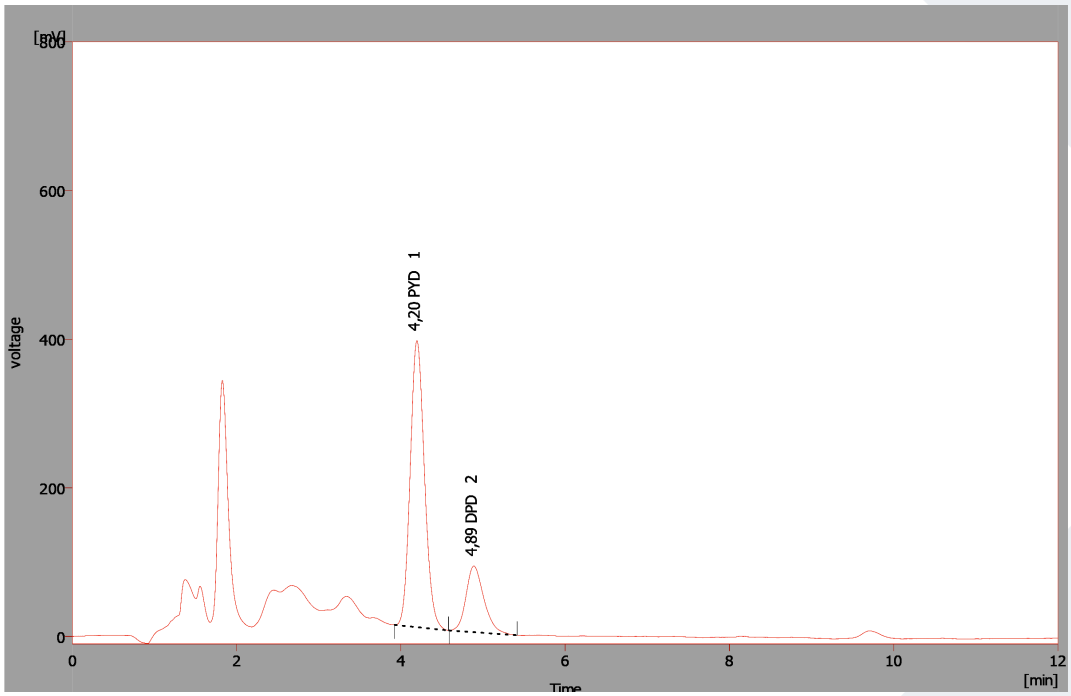
METHOD AT A GLANCE

FLOCHROM® CROSSLINKS IN URINE

The so called “Pyridium Crosslinks”, PYD and DPD, are formed during the collagen maturation. This process is essential for maintaining the structure of collagen fibrils.
When collagen is catabolized as part of normal tissue turnover or due to increased disease-induced degradation of collagen, these compounds are excreted in the urine. The amount of Crosslinks in urine reflects the extent of total collagen degradation and is an important indicator of bone resorption.
Bone turnover, and therefore the excretion of Crosslinks, depends on age: up to the age of 25 the excretion decreases, then it remains relatively constant for several years. Subsequently, increased excretion can be observed, especially in postmenopausal women. Patients with bone disease exhibit significantly greater Crosslinks excretion. The measurement of Crosslinks can be used to monitor the response to treatment of patients with osteoporosis or with Paget's disease and to assess the risk of osteoporosis where a drug treatment would include agents with antiresorptive, antiestrogenic or selective estrogen receptor moderators’ activity. Other conditions for which they have been suggested as biomarkers are arthritis and some types of cancer.



PROVIDE PERSONAL SOLUTIONS

B.S.N. S.r.l. | Biological Sales Network
Tel. +39.0374.351005
Via Coelli, 18 26012 Castelleone (CR)

contact@bsn-srl.it
bsn-srl.it

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HPLC system conditions

Injection volume: 100 µL (variable according to instrumental sensitivity)
Flow rate: 0.5 mL/min
Running time: 12 min
Column heater: 35°C
Fluorescence detector: 290 nm excitation, 400 nm emission
Column conditioning: column should be conditioned for 20 min at flow rate of 0.5 mL/min with mobile phase

Sample preparation

Prepare Reaction Buffer and Washing solution as indicated in IFU.

- Place 1 mL of sample in a screw-capped glass bottle
- Add 1 mL of Hydrolyzing Reagent, vortex and incubate at 120°C overnight
- After cooling, centrifuge for 5 min at 12000 rpm
- Collect 500 µL and transfer in a 10 mL tube
- Add 500 µL of Washing Solution 1 and 2.5 mL of Reaction Buffer as prepared previously
- Vortex, load the entire contents onto an SPE column and percolate under light vacuum
- Add 3 mL of Washing Solution 1 and percolate under gentle vacuum
- Repeat the previous step
- Add 1 mL of Washing Solution 2 and percolate under light vacuum
- Add 1mL of Washing Solution 2 and percolate completely applying high vacuum
- Place 1.5- or 2-mL collection tubes under the SPE columns
- Add 500 µL of Eluent Solution, allow to elute slowly and then apply high vacuum to completely dry the column
- Transfer the eluate into an autosampler vial and inject 100 µL into HPLC system

Performance

ANALYTE	LINEARITY (µg /mL)	LLOD (µg /mL)	LLOQ (µg /mL)	CV% INTRA	CV% INTER
PYP	9.00 – 16.00	238	9.00	2.6 – 10.7	5.9 – 13.1
DPD	2.25 - 360	0.57	2.25	5.3 – 12.6	10.8 – 14.4

Ordering guide

EUH09100	FloChrom® Crosslinks in Urine	100 assays
EUH09051	Controls, lyphil.	2 x 5 x 2.2 mL
EUH09090	Analytical Column	1 pc

CHR-5-22-REV.0

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