

HYDROXYPROLINE IN URINE by UV/VIS FAST – Code Z01610

Chemical characteristics

Hydroxyproline or (2S,4R)-4-Hydroxy-L-pyrrolidine-2-carboxylic acid, is an aminoacid with an hydroxyl group on the pyrrolidine ring, in position 4. Its M.W. is 131,1.

Biochemistry

Hydroxyproline is present almost exclusively in collagen, where represents 40% of the total amount of amino acids. It is formed from proline by a post-translational modification by an enzymatic hydroxylation reaction.

Collagen, main component of bone tissue has a protein structure composed of glycine, proline, hydroxyproline and hydroxylysine. It contains virtually the total content of hydroxyproline and hydroxylysine in the body.

Urinary hydroxyproline is an important marker of bone resorption. Its urinary excretion is an index of the rate of bone resorption. It can increase in osteoporosis, Paget's disease, skeletal metastases, hyperparathyroidism, acute osteomyelitis, osteomalacia.

A large body of evidence highlighted a close relationship between hydroxyproline and direct measurements of bone resorption (bone mineral density, calcium kinetics). In post-menopausal osteoporosis more than 30% of patients have high urinary hydroxyproline values.

Therefore, it is possible to conclude that urinary hydroxyproline is an important marker of bone resorption.



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This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

N° 009	Hydroxyproline in urine by UV/VIS - FAST	06/2024
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TECHNICAL FEATURES

Principle of the Method:

Hydroxyproline is derivatized and injected into the HPLC after acid hydrolysis at 100 °C for 16 h.

<u>Recovery:</u>	100%
<u>Sensitivity:</u>	0,1 mg/l
<u>Linearity:</u>	0,1 – 300 mg/l
<u>Normal 24h urinary values :</u>	10,4 – 22,6 mg/l

<u>Components of the kit :</u>	All the components are ready-to-use, except for Reagent C. Stability: 3 years at 2-8 °C.
Reagent A – Alkaline Solution, 1 x 50 ml	
Reagent B – Buffer Solution, 1 x 50 ml	
Reagent C – Derivatization Solution N°1, 3 x 1,2 ml	<i>To reconstitute with 3 ml of Acetonitrile (not included in the kit). The Reagent C is stable 6 months at 2-8°C after reconstitution.</i>
Reagent D – Derivatization Solution N°2, 1 x 10 ml	
Reagent H – Test Solution, 1 x 5 ml	
Urine Calibrator Liophil, 1 x 5 ml	<u>Code Z01016 (packed separately – see data sheets)</u>
Reagent J – Internal Standard Solution, 1 x 10 ml	
Reagent W – Acidifying Solution, 1 x 10 ml	
Reagent M – Mobile Phase, 3 x 500 ml	

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 20 or 50µl
UV/VIS Detector $\lambda = 495 \text{ nm}$
Chromatograms Recorder

Optional Equipment:

Autosampler
Operational Computer

24 h Urine Collection Procedure:

24 h urine must be collected into a container with 5 ml of HCl 5 M per liter of urine. After collection, 10 ml of urine should be delivered to the lab with the indication of the total diuresis. Delayed analyses require sample freezing at -20 °C or less. Stability of samples: until 2 months.

Warning for the collection procedure : Patients must avoid the intake of foods containing collagen (meat and derivatives, fish, cakes and all the products containing gelatin) at least 24 h before sample collection.

PREANALYTICAL PROCEDURE

Before proceeding with analysis check the analytical system with Test Solution without Internal Standard. Follow this procedure :

Pipette in a tube of 1,5 ml with cap:

- 90 µl of **Reagent B – Buffer Solution**
- 10 µl of **Reagent H –Test Solution**
- 40 µl of **Reagent D – Derivatization Solution N°2**
(verify that pH is ± 9.0)

Close the tube, Vortex for 10 seconds

Incubate at Room Temperature for 45 minutes

INJECTION :

- Inject 20 µl of this solution in the chromatographic system.

Verify that the Test Solution has retention time similar to fig. 1. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system.

You can use again this prepared Solution as a Test for the following analytical session, only if you make some aliquotes of it and store them at $-20\text{ }^{\circ}\text{C}$ (stable about 1 month).

ANALYTICAL PROCEDURE

STEP 1: Acid hydrolysis of samples

- Pipette in a labeled **hydrolysis tube** (with tefloned cap) 500 µl of calibrator, control and patient sample of urine and add 500 µl of 12 N HCl (not included in the kit).
- Close the tubes and place them at 100 °C for 16 h.

