

HIPPURIC ACID and o-m-p- METHYLHIPPURIC ACIDS IN URINE BY UV - FAST – CODE
Z06110

Biochemistry

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. 1).

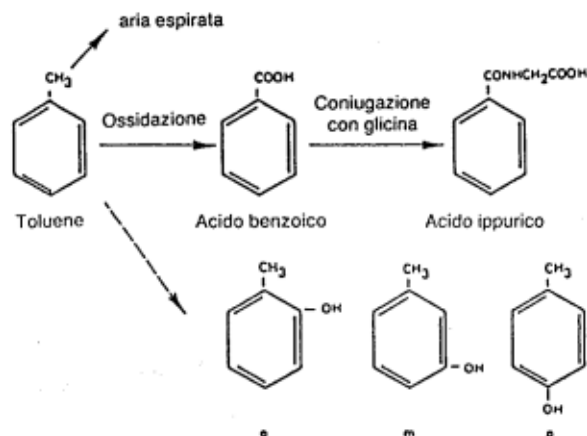


Fig. 1: Biotransformation of Toluene in vivo

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. 2).

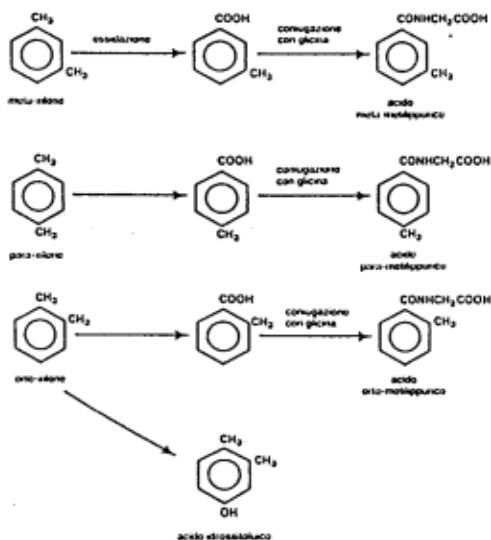


Fig. 2 Biotransformation of Xilene in vivo

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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° 005	Hippuric and o-m-p-Methylhippuric Acids in urine by UV - FAST	08/2024
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TECHNICAL FEATURES

Principle of the Method:

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): 5 µg/ml

Minimum Concentration analyzable (LLOQ) : 15 mg/l

Linearity : 15 – 3000 mg/l

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

Acceptable values in urine of not exposed subjects:

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

Acceptable values in urine after work shift:

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
Ref: ACGIH 2020

<u>Components of the kit:</u>	All the components are ready-to-use. Stability: 3 years at $\leq 20^{\circ}\text{C}$ (except Reagent C stored at 2-8$^{\circ}\text{C}$).
Reagent A – Dilution Solution, 1 x 100 ml	
Reagent C – Test Solution, 1 x 2 ml	<u>Store at 2-8$^{\circ}\text{C}$</u>
Urine Calibrator lyophil, 1 x 5 ml	<u>Code Z06016 (packed separately – see data sheets)</u>
Reagent M – Mobile Phase, 2 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-analytics 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 seconds

INJECTION :

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

Verify that the Test Solution has retention time similar to fig. 3. 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 seconds

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.

HIPPURIC ACID and o-m-p-METHYLHIPPURIC ACIDS IN URINE - Warnings

REAGENT C : TEST SOLUTION

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 seconds

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 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 **itlt 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).**

INJECTION NEEDLE WASHING

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

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
Z60111	Mobile Phase	4 x 500 ml
Z699975902	Poroshell 120 EC C18 Analytical Column (50 x 4,6mm –2,7 μ)	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

HIPPURIC ACID and o-m-p-METHYLHIPPURIC ACIDS IN URINE (Reference Chromatograms)

