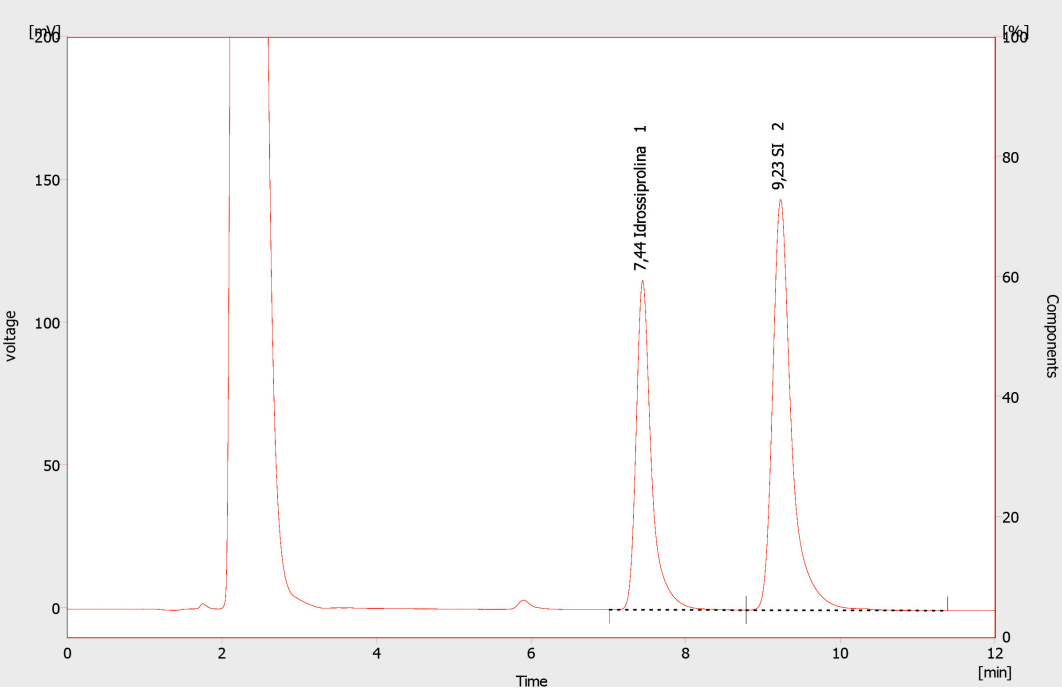
METHOD AT A GLANCE

FLOCHROM® HYDROXYPROLINE IN URINE

4-HydroxyProline is a very abundant amino acid in collagen (about 10%). In normal or pathological cases urinary excretion of hydroxyproline is a reliable indicator of the intensity of collagen of degradation or synthesis and bone remodeling. Urine dosage can be helpful in diagnosis of osteoporosis, bone cancers and other similar pathologies.



HPLC system conditions

Injection volume: 10 µL (variable according to instrumental sensitivity)
Flow rate: 1.2 mL/min
Running time: 12 min
Column heater: Room temperature
Fluorescence detector: 260 nm excitation, 330 nm emission
Column conditioning: column should be conditioned for 10 min at flow rate of 1.0 mL/min with mobile phase

PROVIDE PERSONAL SOLUTIONS

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Sample preparation

- Add 500 µL of urine + 1.5 ml HCl (8 M)* in glass vial, then incubate at 100°C overnight
- Calibration standard doesn't follow the step before, and it is prepared as the hydrolysate as follows:
- Add 200 µL of Reagent A in a 10 mL centrifuge tube
- Add 20 µL of hydrolysate obtained at the step 1 of the procedure or 20 µL of calibration standard and shake well
- Add 100 µL of Internal Standard, mix well
- Add 50 µL of Reagent B, mix well
- Add 50 µL of Reagent C, mix well and wait for 3 min
- Add 100 µL of Reagent D, mix well and wait for 30 sec
- Add 100 µL of Reagent E, mix well and wait for 1 min
- Dilute with 4 mL of solvent mixture (50:50 Acetonitrile/H_2O), mix well
- Transfer 200 µL in autosampler vial and analyze with HPLC technique

*100 mL H_2O + 200 mL HCL 37%

Performance

ANALYTE	LINEARITY (µg /mL)	LLOD (µg /mL)	LLOQ (µg /mL)	CV% INTRA	CV% INTER
Hydroxyproline	2.19 – 183.6	0.66	2.19	2.04 – 6.75	5.2 – 10.9

Ordering guide

EUH05100	FloChrom® Hydroxyproline in Urine	100 assays
EUH05090	Analytical Column	1 pc
EUH05070	Precolumns	5 pcs

CHR-15-19-REV.1

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