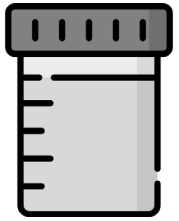
Method Development for Analysis of VOC Metabolites in Urine Using LC-MS/MS

Paramjit Behniwal, Jonathan Gallardo, Jianwen She
Environmental Health Laboratory Branch
Biochemistry Section

BIOMONITORING CALIFORNIA

Volatile Organic Compounds (VOCs)

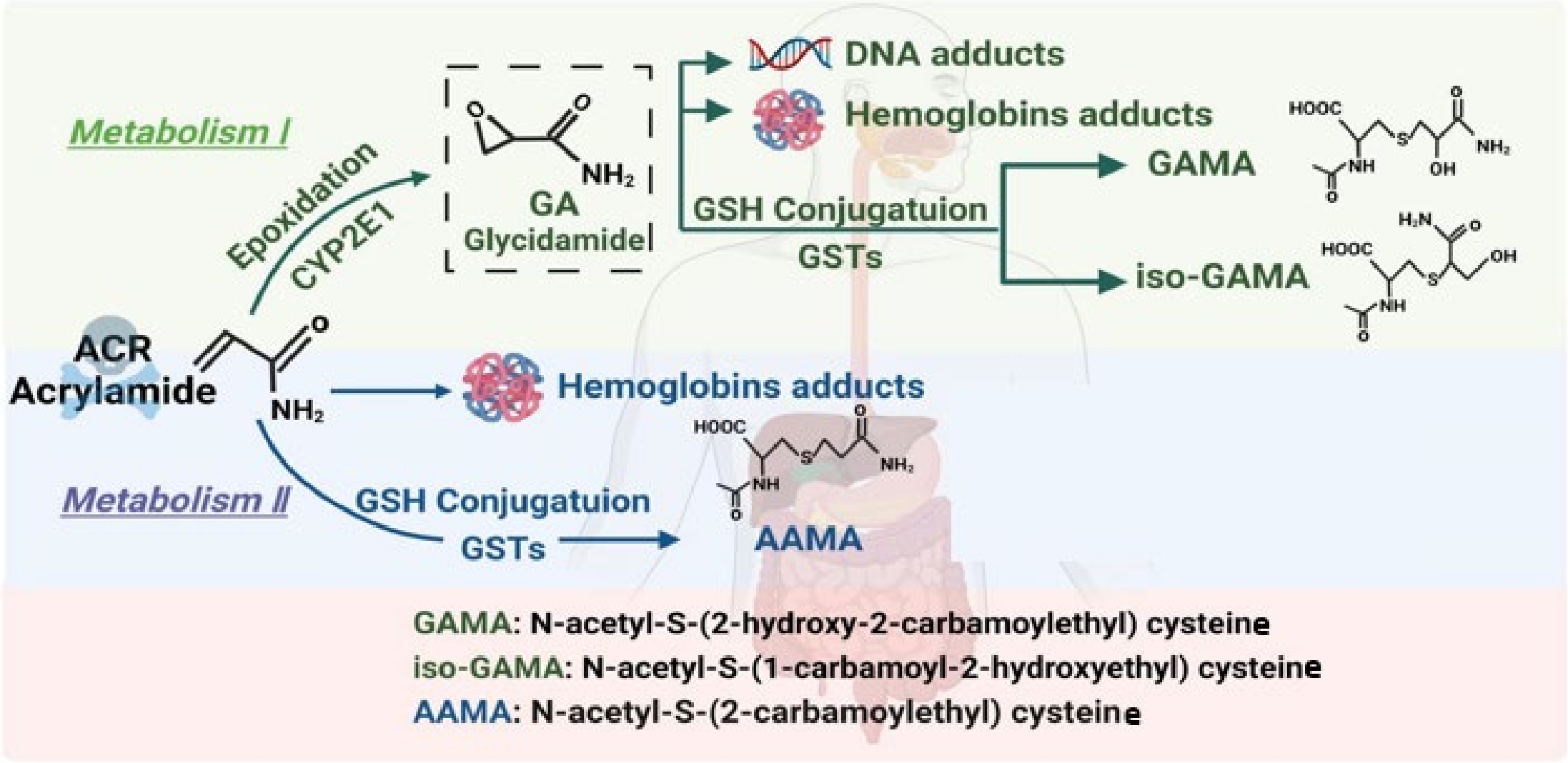
- VOCs are chemicals that can vaporize at room temperature (20◦C) and 1 *atm* of pressure
- They are common air pollutants indoors and outdoors
- Sources of VOCs:
  - Naturally found in the environment
  - Released from manmade sources such as paints, cleaners, cigarette smoke, car exhaust, wood burning and industrial processes such as oil and gas production
- Health effects of VOC exposure:
  - Breathing VOCs can irritate the eyes, nose and throat, can cause difficulty breathing and nausea, and can damage the central nervous system and other organs
  - Some VOCs can cause cancer after long time exposure



