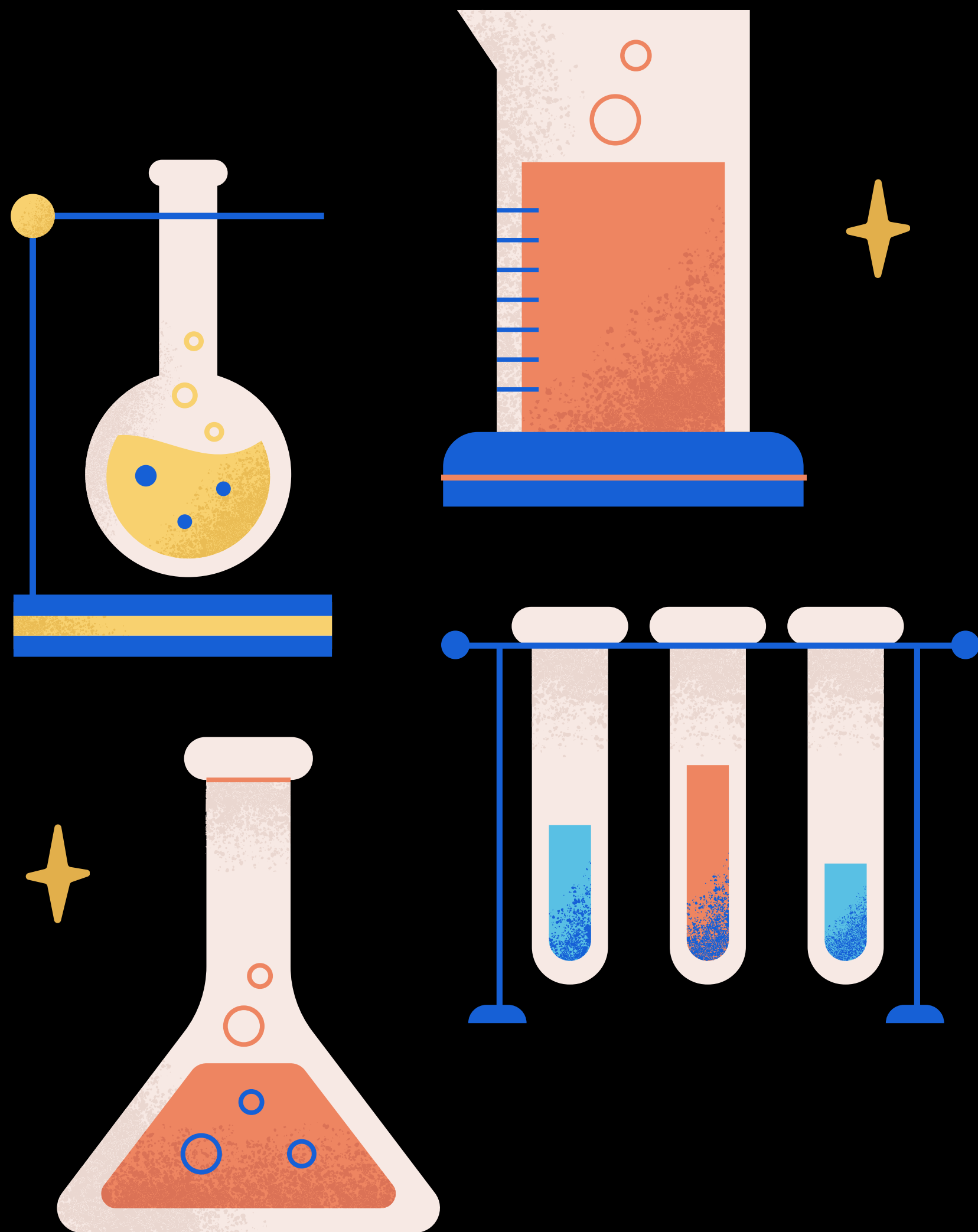
1. HCl demineralization rib (200mg)
2. EDTA deminerlizatoion rib (200mg)
3. Ground bone rib (200mg)



