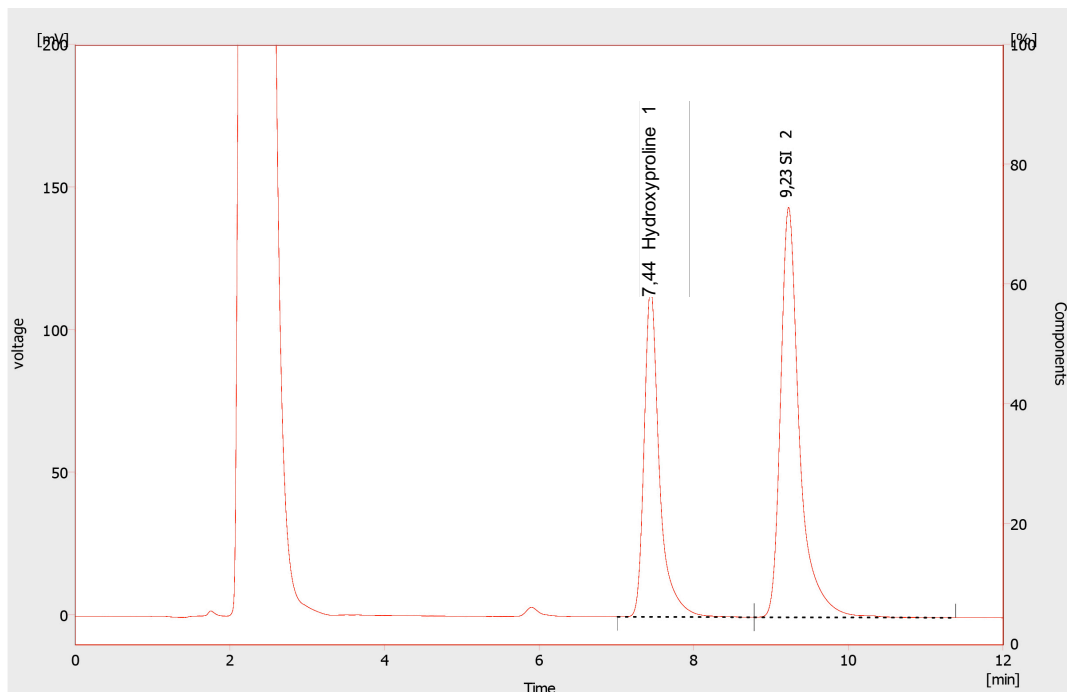


## FLOCHROM<sup>®</sup> HYDROXYPROLINE IN URINE

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  $\mu$ L (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 a flow of 1.0 mL/min with mobile phase

## Sample preparation

• Add 500  $\mu$ L 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  $\mu$ L of Reagent A in a 10 mL centrifuge tube
- Add 20  $\mu$ L of hydrolysate obtained at the step 1 of the procedure or 20  $\mu$ L of calibration standard and shake well
- Add 100  $\mu$ L of Internal Standard, mix well
- Add 50  $\mu$ L of Reagent B, mix well
- Add 50  $\mu$ L of Reagent C, mix well and wait for 3 min
- Add 100  $\mu$ L of Reagent D, mix well and wait for 30 sec
- Add 100  $\mu$ L of Reagent E, mix well and wait for 1 min
- Dilute with 4 mL of solvent mixture (50:50 Acetonitrile/H<sub>2</sub>O), mix well
- Transfer 200  $\mu$ L in autosampler vial and analyze with HPLC technique

\* 100 mL H<sub>2</sub>O + 200 mL HCL 37%

## Performance

ANALYTE	Linearity (mg/L)	LLOD (mg/L)	LLOQ (mg/L)	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