



YOUNG LIN APPLICATION LETTER

Application Note No. : YL-APP-2000004

Subject : Application for Organic Solvent Metabolites, Mandelic, Hippuric, Methyl Hippuric Acid.

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Key Words : Toluene, Xylene, Styrene, PGA, MA, HA, o-,m-,p-MHA

1. Introduction

Toluene and o-,m-,p-xylene are widely used solvents as a thinner or for chemical synthesis in chemical industries. Oxidized toluene in human reacts with glycine to convert as hippuric acid(HA), which is excreted via urine. Xylene isomers is oxidized to o-,m-,p-toluic acid, reacts with glycine to convert as o-,m-,p-methylhippuric acid(MHA), which is excreted via urine. Therefore HA and MHA are used as biomarkers which can recognize how much workers have been exposed to organic solvents. Also, styrene monomer is widely used for production of synthetic resin and exposed level can be determined by the analysis of its metabolites, phenyl glyoxylic acid(PGA), and MA.

Therefore, it is very important to analyze the metabolites excreted through urine in order to determine how much workers are exposed to the organic solvents such as toluene and o-,m-,p-xylene. HPLC is mainly used for this analysis due to simple sample preparation. GC can be used but not widely used due to complex sample preparation

The metabolites were analyzed by ion suppression and ion pairing HPLC.

2. Metabolic process of major organic solvents in human.

	Metabolism	Excreted through urine
Toluene (C_7H_8)	→	Hippuric acid ($C_6H_5CONHCH_2COOH$)
Xylene ($C_6H_4CH_3$) ₂	→	Methylhippuric acid ($CH_3C_6H_5CONHCH_2COOH$)
Styrene ($C_6H_5CH=CH_2$)	→	Mandelic acid ($C_6H_5CH(OH)COOH$) Phenylglyoxylic acid ($C_6H_5COCOCH_2COOH$)

3. Analyzed metabolites

1. Creatinine,
2. Mandelic acid (MA)
3. Hippuric acid (HA)
4. o-Methylhippuric acid (o-MHA)
5. Phenylglyoxylic acid (PGA)
6. m-Methylhippuric acid (m-MHA)
7. p-Methylhippuric acid (p-MHA)

▮ Instruments used.

- Young Lin ACME HPLC
SDV30 LPG Module
M930 Solvent Delivery Pump
M720 UV/Vis Absorbance Detector
- Young Lin Autochro-Win 2.0 plus Data System



