

PHENYLGLYOXILIC ACID / MANDELIC ACID / HIPPURIC ACID /
o-m-p- METHYLHIPPURIC ACIDS IN URINE BY UV - FAST – CODE Z06615

Biochemistry

The determination of **Phenylglyoxilic Acid** (PGA) and **Mandelic Acid** (MDA) is important to determine the risk due to exposure to **Styrene** .

Styrene is metabolised first in **Styrene- Oxide**, subsequently in **Phenylethyleneglycol** and finally in **Phenylglyoxilic Acid** and **Mandelic Acid** (fig. 1) which are eliminated by urine.

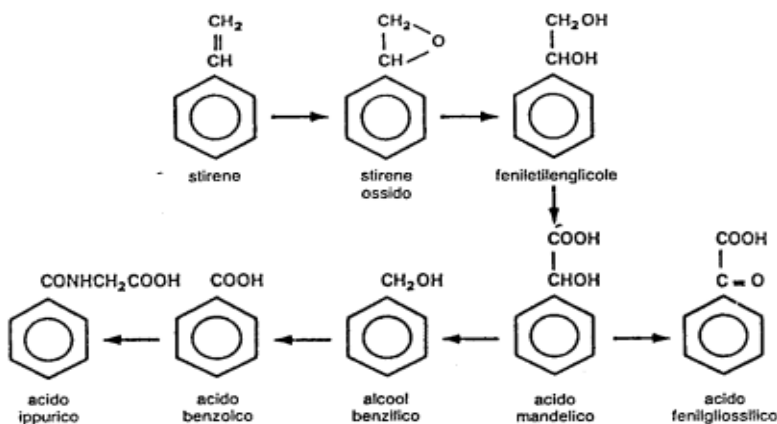


Fig. 1: Biotransformation of Styrene in vivo

The determination of **Hippuric Acid** (HPA) in urine is important to determine the risk due to exposure to **Toluene** .

Toluene is metabolised mostly in **Benzoic Acid** and subsequently combined with **Glycine** is eliminated by urine as **Hippuric Acid** (fig. 2).

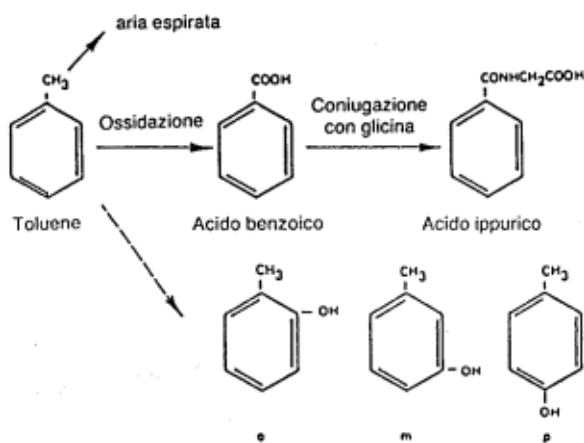


Fig. 2: Biotransformation of Toluene in vivo

N° 002	6 parameters in urine by UV - FAST	06/2024
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The determination of **o-m-p Methylhippuric Acid** (m-MHPA, o-MHPA and p-MHPA) is important to determine the risk due to exposure to a mixture of **o-m-p-Xilene**.

Xilene, which is present as a mixture of three isomers o-m-p, is metabolised in **o-m-p-Methyl-Benzoic** and after conjugation with Glycine are eliminated by urine as a mixture of **o-m-p-Methyl-Hippuric Acids**. (fig. 3).

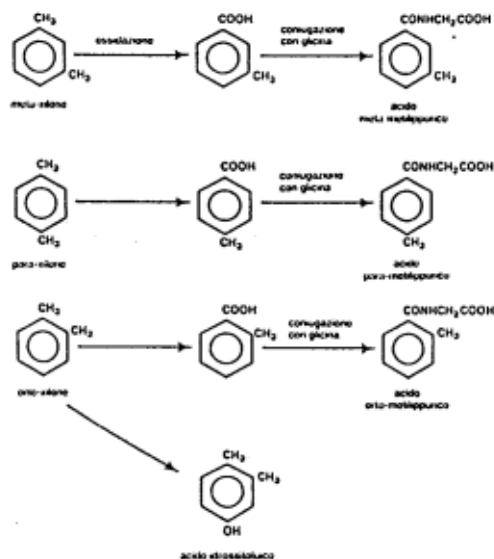


Fig. 3 Biotransformation of Xilene in vivo

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.