VOC Exposure in Humans

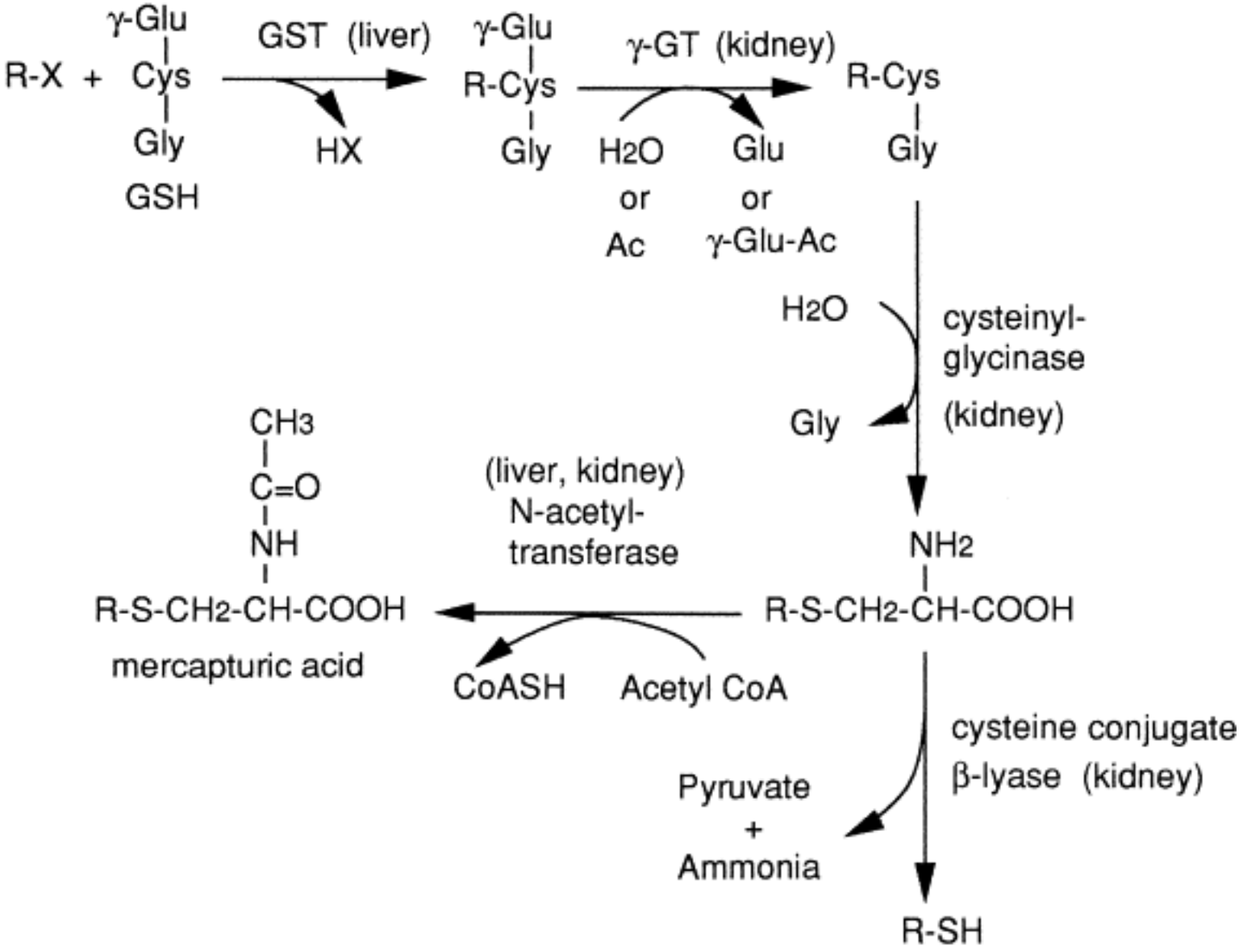
- Once VOCs are absorbed, they are metabolized in the body and excreted in urine, blood, breath, milk, etc.
- VOC urine metabolites are:
  - Used as biomarkers since they are stable and reflect recent exposure to VOCs
  - Excreted mostly as mercapturic acid metabolites and have longer half-life than VOC biomarkers in blood
  - Of interest to Biomonitoring California to assess exposure to VOCs in disproportionately affected communities
  - Have been included as biomarkers of exposure in previous and current Biomonitoring California projects:
    - East Bay Deisel Exposure Project (EBDEP), Stockton Air Pollution Exposure Project (SAPEP), FRESSCA, BiomSPHERE, Intraprogram Pilot Project (IPP7), and California Fire Fighters (CFF)