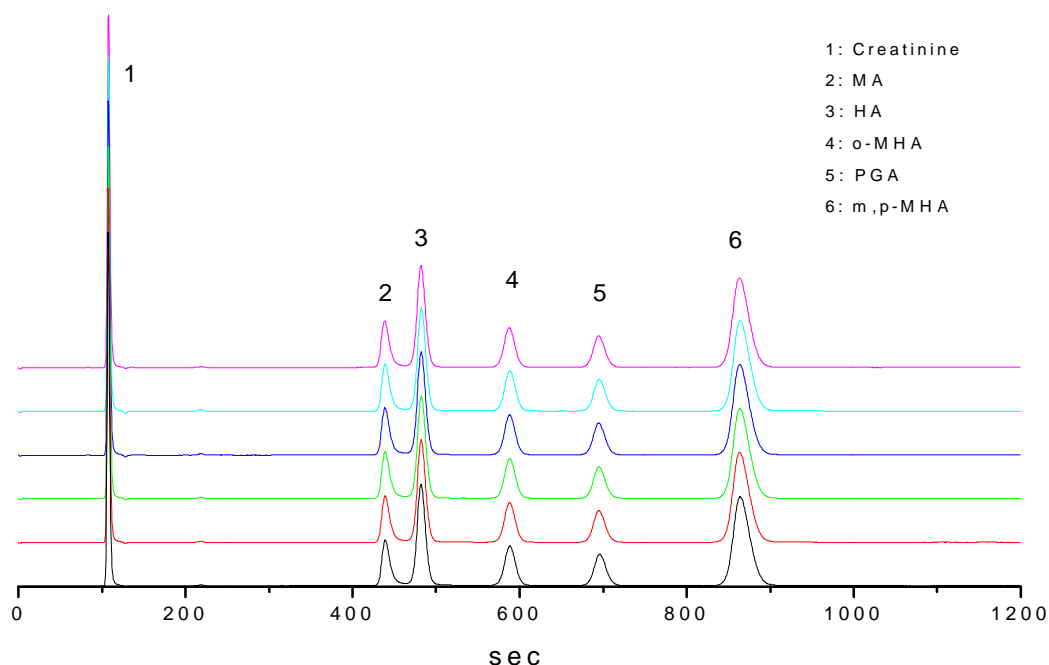
1. Ion pairing method

▮ Analytical conditions and sample preparation

- Column : Metachem Polaris C18-A, 5 μ m, 150 \times 4.6mm
- Eluent : ACN:0.02M TBABr=2:8 (pH 6.10 adjusted with 0.2M NaOH & HCl)
- Detection : UV @210nm
- Flow : 1.0 ml/min
- Injection volume : 20 μ l
- Sample preparation : Urine samples were diluted by eluent and filtered by 0.2 μ m membrane filter. Standards were prepared after dilution with eluent.

▮ Reproducibility for standard sample

Reproducibility for 50 μ g/mL (MA:100 μ g/mL) standard sample is shown on the <Figure 1> and the <Table 1>. Analysis results were overlaid for 6 time injections. Reproducibility for retention time was < 0.1% while area reproducibility was < 1.3%.



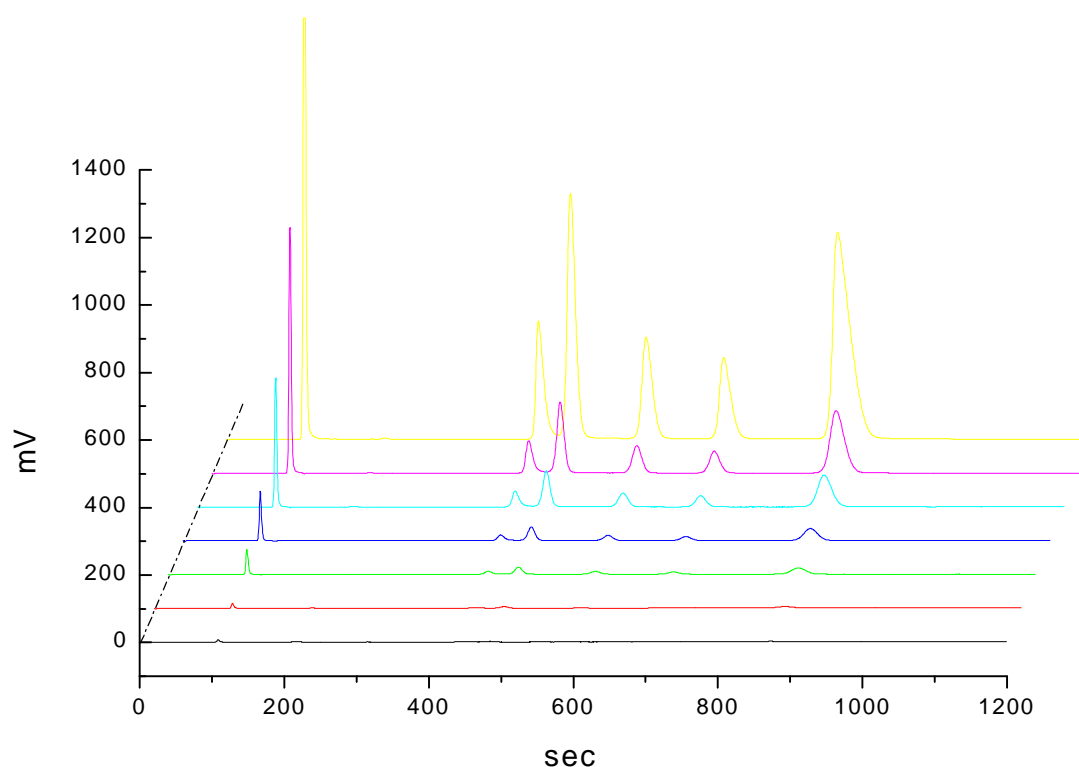
<Figure 1> Overlaid chromatograms for 6 time injections of 50 μ g/mL (MA:100 μ g/mL) standard samples.

<Table 1> Reproducibility of retention time and area for 6 time injections of 50µg/mL(MA:100µg/mL) standard samples.