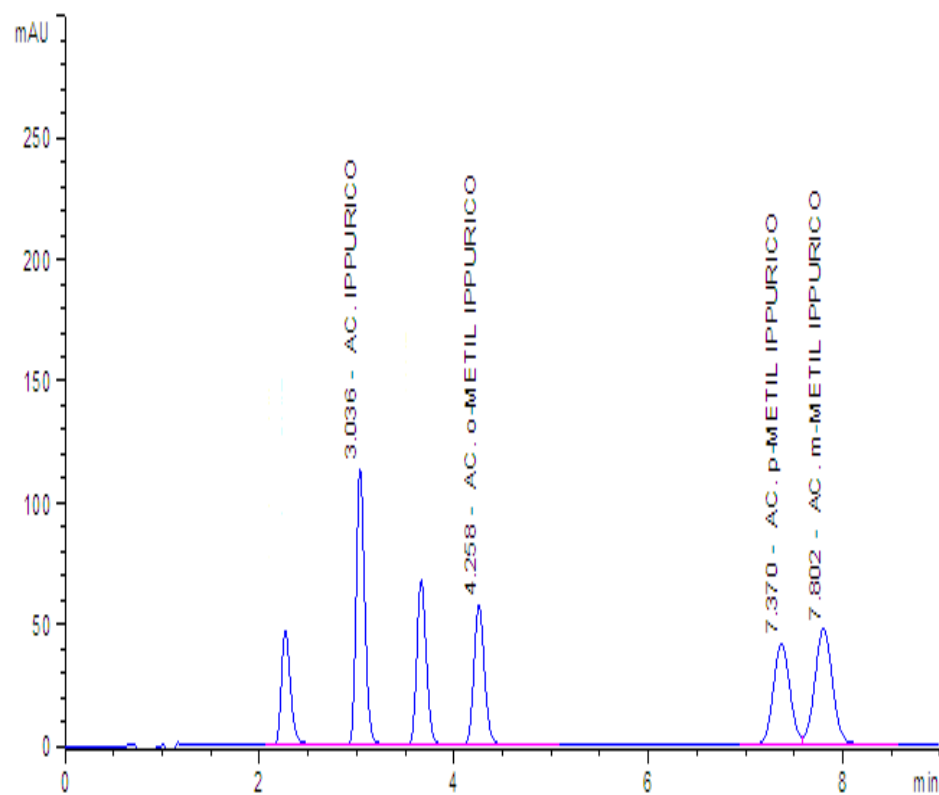


Fig. 3	Test Solution
	R.T. 3.03 Hippuric Acid
	R.T. 4.25 o-Methylhippuric Acid
	R.T. 7.37 p-Methylhippuric Acid
	R.T. 7.80 m-Methylhippuric Acid

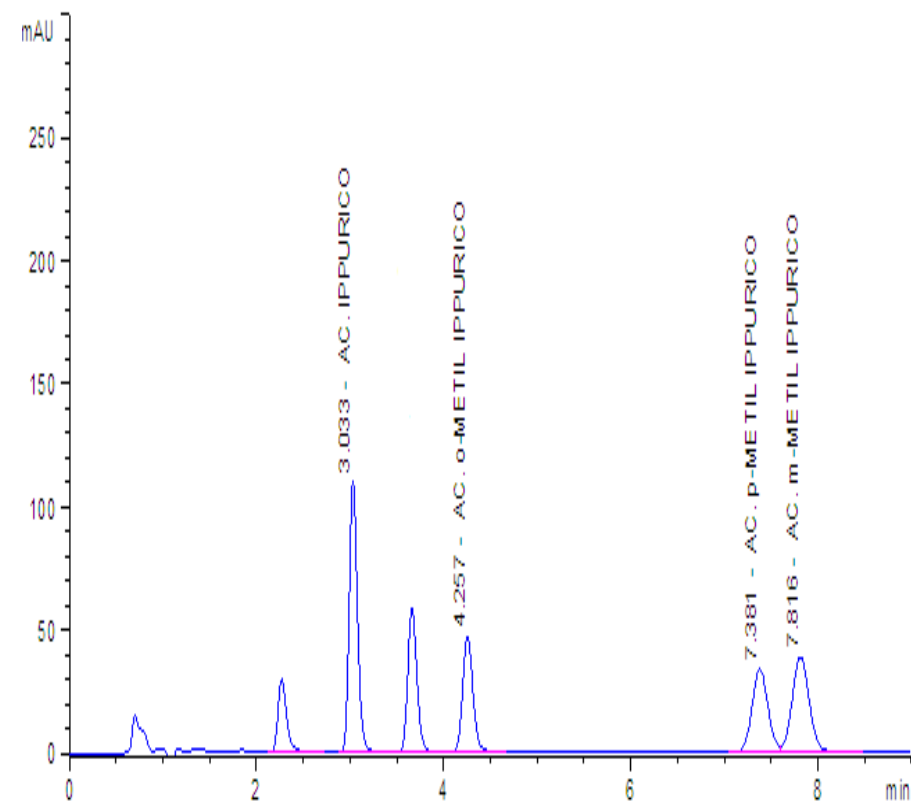


Fig. 4	Calibrator in urine
	R.T. 3.03 Hippuric Acid 1.481,6 mg/l
	R.T. 4.25 o-Methylhippuric Acid 479,4 mg/l
	R.T. 7.38 p-Methylhippuric Acid 485,6 mg/l
	R.T. 7.81 m-Methylhippuric Acid 490,8 mg/l

