



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

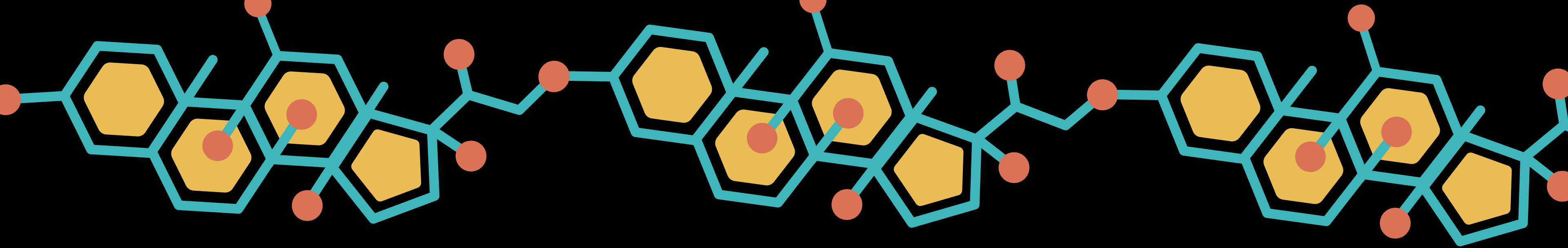
Schrader, S. A., Brewster, K., Hall, R., Giera, M., & Sánchez-López, E. (2024). Recent Advances in LC-MS/MS Analysis of Ancient Hormones. In . doi:10.5281/zenodo.14917697

Version: Not Applicable (or Unknown)

License: [Creative Commons CC BY 4.0 license](https://creativecommons.org/licenses/by/4.0/)

Downloaded from: <https://hdl.handle.net/1887/4198293>

Note: To cite this publication please use the final published version (if applicable).



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

Sarah Schrader,¹ Kevin Brewster,² Rachael
Hall,¹ Martin Giera,² Elena Sánchez-López²

¹ Faculty of Archaeology, Leiden University

² Center for Proteomics and Metabolomics, Leiden University Medical Center



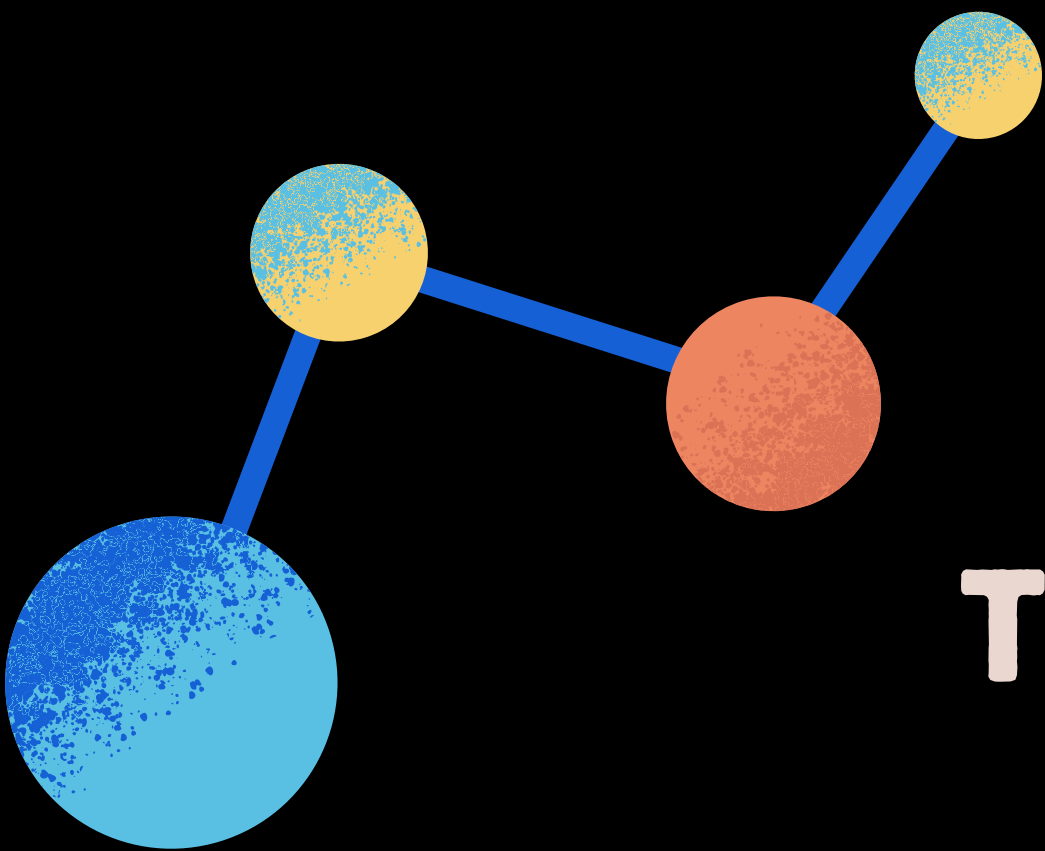
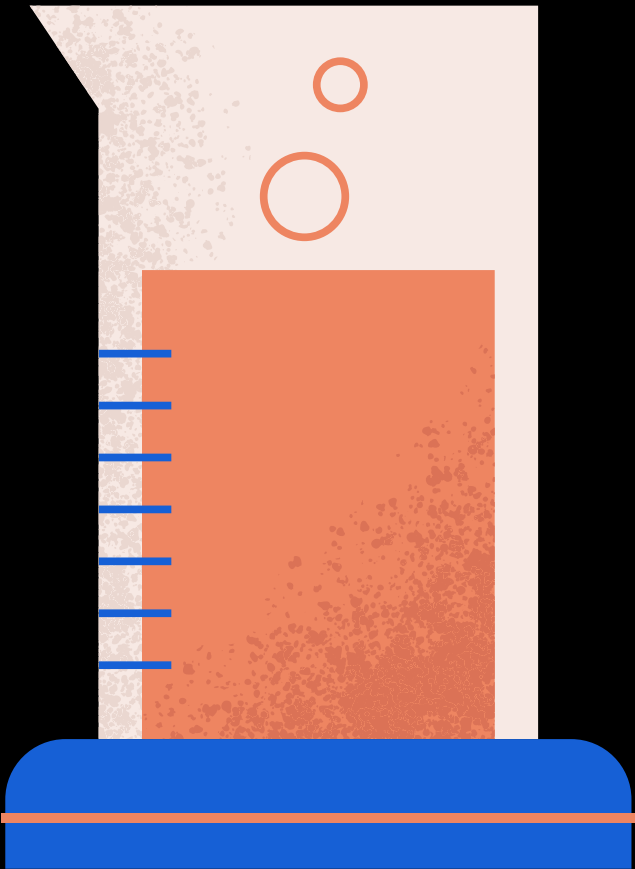


TABLE OF CONTENTS

01	Previous Research
02	The Evolution of Stress Project
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^{a,*}, Steven Thomson^b, Andrew Nelson^a, Christine White^a, Gideon Koren^{b,c,d,h}, Michael Rieder^{b,c,d,e,f,g}, Stan Van Uum^{c,e}

Contents lists available at [ScienceDirect](#)

 **International Journal of Paleopathology**
journal homepage: www.elsevier.com/locate/ijpp 

Integrating cortisol and isotopic analyses of archaeological hair: Elucidating juvenile ante-mortem stress and behaviour 

Emily C. Webb^{a,*}, Christine D. White^a, Stan Van Uum^b, Fred J. Longstaffe^c

BRIEF COMMUNICATION 

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

Leslie Quade¹  | Paul L. Chazot² | Rebecca Gowland¹ 

Contents lists available at [ScienceDirect](#)

 **International Journal of Paleopathology**
journal homepage: www.elsevier.com/locate/ijpp 

Cortisol in deciduous tooth tissues: A potential metric for assessing stress exposure in archaeological and living populations 

Leslie Quade^{a,*}, Miroslav Králík^a, Petra Bencúrová^b, Erin C. Dunn^c

Contents lists available at [ScienceDirect](#)

 **Journal of Archaeological Science: Reports**
journal homepage: www.elsevier.com/locate/jasrep 

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

Elisha Tisdale^{a,*}, Lana Williams^a, John J. Schultz^{a,b}, Sandra M. Wheeler^a

PREVIOUS RESEARCH

- Hair
- Dentine
- Cortisol, estradiol, testosterone

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^{a,*}, Steven Thomson^b, Andrew Nelson^a, Christine White^a, Gideon Koren^{b,c,d,h}, Michael Rieder^{b,c,d,e,f,g}, Stan Van Uum^{c,e}

Contents lists available at [ScienceDirect](#)

 **International Journal of Paleopathology**
journal homepage: www.elsevier.com/locate/ijpp 

Integrating cortisol and isotopic analyses of archaeological hair: Elucidating juvenile ante-mortem stress and behaviour

Emily C. Webb^{a,*}, Christine D. White^a, Stan Van Uum^b, Fred J. Longstaffe^c 

BRIEF COMMUNICATION  **WILEY**

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

Leslie Quade¹  | Paul L. Chazot² | Rebecca Gowland¹ 

Contents lists available at [ScienceDirect](#)

 **International Journal of Paleopathology**
journal homepage: www.elsevier.com/locate/ijpp 

Cortisol in deciduous tooth tissues: A potential metric for assessing stress exposure in archaeological and living populations

Leslie Quade^{a,*}, Miroslav Králík^a, Petra Bencúrová^b, Erin C. Dunn^c 

Contents lists available at [ScienceDirect](#)

 **Journal of Archaeological Science: Reports**
journal homepage: www.elsevier.com/locate/jasrep 

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

Elisha Tisdale^{a,*}, Lana Williams^a, John J. Schultz^{a,b}, Sandra M. Wheeler^a 

PREVIOUS RESEARCH

- Hair
- Dentine
- Cortisol, estradiol, testosterone
- ELISA

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^{a,*}, Steven Thomson^b, Andrew Nelson^a, Christine White^a, Gideon Koren^{b,c,d,h}, Michael Rieder^{b,c,d,e,f,g}, Stan Van Uum^{c,e}

Contents lists available at [ScienceDirect](#)

 **International Journal of Paleopathology**
journal homepage: www.elsevier.com/locate/ijpp 

Integrating cortisol and isotopic analyses of archaeological hair: Elucidating juvenile ante-mortem stress and behaviour

Emily C. Webb^{a,*}, Christine D. White^a, Stan Van Uum^b, Fred J. Longstaffe^c 

BRIEF COMMUNICATION 

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

Leslie Quade¹  | Paul L. Chazot² | Rebecca Gowland¹ 

Contents lists available at [ScienceDirect](#)

 **International Journal of Paleopathology**
journal homepage: www.elsevier.com/locate/ijpp 

Cortisol in deciduous tooth tissues: A potential metric for assessing stress exposure in archaeological and living populations