	Creatinine	MA	HA	o-MHA	PGA	m,p-MHA
RT(min)	1.796	7.314	8.036	9.80533	11.59033	14.4
%R.S.D	0.24	0.064	0.058	0.042	0.056	0.035
Area	2862.692	1070.805	2476.187	1274.700	1109.948	4396.586
%R.S.D	1.21	1.13	0.55	0.43	0.42	0.46

Linearity

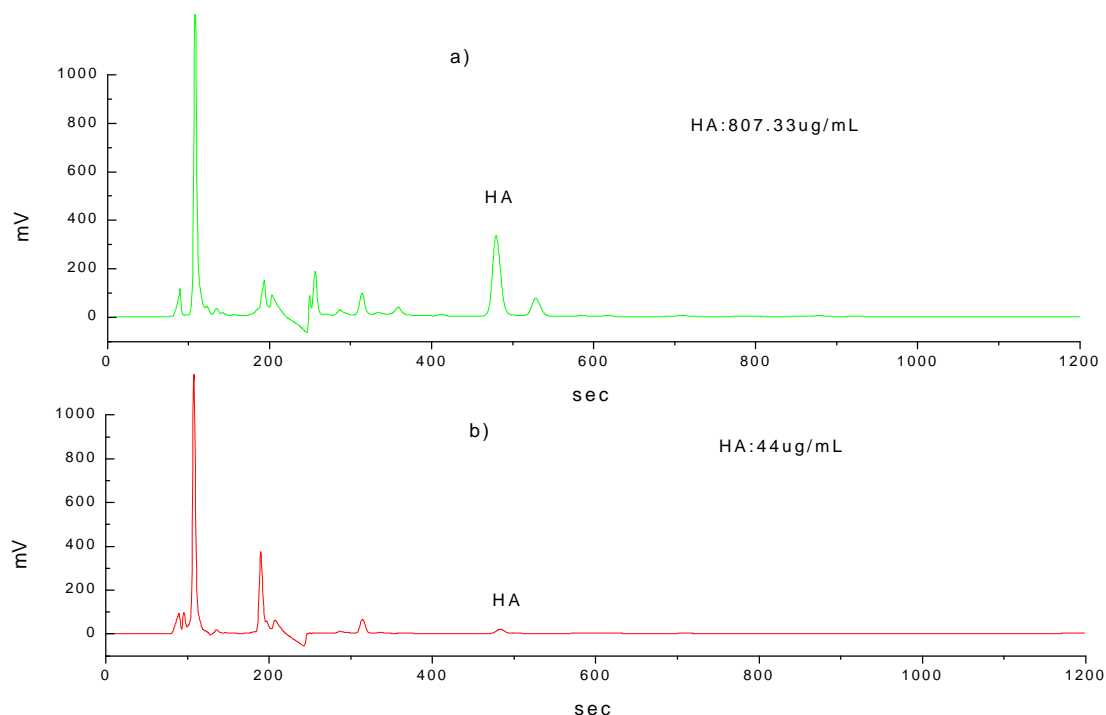
Chromatograms for standard samples from 0.5; 200µg/mL(MA:1; 400µg/mL) are overlaid. Linearity for Creatine of 1~50µg/mL, $r^2=0.999587$, MA of 2~400µg/mL, $r^2=0.999711$, HA of 1~200µg/mL, $r^2=0.999916$, o-MHA of 1~200µg/mL, $r^2=0.999986$, PGA of 1~200µg/mL, $r^2=0.999983$, m,p-MHA of 1~200µg/mL, $r^2=0.999960$ respectively



<Figure 2> Overlaid chromatograms for standard samples from 0.5; 200µg/mL(MA:1; 400µg/mL).

Urine sample analysis

Industrial urine samples were analyzed. <Figure 3> a) was extracted from a worker who had been exposed to organic solvents at industrial works while <Figure 3> b) was extracted from a worker who had been less exposed. Concentration of HA of the exposed worker is 20 times more than less exposed worker.



<Figure 3> Results for industrial urine samples. a) for exposed worker, b) less exposed worker.

