

trans,trans-MUCONIC ACID IN URINE BY UV - FAST - CODE Z23210

BIOCHEMISTRY

The measuring of **trans,trans-MUCONIC Acid** is suggested as a reliable biological marker for monitoring workers exposed to benzene.

Benzene is an industrial chemical product which often appears in mineral oils following combustion processes. For this reason there is a high risk of environmental pollution. Its levels in the air can easily increase due to emissions from coal and oil burning, benzene leak and storage operations, exhaust fumes from cars, evaporation from gasoline service stations, and use of industrial solvents. Smoking is another source of benzene in air since tobacco contains high levels. Acute exposure to benzene can cause drowsiness, dizziness, rapid heart rate, headaches, tremors, confusion, unconsciousness and death.

People who breathe benzene for long periods may have different harmful effects. In fact, it is haematoxic and has been identified as a carcinogen, can bring to anaemia, excessive bleeding and it can be harmful to the immune system and the reproductive organs.

Regarding cancerogenesis, according to the American Department of Health and Human Services (DHHS) benzene is a human carcinogen. Long-term exposure to relatively high levels of benzene in the air can cause leukaemia. For all these reasons monitoring and control of workers exposed to benzene is very important.

Benzene is primarily metabolised by a cythochrome P-450 (CYP-450) to phenolic compounds. The range of metabolic conversion of benzene to t,t-MA is about 2 – 25 % and dependent on the benzene exposure level. Once oxidized, the metabolites play a substantial role in benzene's toxicity by inducing either DNA alkylation or oxidative stress that lead to the above mentioned systemic disorders.

 EUREKA S.R.L. LAB DIVISION
Via M. D'Antona 28
60033 Chiaravalle (AN) - Italy
Tel. +39 071 7450790
eureka-support@sentinel.it
www.eurekakit.com



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	T,t-Muconic Acid in urine by UV - FAST	07/2024
--------	--	---------

TECHNICAL FEATURES

Principle of the Method:

trans,trans-MUCONIC Acid is diluted with a suitable diluent and after purification in a clean up column it is injected into the HPLC System.

Recovery:

92 %

Sensitivity (LLOD):

6,66 µg/l

Minimum Concentration analyzable (LLOQ):

20 µg/l

Linearity:

20 – 10.000 µg/l

Intra-series accuracy (relative error %) :

Ci	Cs
200 µg/l	800 µg/l
1,97%	1,51%

Inter-series accuracy (relative error %) :

Ci	Cs
200 µg/l	800 µg/l
5,38%	3,07%

Reproducibility Intra series (coefficient of variation%):

C LLOQ	Cm	Cs
20 µg/l	400 µg/l	1200 µg/l
5,76%	2,83%	2,02%

Reproducibility Inter series (coefficient of variation%):

C LLOQ	Cm	Cs
20 µg/l	400 µg/l	1200 µg/l
6,41%	4,05%	2,28%

Correlation Coefficient R2 + Dev Std

0,9973 ± 0,0012

Exposure Biological Index – E.B.I. :

500 µg/g creatinine
ACGIH 2020

Metabolic Interferences :

Sorbic Acid : about 0,12 % of Sorbic Acid got with diet is converted in t,t-MA

<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 Reagents E and F.
Reagent A – Dilution Solution, 1 x 100 ml	
Reagent B – Wash Solution N° 1, 1 x 200 ml	
Reagent C – Wash Solution N° 2, 1 x 100 ml	
Reagent D – Eluting Solution, 1 x 100 ml	
Reagent E – Test Solution, 1 x 2 ml	<u>Store at 2-8 °C</u>
Reagent F – Internal Standard, 1 x 1 ml	<u>Store at 2-8 °C</u>
Urine Calibrator lyoph., 1 x 5 ml	<u>Code Z23016 (packed separately – see data sheets)</u>
Reagent M – Mobile Phase, 2 x 500 ml	
Clean up Columns, 100 pcs	

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 20 μl
Spectrofotometric Detector UV/VIS $\lambda=264\text{ nm}$
Chromatograms Recorder

Optional Equipment:

Autosampler
Operational Computer

Urine Collection Procedure:

After work shift, collect 10 ml of urine in a suitable test tube. Delayed analyses require sample freezing at -20°C or less. Stable over 4 weeks.

