

METHOD AT A GLANCE

FLOMASS® *t,t*-MUCONIC ACID AND PHENYLMERCAPTURIC ACID IN URINE

Benzene is an important industrial chemical product, an intermediate in the synthesis of many chemicals, and a natural component of petroleum and gasoline. It is also a ubiquitous environmental pollutant due to its formation in many combustion processes. Industrial emissions and traffic exhaust from burning fossil fuels, as well as personal smoking habits or exposure to environmental tobacco smoke can increase the risk of prolonged exposure to benzene. The main effect of benzene is the decrease of red blood cells, resulting in aplastic anemia and is associated with other blood disorders, also can cause acute myeloid leukemia/acute non-lymphocytic leukemia. For this reason, the International Agency for Research on Cancer (IARC) has included it among the compounds of group 1, carcinogenic to humans. Currently, the Scientific Committee for Occupational Exposure Limits classifies it among genotoxic carcinogens for which the existence of a threshold cannot be sufficiently supported, so even low exposures are considered a significant risk. Benzene causes toxic effects through metabolism. It is metabolized by cytochrome P450 (CYP) enzymes into benzene oxide, which is the source of all other metabolites. PMA and t.t-MA are both minor urinary metabolites of benzene exposure and are recommended for biological monitoring of benzene in the workplace.

Although in the past the total concentration of these two molecules was evaluated, analyzed following an acid hydrolysis of the same, now it is preferable to refer to the free fraction, since there is the possibility that other molecules derived from the metabolism of benzene release t, t-MA and PMA during the hydrolysis process, thus leading to an overestimation of the obtained concentration values.





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HPLC-MS/MS system conditions

Ionization: ESI negative mode MS/MS: specific MRM Injection volume: 2-20 μL (variable according to instrumental sensitivity) Running time: 6.50 min Column heater: 40°C Column conditioning: column should be conditioned for 5 min at chromatographic gradient initial condition. Then, run 3 blank injections (MPA only) using the gradient as indicate in IFU

Sample preparation

- Prepare a mix with 15 μL Internal Standard + 170 μL Diluting Solution sufficient for the number of samples to be analyzed
- Pipette 185 μ L solution mix as previously prepared in a vial
- Add 15 µL sample and vortex
- Transfer the content in an autosampler vial
- Inject 2-20 µL according to instrumental sensitivity and analyze with HPLC-MS/MS technique

Performance

ANALYTE	LINEARITY (ng/mL)	LLOD (ng/mL)	LLOQ (ng/mL)	CV% INTRA	CV% INTER
t,t-Muconic Acid	21.7 - 10000	6.50	21.7	3.3 - 4.0	3.5 – 5.2
Phenylmercapturic Acid	0.93 - 500	0.28	0.93	1.7 -2.5	3.8 – 4.5

Ordering guide

EUM29200	FloMass® t,t-Muconic Acid and Phenylmercapturic Acid in Urine	200 assays
EUM29041	6-Levels Calibrators, lyophil.	2 x 6 x 0.5 mL
EUM27051	2-Levels Controls, lyophil.	2 x 2 x 1.0 mL
EUM00C28	Chromatographic Column	1 pc