Leslie Quade^{a,*}, Miroslav Králík^a, Petra Bencúrová^b, Erin C. Dunn^c 

Contents lists available at [ScienceDirect](#)

 **Journal of Archaeological Science: Reports**
journal homepage: www.elsevier.com/locate/jasrep 

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

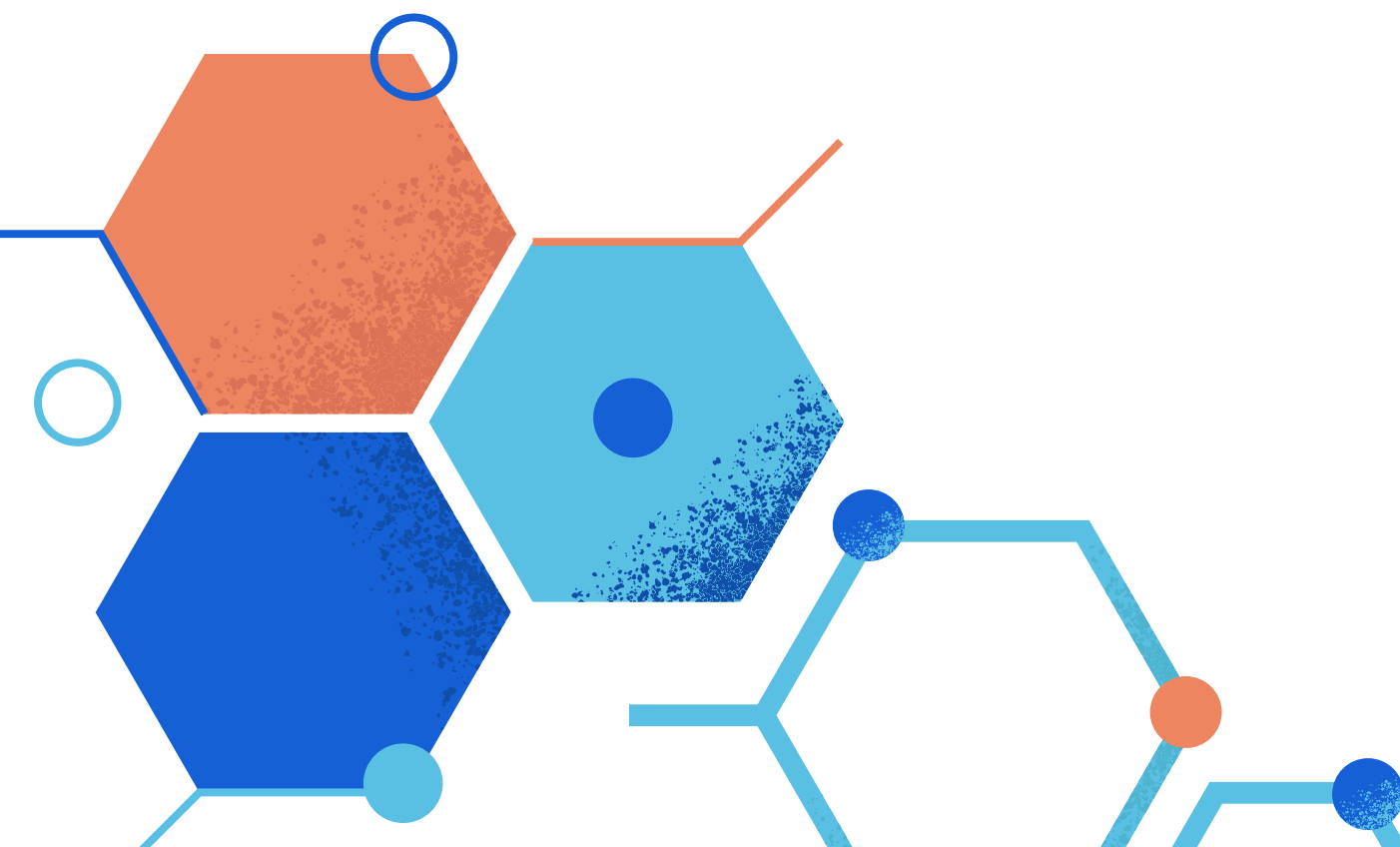
Elisha Tisdale^{a,*}, Lana Williams^a, John J. Schultz^{a,b}, Sandra M. Wheeler^a 



LC-MS/MS

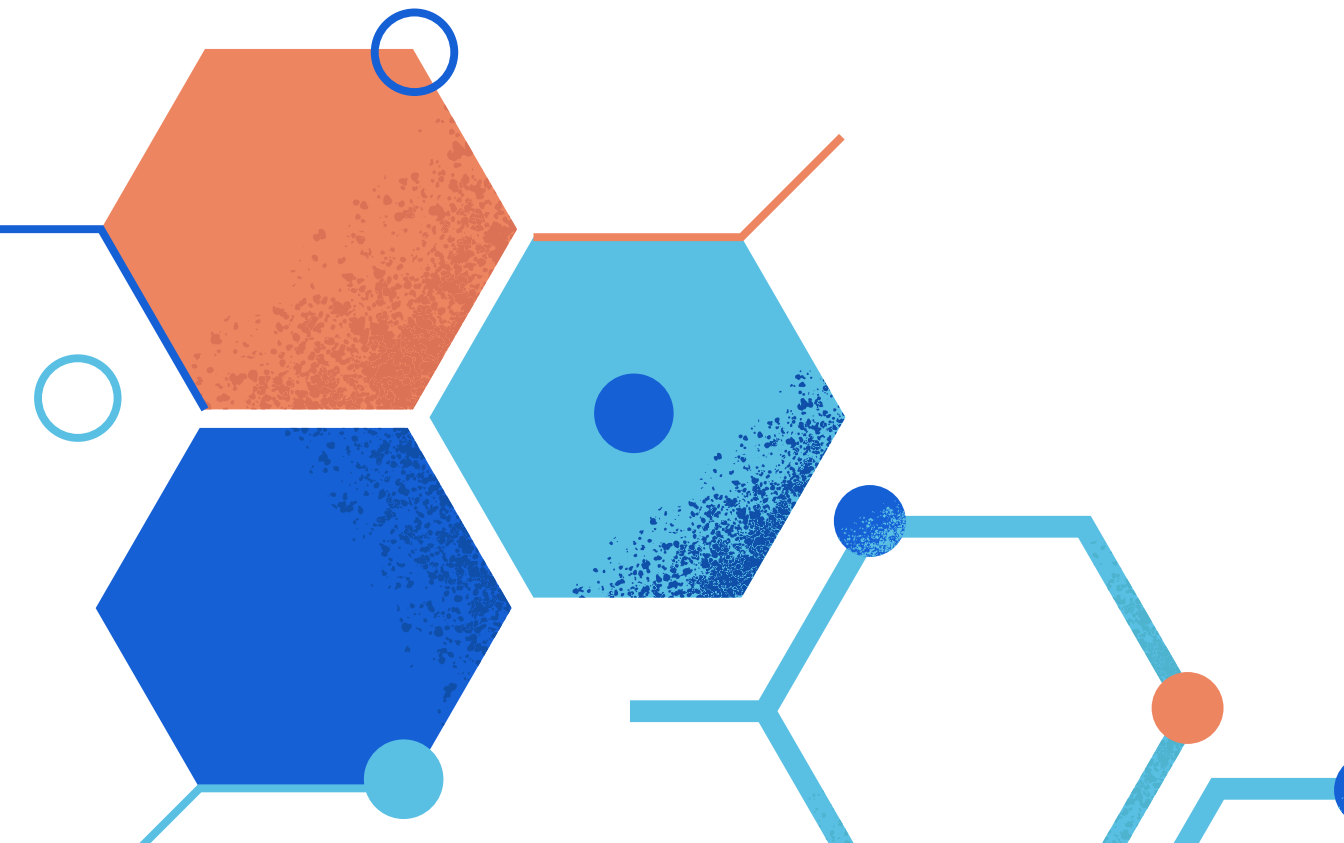
Higher sensitivity, with accuracy, reliability, and precision measurements

THE EVOLUTION OF STRESS





THE EVOLUTION OF STRESS



LC-MS/MS

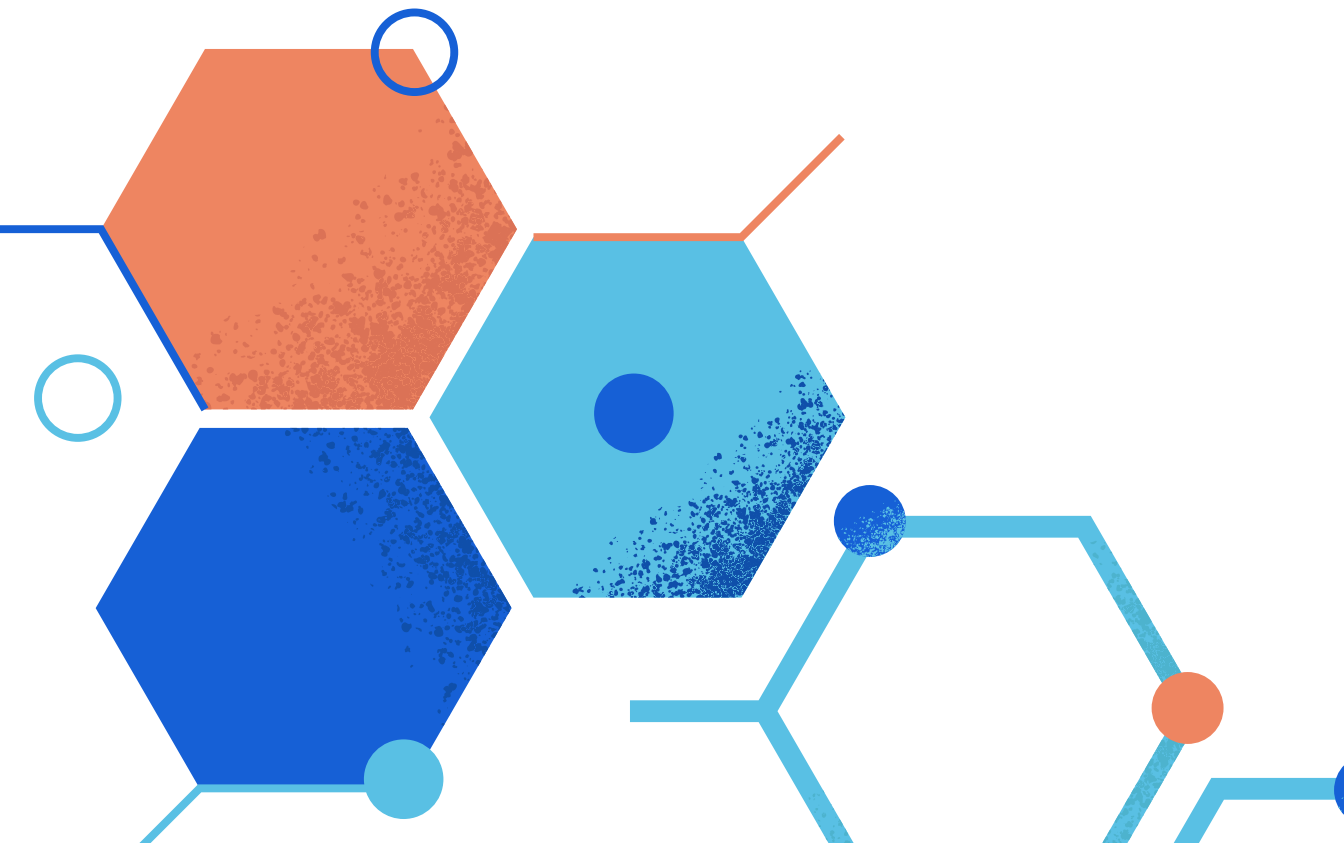
Higher sensitivity, with accuracy, reliability, and precision measurements