HIPPURIC ACID and o-m-p-METHYLHIPPURIC ACIDS IN URINE (Reference Chromatograms)

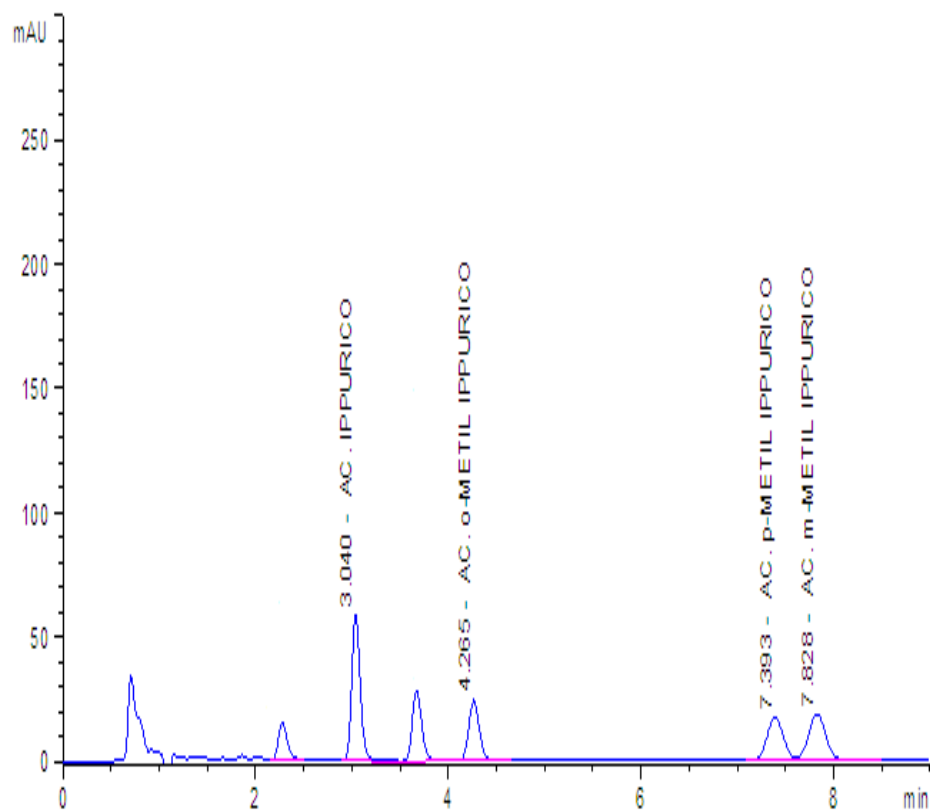


Fig. 5	Control in urine level 1		
	R.T. 3.04	Hippuric Acid	599,9 mg/l
	R.T. 4.25	o-Methylhippuric Acid	176,8 mg/l
	R.T. 7.30	p-Methylhippuric Acid	182,7 mg/l
	R.T. 7.82	m-Methylhippuric Acid	159,7 mg/l

