

t,t-Muconic and s-Phenylmercapturic Acids in urine by LC/MS

Code LC23010





This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD).

The declaration of conformity (CE) is available upon request.



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INTRODUCTION

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 haematotoxic 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.

The acid S-phenylmercapturic (S-PMA) is a urinary metabolite of benzene, which derives from the conjugation of the intermediate benzene-epoxide with glutathione. The metabolite S-PMA was recently added to the list of biological indicators of exposure to benzene. S-PMA was identified as a product of biotransformation of benzene in 1953.

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TECHNICAL FEATURES

PRINCIPLE OF THE METHOD			
The urine sample is diluted and injected in LC-MS/MS.			
RECOVERY:	101,6 – 117,2 %		
SENSITIVITY (LLOD):	t,t-Muconic Acid	20 ng/ml	
	s-Phenylmercapturic Acid	1 ng/ml	
MINIMUM CONCENTRATION ANALYZABLE (LLOQ):	t,t-Muconic Acid	75 ng/ml	
	s-Phenylmercapturic Acid	5 ng/ml	
LINEARITY:	t,t-Muconic Acid	75 – 10.000 ng/ml	
	s-Phenylmercapturic Acid	5 – 10.000 ng/ml	
EXPOSURE BIOLOGICAL INDEX – I.B.E.	t,t-Muconic Acid (t,t-MA) 500 µg/l		
t,t-Muconic Acid	Corresponding to the Average Exposure Value of 4 ppm of Benzene in the air.		
ACCEPTABLE VALUES IN NON-EXPOSED SUBJECTS:	< 0,3 mg/g creatinine (no smokers)		
t,t-Muconic Acid	Ref.: G Ital Med Lav Erg 2002;24:3 Suppl		
METABOLIC INTERFERENCES:	Sorbic Acid : about 0,12 % of Sorbic Acid got with diet is converted in t,t-MA		
t,t-Muconic Acid			
ACCEPTABLE VALUES IN EXPOSED SUBJECTS:	< 25 µg/l		
s-Phenylmercapturic Acid			
Accuracy intra serie (relative error %)	Ci	Cs	
t,t-Muconic Acid:	250 ng/ml	1.000 ng/ml	
	5,94%	2,85%	
Accuracy inter serie (relative error %)	Ci	Cs	
t,t-Muconic Acid:	250 ng/ml	1.000 ng/ml	
	7,54%	3,04%	
Reproducibility intra serie (coefficient of variation %)	C LLOQ	Cm	CUP
t,t-Muconic Acid:	75 ng/ml	500 ng/ml	1.500 ng/ml
	5,80%	4,93%	4,76%
Accuracy intra serie (relative error %)	Ci	Cs	
s-Phenylmercapturic Acid:	10 ng/ml	50 ng/ml	
	3,88%	5,47%	
Accuracy inter serie (relative error %)	Ci	Cs	
s-Phenylmercapturic Acid:	10 ng/ml	50 ng/ml	
	4,52%	3,97%	
Reproducibility intra serie (coefficient of variation %)	C LLOQ	Cm	CUP
s-Phenylmercapturic Acid:	5 ng/ml	5 ng/ml	100 ng/ml

	8,10%	2,63%	2,48%
Reproducibility inter serie (coefficient of variation %) s-Phenylmercapturic Acid:	C LLOQ 5 ng/ml 14,66%	Cm 500 ng/ml 6,63%	CUP 1.500 ng/ml 8,19%
Coefficient of Correlation R2 + Dev Std:	0,9945 ± 0,0021 t,t-Muconic Acid 0,9964 ± 0,0030 s-Phenylmercapturic Acid		

COMPONENTS OF THE KIT (100 TESTS)