Multi-Tissue & Lifecourse Approach

Bone, hair, dentine, fingernails



THE EVOLUTION OF STRESS



LC-MS/MS

Higher sensitivity, with accuracy, reliability, and precision measurements

Multi-Tissue & Lifecourse Approach

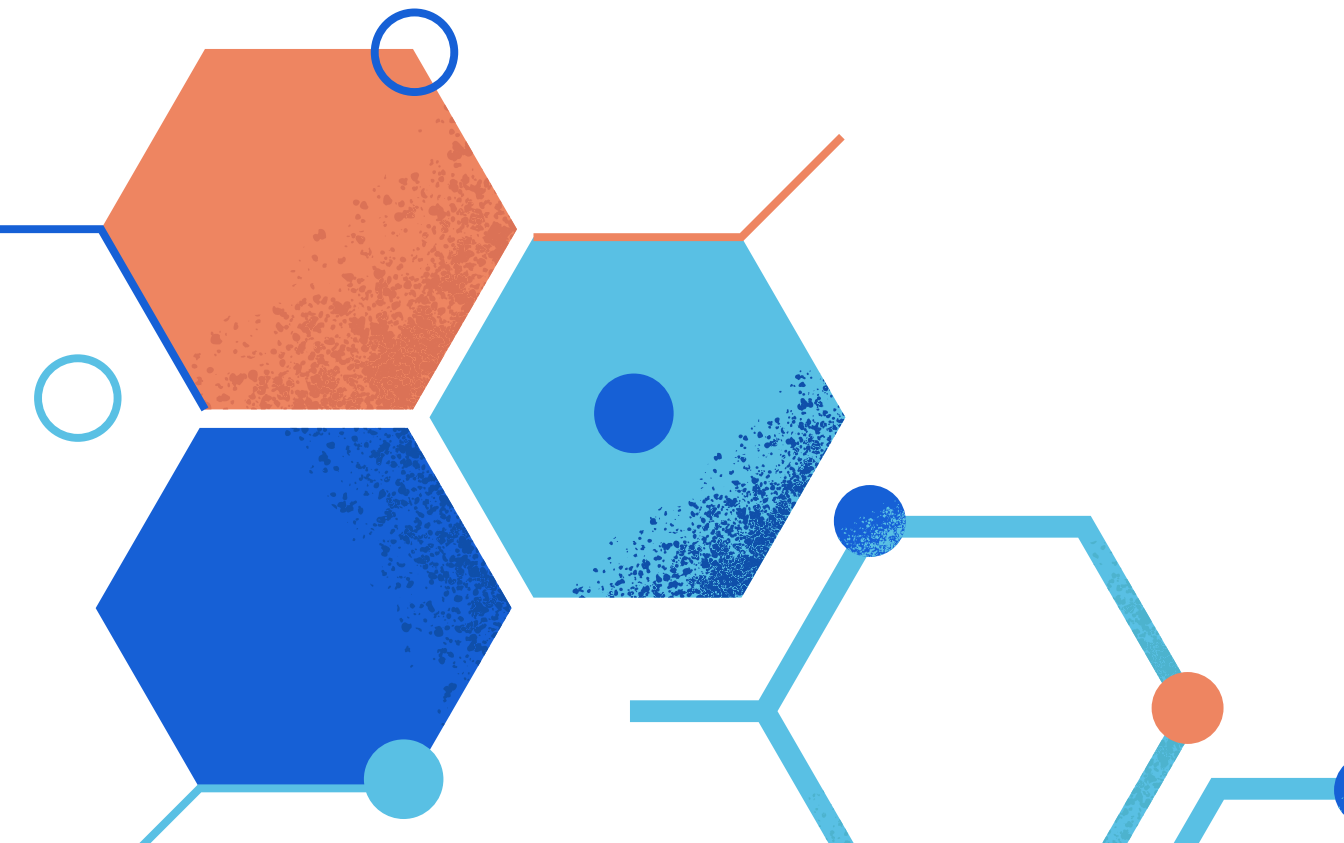
Bone, hair, dentine, fingernails

Inter-/Intra- Individual Variation

Assessing relationship between tissues AND individuals



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



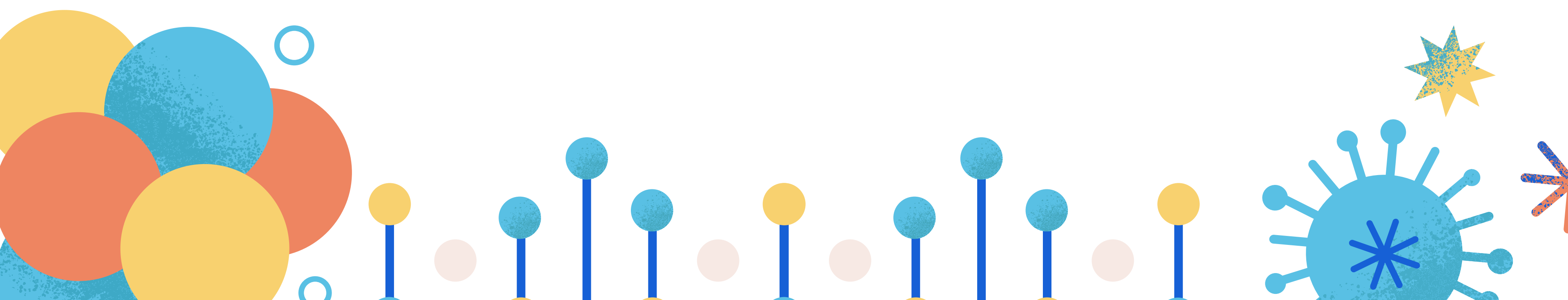
MATERIALS, METHODS & ETHICS

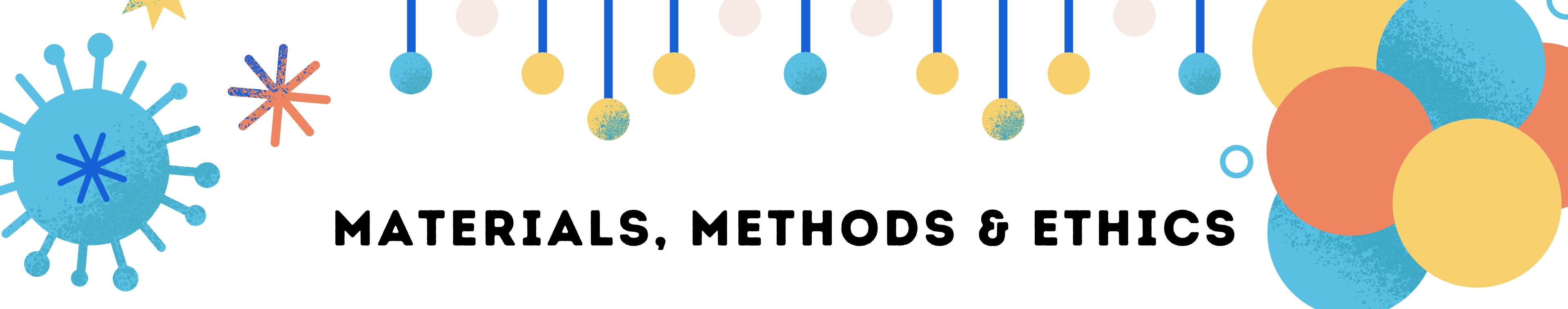
Arnhem

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

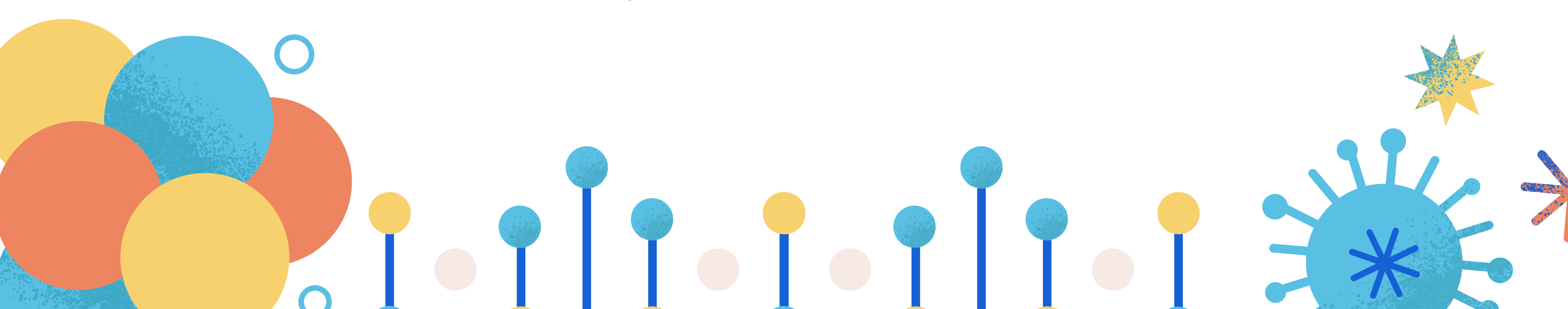
Standard pelvic and cranial traits were
used for the estimation of sex and age.



