

## **FREE 2,5 - HEXANEDIONE IN URINE BY FLUORIMETRY - FAST – CODE Z05910**

### **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.

|             |                                                     |             |
|-------------|-----------------------------------------------------|-------------|
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## **TECHNICAL FEATURES**

### **Principle of the Method:**

**2,5 Hexanedione** is derivatized with a specific reagent and, after purification in a clean up column, it is injected into the HPLC System.

**Recovery :** circa 97,5%

**Sensitivity (LLOD) :** 0,003 µg/ml

**Minimum Concentration analyzable (LLOQ) :** 0,01 µg/ml

**Linearity :** 0,01 – 40,0 µg/ml

### **Accuracy intra serie (relative error %) :**

| <b>Ci</b> | <b>Cs</b> |
|-----------|-----------|
| 0,1 µg/ml | 1 µg/ml   |
| 7,46%     | 2,50%     |

### **Accuracy inter serie (relative error %) :**

| <b>Ci</b> | <b>Cs</b> |
|-----------|-----------|
| 0,1 µg/ml | 1 µg/ml   |
| 13,01%    | 3,74%     |

### **Reproducibility intra serie (coefficient of variation %):**

| <b>C LLOQ</b> | <b>Cm</b> | <b>Cs</b> |
|---------------|-----------|-----------|
| 0,01 µg/ml    | 0,5 µg/ml | 5 µg/ml   |
| 13,98%        | 7,46%     | 1,79%     |

### **Reproducibility inter serie (coefficient of variation %):**

| <b>C LLOQ</b> | <b>Cm</b> | <b>Cs</b> |
|---------------|-----------|-----------|
| 0,01 µg/ml    | 0,5 µg/ml | 5 µg/ml   |
| 8,94%         | 6,07%     | 3,35%     |

**Coefficient of correlation R2 + Dev Std** 0,9945 ± 0,0069

**Acceptable values in urine f.t.f.s.l.** 0,5 mg/l

*Ref: ACGIH 2020*

|                                                                       |                                                                                                            |
|-----------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|
| <b><u>Components of the kit :</u></b>                                 | All the components are ready-to-use. Stability: 3 years at room temperature ( $\leq 20^{\circ}\text{C}$ ). |
| <b>Reagent A</b> – Derivatization Solution, <b>1 x 80 ml</b>          |                                                                                                            |
| <b>Reagent B</b> – Buffer Solution, <b>2 x 80 ml</b>                  |                                                                                                            |
| <b>Reagent C</b> – Conditioning Solution N°1, <b>2 x 100 ml</b>       |                                                                                                            |
| <b>Reagent D</b> – Conditioning Solution N° 2, <b>1 x 200 ml</b>      |                                                                                                            |
| <b>Reagent E</b> – Wash Solution, <b>3 x 200 ml</b>                   |                                                                                                            |
| <b>Reagent F</b> – Eluting Solution, <b>1 x 60 ml</b>                 |                                                                                                            |
| <b>Reagent G</b> – Diluting Solution, <b>1 x 55 ml</b>                |                                                                                                            |
| <b>Reagent H</b> – Test Solution / Chemical Standard, <b>1 x 5 ml</b> | <b><u>See Warnings</u></b>                                                                                 |
| <b>Reagent I</b> – Internal Standard Solution, <b>1 x 5 ml</b>        | <b><u>Store at 2-8 °C</u></b>                                                                              |
| <b>Reagent M</b> – Mobile Phase, <b>3 x 500 ml</b>                    |                                                                                                            |
| <b>Clean-up Columns 100 pcs</b>                                       |                                                                                                            |

**Minimum Instrumental equipment required:**

Isocratic HPLC System with loop of 100  $\mu\text{l}$   
 Fluorimetric Detector  $\lambda_{\text{EX}}=273\text{ nm}$   $\lambda_{\text{EM}}=450\text{ nm}$   
 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  $-20^{\circ}\text{C}$ .  
 It's preferable that pH is lightly acid  
**( 2,0 < pH < 4,0)**