# RESULTS

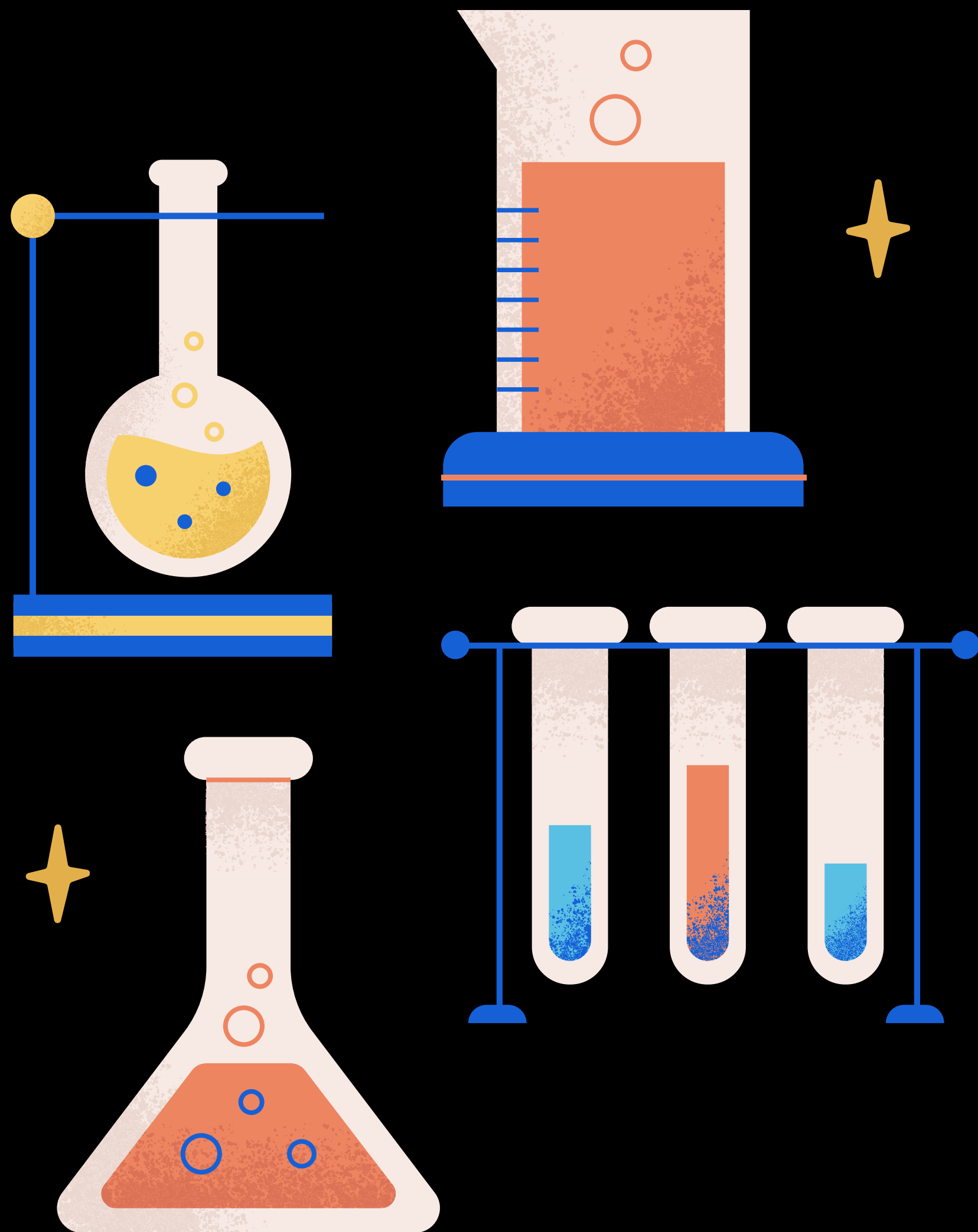
1. ~~HCl demineralization rib (200mg)~~
2. ~~EDTA deminerlizeatoin rib (200mg)~~
3. ~~Ground bone rib (200mg)~~





# RESULTS

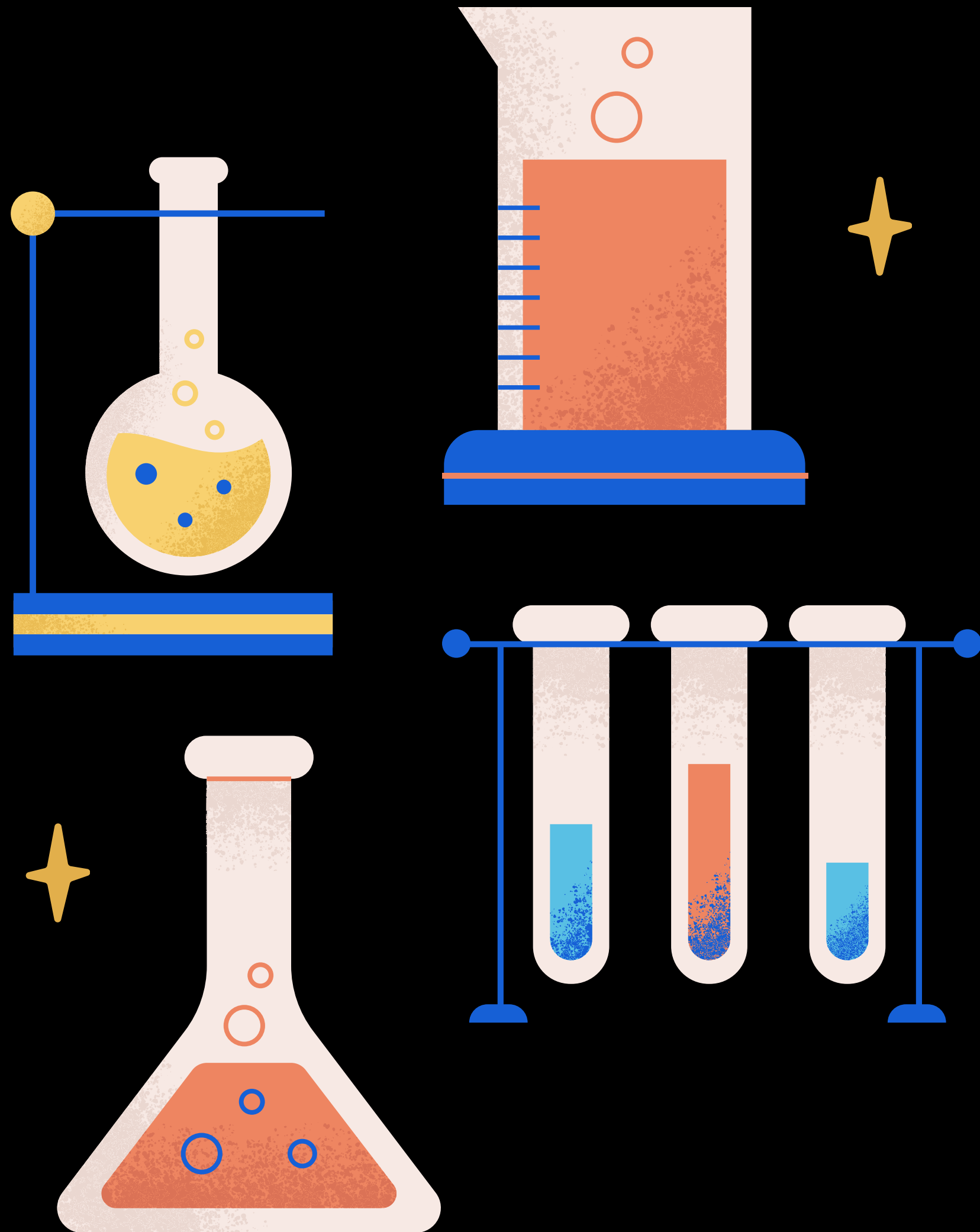
1. ~~HCl demineralization rib (200mg)~~
2. ~~EDTA demineralization rib (200mg)~~
3. ~~Ground bone rib (200mg)~~
4. MeOH extraction femur cortical (200mg)