Reagent A – Internal Standard Solution	1 x 10 ml	Store at -20 °C
Reagent B – Diluting Solution	1 x 100 ml	
Calibrator lyophil. in urine – Level 0	1 x 1 ml	Code LC23016 (Packed separately – see data sheets)
Calibrator lyophil. in urine – Level 1	1 x 1 ml	Code LC23016 (Packed separately – see data sheets)
Calibrator lyophil. in urine – Level 2	1 x 1 ml	Code LC23016 (Packed separately – see data sheets)
Calibrator lyophil. in urine – Level 3	1 x 1 ml	Code LC23016 (Packed separately – see data sheets)
Calibrator lyophil. in urine – Level 4	1 x 1 ml	Code LC23016 (Packed separately – see data sheets)
Calibrator lyophil. in urine – Level 5	1 x 1 ml	Code LC23016 (Packed separately – see data sheets)
Reagent M1 – Mobile Phase M1	2 x 500 ml	
Reagent M2 – Mobile Phase M2	1 x 500 ml	

All reagents are ready to use and stable for 3 years at room temperature, except Reagent A which should be stored at -20 °C.

The storage procedure for the Lyophilic Calibration Standard is described in the dedicated data sheet.

ACCESSORIES and CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC23016	Calibrator in urine for t,t-Muconic/s-Phenylmercapturic Acids	6 x 2 x 1 ml
LC23019	Control in urine for t,t-Muconic/s-Phenylmercapturic Acids - Levels 1, 2	2 x 5 x 1 ml
SK23010	Starter kit for t,t-Muconic/s-Phenylmercapturic Acids in urine	1 Pc
Z695970902	Poroshell EC 120-C18 (100 x 4,6 mm, 4 µm) Analytical Column	1 Pc

MINIMUM INSTRUMENTAL EQUIPMENT REQUIRED

LC-MS/MS Triple Quadrupole with binary pump
MRM mode, ESI negative

OPTIONAL EQUIPMENT

Autosampler
Operational Computer.

URINE COLLECTION AND URINE SAMPLE STORAGE METHODS

End of shift urine. Freeze the sample at -20 °C if the analysis has to be deferred. It remains stable for more than 4 weeks.

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

ANALYTICAL PROCEDURE

STEP 1

Dispense in a vial:

- 9000 µl of **Reagent B – Diluting Solution**
- 1000 µl of **Reagent A – Internal Standard Solution**

Vortex for 10 sec.

The solution should be prepared taking into account the number of samples to be analysed, considering that 900 µl of solution per sample should be used.

N.B.: at this step, the internal standard is stable for 7 days at -20 °C

STEP 2

Dispense in a vial :

- 100 µl of **Sample/Control/Calibrator**
- 900 µl of **Solution prepared in STEP 1**

Vortex for 10 sec.

If the solution should present turbidity, centrifuge at 14.000 rpm for 10 minutes and inject the supernatant.

N.B.: at this step, the sample is stable for 2 days at -20 °C

INJECTION

- Inject 10 µl of solution (or supernatant) in HPLC.

T,t-MUCONIC/S-PHENYLMERCAPTURIC ACIDS IN URINE by LC/MS

Warnings

COLUMN CONDITIONING

Install the new analytical column Poroshell 120 EC-C18 (100 x 4.6 mm, 4 µm), thermostated at 40 °C. Disconnect the detector and pass 30 ml of a solution of **H₂O : Acetonitrile (20 : 80 v / v)** at a flow of 1.0 ml/minute. Do not recycle the washing liquids. Pass 30 ml of a solution of **H₂O : Acetonitrile (80 : 20 v / v)** at a flow rate of 1.0 ml/minute. **Do not recycle wash liquids.** Condition the column with the mobile phase at a flow rate of 1.0 ml/minute and drain the first 20 ml. When finished, inject the chemical test and check the suitability of the separation.

Phase recirculation analysis is NOT possible.

The signal that the mobile phase must be changed is given by the significant increase in detector background noise.

If the T Amb of the Laboratory is >20 °C it is recommended to keep the Mobile Phase at 2-8 °C between an analytical session.

