

CITRATES IN URINE BY UV - FAST – Code Z32510

BIOCHEMISTRY

Citric acid is an organic acid. Among the urine inhibitors it is considered of particular interest because its administration induces an increase of pH and citrate in urine that can be easily measured. It can therefore correct metabolic abnormalities responsible for lithogenesis. In fact, due to its affinity for calcium, it lowers ionic concentration, thus decreasing the urinary saturation of calcium salts.

In addition, the citrate is a direct inhibitor of the nucleation of calcium oxalate and calcium phosphate and aggregation of calcium oxalate. Finally, inducing an alkalinization of urine (pH 6-7), it also becomes an inhibitor of uric acid stones, since at these pH values the majority of uric acid is in dissociated form and is therefore soluble. Urinary alkalinization induced by citrate not only increases the solubility of uric acid, but also of cystine.

Most literature defines hypocitraturia as citrate values less than 320 mg / 24 hours which can be isolated or associated with other metabolic abnormalities. A decline compared to the normal values may increase the risk of stones, or other solids that are formed in the urinary tract or kidney.

Significant differences were found in citraturia by gender and diet. In fact, increased excretion is seen in females because of the higher rate of estrogen (this would explain the lower incidence of gallstones in women of childbearing age), a diet with excess protein, sodium chloride or prolonged fasting.

With this method you can determine the concentration of citric acid in urine.

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CE

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° 002	Citrates in urine by UV - FAST	07/2024
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TECHNICAL FEATURES

Principle of the Method:

Citrates are first diluted with a specific reagent. Then they undergo the procedure of purification by Clean up columns. The resulting solution is injected directly into HPLC.

Recovery : 100%

Sensitivity (LLOD): 15 mg/l

Minimum concentration analysed (LLOQ): 50 mg/l

Reference Values: 320 –1260 mg/24h

Dynamic Range of the Method : 50 – 20.000 mg/l

Accuracy intra serie (relative error %):

Ci	Cs
100 mg/l	1000 mg/l
5,73%	7,37%

Accuracy inter serie (relative error %):

Ci	Cs
100 mg/l	1000 mg/l
8,00%	8,42%

Reproducibility intra serie (coefficient of variation %):

C LLOQ	Cm	Cs
50 mg/l	500 mg/l	2000 mg/l
13,22%	1,69%	1,23%

Reproducibility inter serie (coefficient of variation %):

C LLOQ	Cm	Cs
50 mg/l	500 mg/l	2000 mg/l
14,15%	1,93%	1,44%

Coefficient of correlation R2 + Dev Std : 0,9970 ± 0,0013

<u>Components of the kit :</u>	All the Reagents are ready-to-use and stable 3 years at < 20 °C.
Reagent A – Conditioning Solution N° 1, 1 x 120 ml	
Reagent B – Conditioning Solution N° 2, 1 x 120 ml	
Reagent C – Conditioning Solution N° 3, 1 x 120 ml	
Reagent D – Diluent Solution, 1 x 120 ml	
Reagent E – Wash Solution N°1, 1 x 120 ml	
Reagent F – Wash Solution N°2, 1 x 120 ml	
Reagent G – Eluting Solution, 1 x 120 ml	
Reagent H – Test Solution/Chemical Standard, 1 x 10 ml	<u>Store at 2-8 °C</u>
Reagent I – Internal Standard Solution, 1 x 15 ml	<u>Store at 2-8 °C</u>
Calibrator lyoph. in urine , 1 x 5 ml	<u>Code Z32216 (packed separately – see data sheet)</u>
Reagent K – Wash Solution for Injection Needle, 1 x 200 ml	
Reagent M – Mobile Phase, 4 x 500 ml	
Clean-up Columns , 100 pcs	

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 50 µl.
Spectrophotometric Detector UV $\lambda=210$ nm

Optional Equipment:

Autosampler.
Operational Computer

Urine Collect :

24 hours urine.

Preanalytical Phase :

Sample stable 2 days at 2-8 °C or 30 days at – 20 °C.

PREANALYTICAL PROCEDURE

Pipette in a tube:

- 900 µl of H₂O HPLC grade
- 50 µl of **Reagent H - Test Solution**
- 50 µl of **Reagent I - Internal Standard Solution**

NOTE : Before starting the analytical session it would be better to inject 50 µl of this solution in HPLC to identify the retention time of Citrates, that must be similar to that shown in below Fig. If the Test has given positive result, proceed with the analytical session. If it is not the case, verify the functionality of the analytical system.