# RESULTS

1. ~~HCl demineralization rib (200mg)~~
2. ~~EDTA demineralization rib (200mg)~~
3. ~~Ground bone rib (200mg)~~
4. ~~MeOH extraction femur cortical (200mg)~~



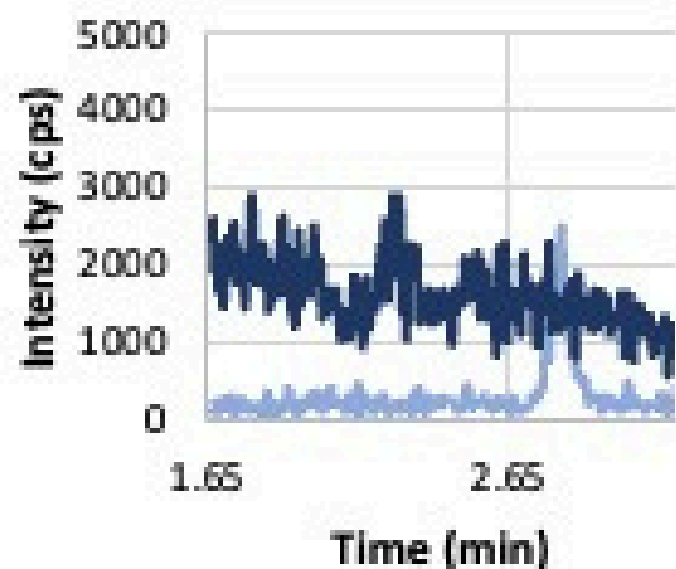


# RESULTS

1. ~~HCl demineralization rib (200mg)~~
2. ~~EDTA demineralization rib (200mg)~~
3. ~~Ground bone rib (200mg)~~
4. MeOH extraction femur cortical (200mg)
5. MeOH extraction femur cortical (300-400mg)
6. MeOH rib (50, 100 mg )
7. MeOH femur cortical (50, 100, 200mg)