VOC Metabolism



Zhao, M., Zhang, B., & Deng, L. (2022). The mechanism of acrylamide-induced neurotoxicity: current status and future perspectives. *Frontiers in Nutrition*, 9, 859189

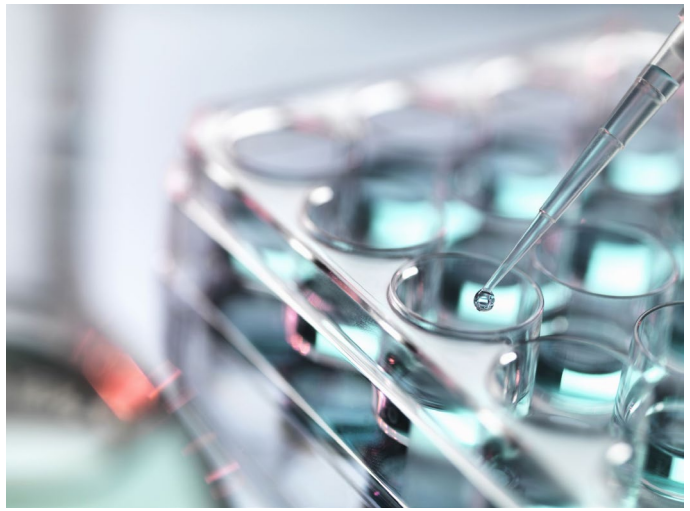
Detoxification of VOCs by glutathione-S-transferase system



VOC Parent and Metabolite Compounds

Parent VOC	VOC Metabolite	Analyte Code
Acrolein	N-Acetyl-S-(2-carboxyethyl)-L-cysteine	CEMA
	N-Acetyl-S-(3-hydroxypropyl)-L-cysteine	HPMA
Acrylamide	N-acetyl-S-(2-carbamoylethyl)-L-cysteine	AAMA
	N-Acetyl-S-(2-hydroxy-3-propionamide)-L-cysteine	GAMA
Acrylonitrile	N-Acetyl-S-(2-cyanoethyl)-L-cysteine	CYMA
	N-Acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine	CYHA
Acrylonitrile, vinyl chloride, ethylene oxide	N-Acetyl-S-(2-hydroxyethyl)-L-cysteine	HEMA
Benzene	N-Acetyl-S-(phenyl)-L-cysteine	PMA
1-bromopropane	N-acetyl-S-(n-propyl)-L-cysteine	BPMA
1,3-butadiene	N-Acetyl-S-(3,4-dihydroxybutyl)-L-cysteine	DHBM
	N-Acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine	MHB3
Carbon disulfide	2-thioxothiazolidine-4-carboxylic acid	TTCA
N, N-Dimethylformamide	N-Acetyl-S-(N-methylcarbamoyl)-L-cysteine	AMCA
Ethylbenzene, styrene	Phenylglyoxylic acid	PHGA
Isoprene	N-Acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine	IPM3
Propylene oxide	N-Acetyl-S-(2-hydroxypropyl)-L-cysteine	HPM2
Styrene	N-Acetyl-S-(1-phenyl-2-hydroxyethyl)-L-cysteine + N-Acetyl-S-(2-phenyl-2-hydroxyethyl)-L-cysteine	PHEM
	Mandelic acid	MADA
Tetrachloroethylene	N-Acetyl-S-(trichlorovinyl)-L-cysteine	TCVM
Toluene	N-Acetyl-S-(benzyl)-L-cysteine	BMA
Trichloroethylene	N-Acetyl-S-(1,2-dichlorovinyl)-L-cysteine	1DCV
	N-acetyl-S-(2,2-dichlorovinyl)-L-cysteine	2DCV
Xylene	2-methylhippuric acid	2MHA
	3-methylhippurric acid + 4-methylhippurric acid	3MHA + 4MHA