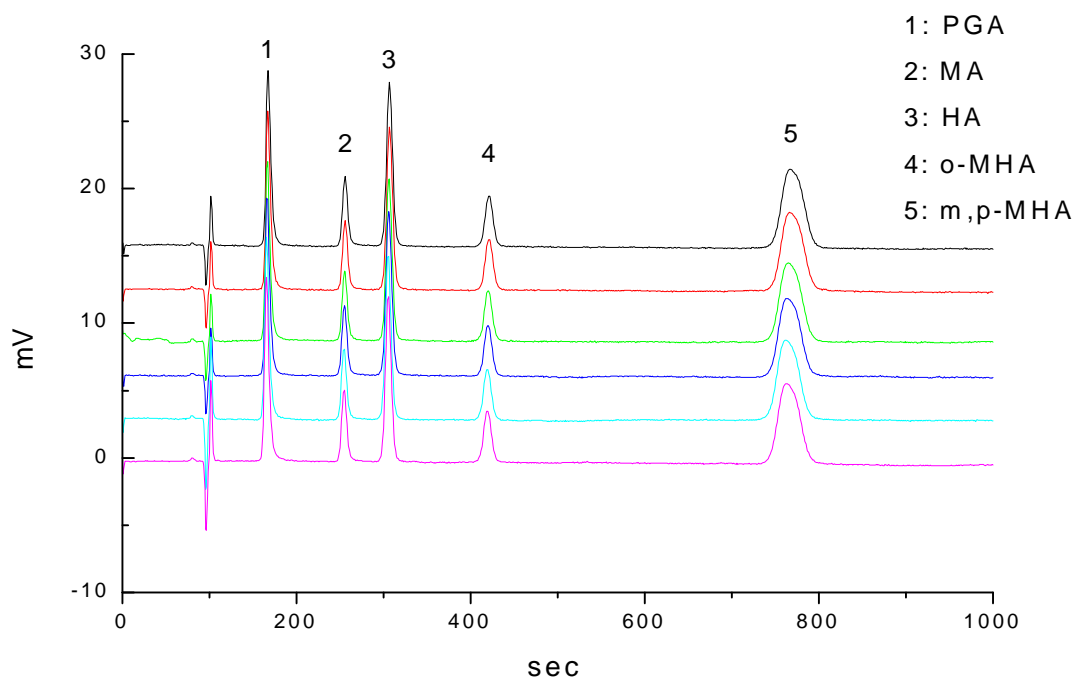
2. Ion suppression method

Analytical conditions and sample preparation

- Column : Metachem Polaris C18-A, 5 μ m, 150 \times 4.6mm
- Eluent : ACN:20mM KH₂PO₄=10:90 (pH 3.3 adjusted with Acetic acid)
- Detection : UV @225nm
- Flow : 1.4 ml/min
- Injection volume : 20 μ l
- Sample preparation : Urine samples were diluted by eluent and filtered by 0.2 μ m membrane filter. Standards were prepared after dilution with eluent.

Reproducibility and detection limit

Reproducibility for 5 µg/mL of PGA and MA and 2.5 µg/mL of HA, o-MHA and m,p-MHA standard sample is shown on the <Figure 4> and the <Table 2>. Analysis results were overlaid for 6 time injections. Reproducibility for retention time was < 0.5% while area reproducibility was < 1.3%. Also detection limit was < 0.3 µg/mL, so sub-ppm level could be analyzed.



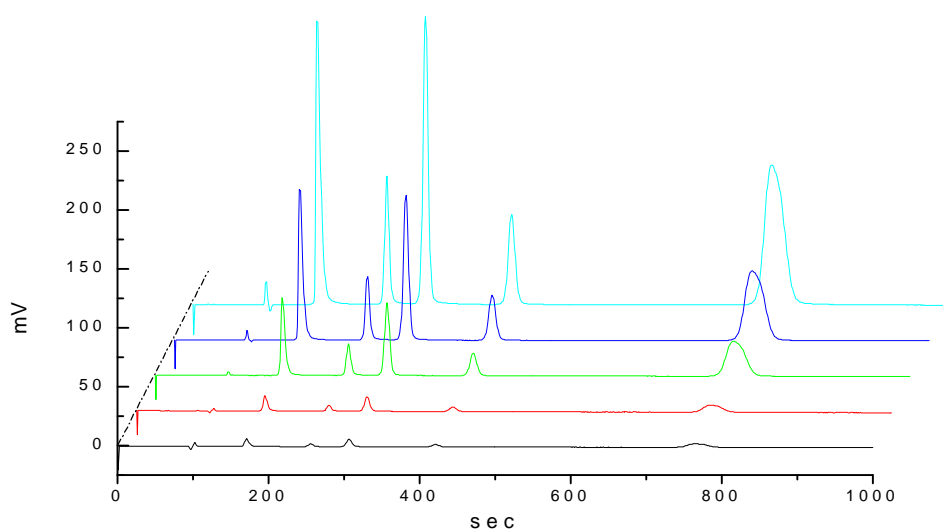
<Figure 4> Overlaid chromatograms for 6 time injections of 5 µg/mL of PGA and MA and 2.5 µg/mL of HA, o-MHA and m,p-MHA standard sample.