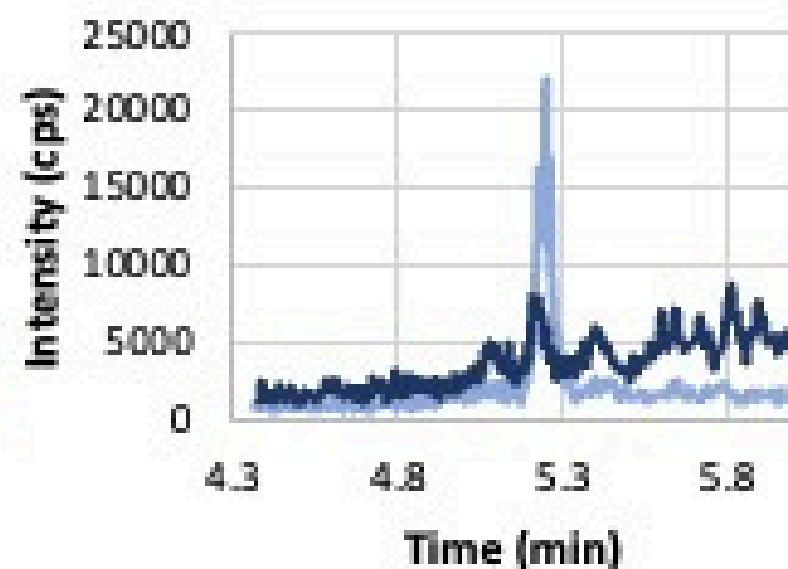
**Cortisol levels in femur**

— Cortisol standard at 0.09 ng/mL  
— Femur 200 mg



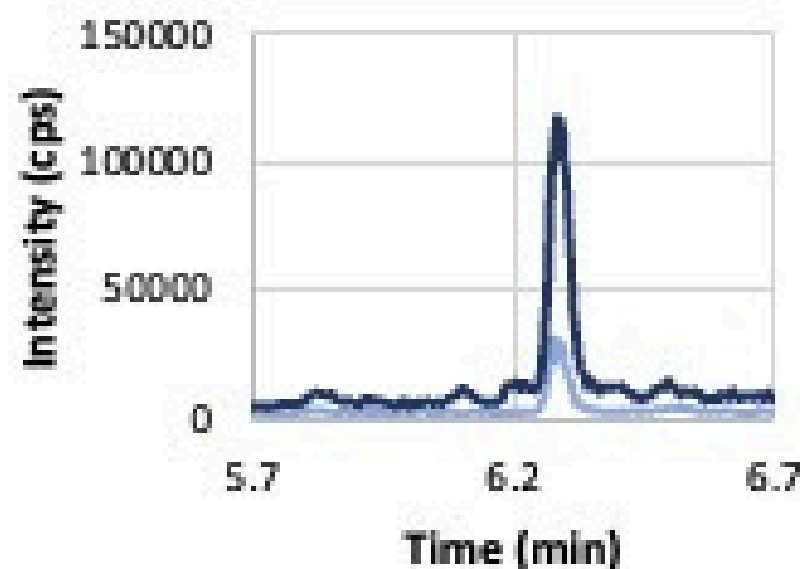
**$\beta$ -estradiol levels in femur**

—  $\beta$ -estradiol standard at 4.5 ng/mL  
— Femur 200 mg



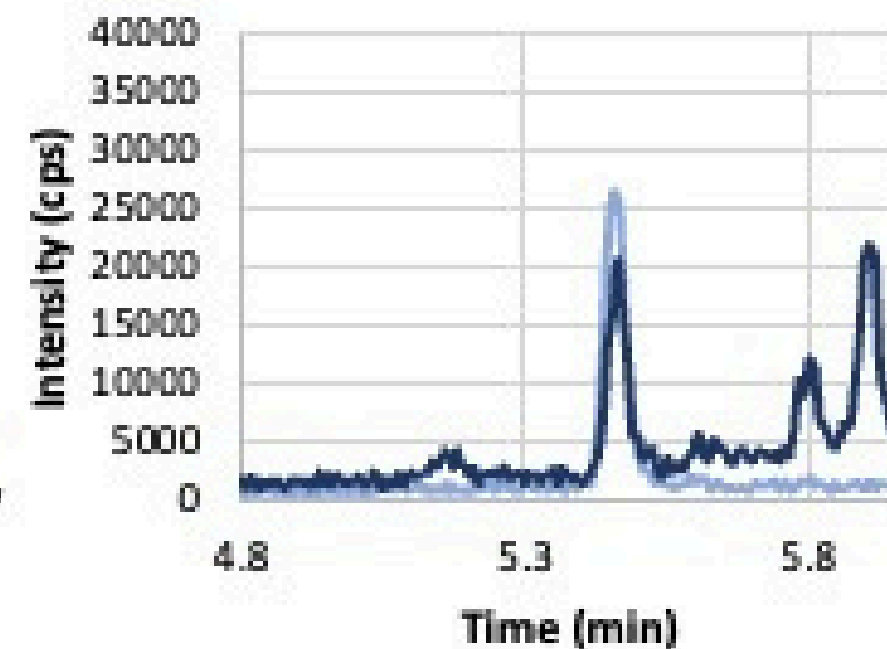
**Progesterone levels in femur**

— Progesterone standard at 0.09 ng/mL  