VOC Method Development



Standard preparation and instrument optimization

- Each standard was individually sourced and weighed to prepare concentrated solutions
- Mass spectrometer (MS) conditions were optimized for each compound
- Mixed intermediate standards were prepared at different concentration ranges per analyte
- Liquid chromatography (LC) method was optimized for all analyte peaks

Quality Control (QC) material preparation

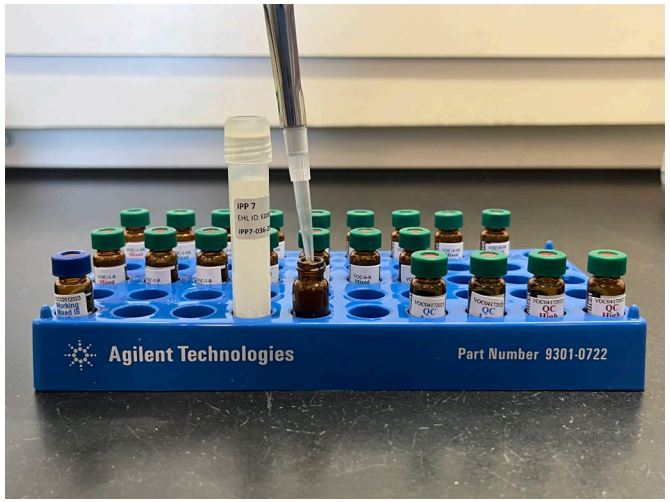
- QC material were prepared in-house by spiking all analytes into synthetic urine
- Two levels of QCs prepared (QC low & QC high)
- N=20 experimental measurements of QC samples taken over 2-4 weeks prior to sample analyses

Validation and sample analysis

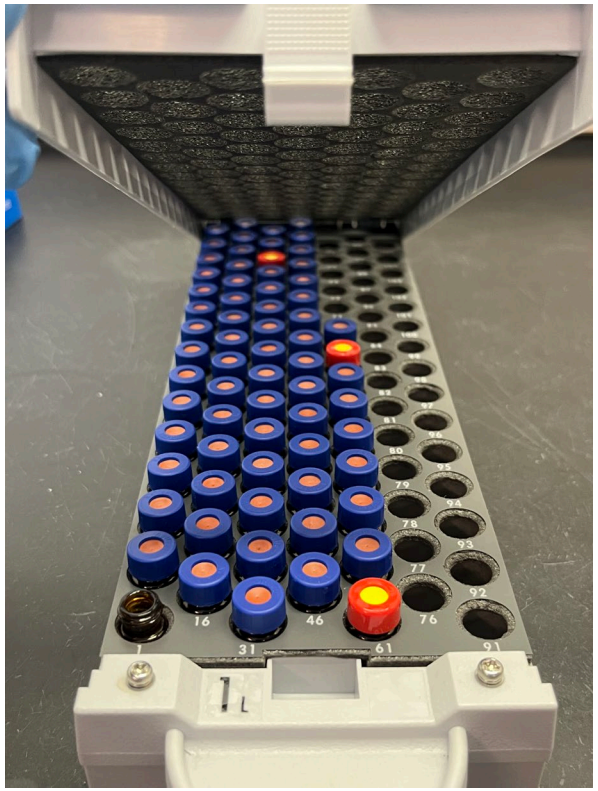
- CDC reference samples and quality control materials were acquired and measured to validate method
- Intraprogram Pilot Project 7 (n=39) and California Fire Fighters (CFF) samples analyzed (n=66) & results are under review

