

1 – HYDROXYPYRENE IN URINE BY FLUORIMETRY- FAST – CODE Z38210

BIOCHEMISTRY

1-Hydroxypyrene is a metabolic product of polycyclic aromatic hydrocarbons (PAH) in human urine. The generic term PAH represents more than 200 aromatic compounds with condensed ring systems. Just as a large number of PAHs have been shown to be highly carcinogenic in animal experiments, exposure to PAH can be a serious danger to human organism as well. Intake of PAHs may occur primarily in the lungs and the gastro-intestinal tract or through the skin as a secondary route.

Commonly, PAHs are created by the incomplete combustion of organic material, e.g. by firing plants or combustion engines. Because of this, environmental pollution caused by PAHs is widespread, especially in urban and industrial centres. Workers in industrial plants, such as in gasworks, cokeworks and those in the aluminium industry, who are exposed to high PAH levels, show elevated rates of lung cancers. Among individual exposition sources, smoking is considered to be the most relevant.

1 – Hydroxypyrene is excreted in urine conjugated with glucuronic acid and with sulfate. To free it for analysis, the sample is hydrolyzed with β -glucuronidase/arilsulfatase for 16 h at 37 °C at pH=5,0. Among PAH metabolites 1-hydroxypyrene is the most important biomarker for the evaluation of occupational related PAH exposure.

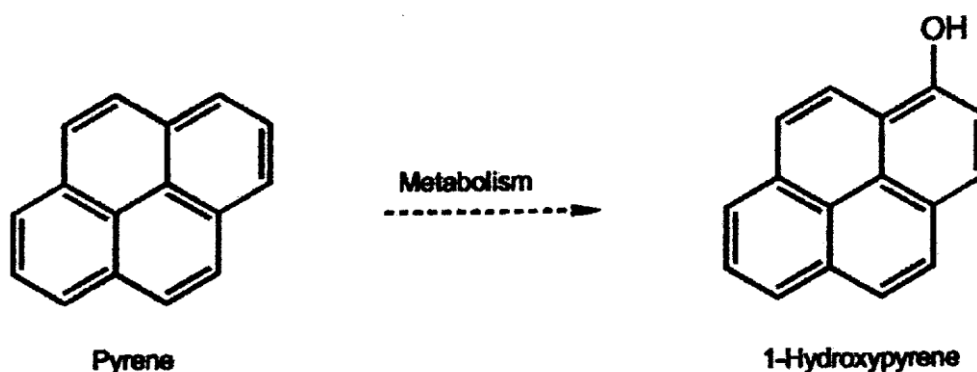


Fig. 1 : metabolism of pyrene to 1-Hydroxypyrene



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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° 004	1-Hydroxypyrene in urine by Fluorimetry - FAST	06/2024
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TECHNICAL FEATURES

Principle of the Method:

1-HYDROXYPYRENE, after enzymatic hydrolysis at pH=5,0 and after purification in a clean up column it is injected into the HPLC System.

Recovery : 99,1%

Sensitivity (LLOD) : 100 ng/l

Minimum concentration analyzable (LLOQ): 300 ng/l

Acceptable values in urine of not exposed subjects: < 1.400 ng/g creatinine
Ref.: *G Ital Med Lav Erg* 2003;25:1

Reference Values : < 2.000 ng/g creatinine
< 2.500 ng/l
Ref: *ACGIH* 2020

Dynamic Range of the Method : 300 – 5.000 ng/l

Accuracy intra serie (relative error %) :

C ₁	C ₂
1100 ng/l	1500 ng/l
1,2%	3,4%

Accuracy inter serie (relative error %) :

C ₁	C ₂
1100 ng/l	1500 ng/l
1,5%	2,8%

Reproducibility intra serie (coefficient of variation %):

C _{LLOQ}	C _{MEDIUM}	C _{SUP}
300 ng/l	2000 ng/l	3000 ng/l
1,0%	0,8%	1,8%

Reproducibility inter serie (coefficient of variation %):

C _{LLOQ}	C _{MEDIUM}	C _{SUP}
300 ng/l	2000 ng/l	3000 ng/l
3,1%	1,2%	1,8%

Coefficient of Correlation R2 + Dev Std: 0,9951 ± 0,0004

<u>Components of the kit :</u>	All the components are ready-to-use and stable 3 years at room temperature ($\leq 20^{\circ}\text{C}$) except Reagent F and G that must be stored at $2-8^{\circ}\text{C}$.
Reagent A – Buffer Solution for Hydrolysis, 1 x 50 ml	
Reagent B – Hydrolysis Enzyme, 5 x 1,0 ml	To reconstitute with 9,0 ml of Reagent A. After reconstitution, the enzyme is stable 1 year at $2-8^{\circ}\text{C}$.
Reagent C – Wash Solution, 1 x 200 ml	
Reagent E – Eluting Solution, 1 x 50 ml	
Reagent F – Internal Standard Solution, 1 x 5 ml	<u>Store at $2-8^{\circ}\text{C}$</u>
Reagent G – Test Solution, 1 x 5 ml	<u>Store at $2-8^{\circ}\text{C}$</u>
Urine Calibrator lyophil. , 2 x 10 ml	<u>Code Z38016 (packed separately – see data sheet)</u>
Reagent M – Mobile Phase, 3 x 500 ml	
Clean-up Columns , 100 pcs	

