

CITRATES IN SERUM and IN URINE – DUAL KIT BY UV – Code Z32410

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 for urine (13 mg/l for serum) 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/serum.

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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° 005	Citrates in urine and in serum – Dual kit by UV	10/2024
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CARATTERISTICHE DEL METODO

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 :

10 mg/l in urine
2 mg/l in serum

Minimum concentration analyzed:

30 mg/l in urine
3 mg/l in serum

Reference Values:

320 – 1.260 mg/24h in urine
13 – 28 mg/l in serum

Dynamic Range of the Method :

30 – 20.000 mg/l in urine
2 – 20.000 mg/l in serum

CV%:

Intra serie: 4,3% Inter serie: 5% in urine
Intra serie: 1,3% Inter serie: 2,3% in serum

Components of the kit :

All the Reagents are ready-to-use and stable for 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**

To use ONLY for determinate citrates in serum. Store at 2-8 °C

Reagent I – Internal Standard Solution, **1 x 15 ml**

Calibrator lyoph. in urine, **1 x 5 ml**

Code Z32216 (packed separately – see data sheets)

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 100 µl.
Spectrophotometric Detector UV $\lambda=210$ nm

Optional Equipment:

Autosampler.
Operational Computer

Urine Collect :

24 hours urine.

Whole Blood Collect :

Serum without gel.

Preanalytical Phase for urine:

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

Preanalytical Phase for serum:

Sample stable for 30 days at – 20 °C.

PREANALYTICAL PROCEDURE DUAL KIT

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 1 µl of this solution in GC to identify the time of retention of Opiates, 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 FOR URINE

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 for 2 days at 2-8 °C

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

Vortex for 10 sec.

INJECTION :

- Inject 100 µl of solution in the HPLC system.

ANALYTICAL PROCEDURE FOR SERUM

STEP 1 : Chemical Standard Preparation

Pipette in a tube of 10 ml :

- 3990 µl of H₂O HPLC grade
- 10 µl of **Reagent H - Chemical Standard**

This is a **Standard Solution** at 25 mg/l

STEP 2 : In centrifuge test tube of 10 ml put:

	Chemical Standard	Sample	Controls
Standard Solution at 25 mg/l	1000 µl		
Sample		1000 µl	
Controls			1000 µl
Reagent D – Diluent Solution	1000 µl	1000 µl	1000 µl
Reagent I – Internal Standard Solution	50 µl	50 µl	50 µl

Vortex for 10 sec.

STEP 3 : 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 4 : 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 5 : 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 6 : 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 for 2 days at 2-8 °C

STEP 7: Dilute the eluate 1:5 with Water HPLC grade

Vortex for 10 sec.

INJECTION :

- Inject 100 µl of solution in the HPLC system.

CITRATES in URINE and in SERUM – DUAL KIT – Warnings

REAGENT H : CHEMICAL STANDARD

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 PL Hi-Plex H 300 x 7,7 mm, 8 μ m, thermostated at 55°C. Disconnect the detector and filter H₂O HPLC grade at 0,6 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 0,6 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 0,6 ml/min at thermostat on. At the end flux H₂O HPLC grade for 60 min at 0,6 ml/min (at thermostat on or off). **Store the column in water HPLC grade and at 2-8 °C.**

AUTOSAMPLER NEEDLE WASHING

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

HPLC PARAMETERS

LOOP	100 μ l
FLOW	0,6 ml/min.
PRESSURE	About 30 bar
COLUMN THERMOSTATATION	55°C

ACCESSORIES and CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z32220	Liquid Calibrator for Citrates	2 x 5 ml
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
Z32411	Mobile Phase	4 x 500 ml
ZPL1170-6830	PL Hi-Plex H (300 x 7,7 mm, 8 μ m) Analytical Column	1 Pc
S90199511	Javelin Prefilters	1 x 10 Pcs
S29057U	Standard glass vials of 2 ml with screw cap for Autosampler	1 x 100 Pcs

CITRATES IN URINE

(Reference Chromatograms)