VOC Method: Simplified Workflow

Step 1. Dilute samples & standards 1:10 in mobile phase

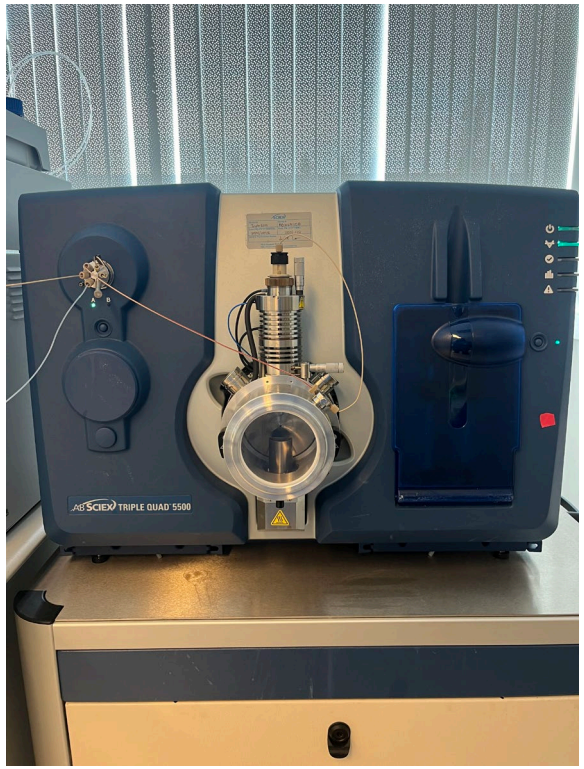


Step 2. Place diluted samples & standards in LC well-plate; program and run



Step 3. Separate analytes through liquid chromatography

Step 4. Collect MRM data to measure analyte in samples



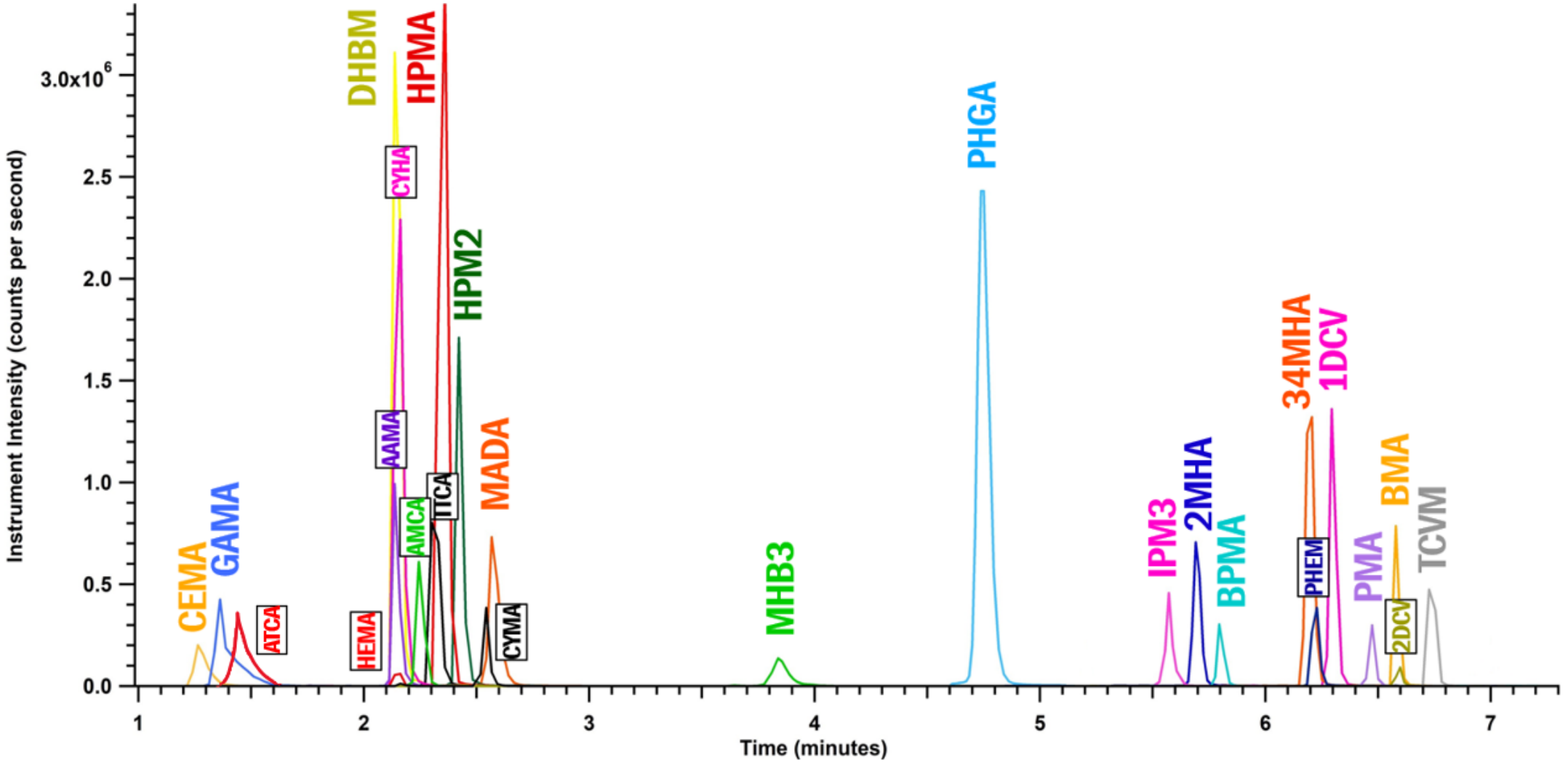
Chromatography conditions for Shimadzu Nexera LC

Parameter	Description		
Column	Acquity UPLC HSS T3		
	1.8 µm × 2.1 mm × 150 mm		
Mobile phase	15mM ammonium acetate (Solvent A)		
	Acetonitrile (Solvent B)		
LC and Needle Wash	LCMS grade water (*weak wash)*		
	25% LCMS grade water		
	25% LCMS grade methanol		
	25% LCMS grade acetonitrile		
	25% LCMS grade isopropanol		
Gradient	Time	Flow Rate	Solvent A:Solvent B
	0 min	250 µL/min	97%:3%
	2 min	250 µL/min	95%:5%
	3 min	300 µL/min	90%:10%
	5 min	300 µL/min	70%:30%
	6.5min	300 µL/min	60%:40%
	7 min	300 µL/min	85%:15%
	7.5 min	300 µL/min	90%:10%
	8 min	300 µL/min	97%:3%
	9 min	300 µL/min	97%:3%