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TECHNICAL FEATURES

Principle of the Method:

Phenylglyoxilic Acid, Mandelic Acid, Hippuric Acid and o-m-p Methylhippuric Acids are analysed after dilution. This solution is then directly injected into HPLC System.

Recovery: >98%

Sensitivity (LLOD): 3,3 mg/l Mandelic Acid and Phenylglyoxilic Acid
5 mg/l Hippuric Acid and Methylhippuric Acids

Minimum Concentration analyzable (LLOQ) : 10 mg/l Mandelic Acid and Phenylglyoxilic Acid
15 mg/l Hippuric Acid and Methylhippuric Acids

Linearity : 10 – 3000 mg/l Mandelic Acid and Phenylglyoxilic Ac.
15 – 3000 mg/l Hippuric Acid and Methylhippuric Acids

Accuracy intra serie (relative error %) Hippuric Acid:

Ci	Cs
300 mg/l	1500 mg/l
4,7%	2,8%

Accuracy inter serie (relative error %) Hippuric Acid:

Ci	Cs
300 mg/l	1500 mg/l
4,2%	4,2%

Reproducibility intra serie (coefficient of variation %) Hippuric Acid:

C LLOQ	Cm	CA
200 mg/l	600 mg/l	1800 mg/l
6,8%	3,0%	0,6%

Reproducibility inter serie (coefficient of variation %) Hippuric Acid:

C LLOQ	Cm	CA
200 mg/l	600 mg/l	1800 mg/l
7,9%	3,2%	2,8%

Coefficient of correlation R2 + Dev. std:

0,9959 ± 0,0024 Hippuric Acid

Accuracy intra serie (relative error %) o-Methylhippuric Acid:

Ci	Cs
300 mg/l	1500 mg/l
5,6%	1,8%

Accuracy inter serie (relative error %) o-Methylhippuric Acid:

Ci	Cs
300 mg/l	1500 mg/l
8,2%	3,9%

Reproducibility intra serie (coefficient of variation %) o-Methylhippuric Acid:

C LLOQ	Cm	CA
200 mg/l	600 mg/l	1800 mg/l
7,7%	2,3%	1,7%

Reproducibility inter serie (coefficient of variation %) o-Methylhippuric Acid:

C LLOQ	Cm	CA
200 mg/l	600 mg/l	1800 mg/l
8,9%	3,7%	0,7%

Coefficient of correlation R2 + Dev. std:

0,9942 ± 0,0025 o-Methylhippuric Acid

Accuracy intra serie (relative error %)
m-Methylhippuric Acid :

Ci	Cs
300 mg/l	1500 mg/l
2,1%	2,6%

Accuracy inter serie (relative error %)
m-Methylhippuric Acid:

Ci	Cs
300 mg/l	1500 mg/l
4,7%	3,2%

Reproducibility intra serie (coefficient of variation %)
m-Methylhippuric Acid:

C LLOQ	Cm	CA
200 mg/l	600 mg/l	1800 mg/l
7,3%	2,6%	0,9%

Reproducibility inter serie (coefficient of variation %)
m-Methylhippuric Acid:

C LLOQ	Cm	CA
200 mg/l	600 mg/l	1800 mg/l
7,5%	3,4%	0,6%

Coefficient of correlation R2 + Dev. std:

0,9963 ± 0,0027 m-Methylhippuric Acid

Accuracy intra serie (relative error %)
p-Methylhippuric Acid :

Ci	Cs
300 mg/l	1500 mg/l
3,2%	2,2%

Accuracy inter serie (relative error %)
p-Methylhippuric Acid:

Ci	Cs
300 mg/l	1500 mg/l
3,5%	4,3%

Reproducibility intra serie (coefficient of variation %)
p-Methylhippuric Acid:

C LLOQ	Cm	CA
200 mg/l	600 mg/l	1800 mg/l
7,0%	2,1%	0,8%

Reproducibility inter serie (coefficient of variation %)
p-Methylhippuric Acid:

C LLOQ	Cm	CA
200 mg/l	600 mg/l	1800 mg/l
7,9%	2,8%	0,5%

Coefficient of correlation R2 + Dev. std:

0,9956 ± 0,0029 p-Methylhippuric Acid

Accuracy intra serie (relative error %)
Phenylglyoxilic Acid :