## **PREANALYTICAL PROCEDURE**

### **Test Solution Preparation**

Pipette in a 10 ml glass tube with cap:

|                                            |         |
|--------------------------------------------|---------|
| <b>Reagent H – Test Solution</b>           | 600 µl  |
| <b>Reagent A – Derivatization Solution</b> | 600 µl  |
| <b>Reagent I – Internal Std Solution</b>   | 40 µl   |
| <b>Reagent B – Buffer Solution</b>         | 1200 µl |

**Vortex for 10 seconds**

- Incubate at 70 °C for 1 h

**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.

**Important :** Don't use this solution to calibrate!

## **ANALYTICAL PROCEDURE**

### **Calibration Standard Preparation**

#### **STEP 1:**

Pipette in a 10 ml tube :

- 900 µl of H<sub>2</sub>O HPLC grade
- 100 µl of **Reagent H - Chemical Standard**

This is a Standard Solution 0,39 mg/l

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

#### **STEP 2 : Derivatization**

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

|                                          | <b>Blank</b> | <b>Calibrator</b> | <b>Samples</b> |
|------------------------------------------|--------------|-------------------|----------------|
| <b>H<sub>2</sub>O HPLC grade</b>         | 600 µl       |                   |                |
| <b>Calibration Solution 0,39 mg/l</b>    |              | 600 µl            |                |
| <b>Sample</b>                            |              |                   | 600 µl         |
| <b>Reagent A – Derivatization Sol.</b>   | 600 µl       | 600 µl            | 600 µl         |
| <b>Reagent I – Internal Std Solution</b> | 40 µl        | 40 µl             | 40 µl          |
| <b>Reagent B – Buffer Sol.</b>           | 1200 µl      | 1200 µl           | 1200 µl        |

**Vortex for 10 sec.**

- Incubate at 70 °C for 1 h

**Cooling**

**N.B.: at this step, the sample is stable 24 hours at 2-8 °C**

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### **STEP 3 : Clean-up column Conditioning**

- Place in Clean-up column 2 ml of **Reagent C – Conditioning Solution N°1**  
*Discharge the percolated liquid*
- Place in Clean-up column 2 ml of **Reagent D – Conditioning Solution N°2**  
*Discharge the percolated liquid*

### **STEP 4 : Calibrator and Samples loading.**

- Dispense the Calibrator and the Samples, previously prepared, into clean up columns.
- Discharge the percolated liquid, if needed a vacuum system could be used for percolation, at speed of 1 ml / min ( drop by drop ).

**Discharge the percolated liquid**

### **STEP 5 : Clean-up columns washing:**

- Wash with 6 ml of **Reagent E – Wash Solution**  
*Discharge the percolated liquid and let dry with a light flux of air*

### **STEP 6 : Eluting:**

- Elute with 600 µl of **Reagent F – Eluting Solution**  
*Collect the eluate and vortex for 10 sec.*

### **STEP 7 : Take 600 µl of eluate and add 500 µl of Reagent G – Diluting Solution**

**Vortex for 20 sec.**

### **INJECTION :**

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

|             |                                                     |             |
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## **FREE 2,5 HEXANEDIONE in URINE - Warnings**

### **REAGENT H : TEST SOLUTION / CHEMICAL STANDARD**

|                        |           |
|------------------------|-----------|
| <b>2,5 Hexanedione</b> | 3,90 mg/l |
|------------------------|-----------|

### **FLUORIMETRIC DETECTOR**

|                         |        |
|-------------------------|--------|
| $\lambda_{Ex}$          | 273 nm |
| $\lambda_{Em}$          | 450 nm |
| <b>SENSITIVITY</b>      | High   |
| <b>INTEGRATION TIME</b> | 4 or 8 |

### **HPLC COLUMN PROTECTION**

To save the analytical column Reverse Phase Zorbax 120 EC-C18 (50 x 4,6mm -3,5 um), the use of Javelin Colfilters (1 x 10 pcs.) cod. S90199511 is obligatory.

