

ACETONE IN URINE BY UV - FAST – CODE Z42210

BIOCHEMISTRY

Acetone is a colorless, mobile, flammable liquid. It is readily soluble in water, ethanol, ether etc., and itself serves as an important solvent. Acetone is the strongest consumer-grade solvent available. It is ideal for thinning fibreglass resin, cleaning fibreglass tools and dissolving two-part epoxies and superglue before hardening. A heavy-duty degreaser, it is useful in the preparation of metal prior to painting; it also thins polyester resins, vinyl and adhesives. It easily removes residues from glass and porcelain.

In the contexts of biological research, buffers that contain Acetone (such as citrate-buffered formalin) use the Acetone to lyse cells for further experimentation.

Acetone is often the primary (or only) component in nail polish remover. Acetonitrile, another organic solvent, is sometimes used as well.

Acetone is an irritant and inhalation may lead to hepatotoxic effects (causing liver damage). The vapours should be avoided. In no circumstance should it be consumed directly or indirectly. Always use goggles when handling Acetone; it can cause permanent eye damage (corneal clouding).

Small amounts of Acetone are metabolically produced in the body, mainly from fat. In Humans, fasting significantly increases its endogenous production (ketosis). Acetone can be elevated in diabetes. Contamination of water, food, or the air can lead to chronic exposure to Acetone. A number of acute poisoning cases have been described. Acetone is not a very toxic compound: it can, however, damage the mucosa of the mouth and can irritate and damage skin. Accidental intake of large amounts of Acetone may lead to unconsciousness and death.

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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.

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TECHNICAL FEATURES

Principle of the Method:

Acetone in the urinary sample is derivatized with a suitable reagent and incubated at 70 °C for 30 minutes. After elution, 10 µl are directly injected into HPLC system.

Recovery :

102,6%
90,5% Recovery LLOQ
106,3% Recovery Cm
111,0% Recovery Ch

Sensitivity (LLOD) :

0,033 mg/l

Minimum Concentration analyzable (LLOQ) :

0,1 mg/l

Linearity :

0,1 - 100 mg/l

Accuracy intra serie (relative error %):

Ci	Cs
0,5 mg/l	5 mg/l
5,49%	4,02%

Accuracy inter serie (relative error %):

Ci	Cs
0,5 mg/l	5 mg/l
7,71%	3,58%

Reproducibility intra serie (coefficient of variation %):

C LLOQ	Cm	CA
0,1 mg/l	1 mg/l	10 mg/l
16,59%	5,32%	1,94%

Reproducibility inter serie (coefficient of variation %):

C LLOQ	Cm	CA
0,1 mg/l	1 mg/l	10 mg/l
17,66%	6,43%	7,08%

Coefficient of correlation R2 + Dev. std:

0,9995 ± 0,0002

Acceptable values in urine after work shift :

< 25 mg/l BEI
Ref.: ACGIH 2020

Components of the kit :

All the components are ready-to-use and stable 3 years at room temperature (≤ 20 °C).

Reagent A – Conditioning Solution 1, **1 x 50 ml**

Reagent E – Conditioning Solution 2, **1 x 50 ml**

Reagent B – Derivatization Solution, **1 x 50 ml**

Reagent C – Stabilizing Solution, **1 x 100 ml**

Reagent D – Wash Solution, **1 x 100 ml**

Reagent F – Eluting Solution, **1 x 80 ml**

Reagent G – Chemical Standard / Test Sol., **1 x 5 ml** **See Warnings Store at 2-8 °C**

Reagent H – Internal Standard Solution, **1 x 20 ml** **Store at 2-8 °C**

Reagent M – Mobile Phase, **2 x 500 ml**

Clean-up Columns, **100 pcs**

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 10 μ l
Spectrophotometric Detector UV/VIS λ =365 nm
Chromatograms Recorder

Optional Equipment:

Autosampler.
Operational Computer

Urine Collection Procedure:

After work shift, collect 10 ml of urine in a suitable test tube. Delayed analyses require sample freezing at -20°C or less. Stable over 4 weeks.

PREANALYTICAL PROCEDURE

Test Solution at 79,1 mg/l Preparation

Pipette in a tube:

- 900 µl of H₂O HPLC grade
- 100 µl of **Reagent G – Test Solution**

This is a Test Solution 79,1 mg/l

Pipette in a 1,5 ml vial:

- 200 µl of **Test Sol. 79,1 mg/l**
- 200 µl of **Reagent B – Derivatization Sol.**

Vortex for 5 sec.

- Incubate at 70 °C for 30 min.

Cooling

INJECTION :

- Inject 10 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.

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ANALYTICAL PROCEDURE

STEP 1 : Chemical Standard 7,91 mg/l Preparation

Pipette in a tube:

- 9,9 ml of H₂O HPLC grade
- 100 µl of **Reagent G - Chemical Standard**

This is a **Chemical Standard** 7,91 mg/l

IMPORTANT : It is important to use standard solution just prepared.