Ci	Cs
300 mg/l	1500 mg/l
5,0%	0,3%

Accuracy inter serie (relative error %)
Phenylglyoxilic Acid:

Ci	Cs
300 mg/l	1500 mg/l
3,3%	1,8%

Reproducibility intra serie (coefficient of variation %)
Phenylglyoxilic Acid:

C LLOQ	Cm	CA
200 mg/l	600 mg/l	1800 mg/l
9,1%	2,3%	0,9%

Reproducibility inter serie (coefficient of variation %)
Phenylglyoxilic Acid:

C LLOQ	Cm	CA
200 mg/l	600 mg/l	1800 mg/l
10,6%	2,4%	1,9%

Coefficient of correlation R2 + Dev. std:

0,9962 ± 0,0023 Phenylglyoxilic Acid

Accuracy intra serie (relative error %) Mandelic Acid:

Ci	Cs
300 mg/l	1500 mg/l
7,5%	2,8%

Accuracy inter serie (relative error %) Mandelic Acid:

Ci	Cs
300 mg/l	1500 mg/l
8,6%	3,8%

Reproducibility intra serie (coefficient of variation %) Mandelic Acid :

C LLOQ	Cm	CA
200 mg/l	600 mg/l	1800 mg/l
6,5%	3,1%	1,0%

Reproducibility inter serie (coefficient of variation %) Mandelic Acid:

C LLOQ	Cm	CA
200 mg/l	600 mg/l	1800 mg/l
9,4%	3,5%	2,7%

Coefficient of correlation R2 + Dev. std:

0,9959 ± 0,0028 Mandelic Acid

Acceptable values in urine of not exposed subjects:

Phenylglyoxilic Acid : < 5 mg / l
Mandelic Acid : < 5 mg / l
Hippuric Acid : < 1500 mg / l
o-m-p Methylhipp. Total Acids : < 1 mg / l

Acceptable values in urine after work shift:

Phenylglyoxilic Acid : < 264 mg / l
Mandelic Acid : < 880 mg / l
Hippuric Acid : < 1600 mg / l
o-m-p Methylhipp. Total Acids : < 1500 mg / l

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

o-m-p Methylhipp. Total Acids : 1,5 g/g creatinine
Mandelic Ac.: 400 mg/g creatinine

*Ref: ACGIH 2020***Components of the kit (500 tests):**

All the components are ready-to-use. Stability: 3 years at room temperature ($\leq 20^{\circ}\text{C}$). Except Reagent C, D, E stored at $2-8^{\circ}\text{C}$.

Reagent A – Dilution Solution, 5 x 100 ml**Reagent C – Test Solution, 1 x 2 ml****Store at 2-8 °C****Urine Calibrator lyophil, 5 x 5 ml****Code Z06016 (packed separately – see data sheets)****Reagent M – Mobile Phase, 10 x 500 ml****Minimum Instrumental equipment required:**

Isocratic HPLC System with loop of 5 μl
Spectrophotometric Detector UV/VIS $\lambda=207\text{ nm}$
Chromatograms Recorder

Urine Collection Procedure:

Before and soon after work shift, collect 10 ml of urine in a suitable test tube.

Pre-analytic phase:

Centrifuge at 4000 rpm for 5 min.
The sample is stable up to 4 weeks at $2-8^{\circ}\text{C}$ or 6 months at -20°C .

PREANALYTICAL PROCEDURE

Test Solution Preparation

Pipette in a tube :

- 990 µl of H₂O HPLC grade
- 10 µl of **Reagent C – Test Solution**

Vortex for 10 sec.

INJECTION :

- Inject 5 µl of this solution in the chromatographic system.

Verify that the Test Solution has retention time similar to fig. 4. 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: Samples Preparation

Pipette in suitable tubes for centrifugation in sequence :

	Blank	Calibrator	Sample
Reagent A – Dilution Solution	1000 µl	1000 µl	1000 µl
Water HPLC grade	10 µl		
Calibrator		10 µl	
Sample			10 µl

Vortex for 10 sec.

N.B.: at this step, the sample is stable 5 days at 2-8 °C

INJECTION :

- Inject 5 µl of this solution in the chromatographic system.