Bibliography= Italian Journal of Occupational and Environmental Hygiene - Suppl. Vol. 3, n.1 - 2012

PREANALYTICAL PROCEDURE

Test Solution at 560 µg/l Preparation.

Dispense in a tube :

- 900 µl of Water HPLC grade
- 100 µl of **Reagent E – Test Solution**
- 10 µl of **Reagent F – Internal Standard Solution** (after dilution as STEP 1 below)

Vortex for 10 seconds

INJECTION :

- Inject 20 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

STEP 1 : Internal Standard Dilution

Dilute **Reagent F – Internal Standard** 1:25 with Water, preparing a volume sufficient to cover the samples of the analytic session

STEP 2: Calibrator, Samples and Controls preparation

As soon as the urine samples are ready for processing check if the pH is >5.0

Dispense in a tube :

	Calibrator	Sample	Controls
Calibrator	200 µl		
Sample		200 µl	
Controls			200 µl
Reagent A – Diluting Solution	800 µl	800 µl	800 µl
Reagent F – Internal Standard Sol.	100 µl	100 µl	100 µl

Vortex for 10 seconds

STEP 3: Sample loading

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

STEP 4: Washing

- Wash with 2 ml of **Reagent B - Wash Solution 1** IN TWO STEPS *discharge the percolated liquid*
- Wash with 1 ml of **Reagent C - Wash Solution 2** *discharge the percolated liquid*

STEP 5: Extraction

- Extract with 1 ml of **Reagent D - Eluting Solution**

Let dry with a light flux of air!

Vortex for 10 seconds

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

INJECTION :

- Inject 20 µl of the eluate into the chromatographic system.

trans,trans-MUCONIC ACID IN URINE - FAST – Warnings

REAGENT E : TEST SOLUTION

trans,trans-MUCONIC ACID	5.600 µg / l
--------------------------	--------------

PARAMETERS OF THE SPECTROFOTOMETRIC DETECTOR

λ	264 nm
GAIN	0,001 AUFS
INTEGRATION TIME	10 sec

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase ZORBAX ECLIPSE PLUS C-18 (4,6 x 100 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 C-18 (4,6 x 100 mm, 3,5 µm). Disconnect the detector and filter 30 ml of H₂O: Acetonitrile (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 : Acetonitrile (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 1,0 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. 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 (20 : 80 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 H₂O : Acetonitrile or Methanol (20 : 80 v / v) .
--

INJECTION NEEDLE WASHING

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

HPLC PARAMETERS

LOOP	20 µl
Recommended Flow rate	1,5 ml / min
Pressure	About 115 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z23016	Urine Calibrator lyophil. for t,t-Muconic Acid	4 x 5 ml
Z38019	Urine multiparametric Control lyophil. for toxic organic compounds, Levels 1,2	2 x 5 x 10 ml
Z23211	Mobile Phase	4 x 500 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

t,t-MUCONIC ACID
(Reference Chromatograms)

