

TOTAL 2,5 –HEXANEDIONE IN URINE BY UV – FAST – Code Z05210

BIOCHEMISTRY

2,5 Hexanedione is an aliphatic diketone. It is a product of biochemical transformation of hexane.

Hexane is a common component of glues used in the production of shoes or leathers. Inhalation is the major way of absorption, while ingestion, eye and skin absorption can also occur. It is then metabolised into 2,5 hexanedione and eliminated by urine.

For these reasons 2,5 hexanedione testing is used to determine the risk of exposure to hexane. Additionally, 2,5 hexanedione levels are related to the quantity of hexane adsorbed.

From a clinical point of view 2,5 hexanedione is considered one of the most important metabolites of hexane in the development of polyneuropathies in leather workers and shoemakers.

CHEMICAL ANALYSIS BY HPLC

2,5 Hexanedione (P.M.=114.14) is an aliphatic volatile diketone. Its molecular structure is not directly detectable by UV determination.

This method allows HPLC urinary determination of 2,5 hexanedione avoiding the extraction and concentration steps.

After acid hydrolysis, 2,5 hexanedione is derivatized with a specific reagent for ketones and then directly injected into the HPLC.

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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° 002	Total 2,5-Hexanedione in urine by UV - FAST	06/2024
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TECHNICAL FEATURES

Principle of the Method:

2,5 Hexanedione-glucuronide is hydrolyzed in acid ambient at 100 °C for 1 hour. After cooling, the derivatization reagent is added and the solution is incubated at 70 °C for 30 minutes. After cooling again, 50 µl of solution are directly injected into the HPLC.

Recovery:

100%

Sensitivity (LLOD):

0,016 µg/ml

Minimum concentration analyzable (LLOQ):

0,05 µg/ml

Linearity:

0,05 - 50,0 µg/ml

Acceptable values in urine of not exposed subjects:

< 0,5 mg/g creatinine

Acceptable values in urine after work shift:

< 5 mg/g creatinine

Rif.: G Ital Med Lav Erg 2002;24:3 Suppl

Accuracy intra serie (relative error %) :

Ci	Cs
0,2 µg/ml	6,25 µg/ml
9,78%	2,18%

Accuracy inter serie (relative error %) :

Ci	Cs
0,2 µg/ml	6,25 µg/ml
9,78%	2,12%

Reproducibility intra serie (coefficient of variation %):

C LLOQ	Cm	Cs
0,05 µg/ml	3,125 µg/ml	12,5 µg/ml
3,61%	2,81%	2,21%

Reproducibility inter serie (coefficient of variation %):

C LLOQ	Cm	Cs
0,05 µg/ml	3,125 µg/ml	12,5 µg/ml
10,05%	3,76%	2,45%

Coefficient of correlation R2 + Dev Std

0,9945 ± 0,0069

Components of the kit :

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

Reagent A - Derivatization solution, **2 x 10 ml**

Reagent B - Test Solution /Chemical Standard 390 mg/l, **1 x 5 ml** **Store at 2-8 °C**

Reagent C - Internal Standard Solution, **1 x 1 ml**

Reagent M - Mobile Phase, **4 x 500 ml**

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 10 μ l
Spectrophotometric Detector UV/VIS $\lambda=334$ nm
Chromatograms Recorder

Optional Equipment:

Autosampler
Operational Computer

Urine Collection Procedure:

Before and soon after work shift, collect 10 ml of urine in a suitable test tube. The sample is stable up to 4 weeks at -20 °C.

PREANALYTICAL PROCEDURE

Test Solution at 39 mg/l Preparation

Pipette in a tube :

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

Pipette in a tube:

- 200 µl of **Solution Test at 39 mg/l**
- 200 µl of **Reagent A – Derivatization solution** **Vortex for 10 seconds**
- Incubate at 70 °C for 30 minutes **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.

ANALYTICAL PROCEDURE

STEP 1 : Preparation of Chemical Standard at 3,9 mg/l

Pipette in a tube :

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

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	Standard	Sample
H₂O HPLC grade	2,0 ml		
Chemical Standard		2,0 ml	
Sample			2,0 ml
Concentrate HCl	0,2 ml	0,2 ml	0,2 ml

Vortex for 10 seconds

STEP 3 : Incubate all the tubes at 100 °C for 1 hour

Cooling at room temperature and **Vortex for 10 seconds**

STEP 4 : Centrifuge at 4.000 rpm for 5 minutes

STEP 5 : Derivatization procedure

- Take 200 µl of Supernatant
- Add 10 µl of **Reagent C – Internal Standard**
- Add 200 µl of **Reagent A – Derivatization solution** **Vortex for 10 seconds**

STEP 6 : Incubate all the tubes at 70 °C for 30 minutes

Cooling at room temperature

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

INJECTION :

- Inject 10 µl of this solution in the chromatographic system.