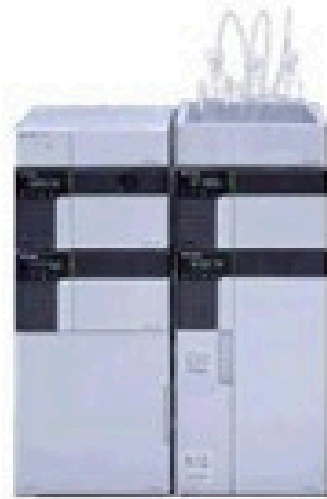


MATERIALS, METHODS & ETHICS

Steroid Extraction

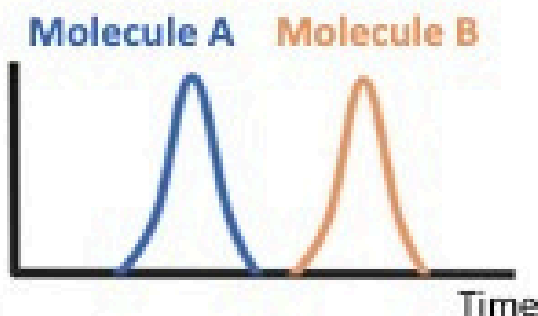
1. HCl: 200mg, demineralize in 100mL 0.01 N HCl (Longin, 1971), 1mL MeOH (Van Uum et al., 2008)
 2. EDTA: 200mg, demineralize in 10% EDTA (Tuross, 2012), 1mL MeOH
 3. Ground Bone: 200 mg, 1mL MeOH
 4. MeOH: 1mL MeOH (Charapata et al., 2018)
- 

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



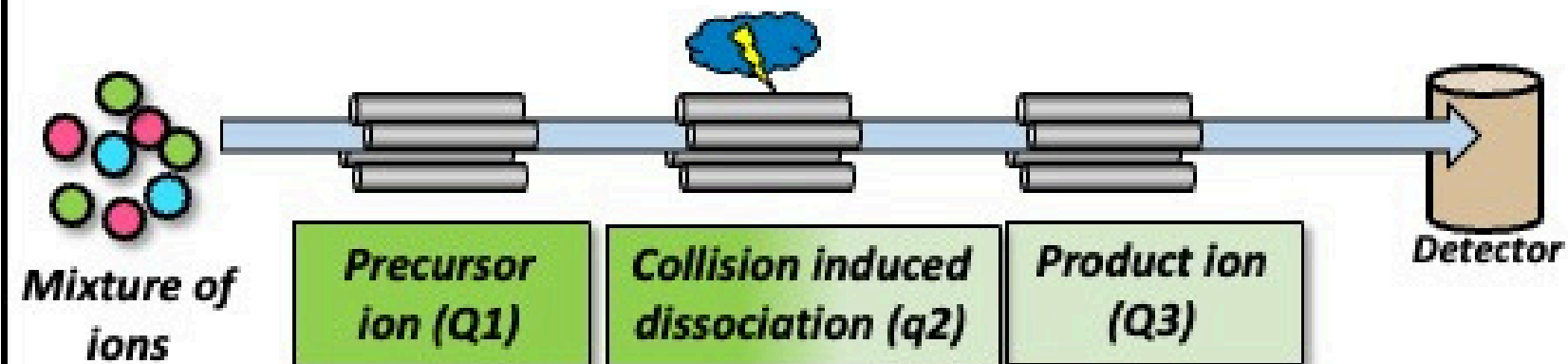
+



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:

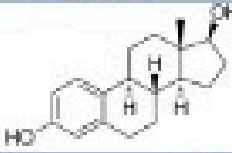
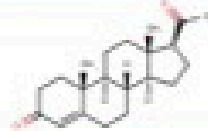
- **Isolation** of each molecule based on its mass-to-charge ratio (m/z) (in Q1)
- **Fragmentation** (in q2), and
- **Isolation of a specific fragment** coming from the parent ion (Q3)



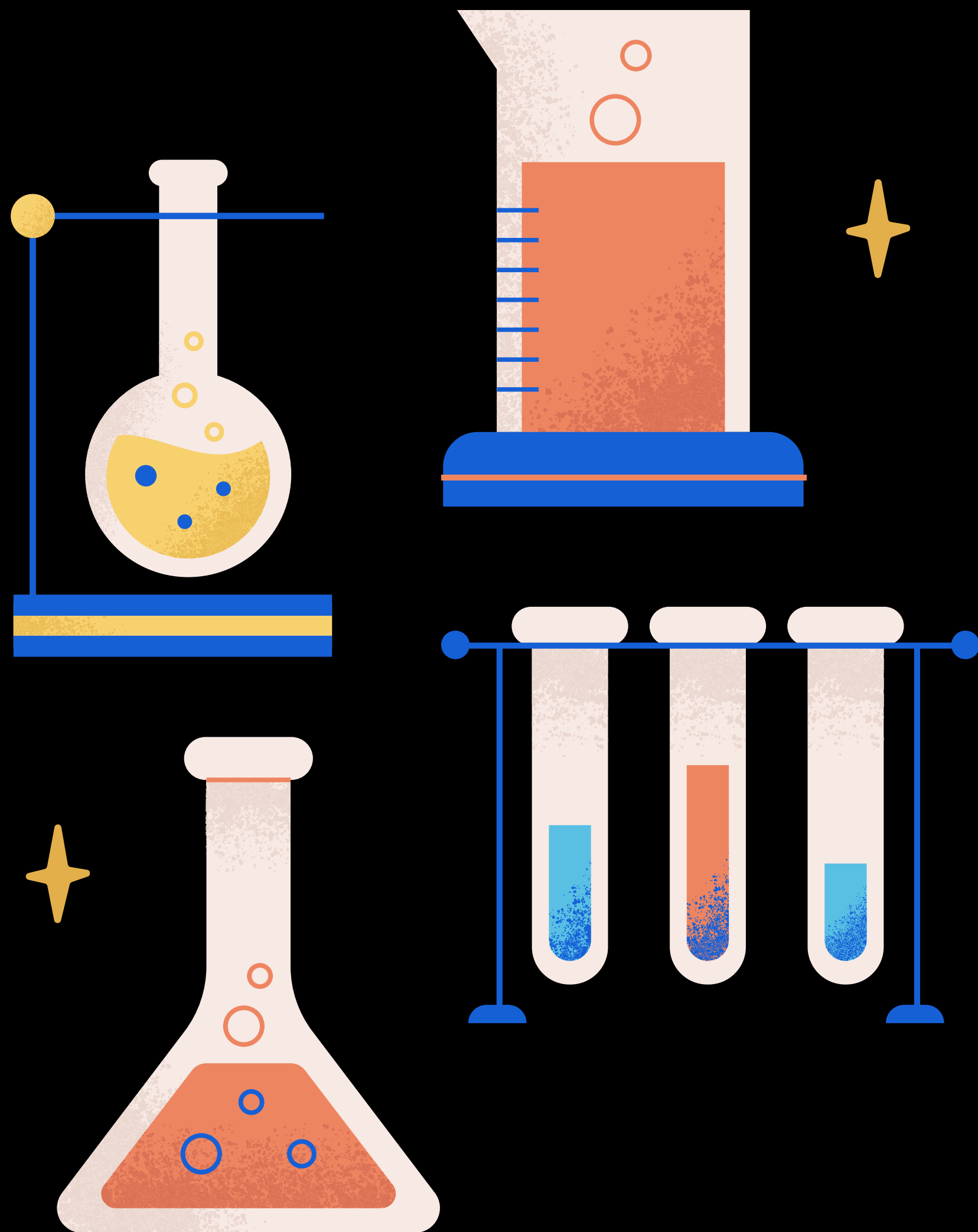
This combination offers **great sensitivity and selectivity** with **absolute quantification**

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

A mixture of **isotopically labeled compounds** (cortisol-d₄, testosterone-d₃ and progesterone-d₉) are used to account for losses during sample preparation and to ensure identification of the steroids of interest

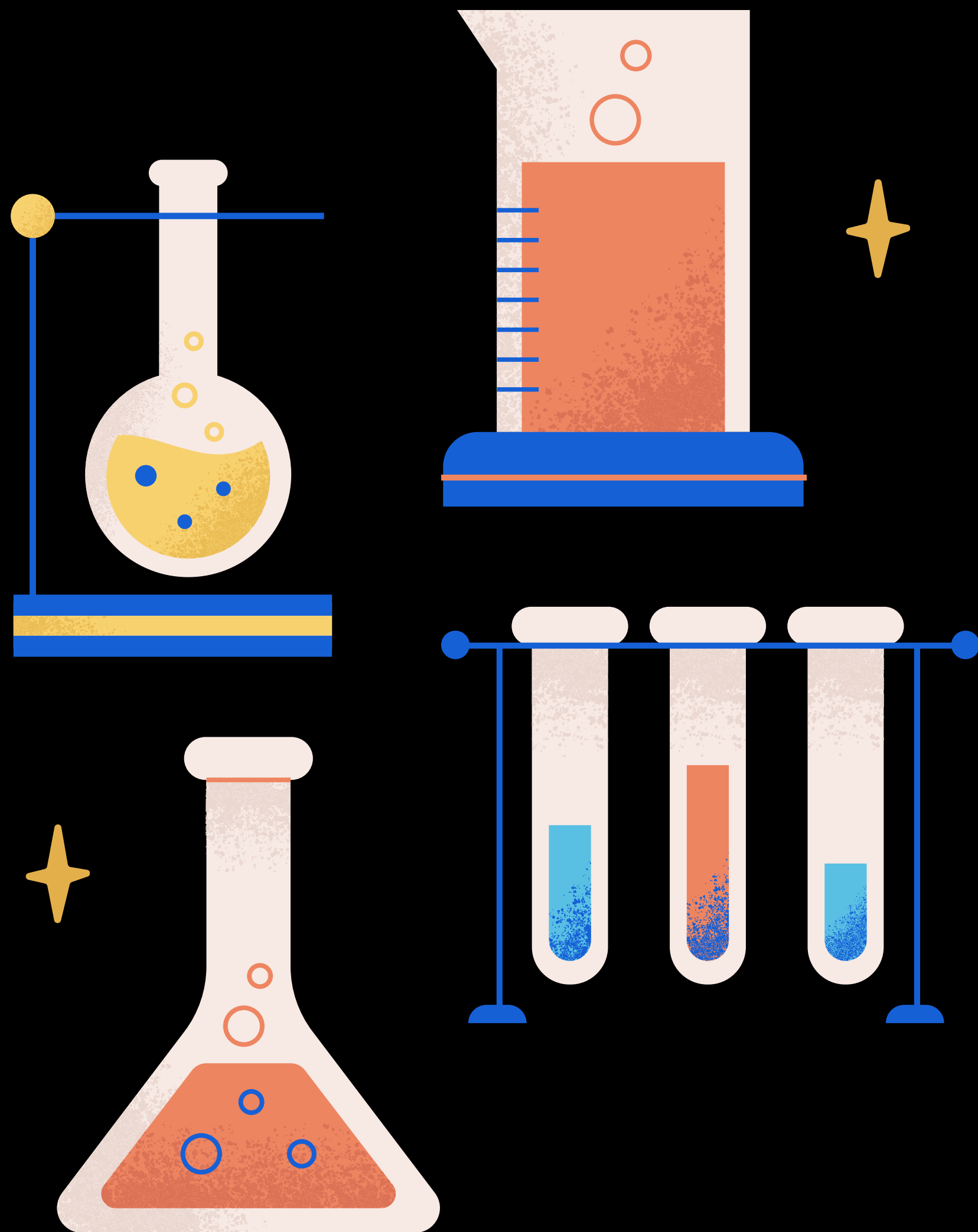
Steroid	Structure	Nominal mass (Da)	Protonated ions (<i>m/z</i>)	Q1 → Q3 transitions
Cortisol		362	363.2	363.3 → 121.1
β-estradiol		272	273.1	237.1 → 107.0
Testosterone		288	289.2	289.1 → 97.0
Progesterone		314	315.2	315.1 → 109.0
Cortisol-d ₄		366	367.2	367.1 → 121.1
Testosterone-d ₃		291	292.2	292.1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