COLUMN CLEANING

Disconnect the detector. Flow 15 ml of **H₂O : Acetonitrile or Methanol (80 : 20 v / v)** at a flow rate of 1.0 ml/minute and discharge. Flush a solution of **H₂O : Acetonitrile or Methanol (20 : 80 v / v)** for 30 minutes and drain. **It is recommended to wash the Analytical Column at the end of each analytical session.** The column should be stored in a solution of **H₂O : Acetonitrile or Methanol (20 : 80 v / v)**.

INJECTION NEEDLE WASHING

Wash with a solution of H₂O / Ethanol or Methanol or Acetonitrile (80 : 20 v/v).

PARAMETERS SET ON WATERS XEVO TQS MICRO

Capillary Voltage (kV)	3,0
Desolvation Gas (L/ora)	800
Desolvation Temperature (°C)	300

FRAGMENTATIONS (optimized on TRIPLE QUADRUPOLE WATERS XEVO TQS MICRO)

Analyte	Q1	Q3	CONE (V)	COLLISION ENERGY
t,t-Muconic Acid (1)	141	52.9	14	10
t,t-Muconic Acid (2)	141	97	10	5
s-Phenylmercapturic Acid (1)	238	108.9	28	12
s-Phenylmercapturic Acid (2)	238	109	28	58
t,t-Muconic Acid -6C13	147	101.9	24	10
s-Phenylmercapturic Acid - D5 (1)	243.1	113.8	18	48
s-Phenylmercapturic Acid - D5 (2)	243.1	114	18	10

GRADIENT SET

Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	FLOW (µl/min)
0	98	2	500
0.5	98	2	500
4.5	10	90	500
5.5	10	90	500
5.6	98	2	500
7.5	98	2	500

T,t-MUCONIC/ S-PHENYLMERCAPTURIC ACIDS IN URINE BY LC/MS

(Reference Chromatograms)

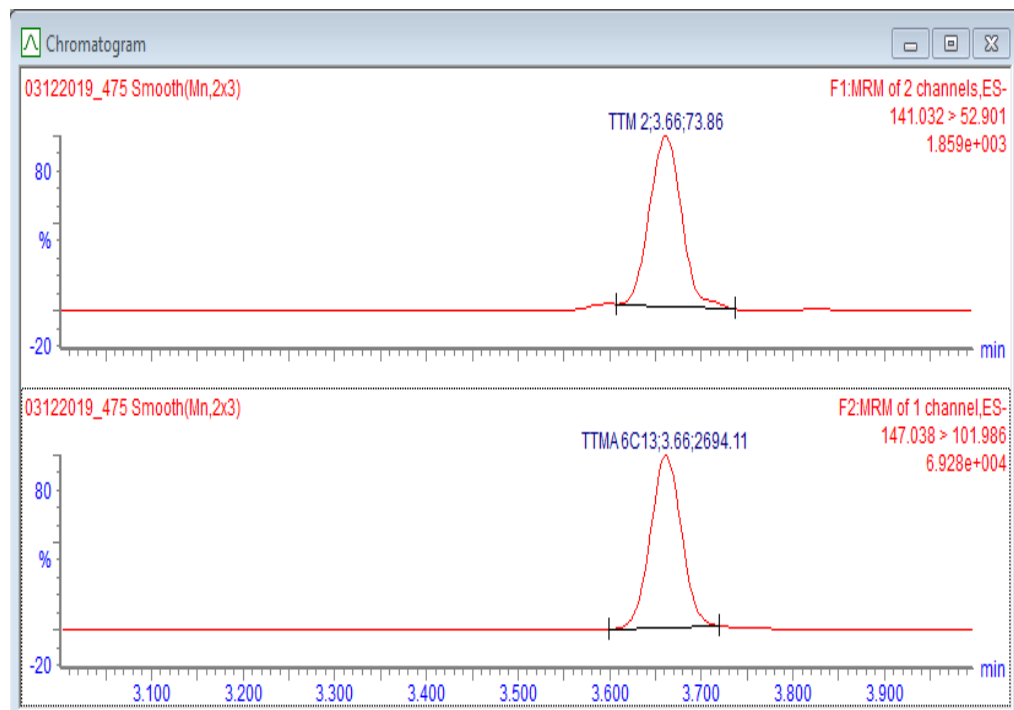


Fig. 1 :	Calibrator in urine
	T,t-Muconic Acid (transition 141 – 53) 750 ng/ml IS