<Table 2> Reproducibility of retention time and area for 6 time injections of PGA and MA and 2.5 µg/mL of HA, o-MHA and m,p-MHA standard sample.

	PGA	MA	HA	o-MHA	m,p-MHA
RT(min)	2.77783	4.25567	5.1085	7.00283	12.73617
%R.S.D	0.48	0.21	0.18	0.18	0.28
Area	88.21383	38.31767	100.8368	41.21483	168.0757
%R.S.D	1.25	1.65	0.80	1.88	1.50
M.D.L (µg/mL)	0.210	0.272	0.072	0.155	0.127

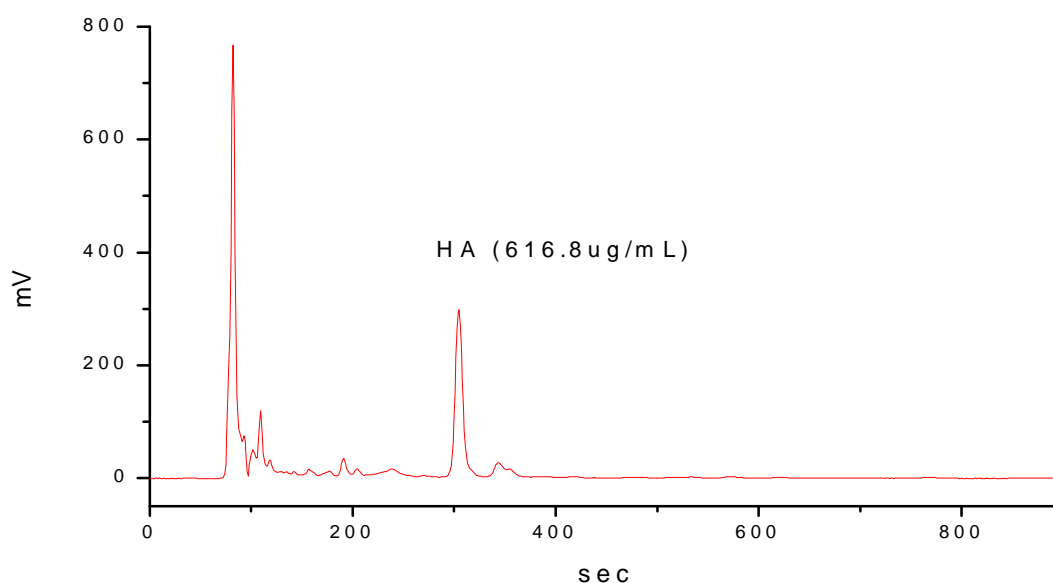
Linearit

Chromatograms for standard samples from 0.5; 200 µg/mL (MA:1; 400 µg/mL) are overlaid. Linearity for PGA of 2.5~100 µg/mL is $r^2=0.999987$, MA of 2.5~100 µg/mL, $r^2=0.999996$, HA of 1.25~50 µg/mL, $r^2=0.999979$, o-MHA of 1.25~50 µg/mL, $r^2=0.999995$, m,p-MHA of 1.25~50 µg/mL, $r^2=0.999974$, respectively



<Figure 5> Overlaid chromatograms for standard samples from 0.5; 200 µg/mL (MA:1; 400 µg/mL).

Urine sample analysis



<Figure 6> Chromatogram for urine sample extracted from the exposed worker.

; **Discussion and conclusion**

Metabolites of organic solvents were analyzed by ion pairing and ion suppression HPLC. Reproducibility for retention time and area and linearity were shown acceptably good for both ion pairing method and suppression method. Also urine sample extracted from exposed workers could be analyzed up to sub-ppm level.