2,5-HEXANEDIONE IN URINE - Warnings

REAGENT B : TEST SOLUTION / CHEMICAL STANDARD

2,5 Hexanedione	390 mg/l
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PARAMETERS FOR THE SPECTROFOTOMETRIC DETECTOR

λ	334 nm
GAIN	0,01 AUFS
INTEGRATION TIME	10 sec

PROTECTION OF THE ANALYTICAL COLUMN

To take care of Analytical Column Zorbax Eclipse Plus C18 4,6 x 100 mm, 3,5 μ m, is obligatory the use of Javelin Prefilters (1 x 10 pcs.) code S90199511.
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HPLC COLUMN CONDITIONING

Install a <u>new</u> analytical C ₁₈ Zorbax Eclipse Plus (4,6 mm x 100 mm, 3,5 μ m). Disconnect the detector and flux 30 ml of H₂O : Acetonitrile (20 : 80 v/v) set flow at 1,0 ml / min. Flux 30 ml of a solution of H₂O : Acetonitrile (80 : 20 v/v) set flow at 1,0 ml/min. Don't recycle the washing solutions. Condition the column with the mobile phase at a flow of 1,0 ml / min. and discharge the first 20 ml. Condition furtherly the column for 30 min. also at recycling phase. Finally inject the Chemical Standard and verify the quality of the HPLC run. It is NOT possible to make analysis at recycling phase. If Room Tem. is > 20° C is better to preserve the Mobile Phase at 2 – 8° C among runs.

COLUMN CLEANING

Disconnect the detector. Flux 15 ml of H₂O : Methanol o Acetonitrile (80 : 20 v/v) set flow at 1,0 ml/min and discharge. Flux a solution made of H ₂ O: Methanol or Acetonitrile (30 : 70 v/v) for 30 min and discharge. It is recommended to wash the Analytical Column at the end of each session. Store the column in a solution of H₂O : Methanol or Acetonitrile (30 : 70 v/v) .

INJECTION NEEDLE WASHING

Water / Ethanol or Methanol or Acetonitrile (80 : 20 v/v).
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HPLC PARAMETERS

LOOP	10 μ l
Recommended Flow Rate	1,8 ml/min.
Pump pressure	About 131 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z05020	Chemical Standard for 2,5 Hexanedione in urine	2 x 5 ml
Z959961902	Zorbax Eclipse Plus C 18 Analytical Column (100 x 4,6mm -3.5 μ m)	1 Pc
S51843550	Clear glass vials with reduced volume from 1,5 ml to 15 μ l	1 x 100 Pcs
S51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 μ l	1 x 100 Pcs

2,5-HEXANEDIONE IN URINE
(Reference Chromatograms)