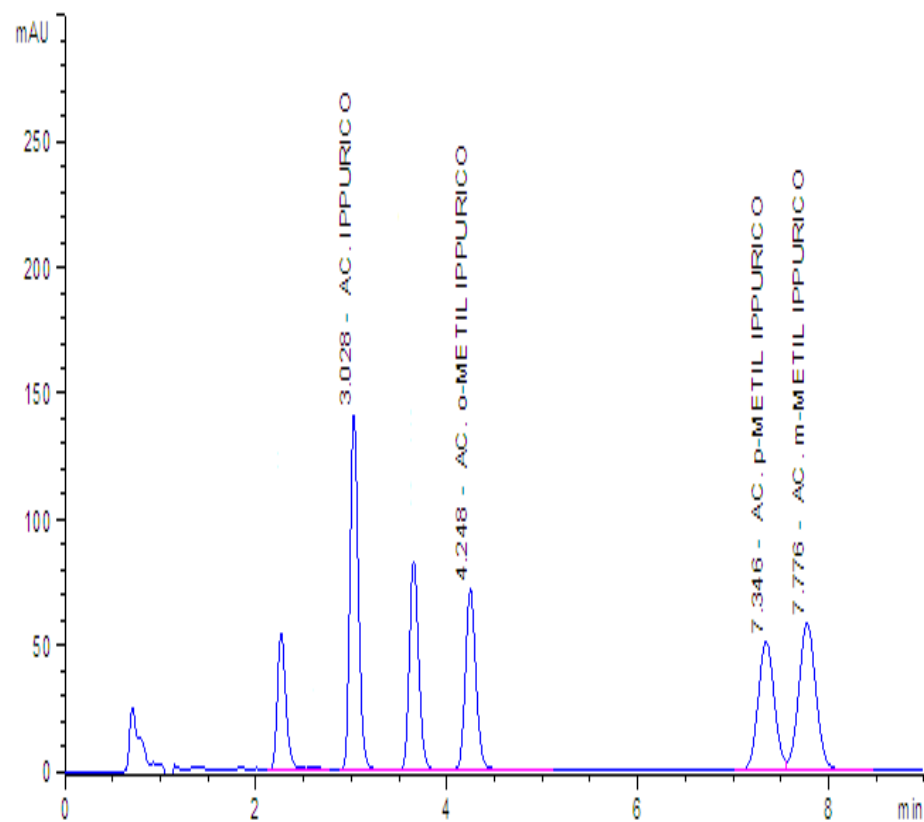


Fig. 6	Control in urine level 2		
	R.T. 3.02	Hippuric Acid	1869,7 mg/l
	R.T. 4.24	o-Methylhippuric Acid	705,4 mg/l
	R.T. 7.34	p-Methylhippuric Acid	756,7 mg/l
	R.T. 7.77	m-Methylhippuric Acid	745,1 mg/l

HIPPURIC ACID and o-m-p-METHYLHIPPURIC ACIDS IN URINE
(Reference Chromatograms)

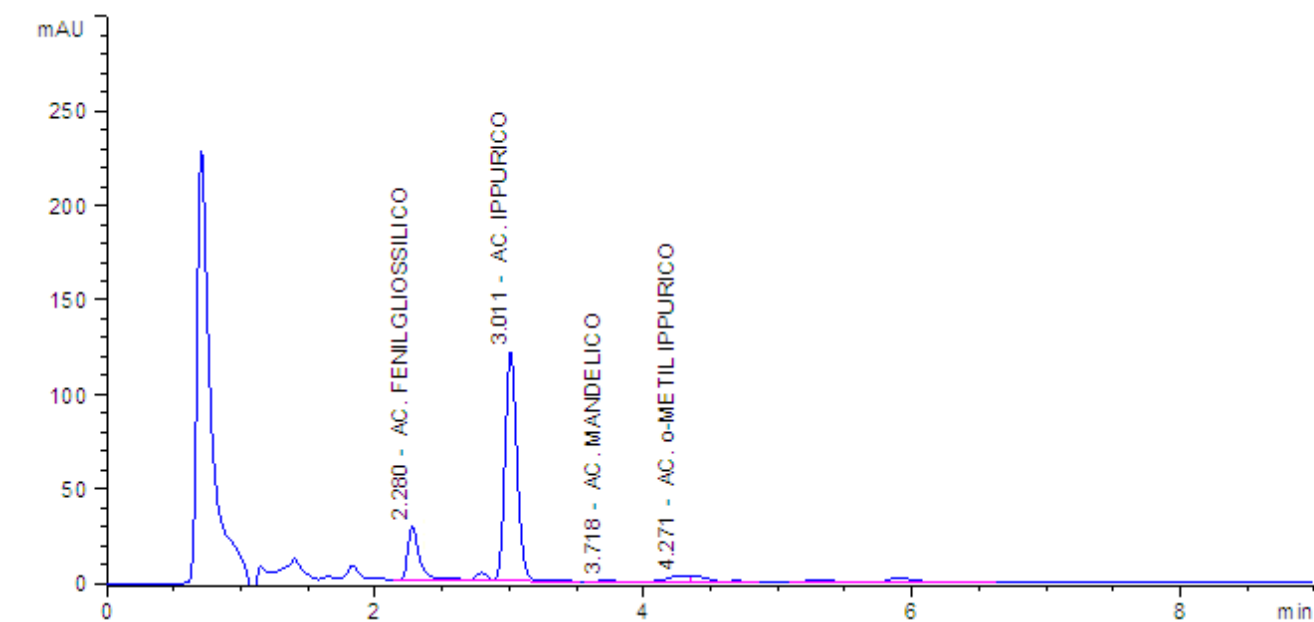


Fig. 7	Urine Sample	
	R.T. 2.28	Phenylglyoxilic Ac. 187,1 mg/l
	R.T. 3.04	Hippuric Acid 1.420,3 mg/l
	R.T. 3.67	Mandelic Acid N.D.
	R.T. 4.25	o-Methylhippuric Acid N.D.
	R.T. 7.30	p-Methylhippuric Acid N.D.
	R.T. 7.82	m-Methylhippuric Acid N.D.