— Femur 200 mg



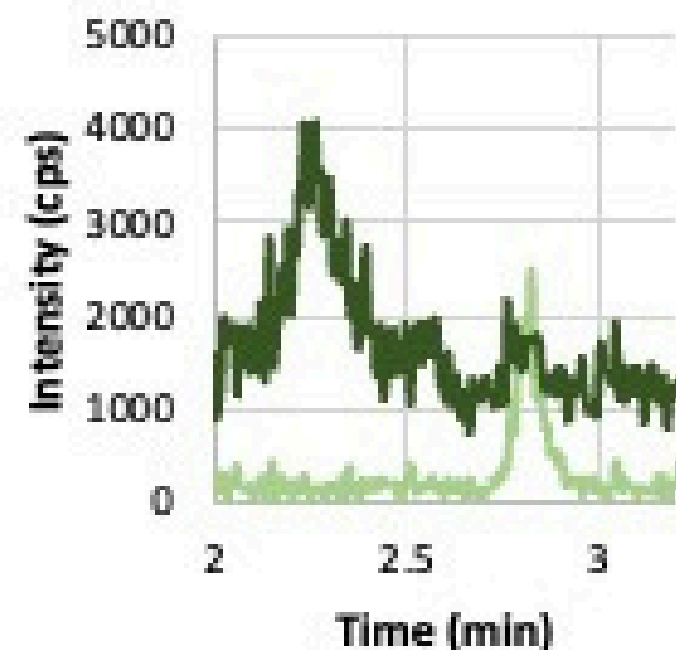
**Testosterone levels in femur**

— Testosterone standard at 0.09 ng/mL  
— Femur 200 mg



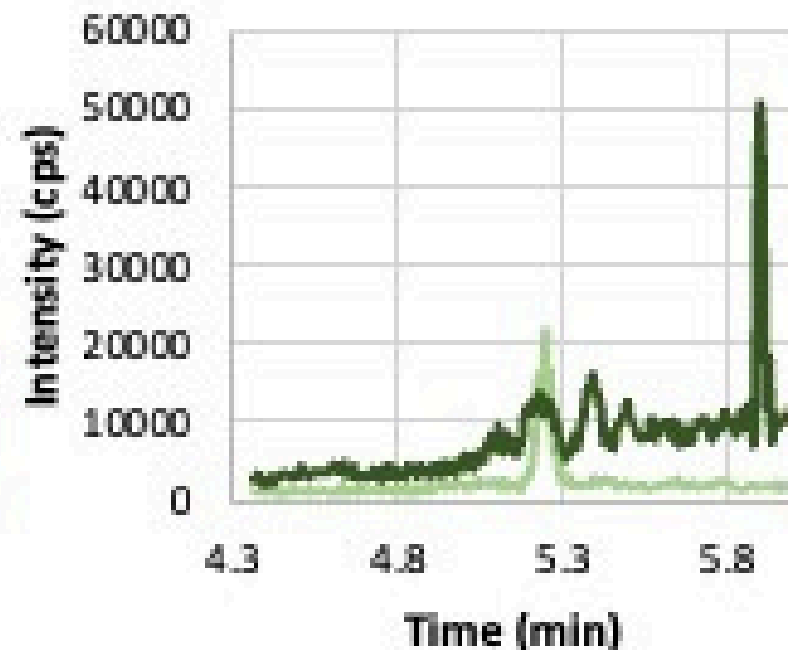
**Cortisol levels in rib**

— Cortisol standard at 0.09 ng/mL  
— Rib 100 mg



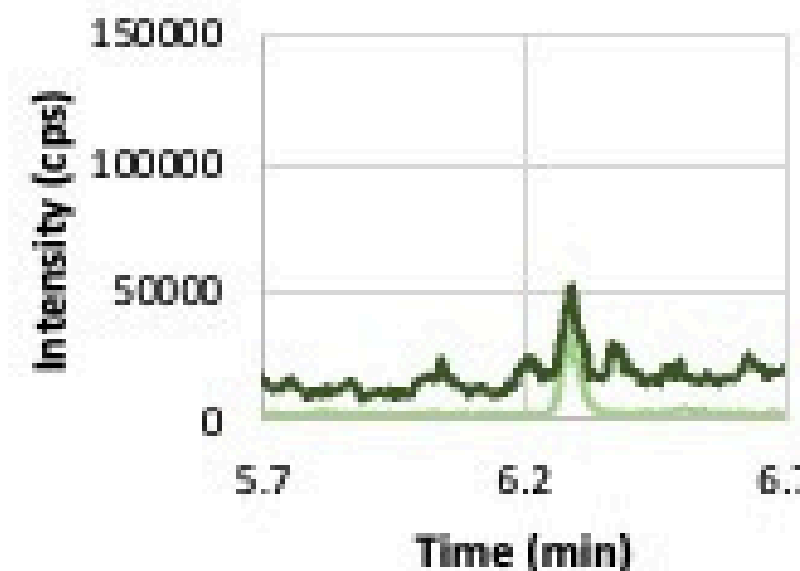