MRM transitions for analytes and internal standards

Parent VOC	Analyte	RT (min)	Quantification Ion Transition	Confirmation Ion Transition	Internal Standard	Quantification Ion Transition
Acrolein	HPMA	2.37	220/91	220/89	HPMA-$^{2}H_6$	226/97
	CEMA	1.25	234//162	234/105	CEMA-$^{13}C_3$	237/162
Acrylamide	GAMA	1.38	249/120	249/128	GAMA-$^{2}H_3$	252/120
	AAMA	2.14	223/104	233/58	AAMA-$^{2}H_4$	237/108
Acrylonitrile	CYHA	2.15	231/84	234/102	DHBM-$^{2}H_7$	257/128
	CYMA	2.6	215/86	215/162	CYMA-$^{2}H_3$	218/165
Acrylonitrile, vinyl chloride,ethylene oxide	HEMA	2.14	206/77	207/75	HEMA-$^{2}H_4$	210/81
Benzene	PMA	6.44	238/109	239/110	PMA-$^{2}H_5$	243/114
1-Bromopropane	BPMA	5.78	204/84	204/75	BPMA-$^{2}H_7$	211/82
1,3-Butadiene	DHBM	2.14	250/121	250/75	DHBM-$^{2}H_7$	257/128
	MHB3	4.07	232/103	233/103	MHB3-$^{2}H_3$	235/103
Carbon Disulfide	TTCA	2.31	162/58	162/33	TTCA-$^{13}C_3$	165/58
N,N-Dimethylformamide	AMCA	2.25	219/162	219/84	AMCA-$^{2}H_3$	222/165
Ethylbenzene, Styrene	PHGA	4.78	149/77	149/105	PHGA-$^{13}C_8$	157/83
Isoprene	IPM3	5.56	246/117	246/87	IPM3-$^{2}H_3$	249/87
Propylene oxide	HPM2	2.47	220/91	221/91	HPM2-$^{2}H_3$	223/91
Styrene	PHEM	6.19	282/153	282/123	PHEM-$^{13}C_6$	288/159
	MADA	2.61	151/107	151/77	MADA-$^{13}C_8$	159/114
Tetrachloroethylene	TCVM	6.72	290/161	290/35	TCVM-$^{13}C_3$	297/165
Toluene	BMA	6.57	252/123	253/124	BMA-$^{2}H_5$	257/128
Trichloroethylene	1DCV	6.28	256/127	258/129	1DCV-^{13}C-$^{2}H_3$	260/127
	2DCV	6.58	257/127	256/127	2DCV-^{13}C-$^{2}H_3$	261/127
Xylene	2MHA	5.68	192/148	192/91	2MHA-$^{2}H_7$	199/155
	3MHA+4MHA	6.17	192/148	192/91	3MHA-$^{2}H_7$ + 4MHA-$^{2}H_7$	199/155

An extracted ion chromatogram of a calibration standard spiked with VOC metabolites



Quality control:

Coefficient of Variance and Spike Recovery

	QC Low				QC High		
Parent VOC	Analyte	Spiked Conc. (ppb)	CV (%)	Average Percent Recovery (%)	Spiked Conc. (ppb)	CV (%)	Average Percent Recovery (%)
Acrolein	HPMA	80	14	114	800	17	125
	CEMA	100	17	90	1000	15	87
Acrylamide	GAMA	50	11	137	500	12	131
	AAMA	12	11	95	120	14	111
Acrylonitrile	CYHA	10	14	95	100	17	142
	CYMA	10	10	110	100	8	108
Acrylonitrile, vinyl chloride,ethylene oxide	HEMA	5	16	105	50	15	112
Benzene	PMA	3.5	11	98	35	14	104
1-Bromopropane	BPMA	10	10	93	100	16	103
1,3-Butadiene	DHBM	50	16	104	500	14	106
	MHB3	10	7	103	100	15	108
Carbon Disulfide	TTCA	50	14	93	500	15	105
N,N-Dimethylformamide	AMCA	40	14	98	400	10	96
Ethylbenzene, Styrene	PHGA	40	7	104	400	10	105
Isoprene	IPM3	5	8	105	50	8	112
Propylene oxide	HPM2	20	17	94	200	17	93
Styrene	PHEM	5	8	99	50	12	102
	MADA	99	8	99	898	8	99
Tetrachloroethylene	TCVM	15	8	100	150	10	104
Toluene	BMA	4	9	109	40	11	105
Trichloroethylene	1DCV	10	10	110	100	8	108
	2DCV	10	10	110	100	8	108
Xylene	2MHA	30	9	107	300	10	108
	3MHA+4MHA	60	9	101	600	6	98

Quality Assessment (QA):

- Reference samples received from the CDC to validate method
- Analyzed 4 QA samples with different concentrations
- 22 analytes out of 24 met results acceptance criteria (except AMCA and DHBM)

Conclusions

- An analytical method was developed for the measurement of VOC metabolites in urine
- The method shows good precision and accuracy: evident through characterization of in-house QC pools and CDC quality assessment samples
- Urine samples were analyzed for the Intraprogram Pilot Project 7 (n=39) and Camp Fire Firefighter Study (n=66). Results are currently under review

THANK YOU!