PHENYLGLYOXILIC / MANDELIC / HIPPURIC ACIDS /
o-m-p-METHYLHIPPURIC ACIDS IN URINE - Warnings

REAGENT C : TEST SOLUTION

Phenylglyoxilic Acid	1000 mg/l
Mandelic Acid	1000 mg/l
Hippuric Acid	1000 mg/l
o-Methyl-hippuric Acid	1000 mg/l
m-Methyl-hippuric Acid	500 mg/l
p-Methyl-hippuric Acid	500 mg/l

REAGENT D : TEST SOLUTION PGA and MDA

Phenylglyoxilic Acid	1000 mg/l
Mandelic Acid	1000 mg/l

REAGENT E : TEST SOLUTION HPA, m-PHPA, o-PHPA and p-PHPA

Hippuric Acid	1000 mg/l
o-Methyl-hippuric Acid	1000 mg/l
m-Methyl-hippuric Acid	500 mg/l
p-Methyl-hippuric Acid	500 mg/l

SPECTROFOTOMETRIC DETECTOR PARAMETERS

λ	207 nm
GAIN	0,01 AUFS
INTEGRATION TIME	10 sec.

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL 120 EC C-18 4,6 x 50 mm, 2,7 μ m, the use of Javelin Prefilters (1 x 10 pcs.) cod. S90199511 is obligatory.

HPLC COLUMN CONDITIONING

Install a <u>new</u> analytical C ₁₈ Reversed-Phase column POROSHELL 120 EC-C18 (50 x 4,6 mm, 2,7 μ m). Disconnect the detector and filter 30 ml of H ₂ O: 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 H ₂ O : 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.
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COLUMN CLEANING

Disconnect the detector. Flux 30 ml of H ₂ O : Acetonitrile or Methanol (80 : 20 v / v) set flow of 1,0 ml/minute and discharge. Flux a solution of H ₂ O : 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 H ₂ O : Acetonitrile or Methanol (30 : 70 v / v).
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INJECTION NEEDLE WASHING

Wash with a solution of Water / Ethanol or Methanol or Acetonitrile (80 : 20 v/v).
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HPLC PARAMETERS

LOOP	5 μ l
Recommended Flow rate	0,8 ml / min
Pressure	About 80 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z06016	Urine multiparametric Calibrator lyophil. for 6 parameters	4 x 5 ml
Z38019	Urine multiparametric Control lyophil. for Toxic Organic Compounds - Levels 1, 2	2 x 5 x 10 ml
Z06611	Mobile Phase	4 x 500 ml
Z699975902	Poroshell 120 EC C18 Analytical Column (50 x 4,6mm –2,7 u)	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

PHENYLGLYOXILIC / MANDELIC / HIPPURIC / o-m-p-METHYLHIPPURIC ACIDS IN URINE
(Reference Chromatograms)