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



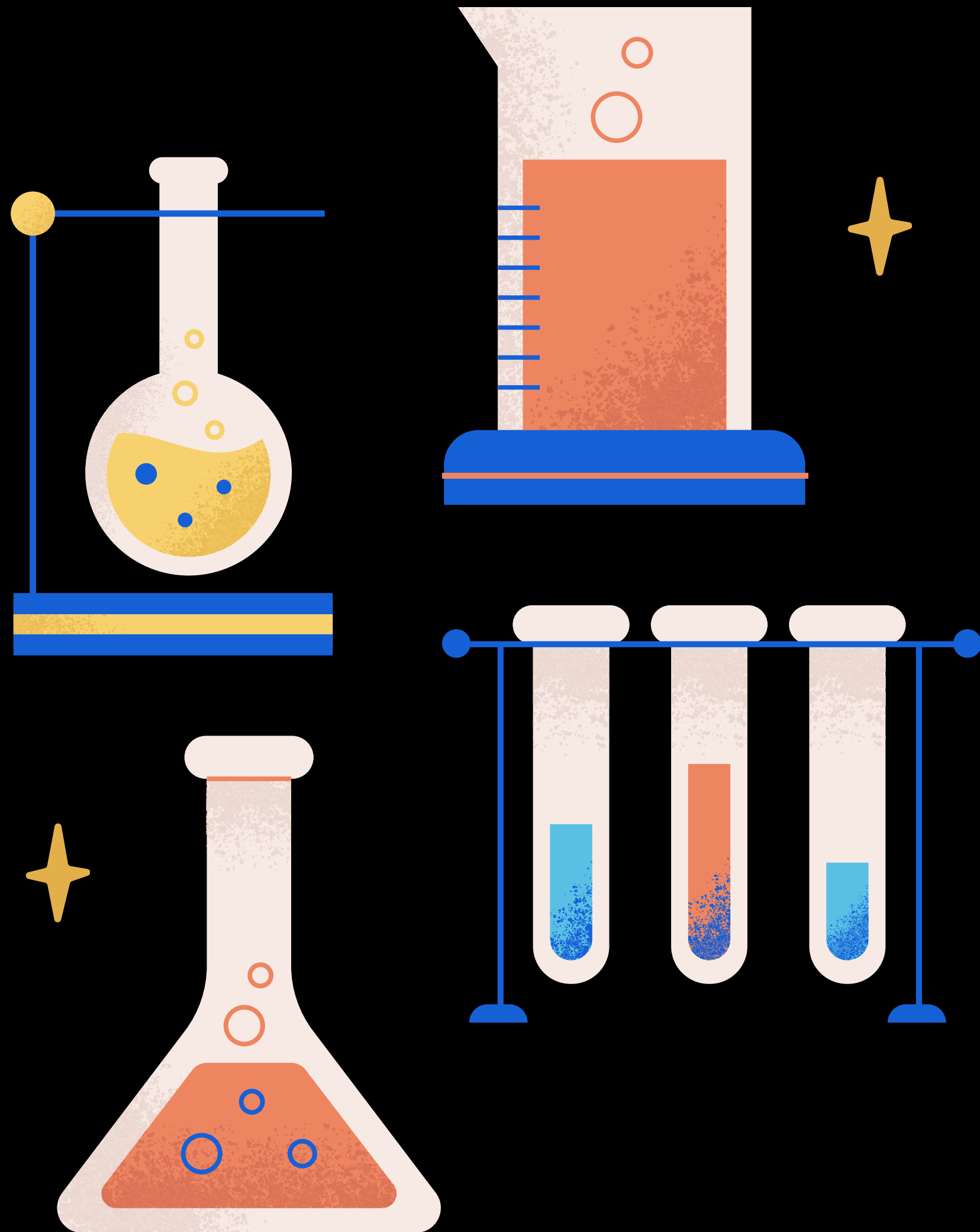
RESULTS

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



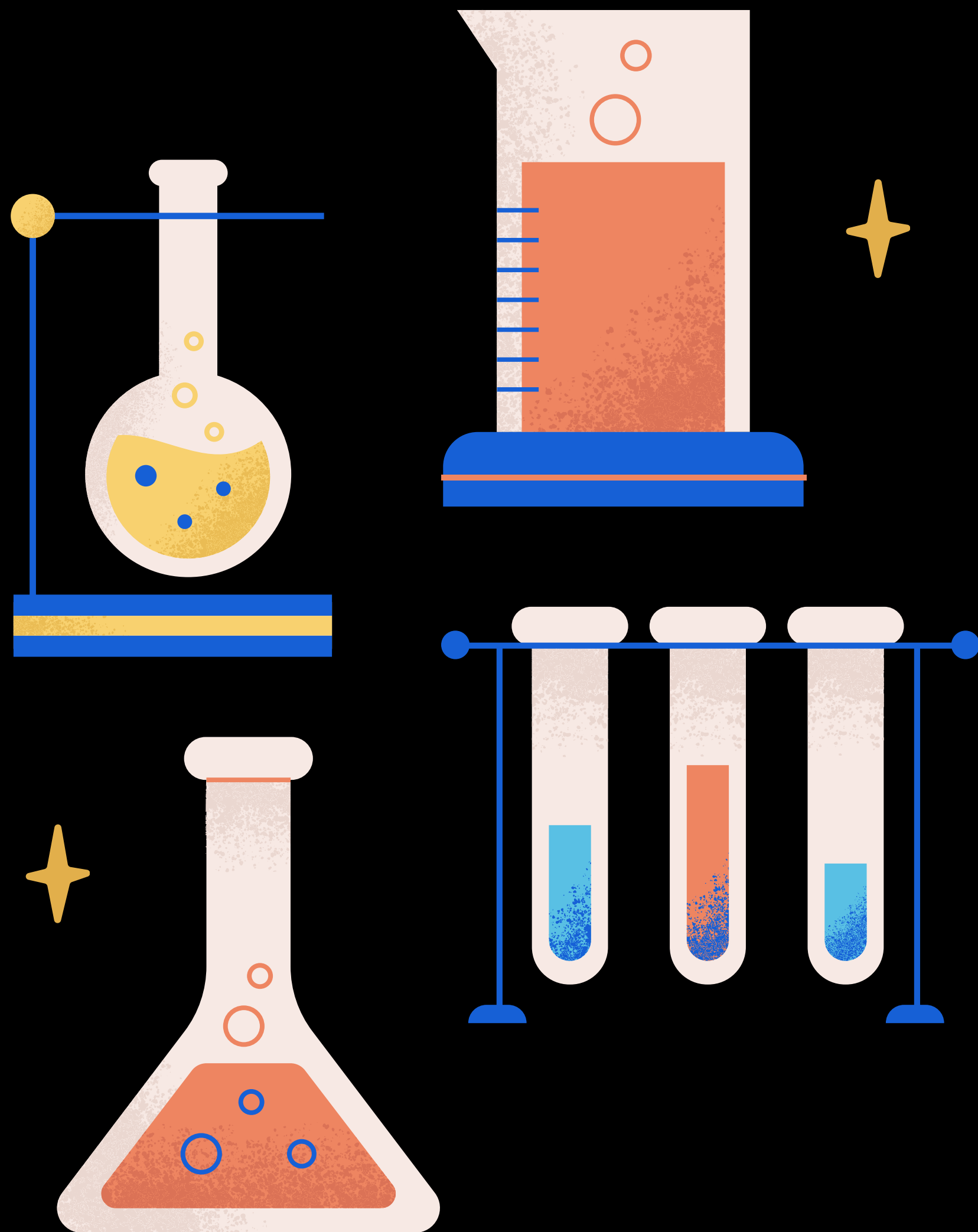
RESULTS

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



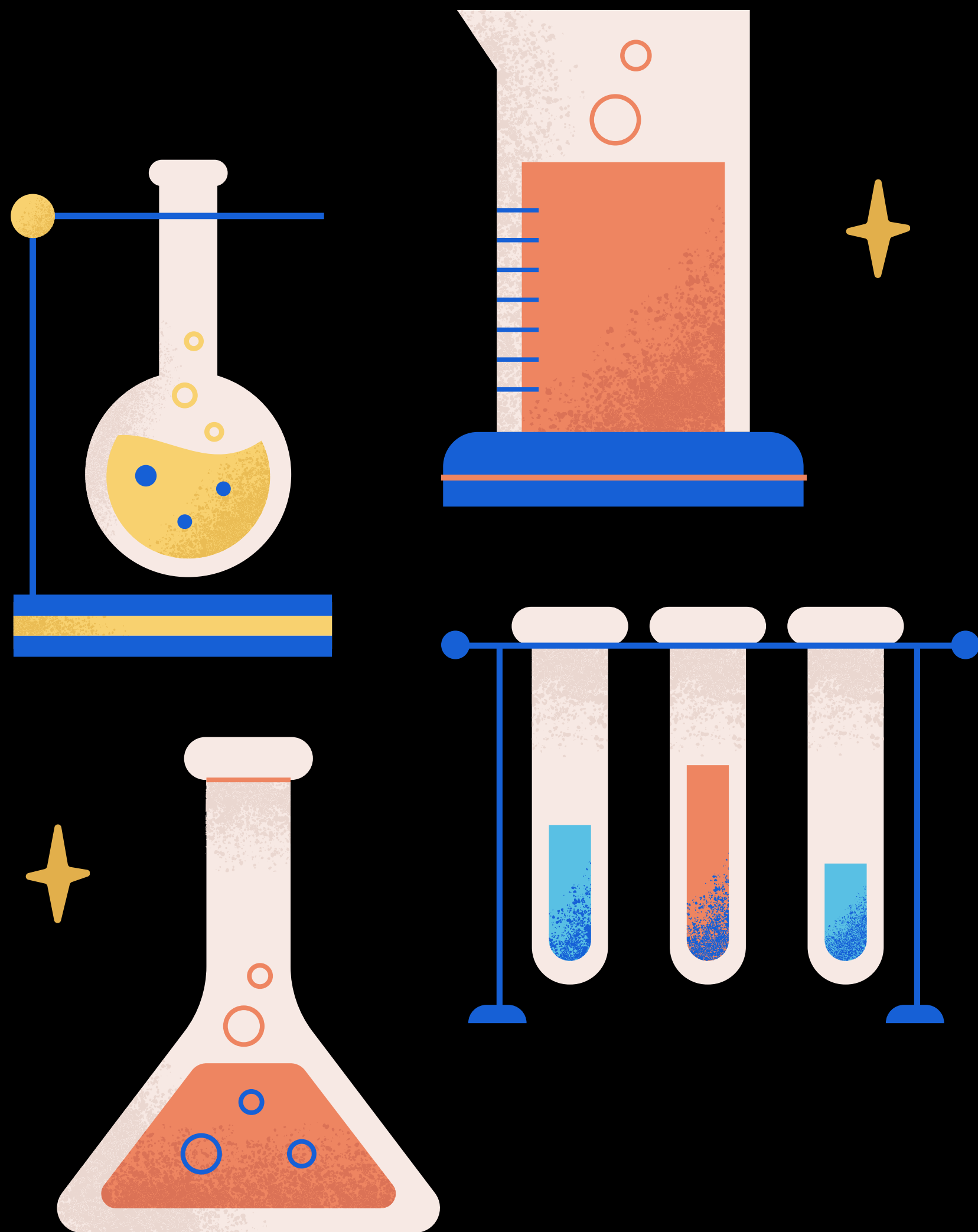
RESULTS

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)~~

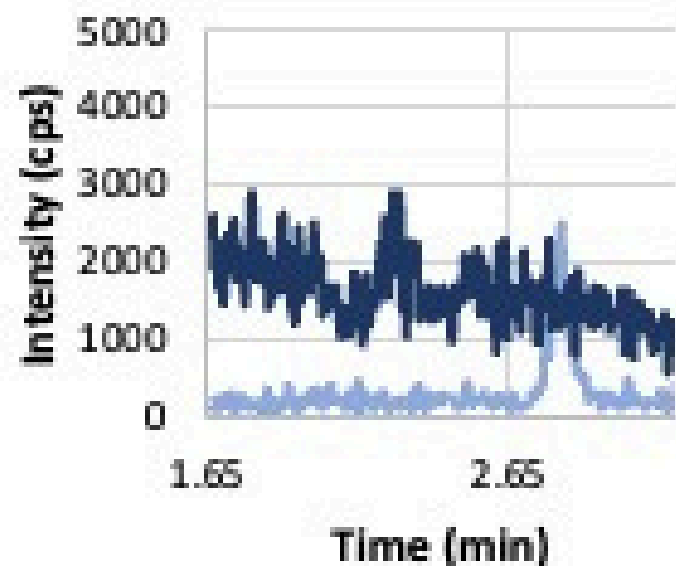


RESULTS

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)