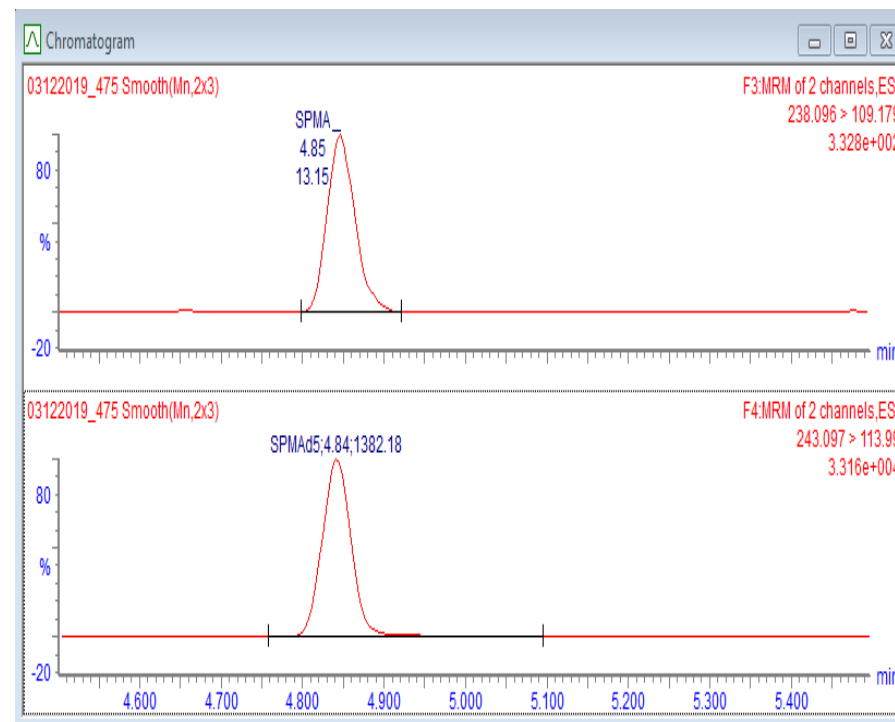


Fig. 2 :	Calibrator in urine
	S-Phenylmercapturic Acid (transition 238 – 109) 50 ng/ml IS

T,t-MUCONIC/ S-

PHENYLMERCAPTURIC ACIDS IN URINE BY LC/MS

(Reference Chromatograms)