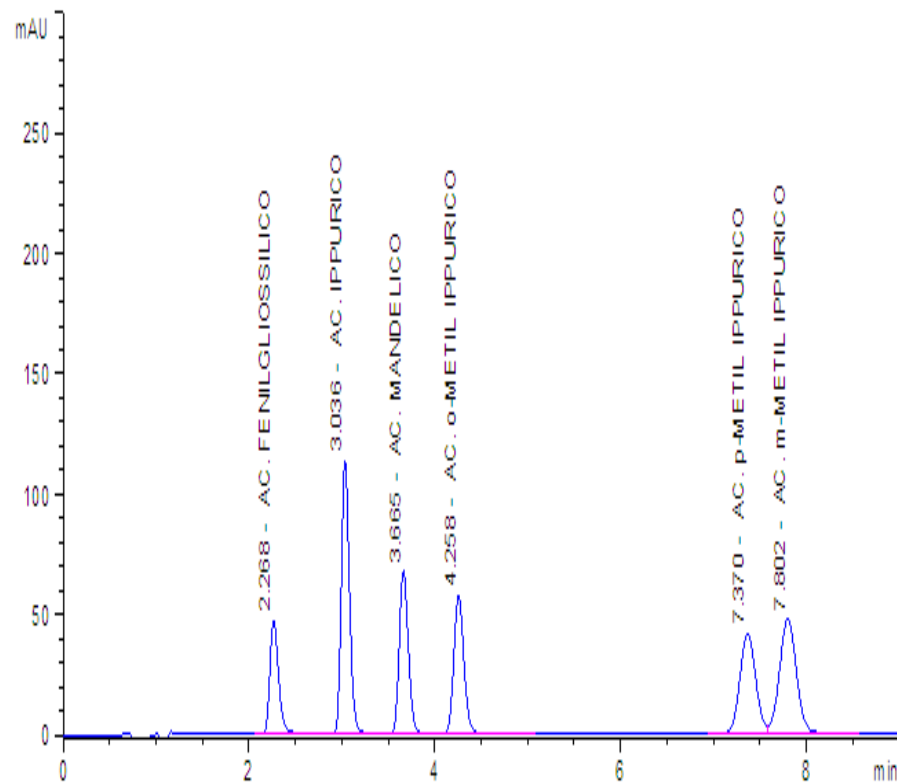


Fig. 3	Test Solution
	R.T. 2.68 Phenylglyoxilic Acid
	R.T. 3.03 Hippuric Acid
	R.T. 3.66 Mandelic Acid
	R.T. 4.25 o-Methylhippuric Acid
	R.T. 7.37 p-Methylhippuric Acid
	R.T. 7.80 m-Methylhippuric Acid

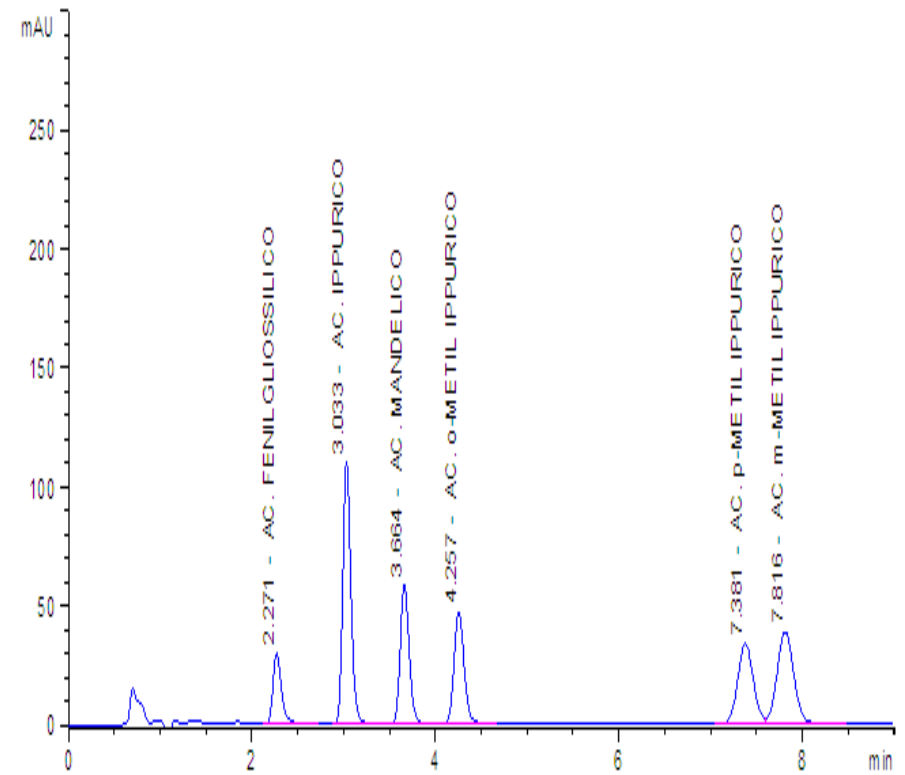


Fig. 4	Calibrator in urine
	R.T. 2.27 Phenylglyoxilic Acid 219,8 mg/l
	R.T. 3.03 Hippuric Acid 1.481,6 mg/l
	R.T. 3.66 Mandelic Acid 894,6 mg/l
	R.T. 4.25 o-Methylhippuric Acid 479,4 mg/l
	R.T. 7.38 p-Methylhippuric Acid 485,6 mg/l
	R.T. 7.81 m-Methylhippuric Acid 490,8 mg/l

PHENYLGLYOXILIC / MANDELIC / HIPPURIC / o-m-p-METHYLHIPPURIC ACIDS IN URINE
(Reference Chromatograms)