**$\beta$ -estradiol levels in rib**

—  $\beta$ -estradiol standard at 4.5 ng/mL  
— Rib 100 mg



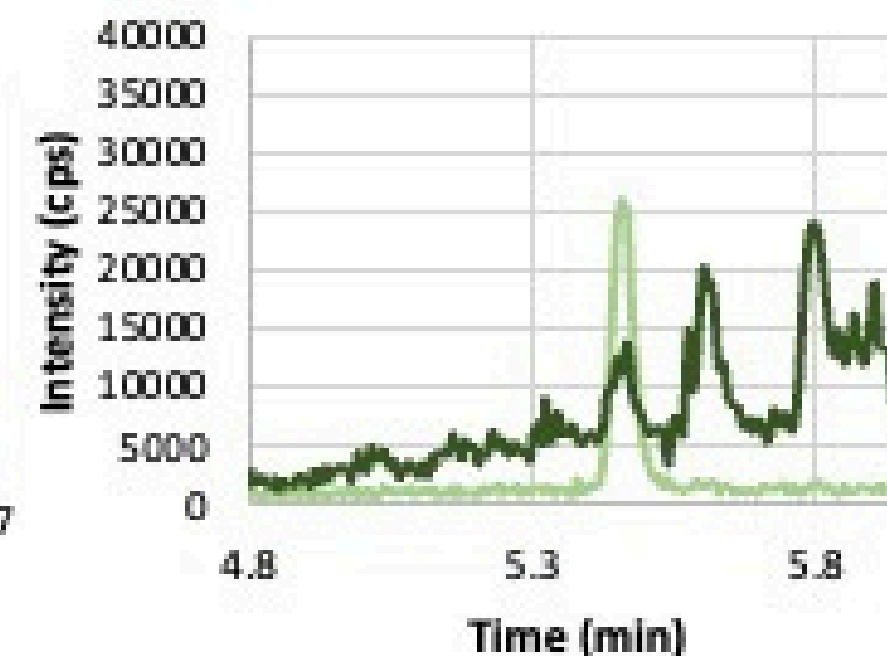
**Progesterone levels in rib**

— Progesterone standard at 0.09 ng/mL  
— Rib 100 mg

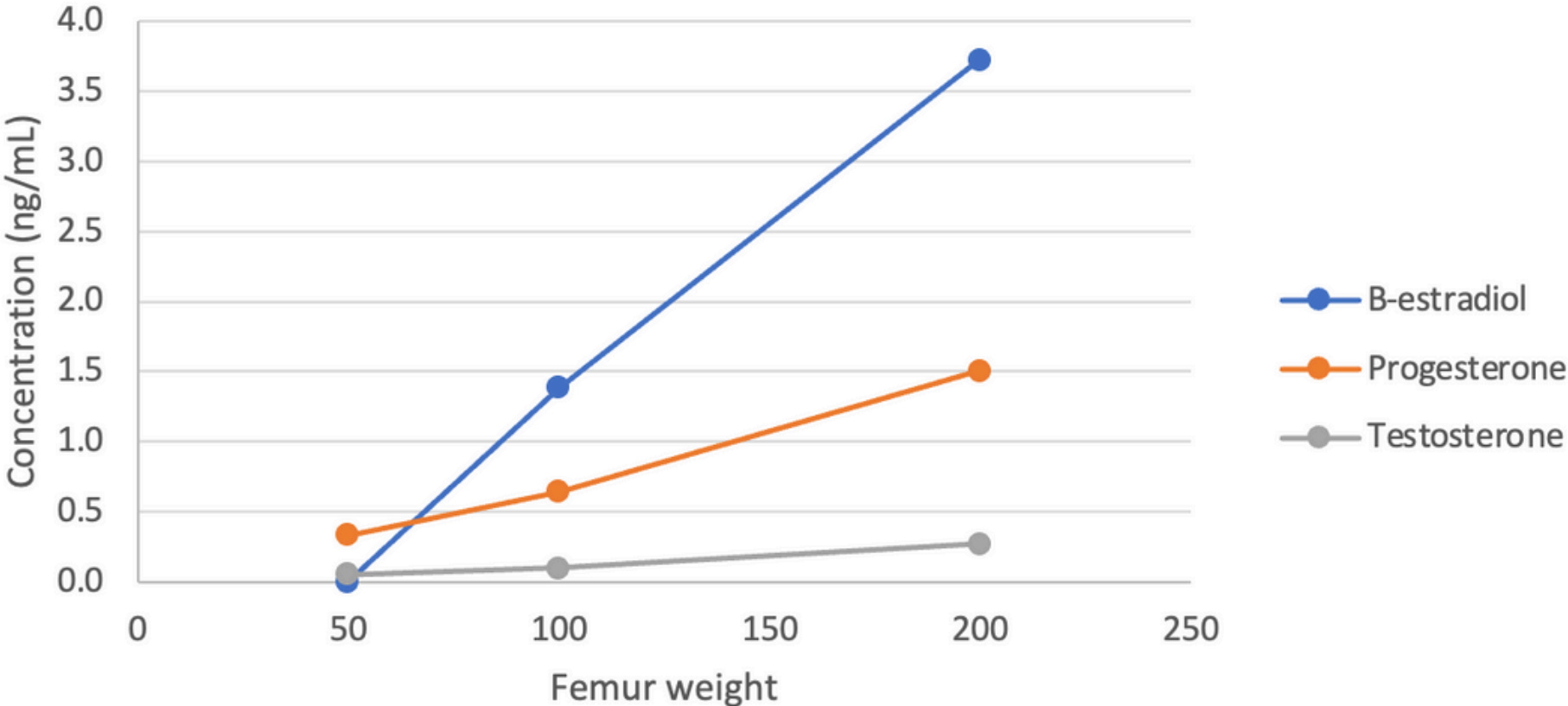


**Testosterone levels in rib**

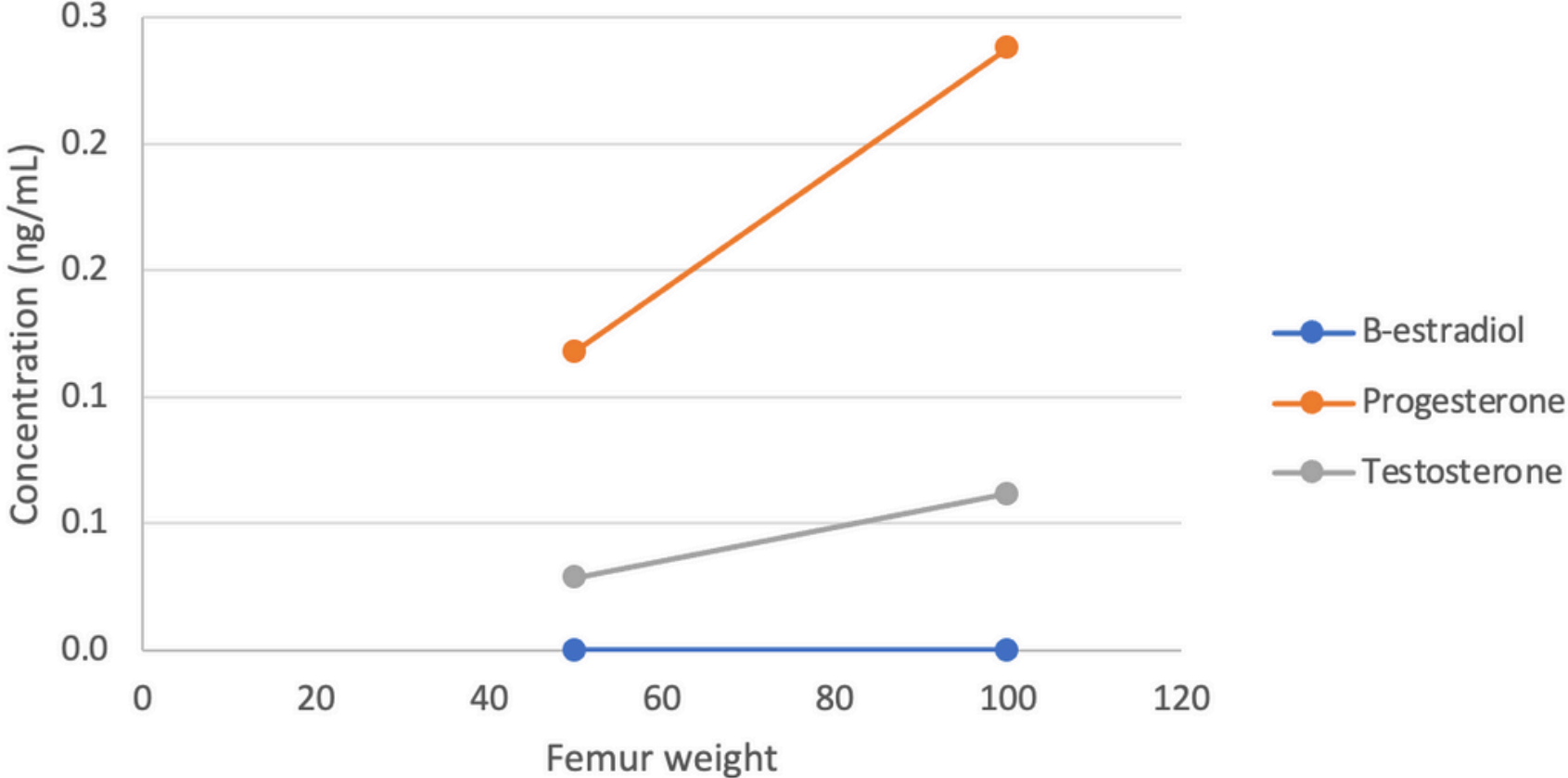
— Testosterone standard at 0.09 ng/mL  
— Rib 100 mg