STEP 2 : Samples Preparation

Pipette in suitable glass-tubes with Teflon screw cap for hydrolysis in sequence:

	Blank	Chemical Standard	Samples
H₂O HPLC grade	1,0 ml		
Chemical Standard 7,91 mg/l		1,0 ml	
Samples			1,0 ml
Reagent H – Internal Std Sol.	0,2 ml	0,2 ml	0,2 ml
Reagent B – Derivatization Sol.	0,5 ml	0,5 ml	0,5 ml

Vortex for 5 sec.

STEP 3 : Incubate at 70 °C for 30 minutes, cooling at Room Temperature and add 1 ml of **Reagent C – Stabilizing Sol.**

Vortex for 5 sec.

STEP 4 : Clean-up Columns Conditioning

- Prepare Clean up columns per each Sample, Calibrator and Control.
- Conditionate Clean-up Columns with 0,5 ml of **Reagent A – Conditioning Sol. 1** and discharge the percolated liquid.

STEP 5 : Conditionate Clean-up Columns with 0,5 ml of **Reagent E – Conditioning Sol. 2** and discharge the percolated liquid.

STEP 6 : Dispense 2,7 ml of sample, previously prepared, into clean up columns.

STEP 7 : Wash Clean-up Columns with 1,0 ml of **Reagent D – Wash Sol. N°1** and discharge the percolated Liquid
Let dry with a flux of air!

STEP 8 : Elute with 0,8 ml of **Reagent F – Eluting Sol.**

STEP 9 : Add 0,2 ml of Water HPLC grade at the eluate.

Vortex for 5 sec.

N.B.: at this step, the sample is stable 3 days at 2-8 °C

INJECTION :

- Inject 10 µl of the eluate into the chromatographic system.

N.B.: If the Blank presented the peak of Acetone, proceed to identify the quantitative difference between the areas / heights of the peaks of Acetone in Calibrators/Samples/Controls and blank.

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ACETONE - Warning

REAGENT G : CHEMICAL STANDARD / TEST SOLUTION

ACETONE	791 mg/l
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SPECTROPHOTOMETRIC DETECTOR PARAMETERS

λ	365 nm
GAIN	0,01 AUFS
INTEGRATION TIME	10 sec.

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL 120 EC-C18 (50 x 4,6 mm, 2,7 μ m), the use of Javelin Colfilters (1 x 10 pcs.) cod. S90199511 is obligatory.

HPLC COLUMN CONDITIONING

Install a new analytical C₁₈ Reversed-Phase column POROSHELL 120 EC-C18 (50 x 4,6 mm, 2,7 μ m).
Disconnect the detector and filter 30 ml of **H₂O : Methanol (20 : 80 v / v)** set flow of 1,0 ml/minute. **Don't recycle the washing solutions.** Flux 30 ml of a solution of **H₂O : Methanol (80 : 20 v / v)** at flow of 1,0 ml/minute. **Don't recycle the washing solutions.** Condition the column with the mobile phase at a flow of 1 ml/minute and discharge the first 20 ml. Finally inject the Chemical Standard and verify the quality of the HPLC run.
And it is possible to make analysis at recycling phase, as long as you have the foresight to filter the same mobile phase with 0.22 μ filter before each analytical run. The signal that the mobile phase must be changed is given by the significant increase of the background noise of the detector Fluorimetric. 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 30 ml of **H₂O : Acetonitrile or Methanol (80 : 20 v / v)** set flow of 1,0 ml/minute and discharge. Flux a solution of **H₂O : Acetonitrile or Methanol (30 : 70 v / v)** for 30 minutes discharge it **It is recommended to perform the washing of Analytical Column at the end of each analytical run.** Store the column in a solution of **H₂O : Acetonitrile or Methanol (30 : 70 v / v).**

HPLC PARAMETERS

LOOP	10 μ l
FLOW	1,2 ml/min.
PRESSURE	About 100 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z42020	Chemical Standard for Acetone	2 x 5 ml
Z699975902	Poroshell 120-EC C18 (150 x 4,6mm -4 μ m) Analytical Column	1 Pc
S90199511	Javelin Colfilters	1 x 10 Pcs
S29057U	Standard glass vials of 2 ml with screw cap	1 x 100 Pcs
Z1636/26	Pirex Tubes of 10 ml with cap SWL (for hydrolysis, till 300 °C)	1 x 40 Pcs



ACETONE IN URINE

(Reference Chromatograms)