ANALYTICAL PROCEDURE

STEP 1 : In centrifuge test tube of 10 ml put:

	Calibrator	Sample	Controls
Calibrator	200 µl		
Sample		200 µl	
Controls			200 µl
Reagent D – Diluent Solution	800 µl	800 µl	800 µl
Reagent I – Internal Standard Solution	50 µl	50 µl	50 µl

Vortex for 10 sec.

STEP 2 : Clean-up Columns Conditioning:

- Pour out in column 1,0 ml of **Reagent A – Conditioning Solution 1**
Slowly at 1 ml/min, don't dry. Discharge the percolated liquid.
- Pour out in column 1,0 ml of **Reagent B – Conditioning Solution 2**
Slowly at 1 ml/min, don't dry. Discharge the percolated liquid.
- Pour out in column 1,0 ml of **Reagent C – Conditioning Solution 3**
Slowly at 1 ml/min, don't dry. Discharge the percolated liquid.

STEP 3 : Preparation of the Sample, Calibrator and Controls in clean-up columns:

- Pour the whole sample and the Calibrator, previously prepared, in the Clean-up columns and discharge in a flow of 1 ml/ min (droop by drop).

STEP 4 : Clean-up Columns Washing:

- Wash with 1,0 ml of **Reagent E – Wash Solution 1**
Discharge the percolated liquid and dry with a light flux of air.
- Wash with 1,0 ml of **Reagent F – Wash Solution 2**
Discharge the percolated liquid and dry with a light flux of air.

STEP 5 : Eluting:

- Elute with 1,0 ml of **Reagent G – Eluting Solution**
Slowly at 1 ml/min. Dry and collect the eluate.

Vortex for 10 sec.

N.B.: at this step, the sample is stable 2 days at 2-8 °C

STEP 6: Dilute the eluate 1:5 with Water HPLC grade

Vortex for 10 sec.

INJECTION :

- Inject 50 µl of solution in the HPLC system.

CITRATES IN URINE – Warnings

REAGENT H : TEST SOLUTION

Citrates	10.000 mg/l
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SPECTROPHOTOMETRIC DETECTOR PARAMETERS

λ	210 nm
GAIN	0,001 AUFS
INTEGRATION TIME	10 sec

COLUMN CONDITIONING

Install a new analytical Hypersil Gold AQ 150 x 4,6 mm, 3 μ m, thermostated at 55°C. Disconnect the detector and filter H₂O HPLC grade at 1 ml/min for 15 min. **Don't recycle the washing solutions.** Filter the mobile phase with a vacuum system and a suitable filter of 0,22 μ . Condition the column with the mobile phase at a flow of 1 ml / min. for 30 min at thermostat on.
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. Flux H₂O HPLC grade for 60 min at 1 ml/min at thermostat on. At the end flux H₂O HPLC grade for 60 min at 1 ml/min (at thermostat on or off).

AUTOSAMPLER NEEDLE WASHING

Use **Reagent K** – Wash Solution for Injection Needle.

HPLC PARAMETERS

LOOP	50 μ l
FLOW	1 ml/min.
PRESSURE	About 30 bar
COLUMN THERMOSTATATION	55°C

ACCESSORIES and CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z32216	Calibrator in urine for Citrates/Oxalate	4 x 5 ml
Z32219	Control in urine for Citrates/Oxalate – Levels 1 and 2	2 x 5 x 5 ml
ZTF25303154630	Analytical Column Hypersil Gold AQ 150 x 4,6 mm, 3 μ m	1 Pc
S29057U	Standard glass vials of 2 ml with screw cap	1 x 100 Pcs

CITRATES IN URINE

(Reference Chromatograms)