### **HPLC COLUMN CONDITIONING**

Install a new analytical Zorbax 120 EC-C18 (50 x 4,6mm -3,5 um). Disconnect the detector and filter 30 ml of H<sub>2</sub>O: Acetonitrile ( 20 : 80 v/v ) solution and subsequently 30 ml of H<sub>2</sub>O per HPLC, set flow at 1,0 ml / min. **Don't recycle the washing solutions.** Condition the column with a solution of H<sub>2</sub>O: Acetonitrile ( 80 : 20 v/v ) and subsequently with Mobile Phase, set flow at 1,0 ml / min. **Don't recycle the washing solutions** and discharge the first 20 ml.  
**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 15 ml of H<sub>2</sub>O : **Acetonitrile or Methanol (80 : 20 v / v)** set flow at 1,0 ml/min and discharge. Flux a solution made of H<sub>2</sub>O : **Methanol or Acetonitrile (30 : 70 v/v)** for 30 min and discharge. **It's recommended washing the analytical column at the end of each run.** Store the column in a solution of H<sub>2</sub>O : **Acetonitrile or Methanol (30 : 70 v / v)**.

### **INJECTION NEEDLE WASHING**

Wash with a solution of H<sub>2</sub>O / Ethanol or Methanol or Acetonitrile (80 : 20 v/v).

### **HPLC PARAMETERS**

|                 |              |
|-----------------|--------------|
| <b>LOOP</b>     | 10 µl        |
| <b>FLOW</b>     | 0,8 ml/min.  |
| <b>PRESSURE</b> | About 40 bar |

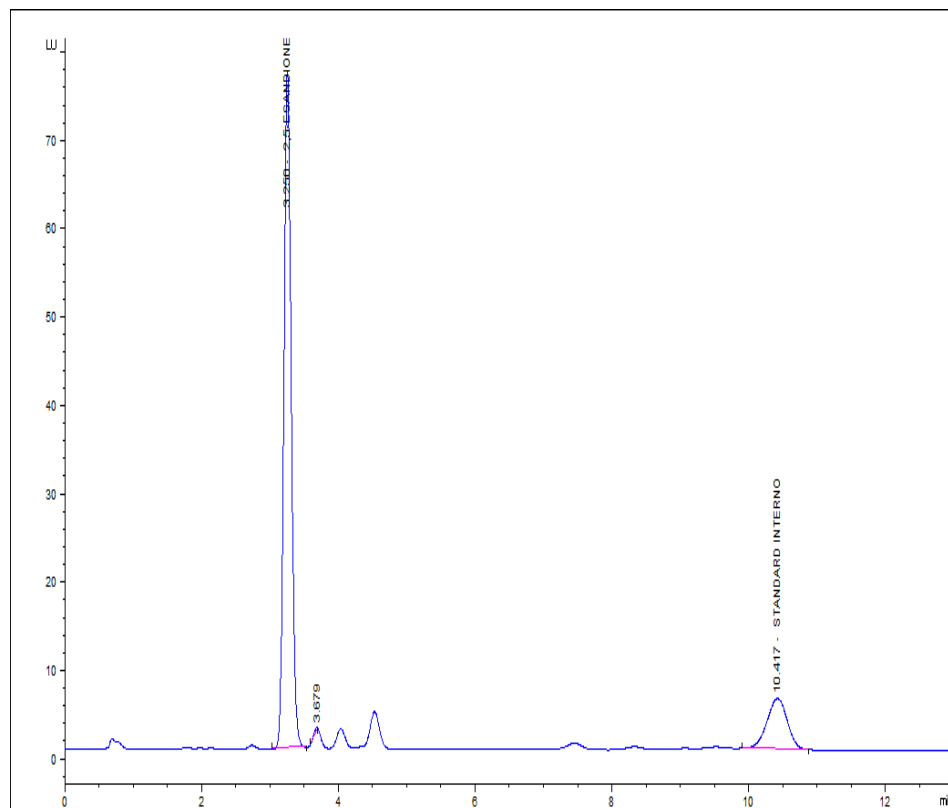
### **ACCESSORIES AND CONSUMABLES**

| <b>CODE</b>       | <b>DESCRIPTION</b>                                         | <b>PACKAGING</b> |
|-------------------|------------------------------------------------------------|------------------|
| <b>Z05020</b>     | Chemical Standard for 2,5 Hexanedione in urine             | 2 x 5 ml         |
| <b>S959943902</b> | Zorbax 120 EC- C 18 Analytical Column (50 x 4,6mm -3,5 um) | 1 Pc             |
| <b>S90199511</b>  | Javelin Colfilters                                         | 1 x 10 Pcs       |
| <b>S29057U</b>    | Standard glass vials of 2 ml with screw cap                | 1 x 100 Pcs      |

