Sciex

Toxicology

Application Note : 2101

Analysis of Urine Biomarker for Toluene, Xylene and Styrene Exposure:

Biomarker Screening in Urine at 8 Seconds per Sample Using LUXON-MS/MS

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Introduction

In various industrial production plants, workers can be exposed to organic compounds like toluene, xylene and styrene. The chronic exposure to these compounds has been linked to severe impacts on the central nervous system. The measurement of their major metabolites in urine would provide an important indication of the overall level of occupational exposure to these widely used compounds. Toluene, xylene and styrene are metabolized and excreted in the urine as hippuric acid (HA), methylhippuric acids (MHA) and mandelic acid (MA), respectively.

Our goal for this application note is to use an automated sample preparation method for the simultaneous quantification of creatinine, hippuric acid, methylhippuric acid and mandelic acid in urine using a single operation in LUXON-MS/MS.

LUXON-MS/MS offers specificity combined with an ultra-fast analysis for an unrivaled screening method. To develop this application, we focused on performing a quick and simple sample preparation. Metabolites are analyzed **simultaneously** with **quantitative** screening results obtained in less than 8 seconds per sample. Each metabolite has been screened based on the WHO (World Health Organization) detection range.

Luxon Ionization Source

The Luxon Ion Source® (**Figure 1**) is the second-generation sample introduction and ionization source based on the LDTD® technology for mass spectrometry. Luxon Ion Source® uses Fiber-Coupled Laser Diode (**Figure 2**) to obtain unmatchable thermal uniformity providing more precision, accuracy and speed. The process begins with dry samples which are rapidly evaporated using indirect heat. The thermally desorbed neutral molecules are carried into a corona discharge region. High efficiency protonation and strong resistance to ionic suppression characterize this type of ionization and is the result of the absence of solvent and mobile phase. This thermal desorption process yields high-intensity molecular ion signal in less than 1 second sample-to-sample and allows working with very small volumes.

Figure 1 - Luxon Ion Source®

Figure 2 - Schematic of the Luxon Ionization Source

Sample Preparation Method

Sample Collection

Urine samples were collected and transferred into barcoded tubes, readable by the Azeo extraction system.

Automated Sample Extraction

Each barcoded vial was scanned by the Azeo Liquid Handler and an automatic batch file was created. The Azeo extraction system (**Figure 3**) is used to extract the samples using the following conditions:

- 20 µL of urine sample were transferred from the vials to a deep-well plate placed in the Lumo Vortexer
- 300 µL of Internal standard were added to each sample
  - Mix
- 300 µL of Dilution buffer and 2.5 µL urine/internal standard mixtures were added into a deep-well plate
  - Mix
- Spot 2.5 µL final dilution onto a LazWell™96 plate
  - Dry 3 minutes at 40°C in the Aura LazWell Dryer

Figure 3 - Automated extraction system

LDTD®-MS/MS Parameters

LDTD

Model: Luxon S-960, Phytronix
Carrier gas: 6 L/min (air)
Laser pattern:

- 3-second ramp to 65% power
- Hold 2 seconds at 65% power

MS/MS

MS model: Q-Trap System® 5500, Sciex
Scan Time: 25 msec
Total run time: 8 seconds per sample
Ionization: APCI
Analysis Method: Negative MRM mode

Table 1 - MRM transitions for Luxon-MS/MS

	Transition	CE
Creatinine	112 → 68	-32
Creatinine-d_3	115 → 68	-32
Mandelic acid	151→ 107	-15
Hippuric acid	178 → 134	-25
Hippuric acid-d_5	183 → 139	-25
Methylhippuric acid	192 → 148	-25

Results and Discussion

Screening range (µg/mL)

The screening range for each metabolite can be found in **Table 2**.

Table 2 – Metabolites Calibration Ranges

Analyte	(µg/mL)
Creatinine	200 - 4000
Mandelic acid (MA)	100 - 2000
Hippuric acid (HA)	100 - 2000
Methylhippuric acid (MHA)	100 - 2000

Validation Test

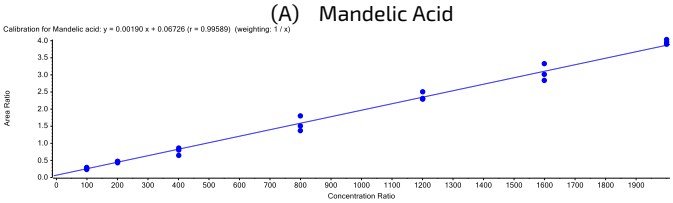
Calibration curves were prepared in a methanol:water (3:7) solution in which the calibration curve samples were diluted. Replicate extractions were deposited onto a LazWell™ plate and dried before analysis. The peak area against the internal standard (IS) ratio was used to normalize the signal.