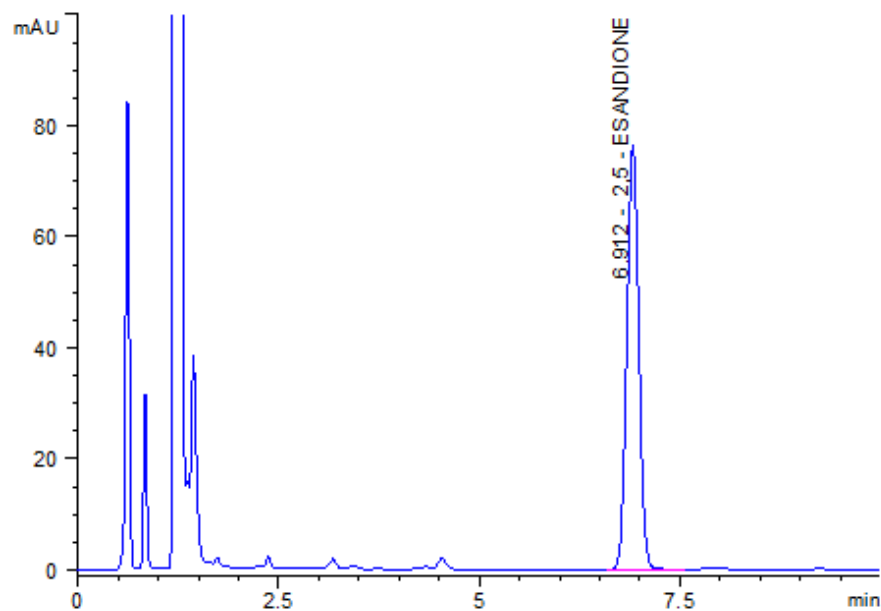


Fig. 1 :	Test Solution
	R.T. 6.91 2,5 Hexanedione

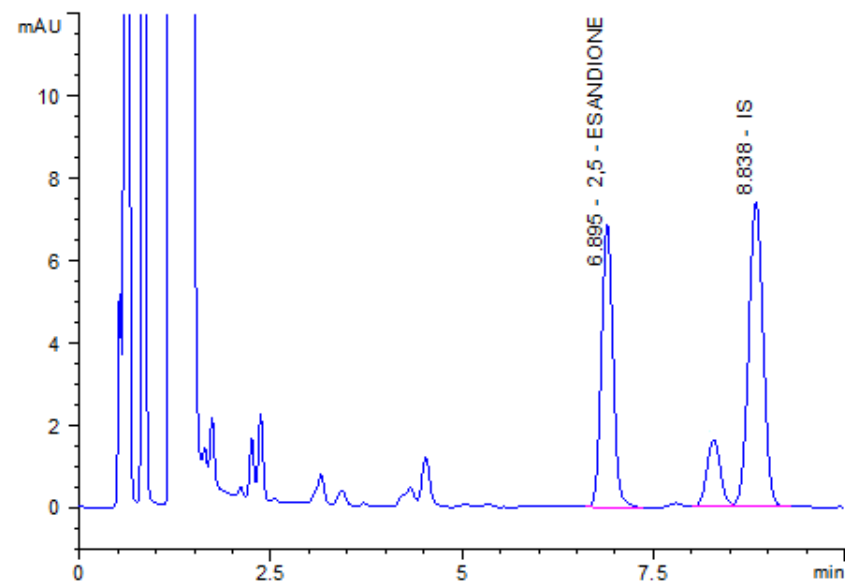


Fig. 2 :	Calibration standard in aqueous solution
	R.T. 6.89 2,5 Hexanedione 3,9 mg/l
	R.T. 8.83 Internal Standard

2,5-HEXANEDIONE IN URINE
(Reference Chromatograms)

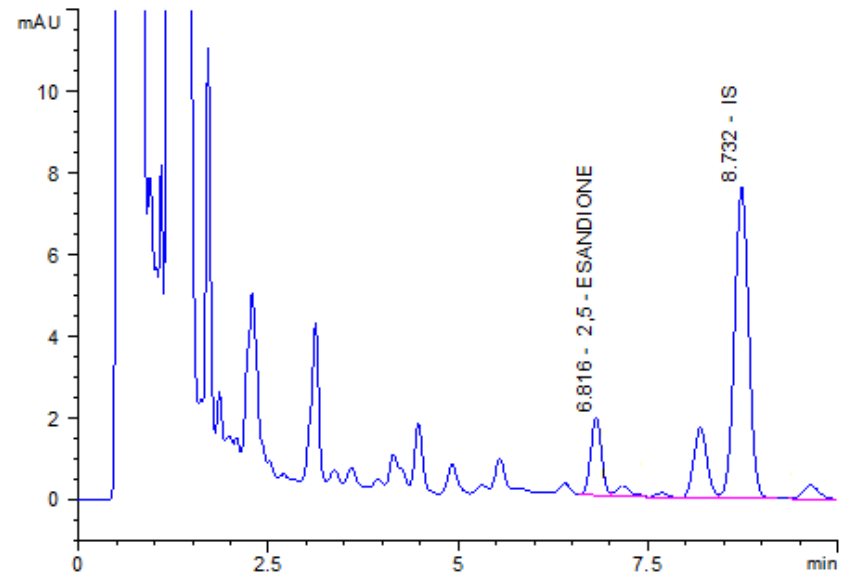


Fig. 3 :	Urine Sample		
	R.T. 6.81	2,5 Hexanedione	0,98 mg/l
	R.T. 8.73	Internal Standard	