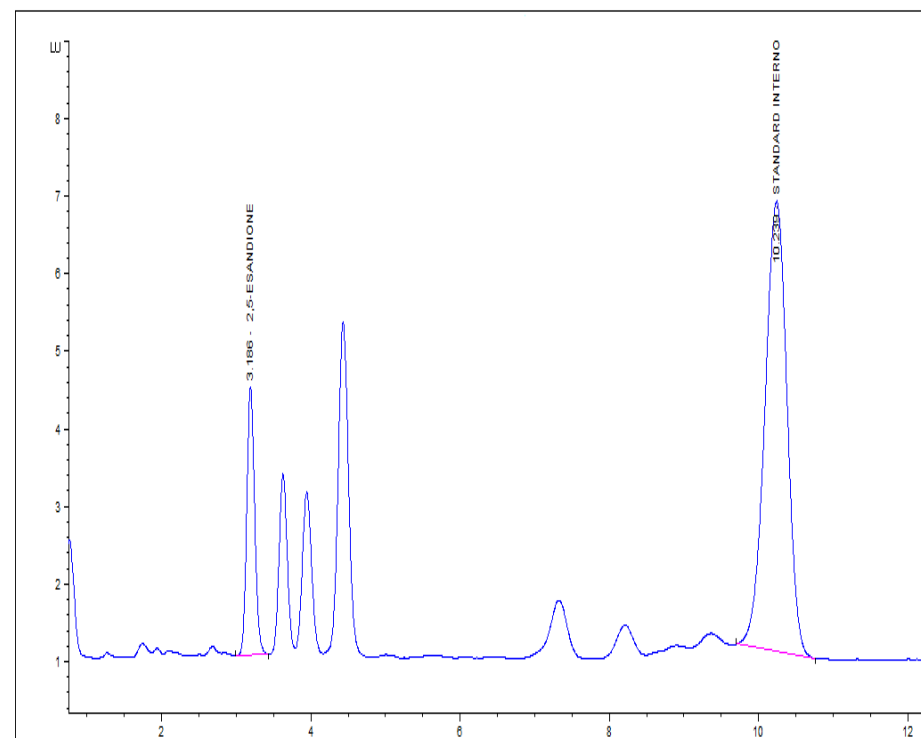


# FREE 2,5 HEXANEDIONE IN URINE

(Reference Chromatograms)