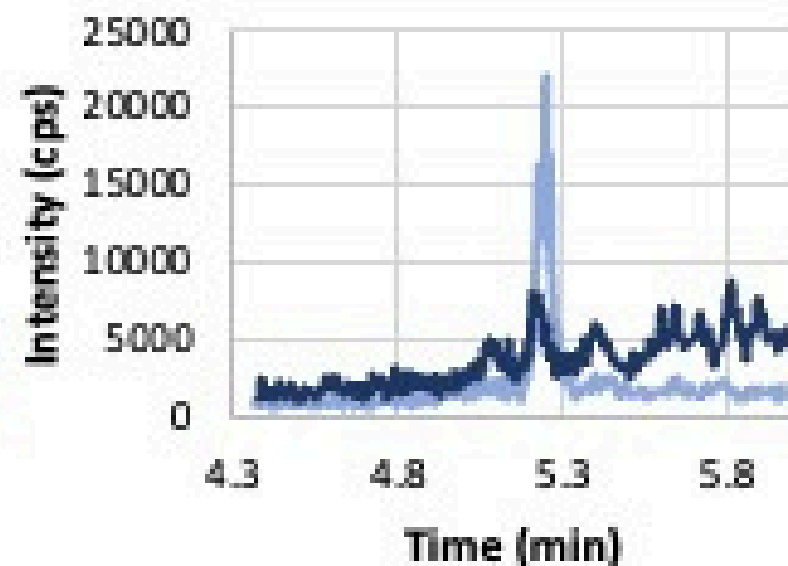
Cortisol levels in femur

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



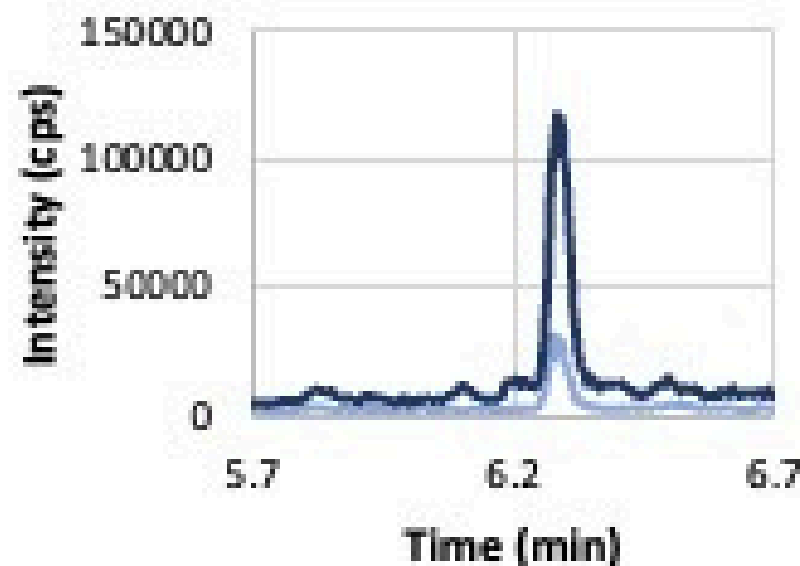
β -estradiol levels in femur

— β -estradiol standard at 4.5 ng/mL
— Femur 200 mg



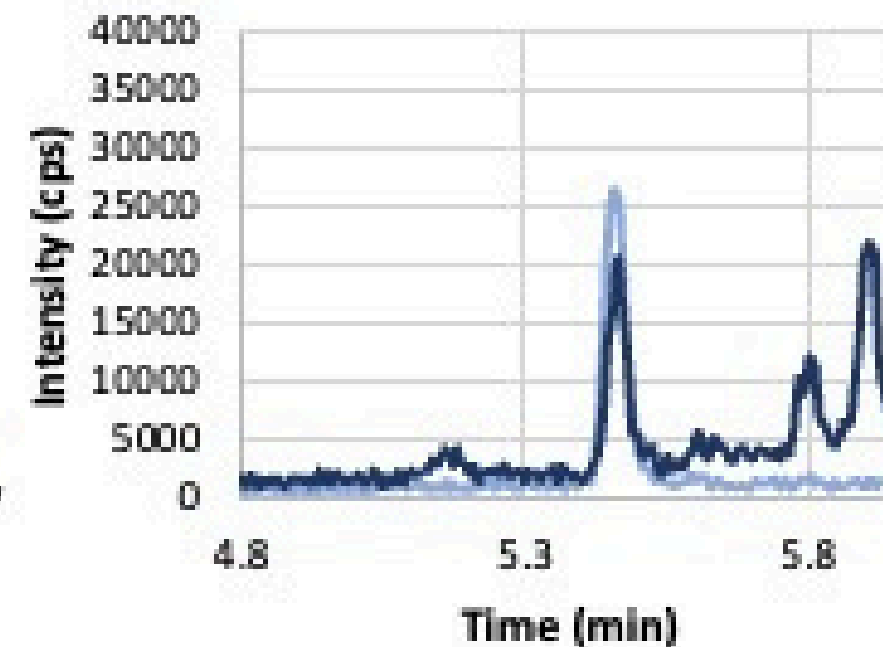
Progesterone levels in femur

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



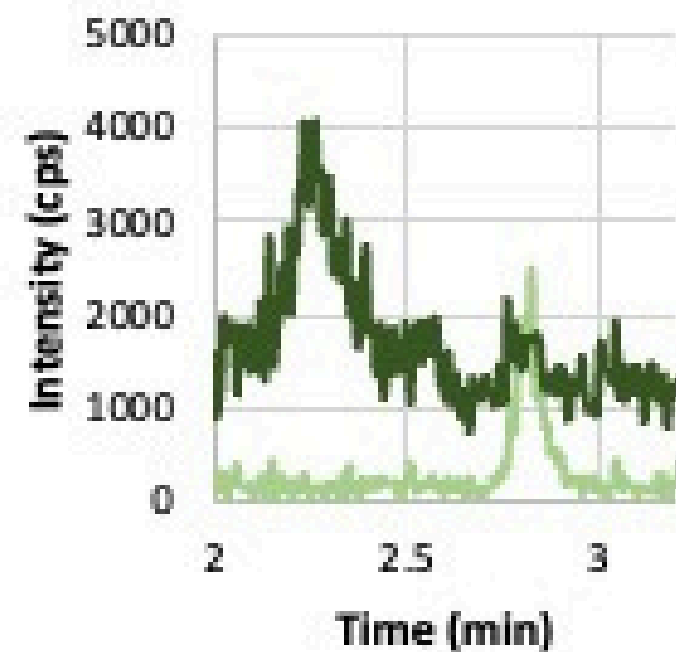
Testosterone levels in femur

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