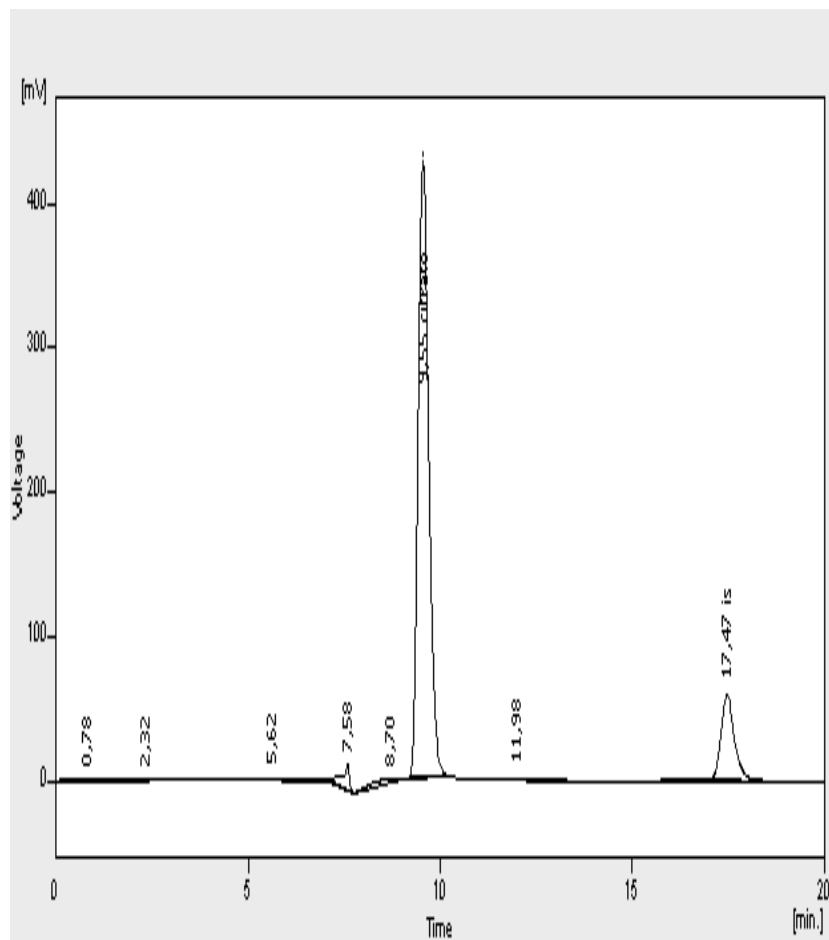


Fig. 1 :	Test Solution
	R.T. 9.55 Citrate
	R.T. 17.46 Internal Standard

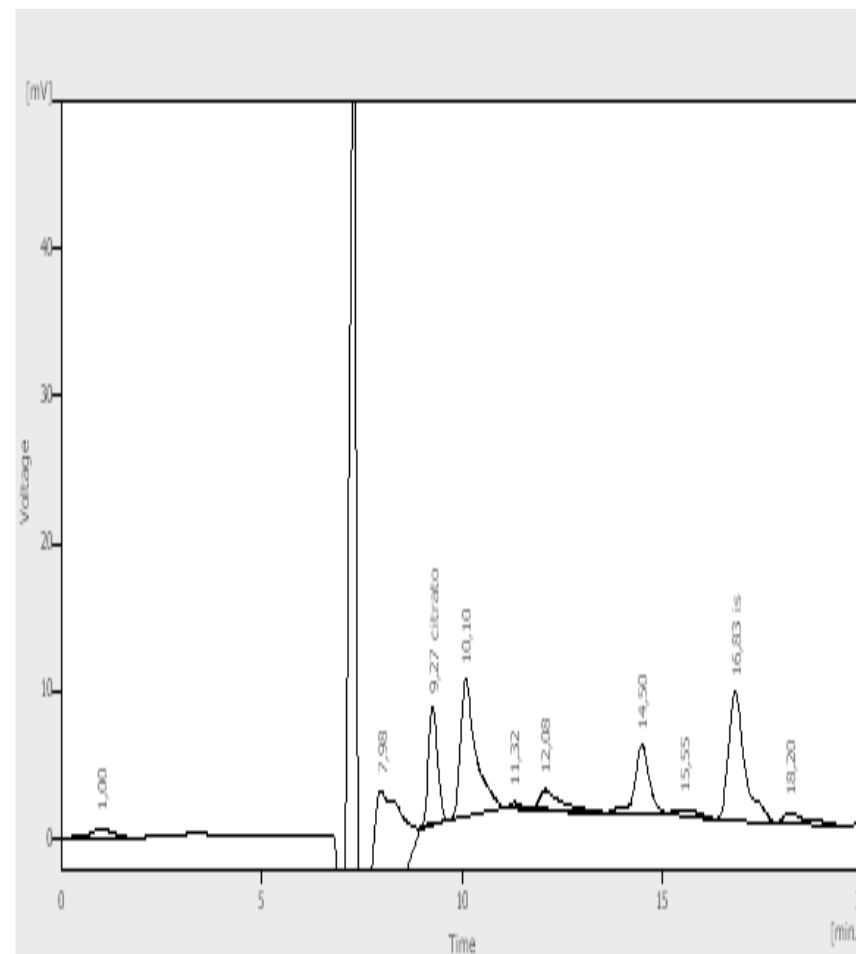


Fig. 2 :	Urine Calibrator
	R.T. 9.27 Citrate 525 mg/l
	R.T. 16.82 Internal Standard

CITRATES IN SERUM

(Reference Chromatograms)