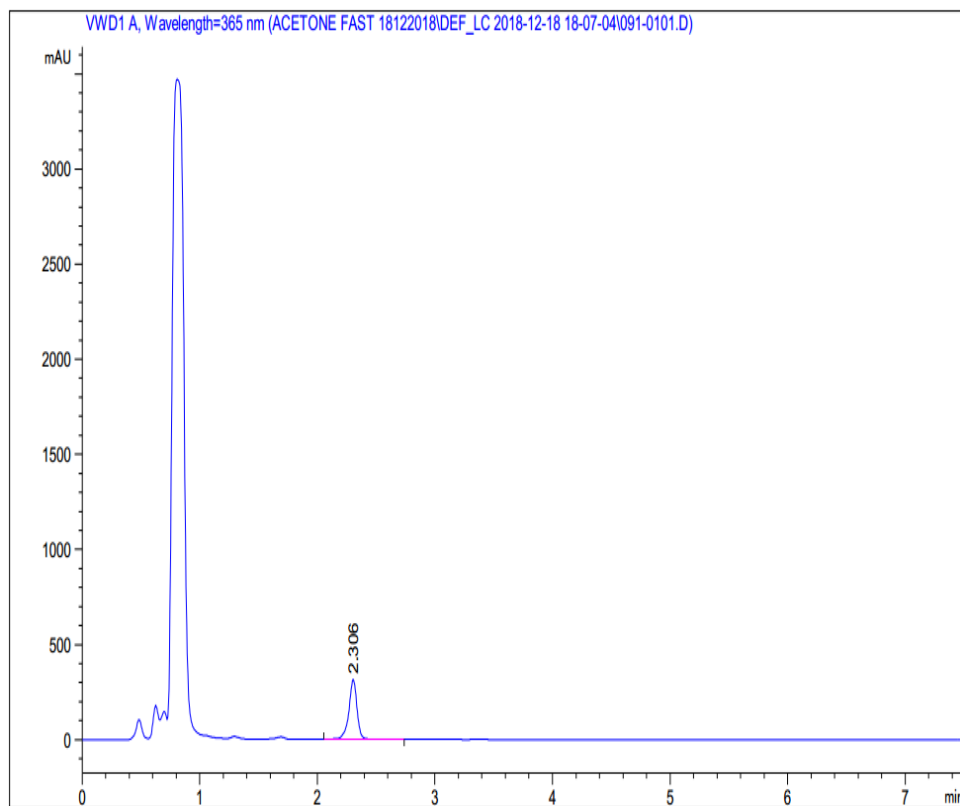


Fig. 1 :	Test Solution
	R.T. 2.306 Acetone

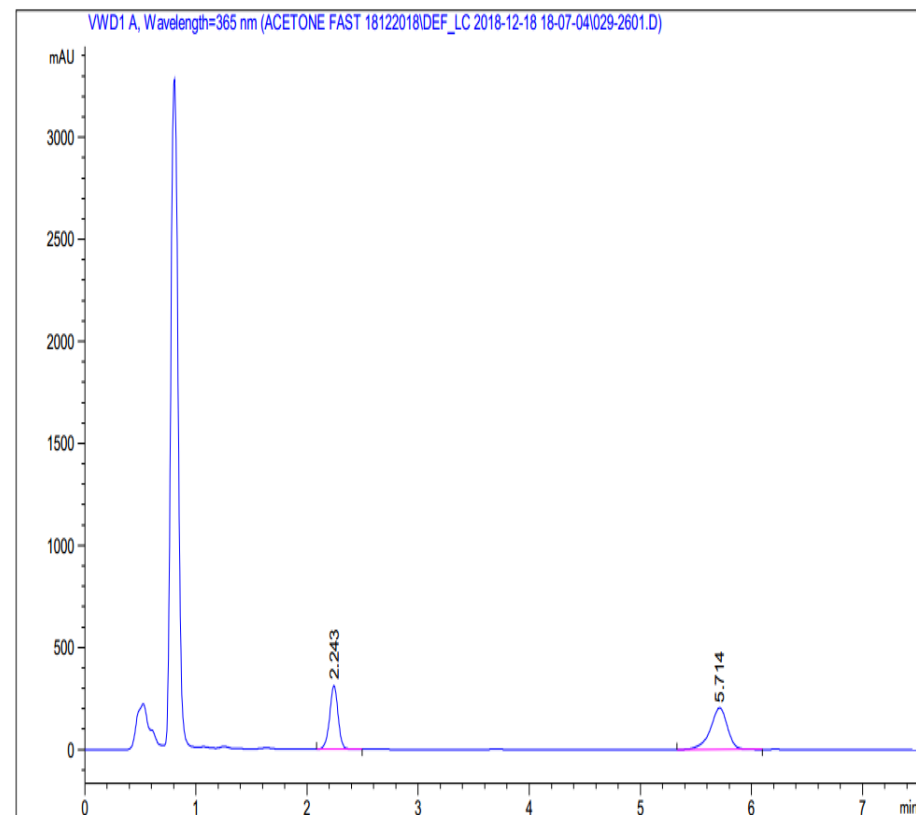


Fig. 2 :	Enriched urine sample
	R.T. 2.243 Acetone
	R.T. 5.714 Internal Standard