STEP 2: Preparation of samples for derivatization

Cooling. **The hydrolyzate may be quoted and stored at - 20 ° C. Stable about 1 month.**

Pipette in a plastic 10 ml tube:

- 100 µl of hydrolysed urine
- 500 µl of **Reagent A** - Alkaline Solution with Internal Standard **Warnings:** Presence of CO₂ bubbles
- 40 µl of KOH concentrate 30% (not included in the kit)
- 500 µl of **Reagent B** – Buffer Solution
- 100 µl of **Reagent J** – Internal Standard Solution

Vortex for 10 seconds

Check that the solution has a pH at 9.0

STEP 3: Derivatization

Transfer in a 2,0 ml glass vial with cap :

- 100 µl of the final solution
- 100 µl of **Reagent C** – Derivatization Solution N°1 (after reconstitution with 3 ml of Acetonitrile)

Vortex for 10 seconds. Wait 1 minute

Incubate at Room Temperature for 5 minutes

Add:

- 100 µl of **Reagent D** – Derivatization Solution N°2
- Close the conical test tube

Vortex for 10 seconds

Incubate at Room Temperature for 45 minutes

Add :

- 100 µl of **Reagent W** - Acidifying Solution

Vortex for 10 seconds

STEP 4: Centrifuge at 5000 rpm for 5 minutes

The solution is stable over 48 hours if stored in a closed tube.

In a dark place and at room temperature.

INJECTION :

Inject 20 µl of the solution into the chromatographic system.

URINARY HYDROXYPROLINE FAST– Warnings

REAGENT H : TEST SOLUTION

HYDROXYPROLINE	About 20 mg/l
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PARAMETERS FOR THE SPECTROPHOTOMETRIC DETECTOR - VARIAN

λ	495 nm
GAIN	Low or Medium
INTEGRATION TIME	4 or 8

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL EC C18 (50 x 4,6 mm – 2,7 µ), the use of Javelin Prefilters (1 x 10 pcs), code S90199511 is obligatory.

HPLC COLUMN CONDITIONING

Install a new analytical column Reverse Phase POROSHELL EC C18 (50 x 4,6 mm – 2,7 µ). Disconnect the detector and flush a solution of H₂O : Acetonitrile (20 : 80 v/v) set flow at 0,8 ml / min for 25 min. **Don't recycle the washing solutions.** Condition the column with the mobile phase at a flow of 1ml / min. for 30 min. Condition further on the column for 30 min. also at recycling phase. Finally inject the derivatized Chemical Standard and verify the quality of the HPLC run.

It is NOT possible to make analysis at recycling phase.

If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

COLUMN CLEANING

Disconnect the detector. Flush 30 ml of H₂O: methanol or Acetonitrile (80: 20 v/v) and download. Flush a solution of H₂O: methanol or Acetonitrile (70: 30 v/v) for 30 minutes for download. When the column will be used again, pass 15 ml of H₂O: methanol (20: 80 v/v) before made conditional with the Mobile phase. It is recommended to wash the analytical column at the end of each session.

HPLC PARAMETERS

LOOP	50 µl
Flow	1 ml / min
Pressure	About 100 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z01016	Urine Calibrator for Hydroxyproline	4 x 5 ml
Z10559	Urine Control for Biogenic Amines - Levels 1 and 2	2 x 5 x 5 ml
Z12611	Mobile Phase	4 x 500 ml
Z699975902	Poroshell 120, EC-C18 (50 x 4,6 mm - 2,7 um) Analytical Column	1 Pc
S90199511	Javelin Prefilters	1 x 10 Pc
S51843550	Clear glass vials with reduced volume from 1,5 ml to 15 ul	1 x 100 Pcs
S51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 ul	1 x 100 Pcs
Z1636/26	10 ml Pirex Tubes with cap SWL (for hidrolisis, till 300 °C- washable and reusable)	1 x 40 Pcs



HYDROXYPROLINE IN URINE by UV/VIS FAST (Reference Chromatograms)