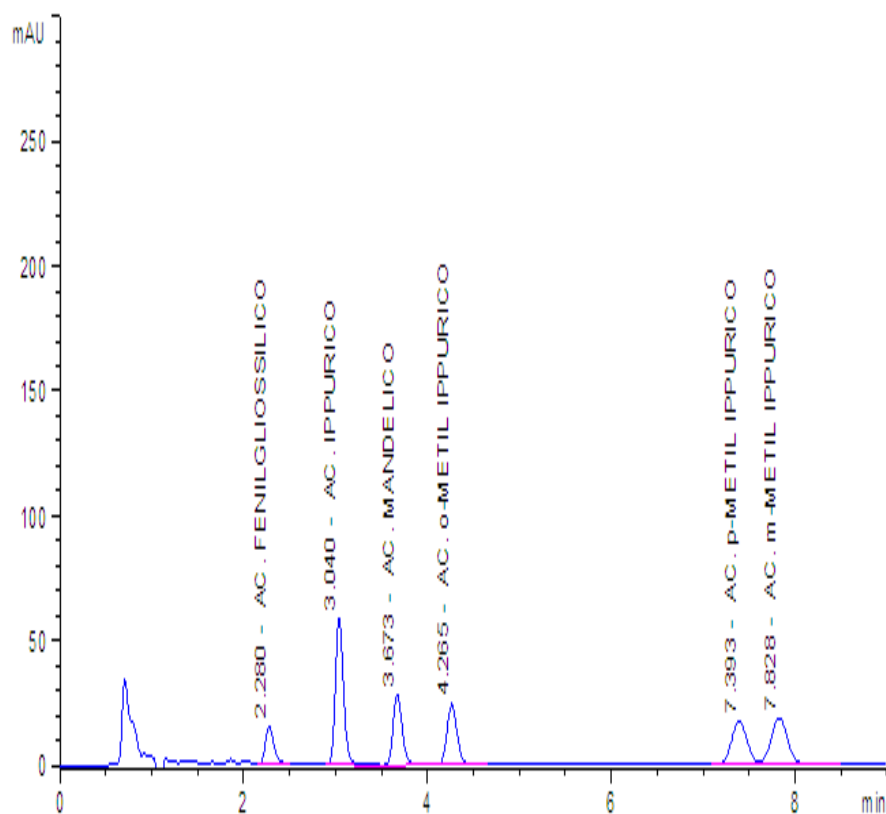


Fig. 5	Control in urine level 1		
	R.T. 2.28	Phenylglyoxilic Acid	89,2 mg/l
	R.T. 3.04	Hippuric Acid	599,9 mg/l
	R.T. 3.67	Mandelic Acid	301,0 mg/l
	R.T. 4.25	o-Methylhippuric Acid	176,8 mg/l
	R.T. 7.30	p-Methylhippuric Acid	182,7 mg/l
	R.T. 7.82	m-Methylhippuric Acid	159,7 mg/l

