Determination of *t,t*-Muconic Acid in Urine via Automated Sample Preparation and HPLC

Application Note CL0412

This application was performed in collaboration with Martin Knirsch, RECIPE, Chemicals + Instruments GmbH (knirsch@recipe.de).

Keywords

Gilson GX-271 ASPEC™ with 406 Dual Syringe Pump, TRILUTION® LH v3.0 Liquid Handling Software, Solid Phase Extraction, SPE, Gilson GX-271 Liquid Handler, TRILUTION ® LC v2.1 Liquid Chromatography Software, human biomonitoring kit

Introduction

Benzene is one of the most widely used industrial chemicals, is a component of mineral oil, and is formed in the pyrolysis of organic material. The predominant sources of emission are the exhaust gases and vapors of road traffic and the petrochemical industry. Due to its high volatility, benzene is ubiquitously distributed in the ambient air. Tobacco smoke was found to be a further important factor determining exposure to benzene.

Benzene is classified as a toxic substance (hematoxic) and a potent human carcinogen. For this reason, an exposure to benzene is a serious danger to the health of the exposed subject. For the biological monitoring of occupational and environmental benzene levels, *trans,trans*-muconic acid (*tt*MA) has been established as a suitable biomarker. *tt*MA is a urinary metabolic product of benzene.

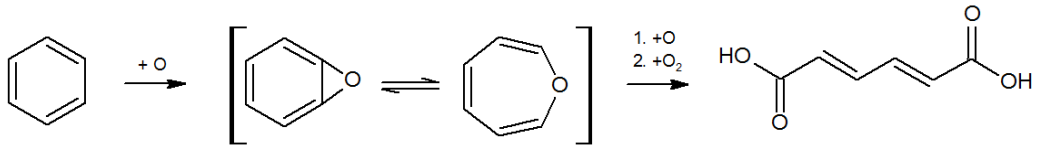

Figure 1. Metabolism of Benzene to *t,t*-Muconic Acid

Gilson instrumentation was utilized to automate both the sample preparation and analysis components of the ClinTox® *t,t*-muconic acid human biomonitoring kit from RECIPE, Chemicals + Instruments GmbH (www.recipe.de).

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Materials & Methods

In the kit, the *trans,trans*-muconic acid (ttMA) is extracted from a urine sample through a short sample preparation procedure. An aliquot of the urine sample is diluted and spiked with an internal standard. Afterwards the entire diluted aliquot is applied to a solid phase extraction column, adsorbing the *tt*MA to the resin. Co-adsorbed interfering substances are washed from the resin with an aqueous washing solution, followed by the *tt*MA being eluted off the column into a collection vial. The entire sample preparation procedure, including initial dilution and transfer of the prepared sample to a final vial, is automated using Gilson's GX-271 ASPEC™ system with 406 Dual Syringe Pump and orbital shaker on bed via TRILUTION® LH v3.0 Liquid Handling Software.

Figure 2. Gilson GX-271 ASPEC™ with 406 Dual Syringe Pump.

A portion of the solid phase extraction eluate is injected onto the HPLC system. A special reversed-phase column is used for the separation, and the analytes are detected using an ultraviolet wavelength. The analysis was run using TRILUTION LC v2.1 Liquid Chromatography software on a GX-271 Liquid Handler system with 402 Dual with Tee Syringe module and GX Direct Injection Module (Figure 3). A 152 UV-VIS detector and 306 series binary mobile phase pumping system were utilized. A column heater was used to maintain HPLC column temperature. Integration was done automatically through the TRILUTION LC software.

Figure 3. Gilson GX-271 Liquid Handler with 402 Dual with Tee Syringe Pump, 306 Mobile Phase Pumps, and a 152 UV-VIS Detector.

