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

Application Note CL0412

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.

Introduction

Benzene, one of the most widely used industrial chemicals, is a component of mineral oil and can be 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 is also a contributing factor for exposure to benzene.

Benzene is classified as a toxic substance (hematoxic) and a potent human carcinogen. For this reason, 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 (ttMA) has been established as a suitable biomarker. ttMA is a urinary metabolic product of benzene (Figure 1).

Figure 2. Gilson GX-271 ASPEC™ System

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). The automated solid phase extraction (SPE) instrumentation is shown in Figure 2.

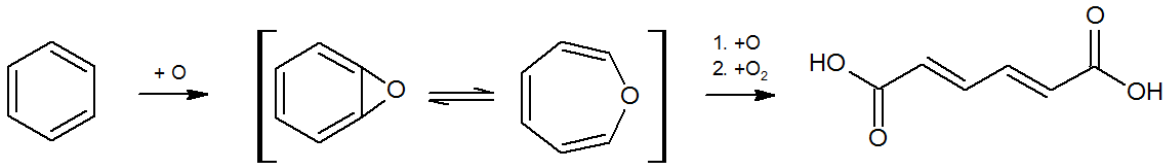

Figure 1. Metabolism of Benzene to t,t-Muconic Acid

Gilson, Inc.
Middleton, WI 53562 USA
Telephone: 800-445-7661

Materials & Methods

SPE Materials

- NanoPure Water
- ClinTox® Complete Kit for t,t-Muconic Acid in Urine (RECIPE Chemicals + Instruments GmbH, Munich Germany)
- 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

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

SPE Method

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

HPLC Equipment and Conditions

A Gilson GX-271 Liquid Handler was equipped with Dual with Tee Syringe module and GX Direct Injection Module. Gilson 152 UV-VIS detector and 306 series binary mobile phase pumping system. The analysis was run using TRILUTION LC Liquid Chromatography software.

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

Figure 3. TRILUTION® LH SPE Method

Gilson, Inc.
Middleton, WI 53562 USA
Telephone: 800-445-7661

Results and Discussion

Automation of the RECIPE ClinTox® t,t-Muconic Acid in Urine kit was performed using Gilson equipment and software. 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 ttMA to the resin. Co-adsorbed interfering substances are washed from the resin with an aqueous washing solution, followed by the ttMA 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, was automated using Gilson's GX-271 ASPEC™ system and orbital shaker on bed via TRILUTION® LH v3.0 Liquid Handling Software (Figure 3).

After preparation, a portion of the solid phase extraction eluate was injected in triplicate 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 Liquid Chromatography software on a GX-271 Liquid Handler system with 402 Dual with Tee Syringe module and GX Direct Injection Module. 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.

Results from a typical chromatogram are shown in Figure 4. The results shown in Table 1 indicate that the intra- and inter-assay performance and precision were similar to or better than results obtained with samples prepared using the manual method.

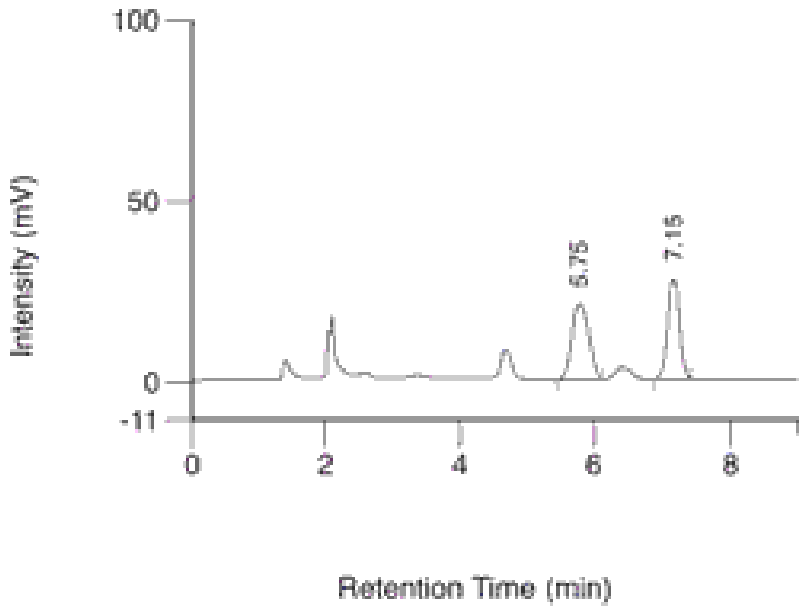

Figure 4. Example Chromatogram of the ClinChek® Level II Urine Control Sample. t,t-Muconic acid eluted from the HPLC column at 5.75 minutes. The peak at 7.15 minutes is an internal standard.

Gilson, Inc.
Middleton, WI 53562 USA
Telephone: 800-445-7661

Assay Performance Parameters*	Gilson Automation Results			Manual Results
	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%			

Table 1. Assay Performance Parameters

Acknowledgments

This application was performed in collaboration with Martin Knirsch, RECIPE, Chemicals + Instruments GmbH

Summary and Conclusions

- The RECIPE ClinTox® t,t-Muconic Acid in Urine kit was successfully automated using Gilson instrumentation. The entire sample preparation procedure, including initial dilution and transfer of the prepared sample to a final vial, was automated using Gilson's GX-271 ASPEC™ system and orbital shaker on bed via TRILUTION® LH v3.0 Liquid Handling Software.
- TRILUTION LC Liquid Chromatography software was used to control and automatically integrate the GX-271 Liquid Handler system with dual syringe pump, GX Direct Injection Module, column heater, binary mobile phase pumping system, and 152 UV-VIS detector.
- Recovery values and intra- and interassay precision were similar or better than values obtained from manual sample preparation.