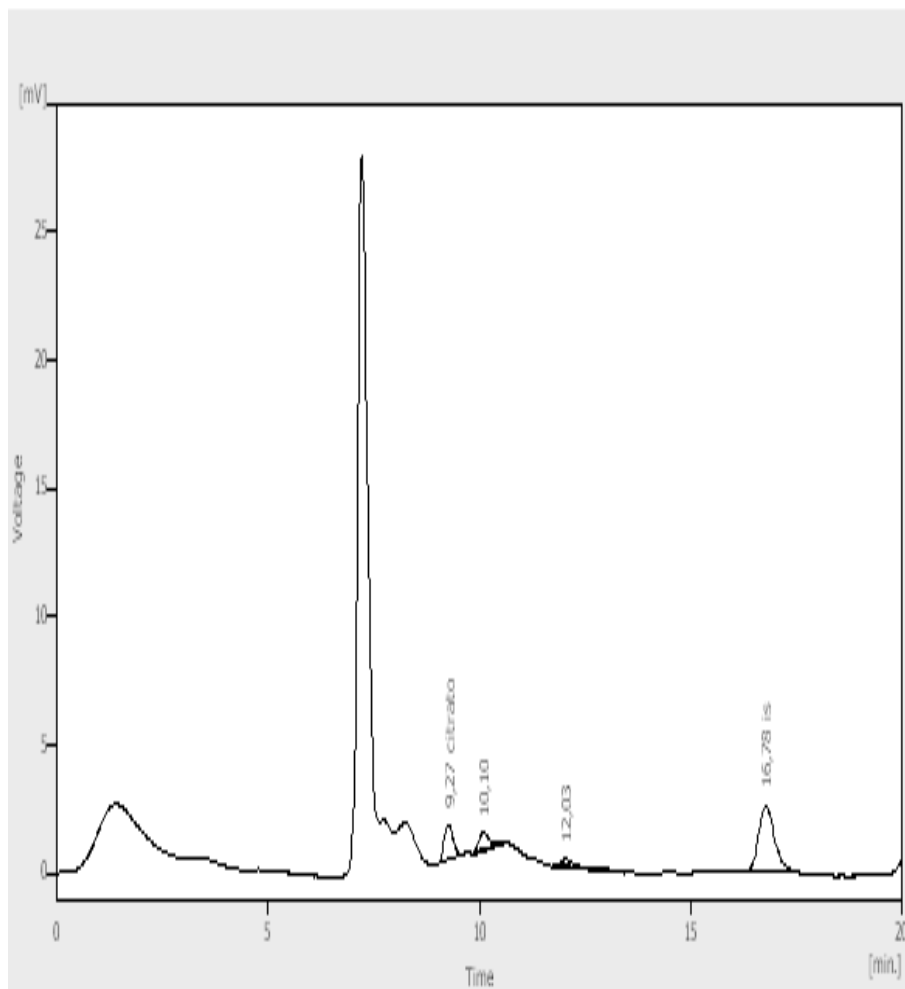


Fig. 3 :	Serum Calibrator
	R.T. 9.27 Citrate 16,6 mg/l
	R.T. 17.78 Internal Standard