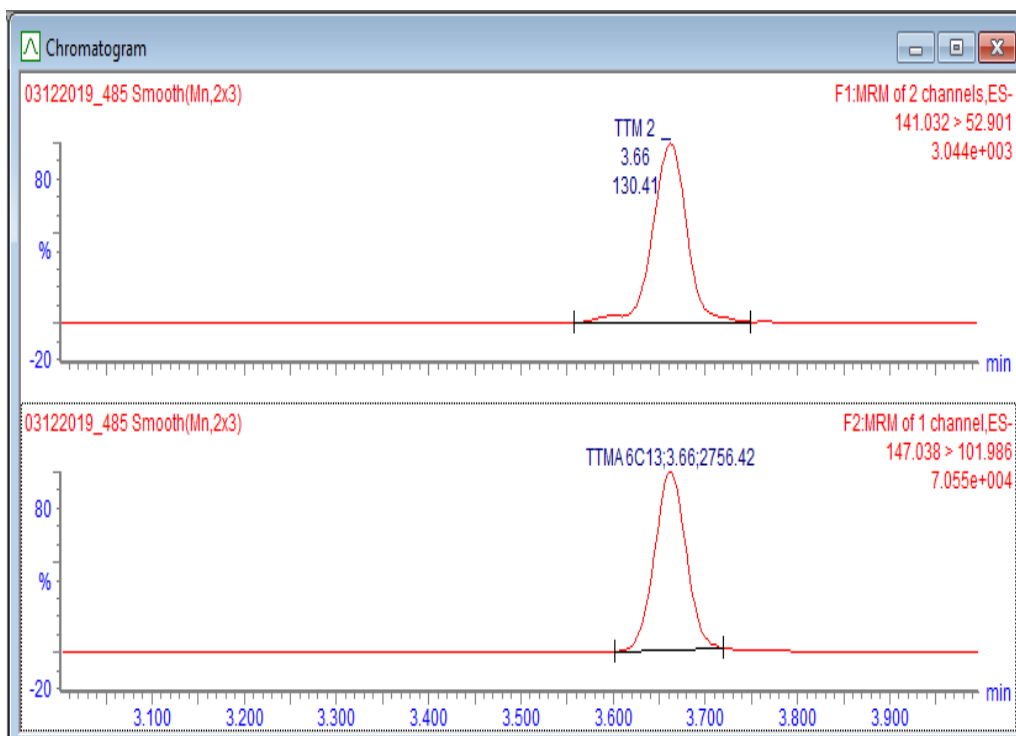


Fig. 3 :	Control in urine Level 2
	T,t-Muconic Acid (transition 141 – 53) 1250 ng/ml IS

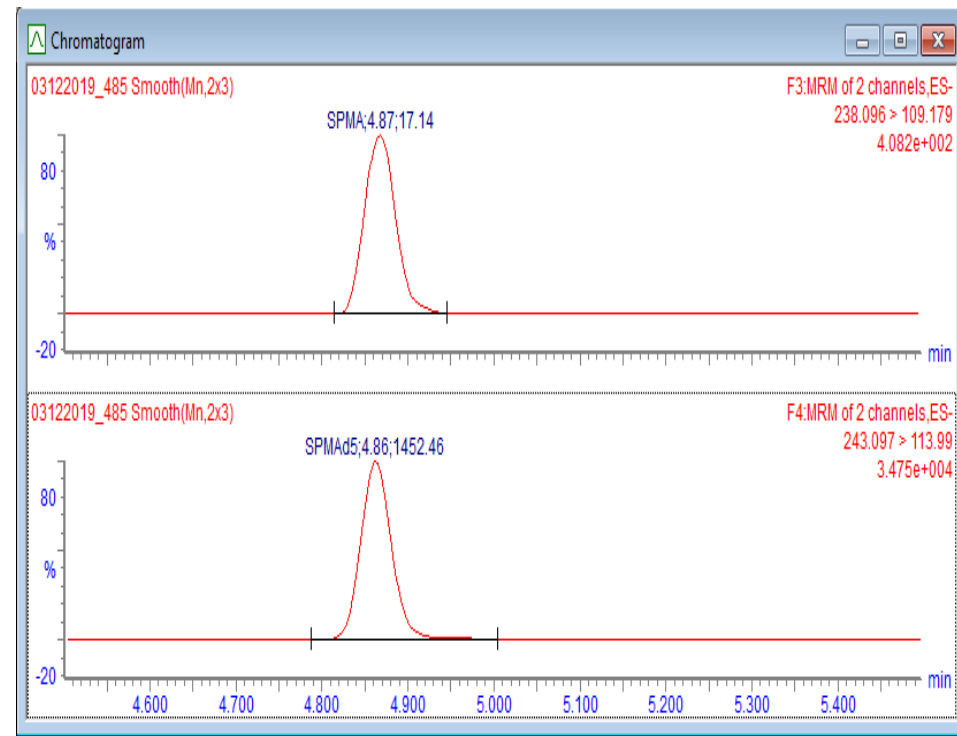


Fig. 4 :	Control in urine Level 2
	S-Phenylmercapturic Acid (transition 238 – 109) 80 ng/ml IS



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