Samples and Solvents:

- NanoPure Water
- ClinTox® Complete Kit for *t,t*-Muconic Acid in Urine
    - Contains urine calibrator, all standard solutions, internal standard solution, SPE solvents, and HPLC Mobile Phase
- ClinTox® Analytical Column
- ClinChek® Urine Control, Level I & II
- Human Urine Sample
- Nitric Acid (conc.) (used in passivation of system)
- Isopropanol (used in passivation of system)

Sample Dilution Method

- Transfer 1 mL sample to vial
- Transfer 150 µL internal standard (IS) to vial
- Transfer 1 mL Reagent B to vial
- Vortex on orbital shaker at 600 rpm for 15 seconds

SPE Method (Figure 4)

- Condition:
  - 3 mL Reagent A
  - 3 mL Reagent B
- Load:
  - 2150 µL (entire) diluted sample
- Wash:
  - 1.5 mL Reagent C
  - 1.5 mL Reagent C
- Dry
- Elute:
  - 1.5 mL Reagent D
  - 1.5 mL Reagent D
  - Collected into same tube
  - Large Air Push at end of second elution
- Transfer eluate (3mL) to vial, vortex on orbital shaker at 600 rpm for 15 seconds

Figure 4. TRILUTION LH SPE Method.

HPLC Conditions

- Mobile Phase Flow Rate: 1 mL/min
- Injection Volume: 50 µL (50 µL sample loop, 2x total loop overfill)
- Run Time: 18 minutes
  - 0 – 9 min 100% Mobile Phase A
  - 9.1 – 15 min 100% Mobile Phase B
  - 15.1 – 18 min 100% Mobile Phase A
- Analytical Column: Heated at 35°C
- Detector: 264 nm (at both 0.01 and 0.05 sensitivity); 5 mm pathlength
- Integration: Front slope: 70; Back Slope: 50; Horizontal Baseline

All equipment was passivated prior to running per the ClinTox® kit instruction manual.

Results

Automation of the RECIPE ClinTox® *t,t*-Muconic Acid in Urine kit was performed using Gilson equipment and software. Samples were prepared and then injected in triplicate onto the HPLC system.

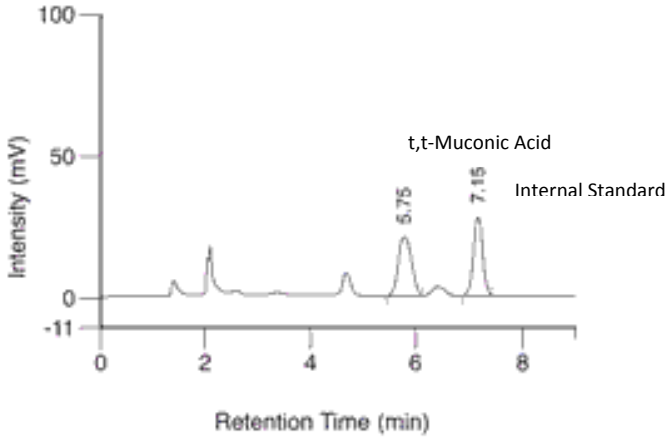

Figure 5. Example Chromatogram of the ClinChek® Level II Urine Control Sample.

Table 1. Assay Performance Parameters

	Gilson Automation Results			Manual Results
Assay Performance Parameters*	Day 1 (0.01 sensitivity)	Day 2 (0.01 sensitivity)	Day 2 (0.05 sensitivity)	Kit Data
Internal Standard SPE Recovery	78%	72%	70%	75-80%
Intraassay Precision (%CV *tt*MA)	4.1%	2.4%	2.5%	5.9%
Interassay Precision (average %CV *tt*MA)	3.0%			5.5%
Intraassay Precision (%CV IS)	4.3%	2.7%	2.9%	
Interassay Precision (average %CV IS)	3.3%			

*Assay LOD = 0.07 mg/l; Assay LOQ = 0.24 mg/l

Summary

The RECIPE ClinTox® *t,t*-Muconic Acid in Urine kit was successfully automated using Gilson instrumentation. Recovery values, as well as intra- and interassay precision, were comparable, if not better than the values obtained from manual sample preparation.

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