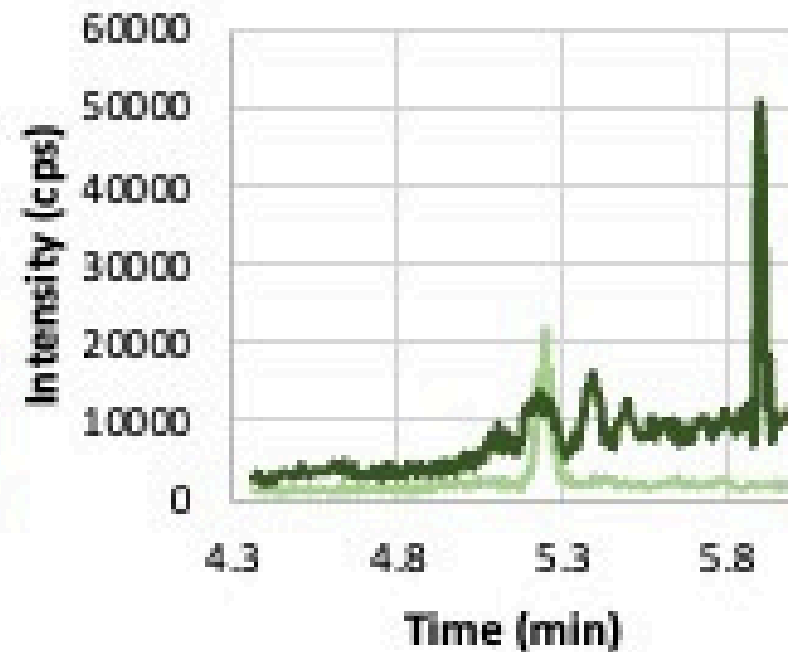
Cortisol levels in rib

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



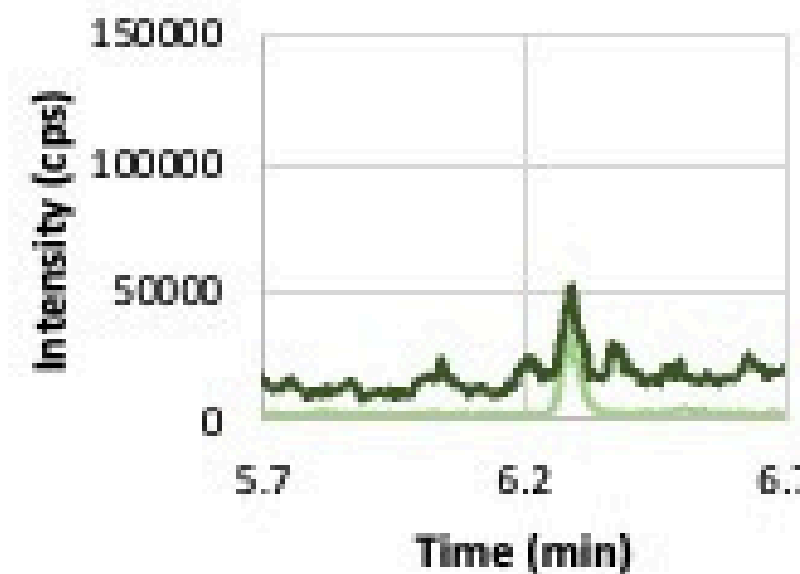
β -estradiol levels in rib

— β -estradiol standard at 4.5 ng/mL
— Rib 100 mg



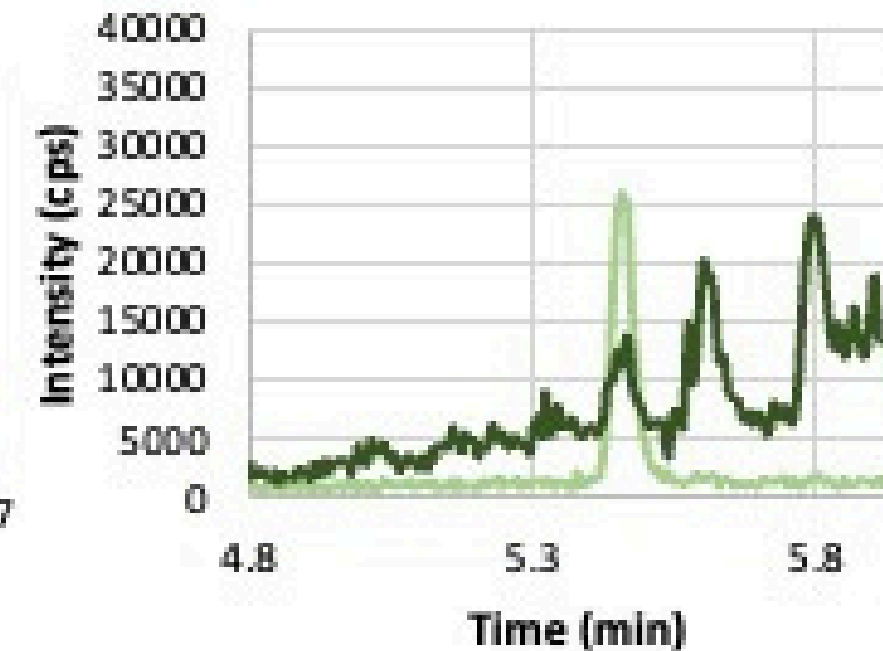
Progesterone levels in rib

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

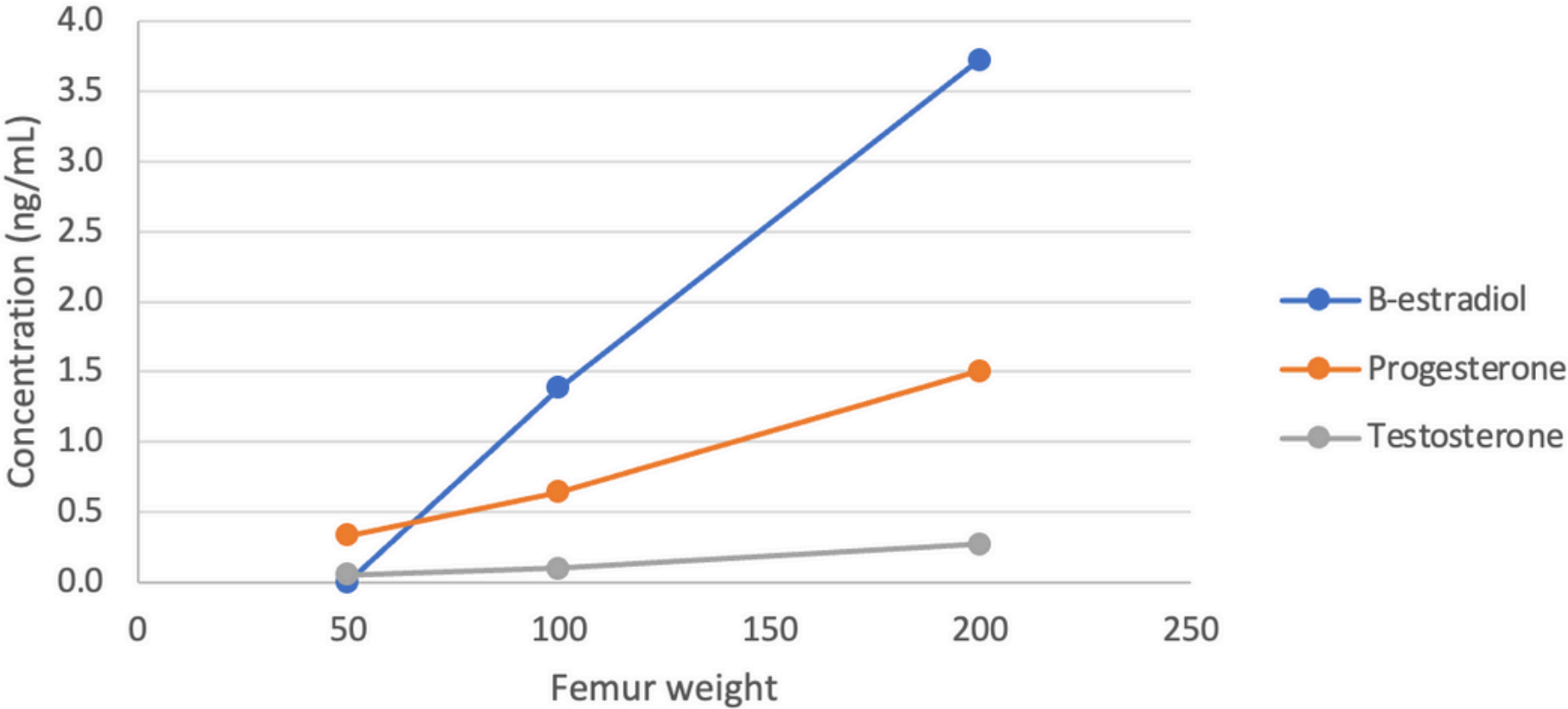


Testosterone levels in rib

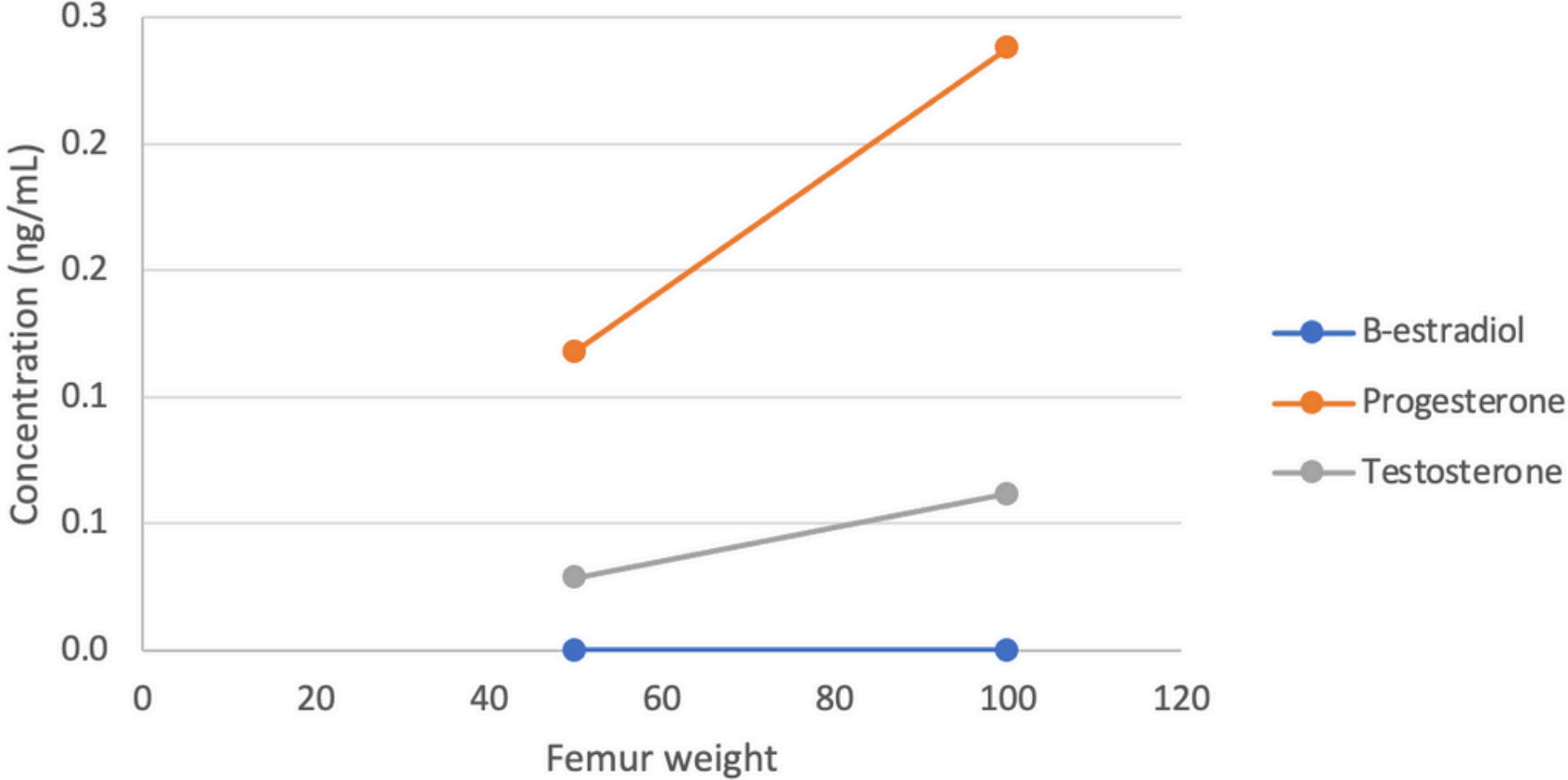
— Testosterone standard at 0.09 ng/mL
— Rib 100 mg