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of $10\mu\text{l}$
 Fluorimetria Detector $\lambda_{\text{Ex}} = 242 \text{ nm}$ $\lambda_{\text{Em}} = 388 \text{ nm}$
 Chromatograms Recorder

Urine Collection Procedure:

Before work shift *or*
 After work shift *or*
 End of the week and after work shift urine
 No preservative
 10 ml of urine should be delivered to the laboratory

Preanalytical Phase :

Centrifuge at 4000 rpm for 5 min. Delayed analyses require the sample is stored at $2-8^{\circ}\text{C}$ or less.
 Stable 2 weeks at $2-8^{\circ}\text{C}$.
 Stable 2 months at -20°C .

PREANALYTICAL PROCEDURE

Pipette in a tube :

- 900 µl of H₂O HPLC grade
- 50 µl of **Reagent G - Test Solution**
- 50 µl of **Reagent F - Internal Standard Solution**

Vortex for 10 sec.

INJECTION :

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

Verify that the Test Solution has retention time similar to fig. 2. 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

STEP 1 : Enzyme Reconstitution

The **Reagent B – Hydrolysis Enzyme** is reconstituted with 9,0 ml of **Reagent A – Buffer Solution for Hydrolysis**. **After reconstitution, the enzyme is stable 1 year at 2–8 °C.**

STEP 2 : Samples Hydrolysis

Pipette in pyrex 10 ml tube.

	Calibrator	Sample
Calibrator	1,0 ml	
Sample		1,0 ml
Reagent B – Hydrolysis Enzyme (reconstituted)	400 µl	400 µl

Verify that pH is $\approx$ 5,0 – Eventually correct it

Vortex for 20 sec.

Incubate at 60 °C for 3 hours

STEP 3 : Samples, Calibrator and Controls loading in Clean-up Columns:

- Add to each hydrolyzed sample 50 µl of **Reagent F – Internal Standard Solution**
- Put 1450 µl of Samples, Calibrator or Controls into Clean-up Columns *Discharge the percolated liquid*

STEP 4 : Clean-up Columns washing:

- Put 2,0 ml of **Reagent C – Wash Sol.** into Clean-up Columns *Discharge the percolated liquid*

STEP 5 : Eluting:

- Elute with 500 µl of **Reagent E – Eluting Solution** *Collect the eluate*

Vortex for 10 sec.

N.B.: at this step, the sample is stable 7 days at 2-8 °C

INJECTION :

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

1-HYDROXYPYRENE - Warnings

REAGENT G : TEST SOLUTION

1 – HYDROXYPYRENE	about 54.000 ng/l
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Conversion coefficients : 1,0 µmoli = 218,25 µg of 1-Hydroxypyrene / 1,0 mg = 4,58 µmoli of 1-Hydroxypyrene

FLUORIMETRIC DETECTOR PARAMETERS

λ_{Ex}	242 nm
λ_{Em}	388 nm
GAIN	1 x 100
INTEGRATION TIME	10 sec.

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase ZORBAX ECLIPSE PLUS C18 (100 x 4,6 mm, 3,5 µm), the use of Javelin Colfilters (1 x 10 pcs.) cod. S90199511 is obligatory.

HPLC COLUMN CONDITIONING

Install a <u>new</u> analytical C ₁₈ Reversed-Phase column ZORBAX ECLIPSE PLUS C18 (100 x 4,6 mm, 3,5 µm). Disconnect the detector and filter 30 ml of H₂O : Acetonitrile (50 : 50 v / v) pH=3 set flow of 1,0 ml/minute. Don't recycle the washing solutions. Condition the column with the mobile phase for 30 minutes at a flow of 1,0 ml/minute and discharge the first 20 ml. Finally inject the Chemical Standard and verify the quality of the HPLC run. It is NOT possible to make analysis at recycling phase. 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 15 ml of H₂O : Acetonitrile (50 : 50 v / v) set flow of 1,0 ml/minute for 30 minutes and discharge. 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 (50 : 50 v / v) .
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INJECTION NEEDLE WASHING

Wash with a solution of Water / Acetonitrile (80 : 20 v/v).