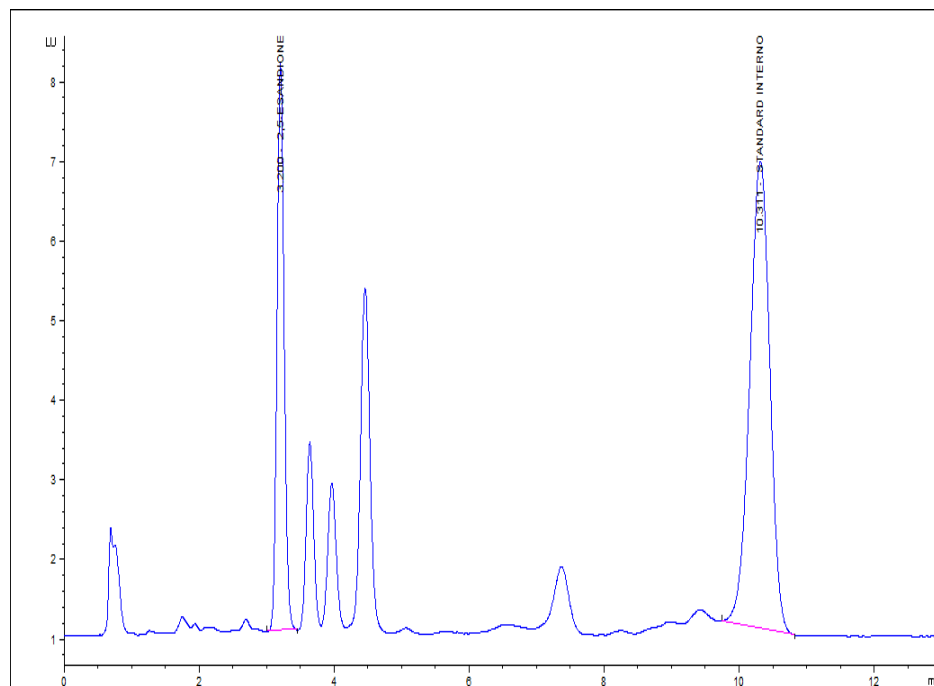
|               |                      |
|---------------|----------------------|
| <b>Fig. 1</b> | <b>Test Solution</b> |
| R.T. 3.2      | 2,5-Hexanedione      |
| R.T. 10.4     | Internal Standard    |



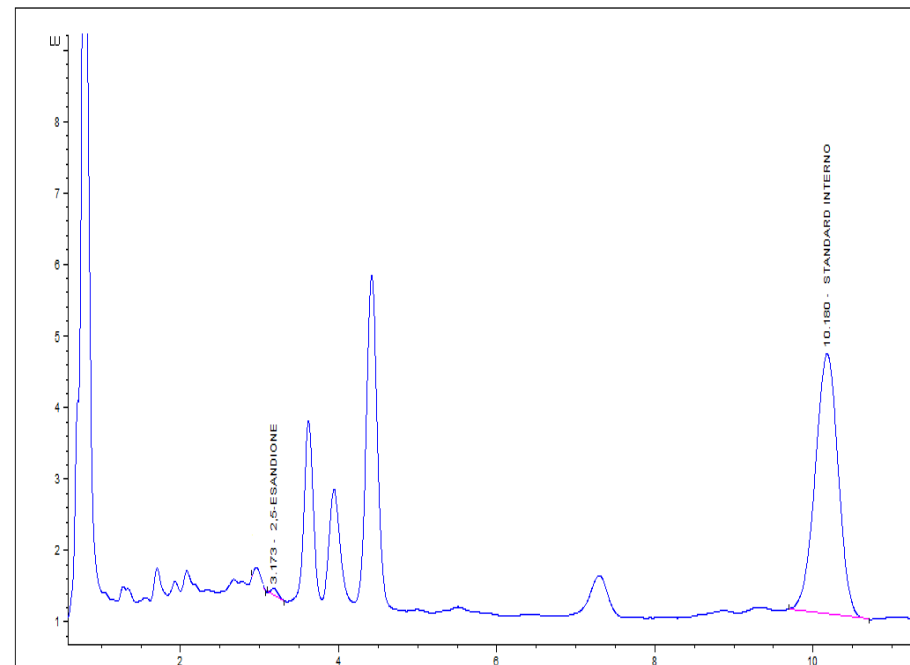
|               |                            |
|---------------|----------------------------|
| <b>Fig. 2</b> | <b>Aqueous Low Control</b> |
| R.T. 3.1      | 2,5-Hexanedione 0,19 mg/L  |
| R.T. 10.2     | Internal Standard          |

# FREE 2,5 HEXANEDIONE IN URINE

(Reference Chromatograms)



|               |                                     |
|---------------|-------------------------------------|
| <b>Fig. 3</b> | <b>Aqueous Calibration Standard</b> |
| R.T. 3.2      | 2,5-Hexanedione 0,39 mg/L           |
| R.T. 10.3     | Internal Standard                   |



|               |                     |
|---------------|---------------------|
| <b>Fig. 4</b> | <b>Urine Sample</b> |
| R.T. 3.1      | 2,5-Hexanedione     |
| R.T. 10.1     | Internal Standard   |