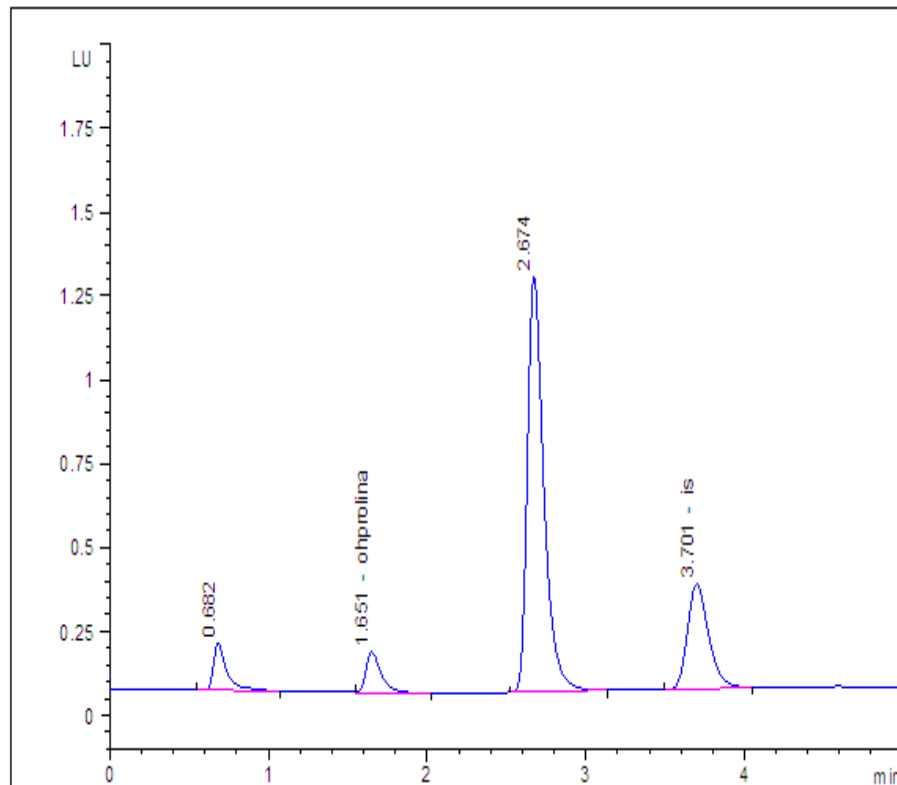


Fig. 1 :	Urine Calibrator		
	R.T. 1.65	Hydroxyproline	19,6 mg/l
	R.T. 3.70	Internal Standard	

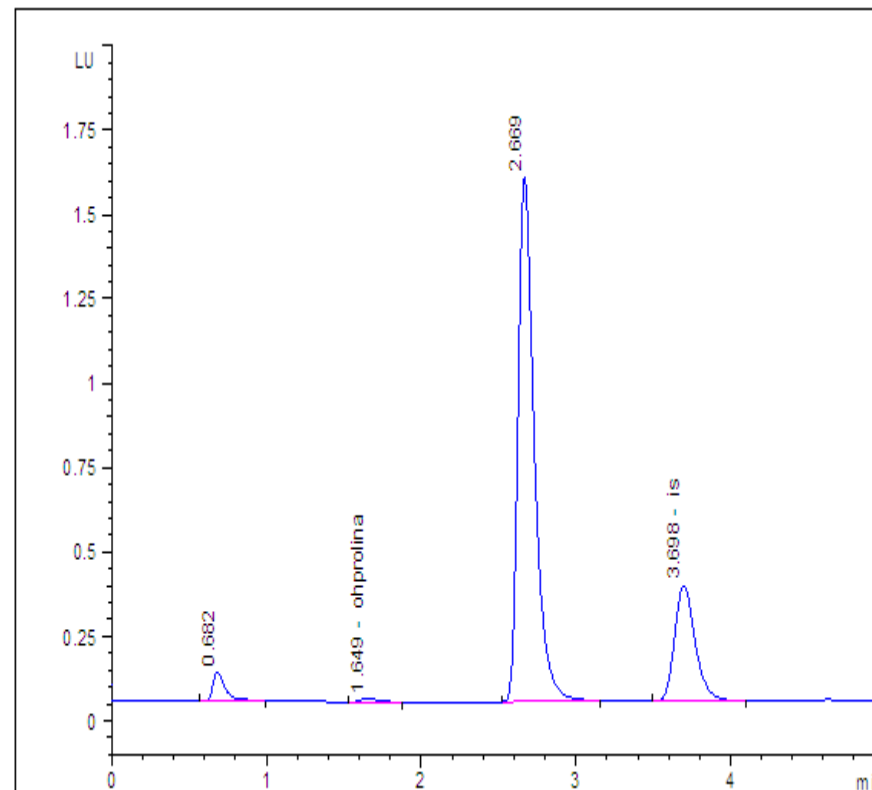


Fig. 2 :	Urine Control		
	R.T. 1.65	Hydroxyproline	1,5 mg/l
	R.T. 3.69	Internal Standard	