Linearity

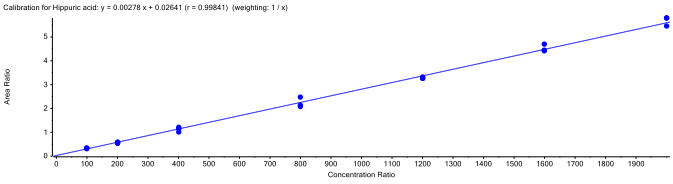
The calibration curves were plotted using the peak area ratio and the nominal concentration of standards. For the linearity test, the following acceptance criteria was used:

- Linear regression (r) must be ≥ 0.995

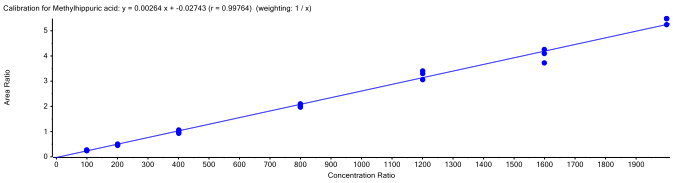
(A) Mandelic Acid



(B) Hippuric Acid



(C) Methylhippuric Acid



(D) Creatinine

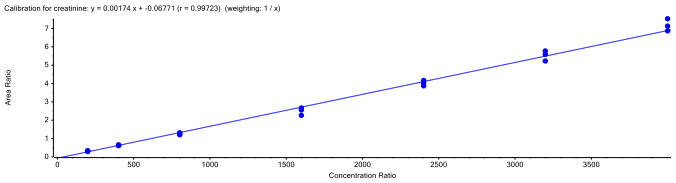


Figure 4 – Calibration curve

Error! Reference source not found. shows the calibration curve for MA (A), HA (B), MHA (C) and creatinine (D).

Precision and Accuracy

For the accuracy and precision evaluation, the following acceptance criteria were used:

- Each concentration must not exceed 15% CV
- Each concentration must be within 100 ± 15% of the nominal concentration

For the inter-run precision and accuracy experiment, each standard was analyzed in triplicate, on three different days. **Table 3** shows the inter-run precision and accuracy results for Hippuric acid. The obtained %CV was below 15% and the accuracy was within 15% of nominal value. Similar results were obtained for the other metabolites.

Table 3 - Inter-Run Precision and Accuracy of Hippuric Acid

Hippuric acid	S1	S2	S3	S4	S5	S6	S7
Conc (µg/ml)	100	200	400	800	1200	1600	2000
N	9	9	9	9	9	9	9
Mean (µg/mL)	107	190	397	792	1169	1613	2033
SD	8,2	9,4	32,8	74,8	11,1	53,9	69
%CV	7,7	4,9	8,3	9,4	1,0	3,3	3,4
%Nom	106,9	95,2	99,1	99,0	97,4	100,8	101,6

Multi-Matrix Evaluation

Urine samples were collected from six volunteers. Samples were screened to verify the endogenic value of each metabolite. To study the matrix effect, urine samples were spiked with metabolites at final concentration addition of 667 µg/mL. **Table 4** shows the screening results of all metabolites. An addition of 667 µg/mL was obtained for each matrix within ± 15% of nominal value.

Table 4 – Multi-Matrix Evaluation Results

Sample	MA Endo (µg/mL)	MA Spike (µg/mL)	HA Endo (µg/mL)	HA Spike (µg/mL)	MHA Endo (µg/mL)	MHA Spike (µg/mL)	Creatinine (µg/mL)
U1	179	942	220	962	21	776	1657
U2	137	872	75	746	75	699	2129
U3	89	802	96	755	26	599	1413
U4	116	857	418	1101	22	642	772
U5	179	928	114	802	28	622	1240
U6	38	755	152	780	23	603	647

Conclusion

The Luxon Ion Source® combined with Sciex Q-Trap 5500 mass spectrometer system allows ultra-fast (**8 seconds per sample**) analysis of the urine metabolites of Toluene, Xylene and Styrene. This method allows the quantification of urine dilution biomarker, creatinine, simultaneously.

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