HPLC PARAMETERS

LOOP	10 µl
FLOW	1,5 ml/min.
PRESSURE	About 130 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z38016	Urine Calibrator lyophil. for 1-Hydroxypyrene	4 x 10 ml
Z38019	Urine multiparametric Control lyophil. for toxic organic compounds, Levels 1,2	2 x 5 x 10 ml
Z959961902	Zorbax Eclipse Plus C18 (100 x 4,6mm –3,5 u) 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

1 – HYDROXYPYRENE IN URINE (Reference Chromatograms)

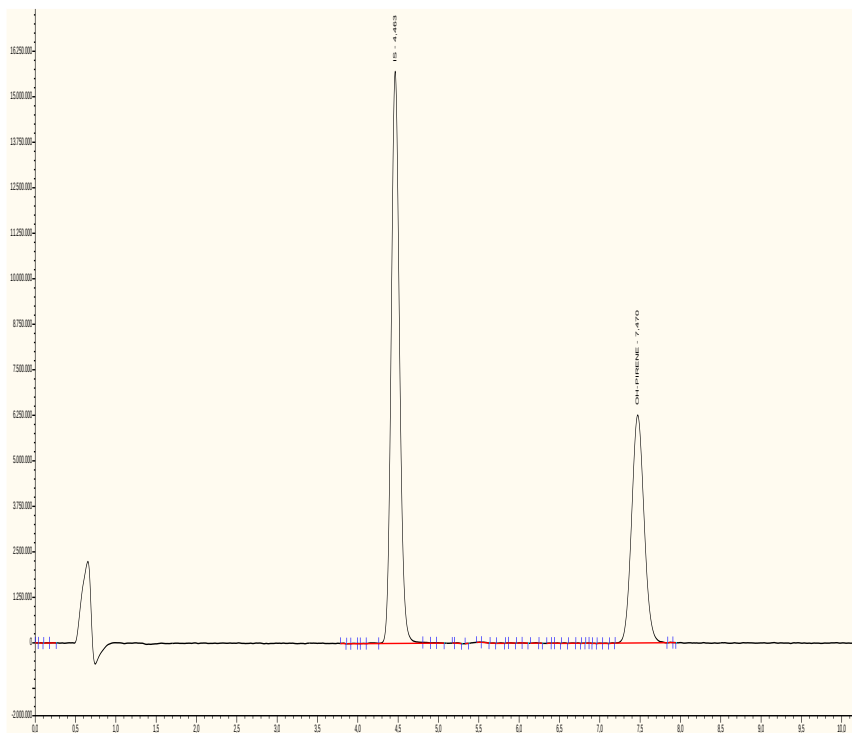


Fig. 2	Test Solution
	R.T. 4.46 Internal Standard
	R.T. 7.47 1-Hydroxypyrene

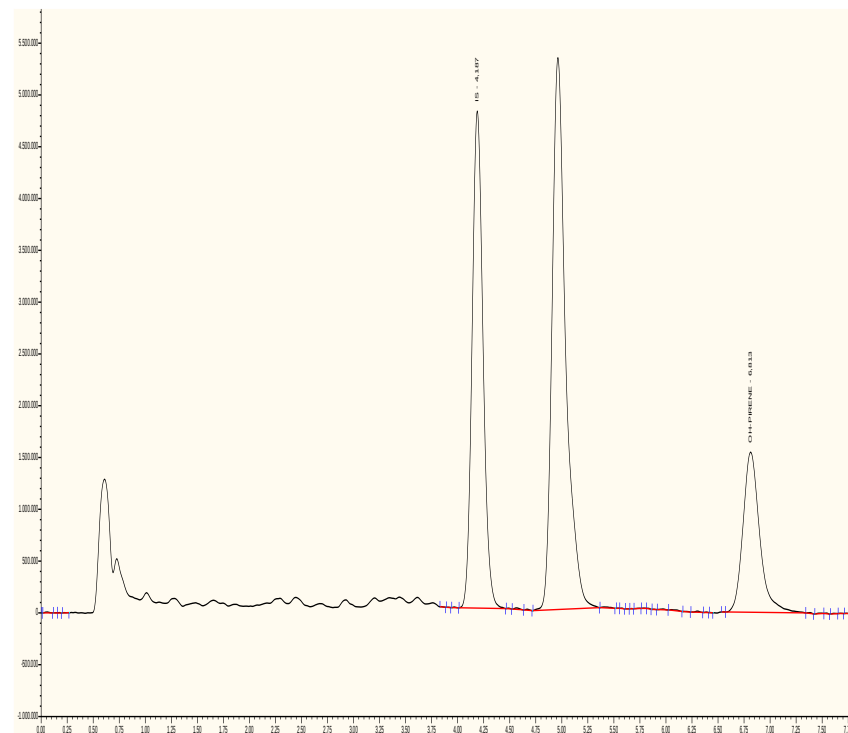


Fig. 3	Urine Calibrator
	R.T. 4.18 Internal Standard
	R.T. 6.81 1-Hydroxypyrene

1 – HYDROXYPYRENE IN URINE (Reference Chromatograms)