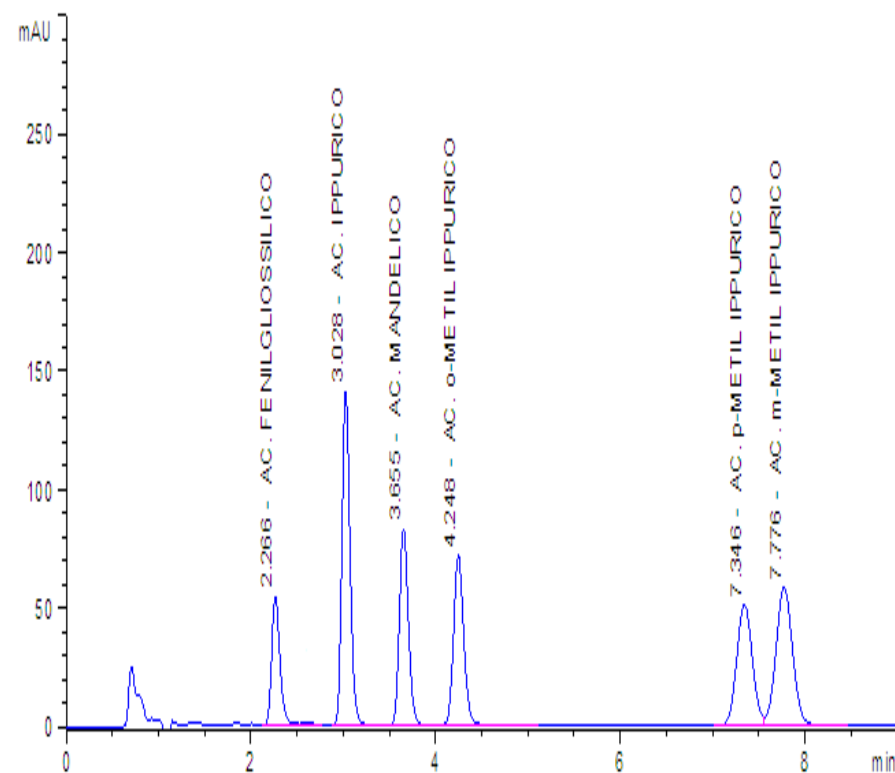


Fig. 6	Control in urine level 2		
	R.T. 2.26	Phenylglyoxilic Acid	442,2 mg/l
	R.T. 3.02	Hippuric Acid	1869,7 mg/l
	R.T. 3.65	Mandelic Acid	1258,8 mg/l
	R.T. 4.24	o-Methylhippuric Acid	705,4 mg/l
	R.T. 7.34	p-Methylhippuric Acid	756,7 mg/l
	R.T. 7.77	m-Methylhippuric Acid	745,1 mg/l

PHENYLGLYOXILIC / MANDELIC / HIPPURIC / o-m-p-METHYLHIPPURIC ACIDS IN URINE
(Reference Chromatograms)

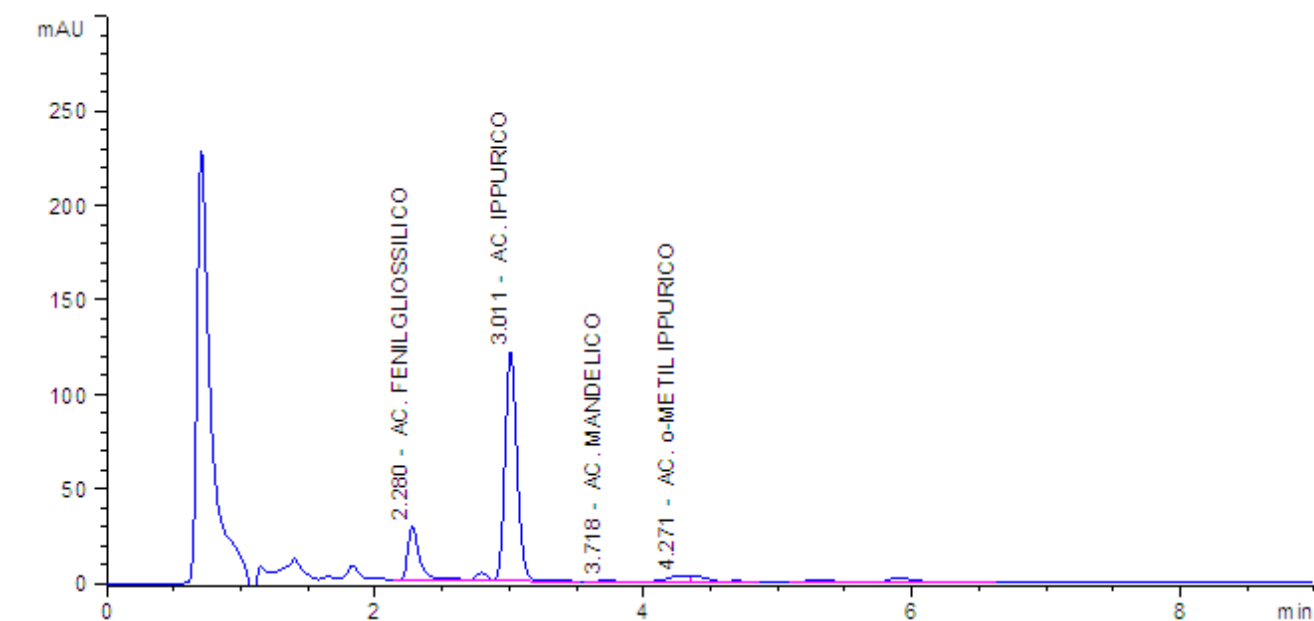


Fig. 7	Urine Sample		
	R.T. 2.28	Phenylglyoxilic Ac.	187,1 mg/l
	R.T. 3.04	Hippuric Acid	1.420,3 mg/l
	R.T. 3.67	Mandelic Acid	N.D.
	R.T. 4.25	o-Methylhippuric Acid	N.D.
	R.T. 7.30	p-Methylhippuric Acid	N.D.
	R.T. 7.82	m-Methylhippuric Acid	N.D.