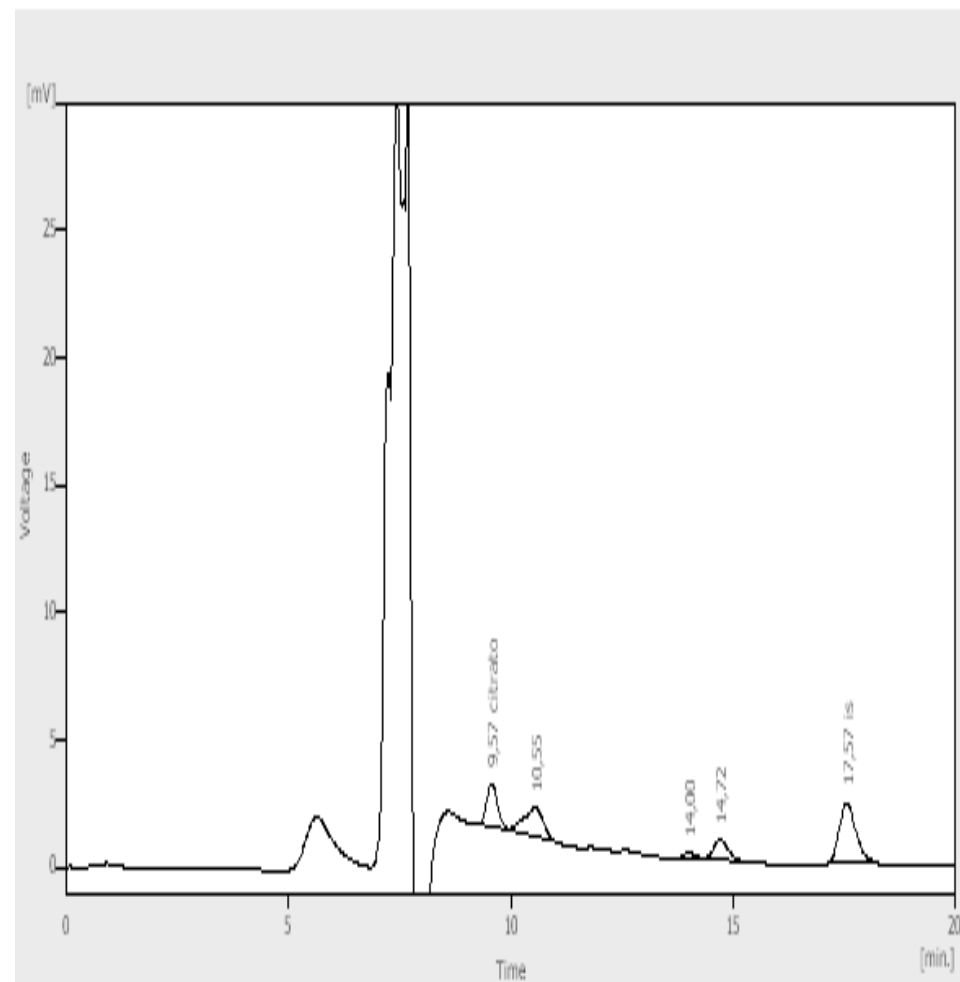


Fig. 4 :	Serum Calibrator
	R.T. 9.57 Citrate 25 mg/l
	R.T. 17.57 Internal Standard