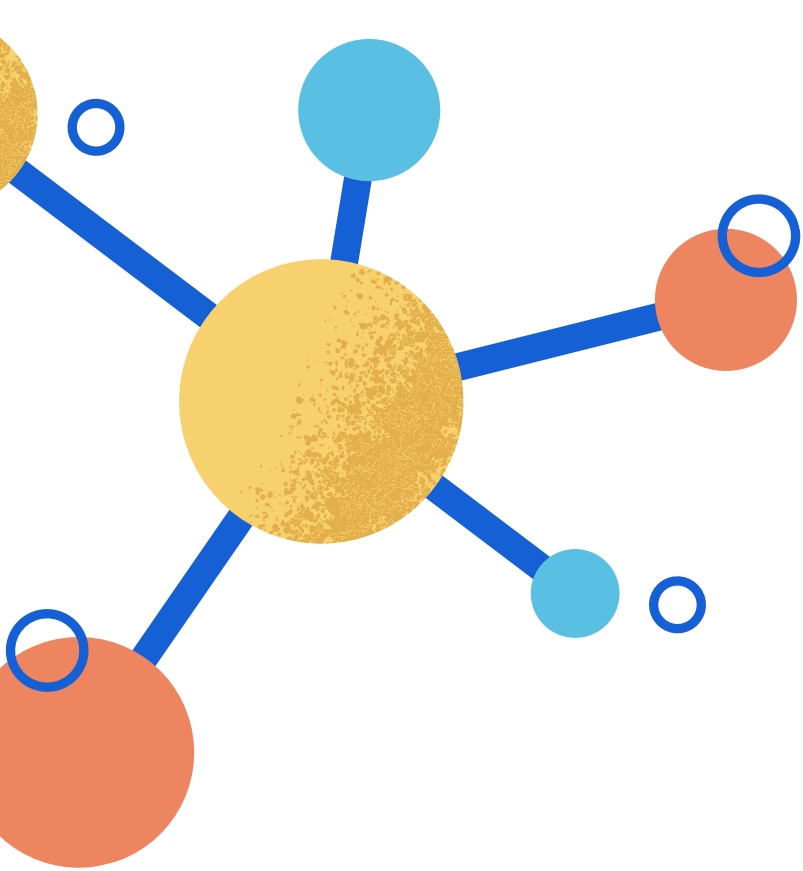


Concentration steroids vs femur weight



Concentration steroids vs Ribs weight

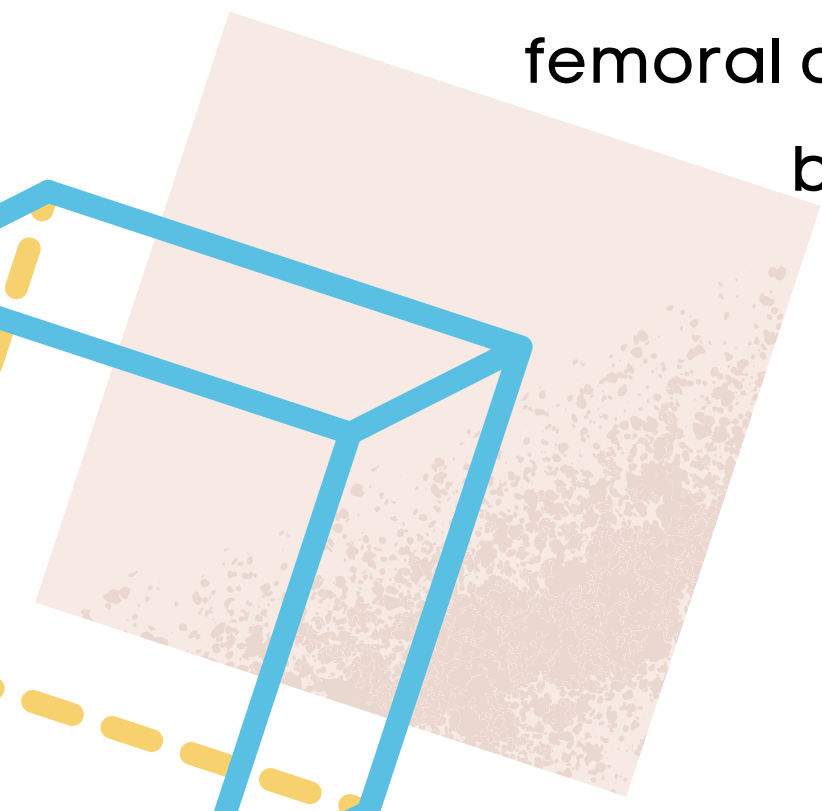




**Cortisol**

Cortisol does not appear to  
be present/detectable in  
femoral cortical or rib

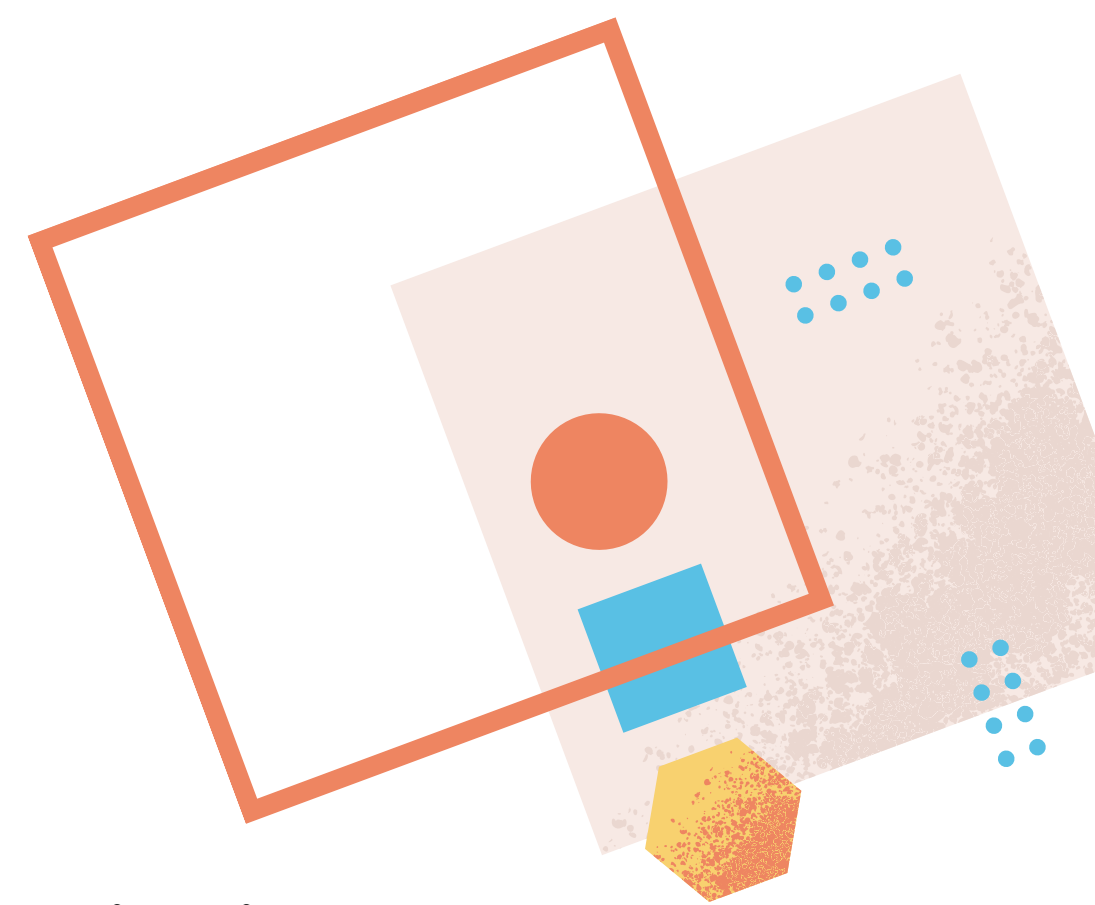
bone



# SUMMARY

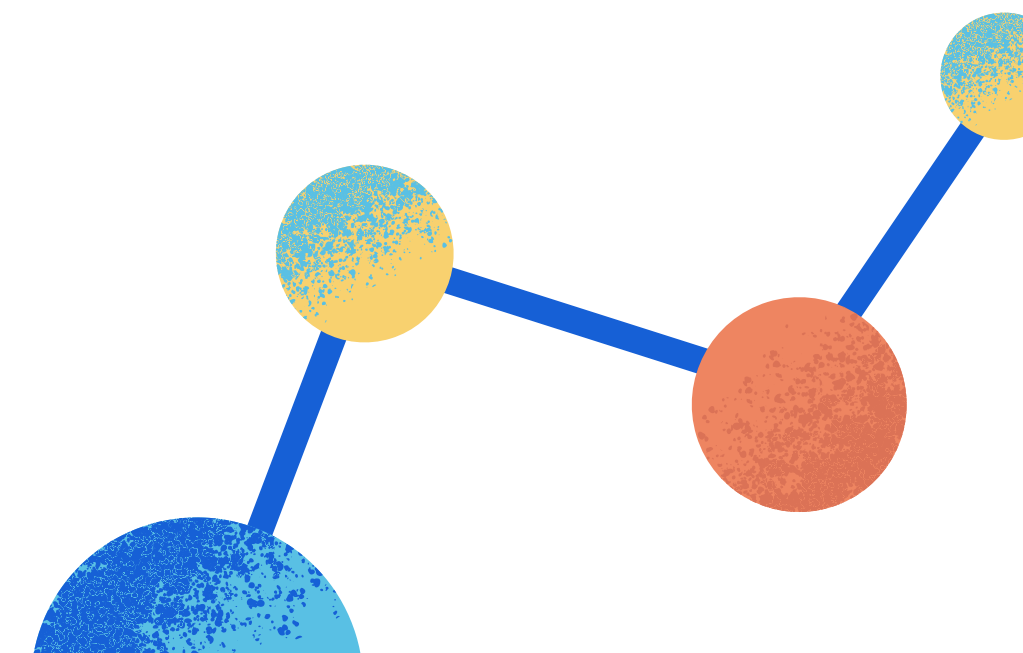
**Other Steroids**

Testosterone, estradiol,  
and progesterone can all  
be reliably quantified



**Implications**

Paleohormonal analysis of  
archaeological material



The background is black with several decorative elements. In the top right and bottom left, there are interlocking gears in yellow, orange, and light blue. On the left and right sides, there are large, light blue circular areas with a dark blue border. Inside these circles are various abstract shapes: yellow stars, white stars, yellow dots, blue dots, and yellow branching structures. The title 'NEXT STEPS' is centered in the upper half of the image in a bold, white, sans-serif font.

# NEXT STEPS

- 1 Intra-/Inter- individual variation
- 2 Sex/Age Variability
- 3 Other tissues
- 4 Other populations





# THANK YOU!

S.A.SCRADER@ARCH.LEIDENUNIV.NL  
@DRSARAHSCRADER

WE WOULD LIKE TO THANK MARTIJN DEFILET, GEMEENTE ARNHEM,  
AND THE ARNHEM C FOR ALLOWING US TO ANALYZE THESE  
REMAINS.

THIS PRESENTATION IS PART OF THE PROJECT EVOLUTION OF  
STRESS (406.XS.01.118) OF THE RESEARCH PROGRAMME OPEN  
COMPETITION, WHICH IS FINANCED BY THE DUTCH RESEARCH  
COUNCIL (NWO)