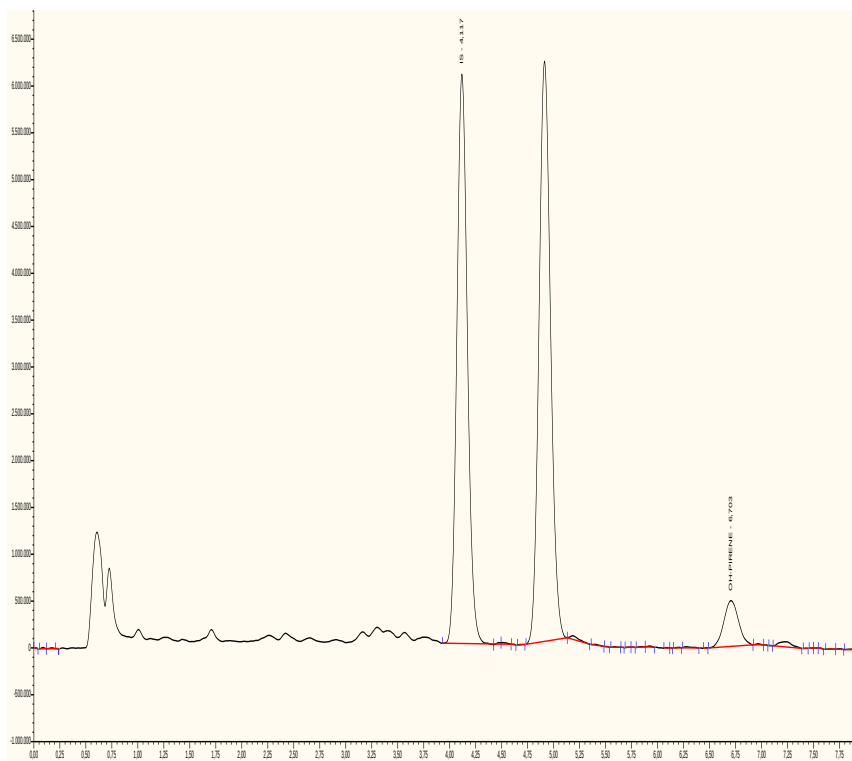


Fig. 4	Low Control
	R.T. 4.11 Internal Standard
	R.T. 6.70 1-Hydroxypyrene

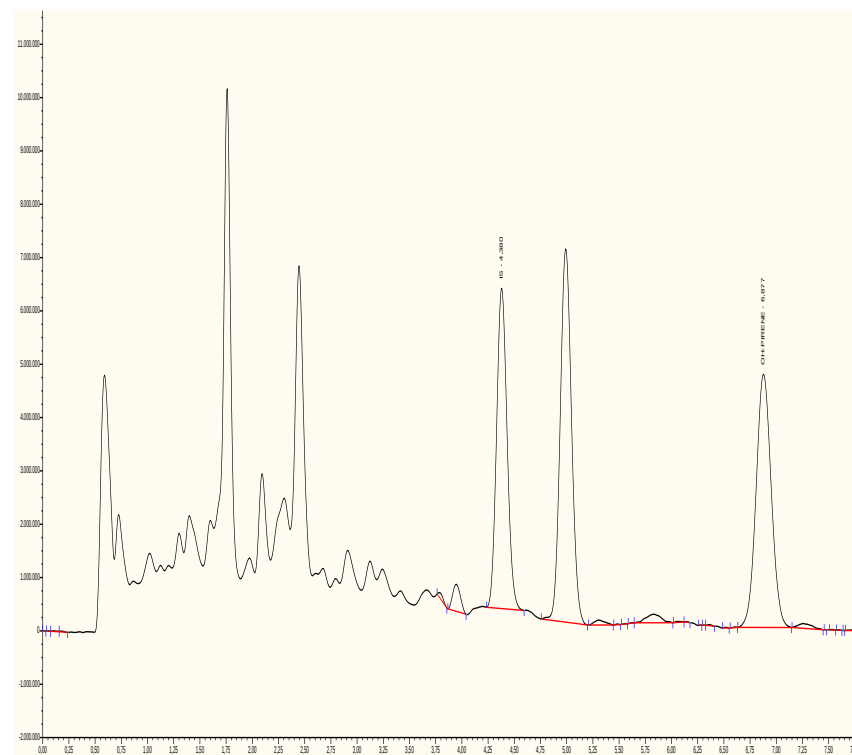


Fig. 5	Urine Sample
	R.T. 4.38 Internal Standard
	R.T. 6.87 1-Hydroxypyrene 1500 ng/l