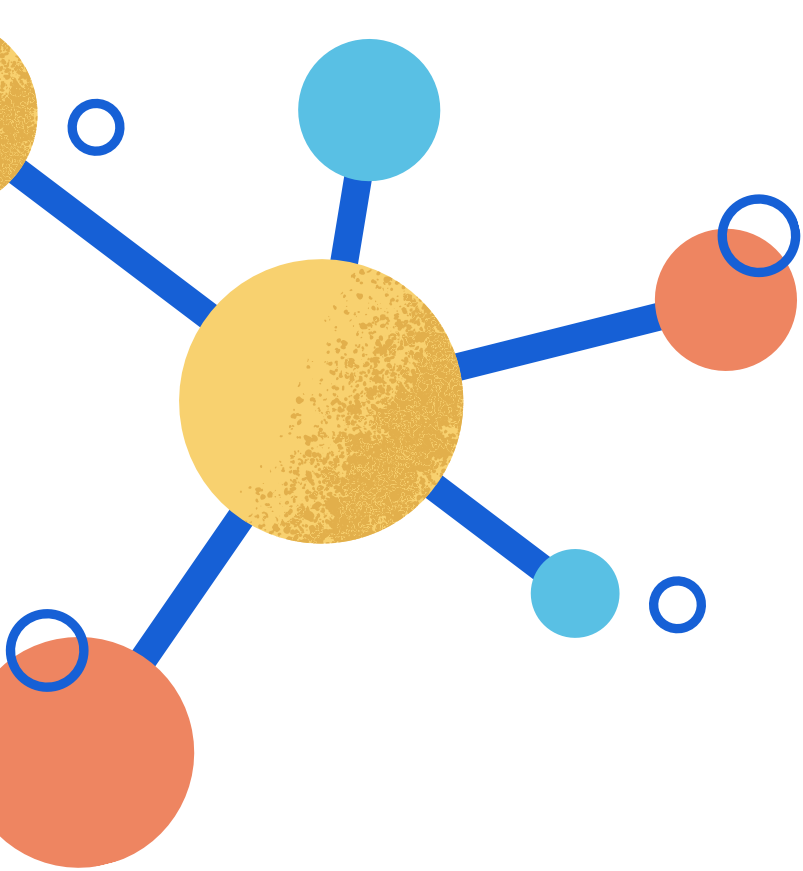


Concentration steroids vs femur weight



Concentration steroids vs Ribs weight

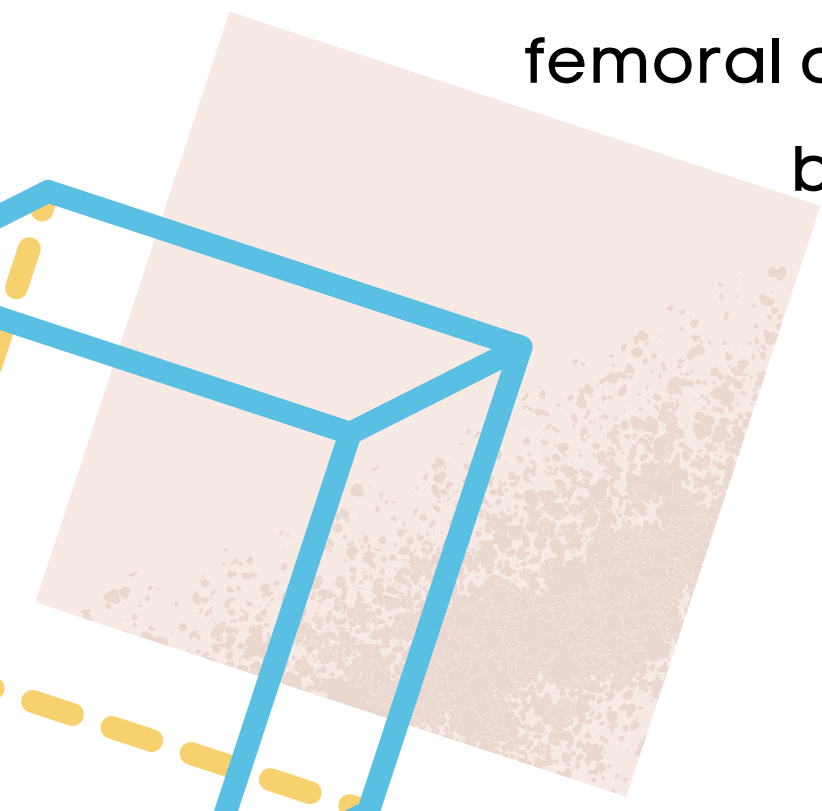




Cortisol

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

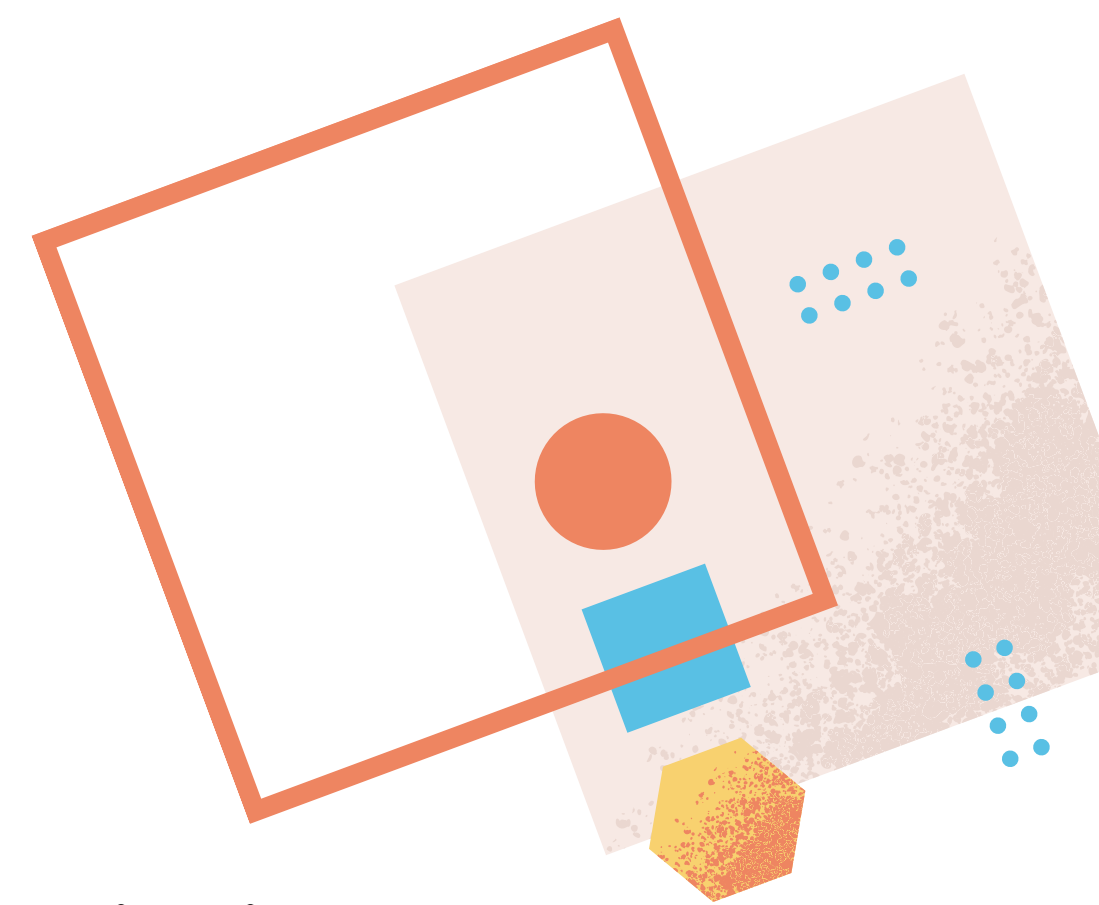
bone



SUMMARY

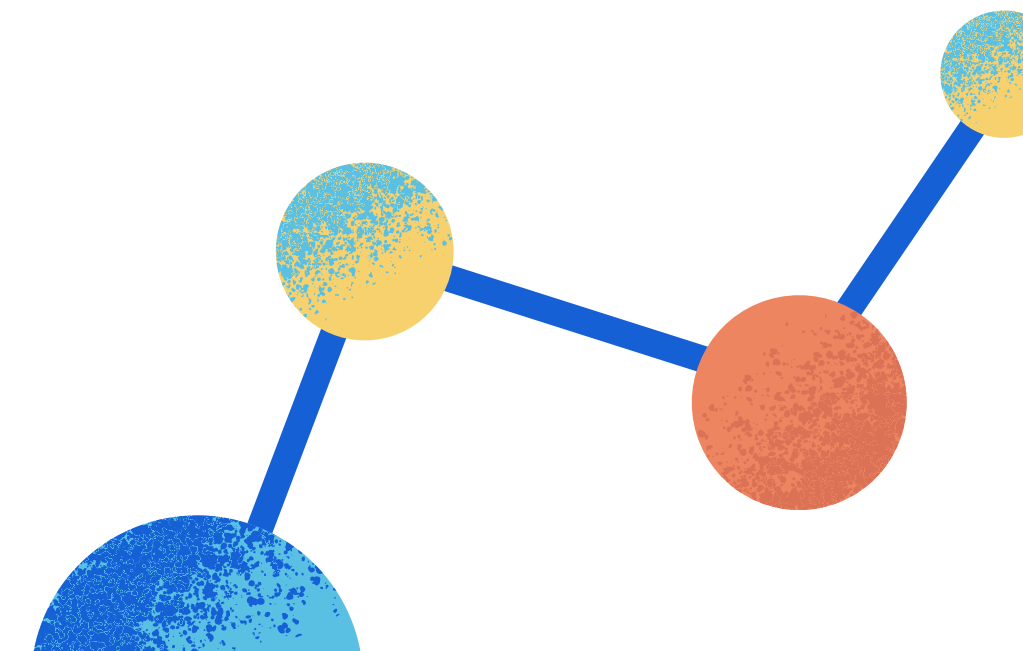
Other Steroids

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 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)