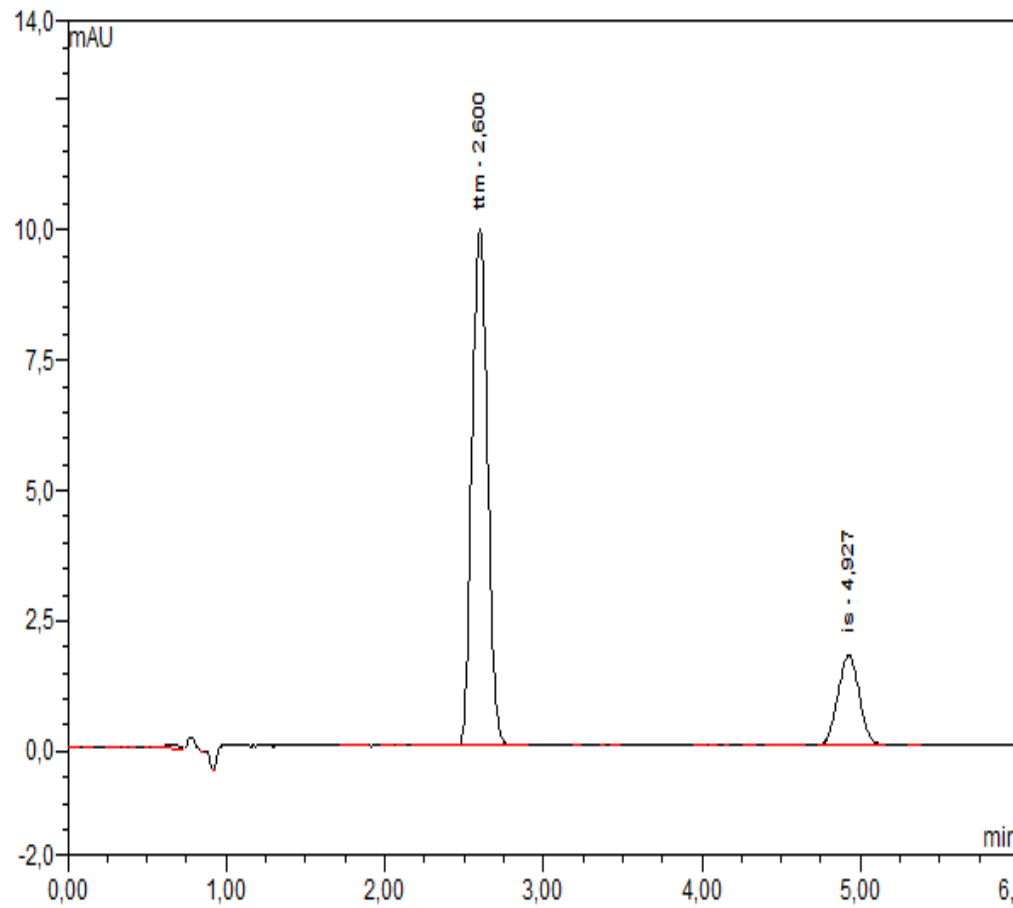


Fig. 1	Test Solution
	R.T. 2.60 t,t-Muconic Ac.
	R.T. 4.92 Internal Standard

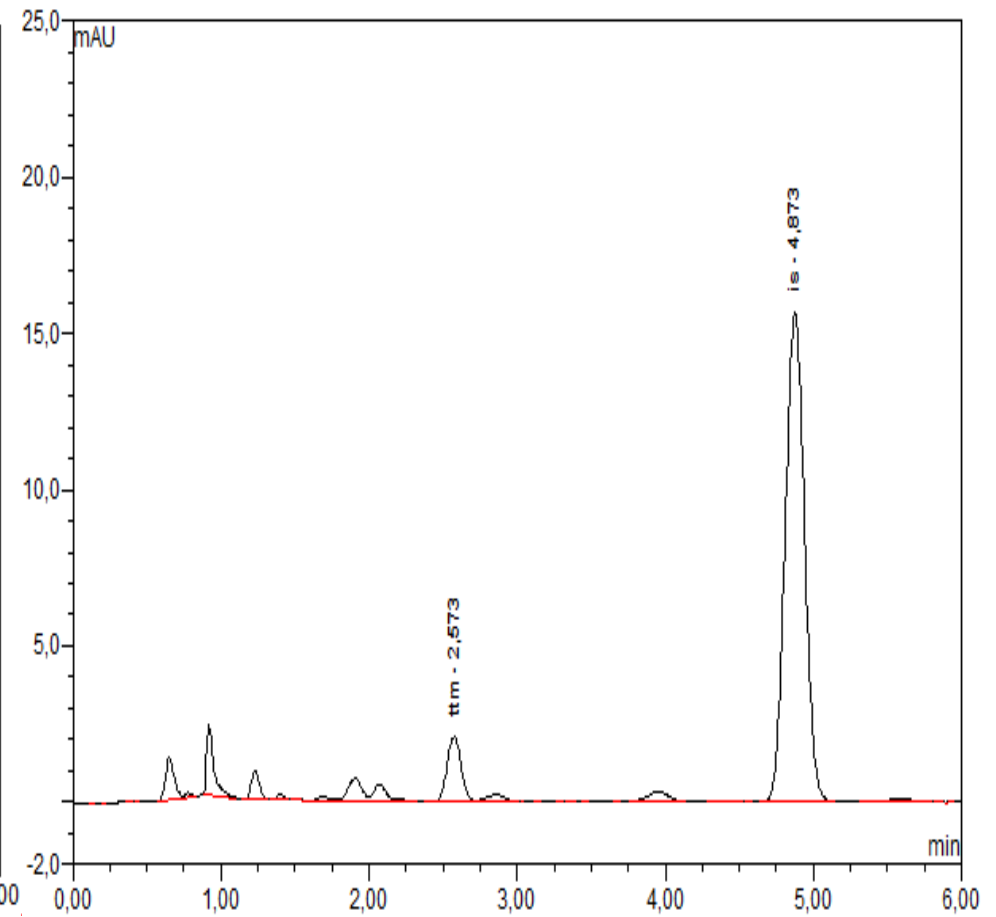


Fig. 2	Urine Calibrator
	R.T. 2.57 t,t-Muconic Ac. 660 µg/l
	R.T. 4.87 Internal Standard

t,t-MUCONIC ACID
(Reference Chromatograms)

