Analysis of 18 Urinary Mercapturic Acids by two Multiplex-LC-MS/MS Methods

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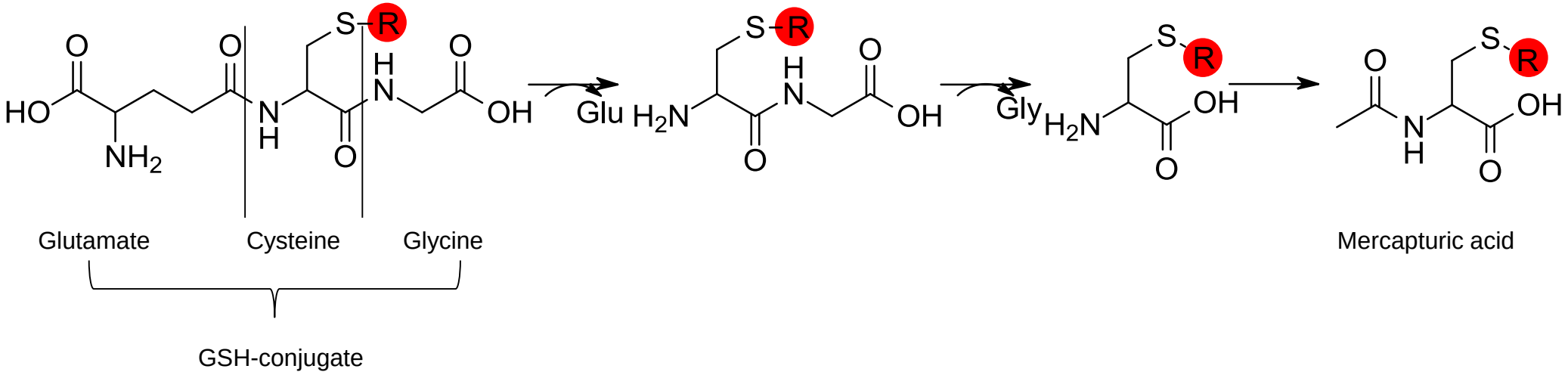
Mercapturic acid pathway

- Mercapturic acids (MAs) formed from glutathione (GSH)-S-conjugates with toxicants in several (enzymatically catalyzed) steps
- MAs: detoxification products ⇨ Biomarkers of exposure to several toxic compounds (reactive intermediates)



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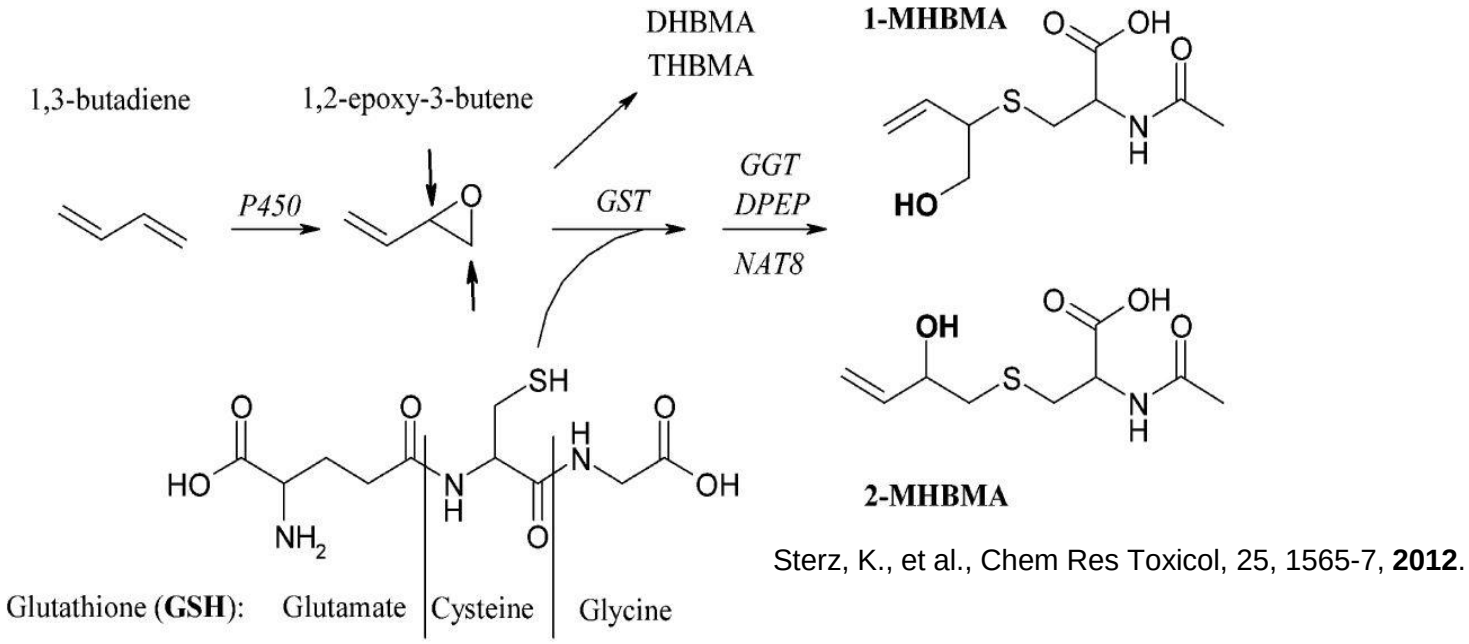