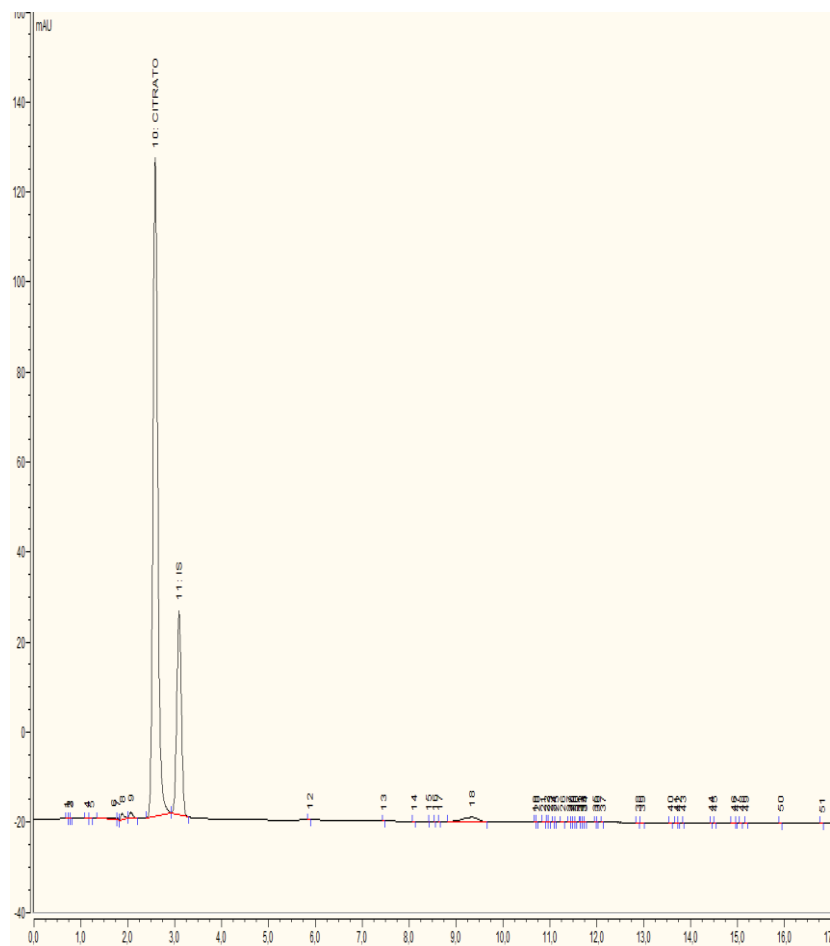


Fig. 1 :	Test Solution
	R.T. 2.7 Citrate
	R.T. 3.2 Internal Standard

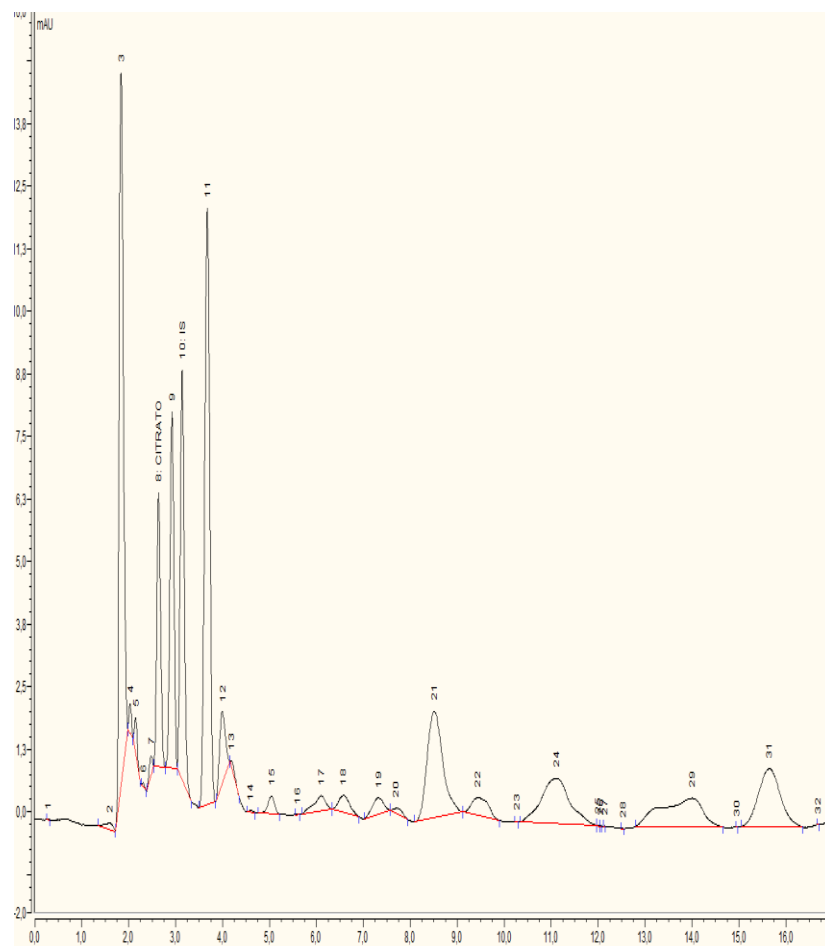


Fig. 2 :	Urine Calibrator
	R.T. 2.7 Citrate 350 mg/l
	R.T. 3.2 Internal Standard