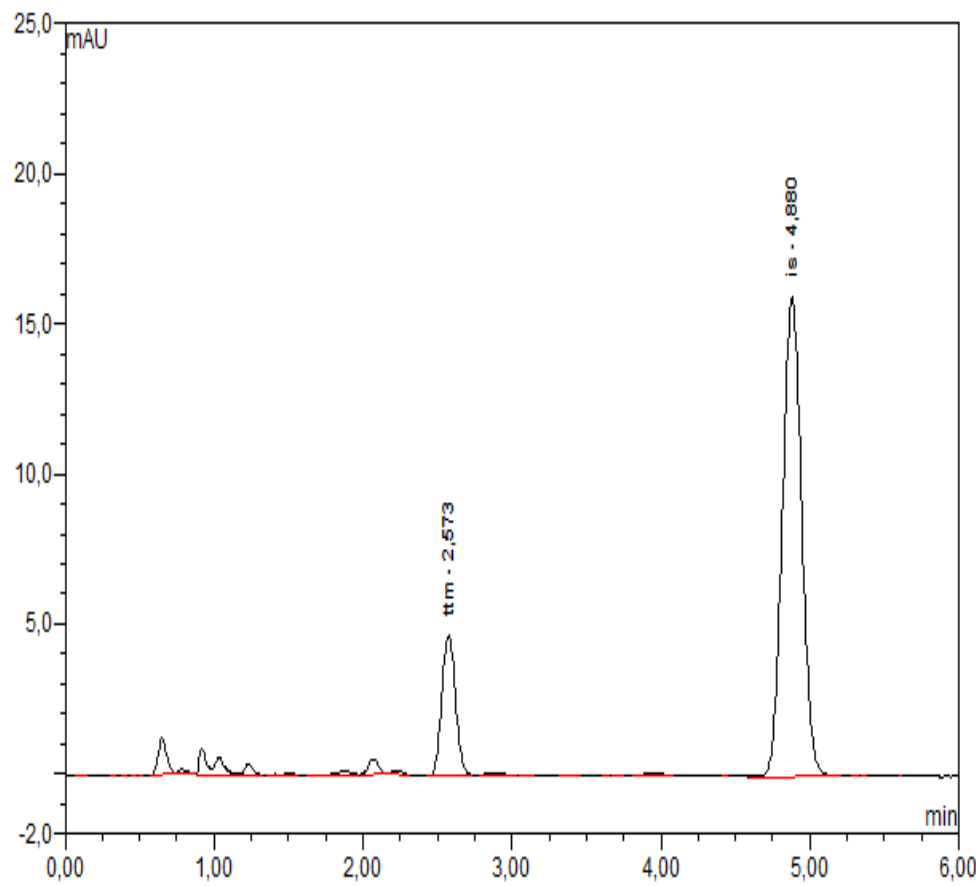


Fig. 3	Urine Control level 2
	R.T. 2.57 t,t-Muconic Ac. 1635,4 µg/l
	R.T. 4.88 Internal Standard

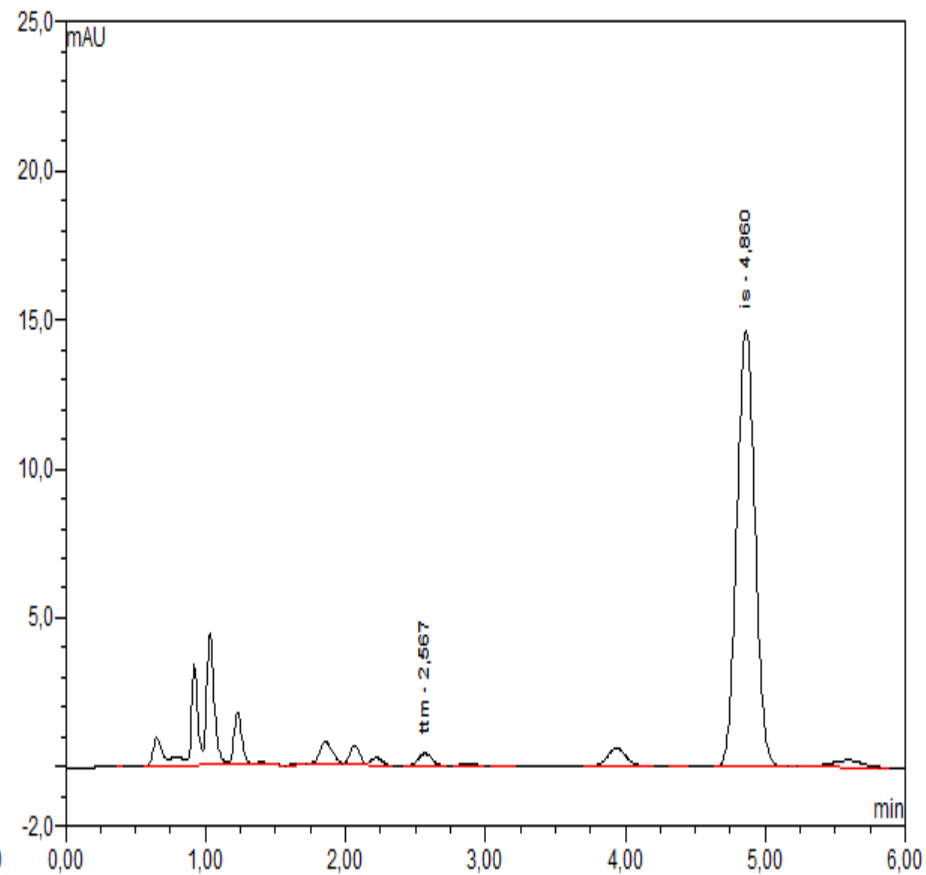


Fig. 4	Urine sample not pathological
	R.T. 2.56 t,t-Muconic Ac. 143,2 µg/l
	R.T. 4.86 Internal Standard