Sterz, K., et al., Chem Res Toxicol, 25, 1565-7, **2012**.

18 Mercapturic acids as Biomarkers of Exposure to Harmful Constituents in Tobacco Smoke (FDA List)

Parent Compound	N-Acetyl-S-[...]-cysteine	Abbrev.	Toxicity
Acrylamide	-2-carbamoylethyl-	AAMA	Carcinogen (CA)
	-2-carbamoyl-2-hydroxyethyl-	GAMA	
Acrylonitrile	-2-cyanoethyl-	CEMA	CA, respiratory tox. (RT)
Acrylonitrile / Ethylene oxide / Vinyl chloride	-2-hydroxyethyl-	HEMA	CA, RT
Acrolein	-3-hydroxypropyl-	3-HPMA	RT, cardiovascular tox. (CT)
Benzene	-(S)-phenyl-	SPMA	CA, CT
1,3-Butadiene	-3,4-dihydroxybutyl-	DHBMA	CA, RT
	-1-hydroxymethyl-2-propenyl- / 2-hydroxyl-3-butenyl-	MHBMA 1/2	
Crotonaldehyde	-3-hydroxypropyl-1-methyl-	HMPMA	CA
	-3-carboxy-1-methyl-ethyl-	CMEMA	
N,N-dimethylformamide	-methylcarbamoyl-	AMCC	Liver toxic, teratogenic[1]
Propylene oxide	-2-hydroxypropyl-	2-HPMA	CA, RT
Styrene	-1/2-phenyl-2-hydroxyethyl-	PHEMA 1/2	CA
Toluene	-(S)-benzyl-	SBMA	RT
Alkylating agents	-methyl- / -ethyl-	MMA / EMA	CA

1 Rodgman, A., et al., The chemical components of tobacco and tobacco smoke. CRC, Boca Raton, **2008**

Column-switching LC-MS/MS: Multi-method I

(CEMA, HEMA, SPMA, SBMA, HMPMA, CMEMA, PHEMA, MMA, EMA)

Sample preparation

1. Add internal standards (D_x-authentic stds; $^{13}C_6$-PHEMA) to 500µL urine and acidify with HCl
2. After 5 min incubation, add ammonium formate / NaOH
3. Centrifugation

LC-MS/MS

- LC: Agilent1200; MS/MS: API5000; ESI^-, scheduled MRM mode; Run time: 18 min
- Luna C8, 150x4.6mm, 3µ
- RAM: LiChrospher ADS RP-8, 25µ, exclusion: 15 kDa

Method characteristics

Analyte	CEMA	HEMA	SPMA	SBMA	HMPMA	CMEMA	PHEMA1	PHEMA2	MMA	EMA
LOD [ng/mL]	0.08	0.06	0.005	0.027	0.49	1.91	0.03	0.13	0.88	0.008
Range [ng/mL]	0.25-1250	0.20-100	0.02-100	0.10-500	5.0-2500	5.0-2500	0.10-50	0.40-200	2.5-1250	0.03-150

Column-switching LC-MS/MS: Multi-method I

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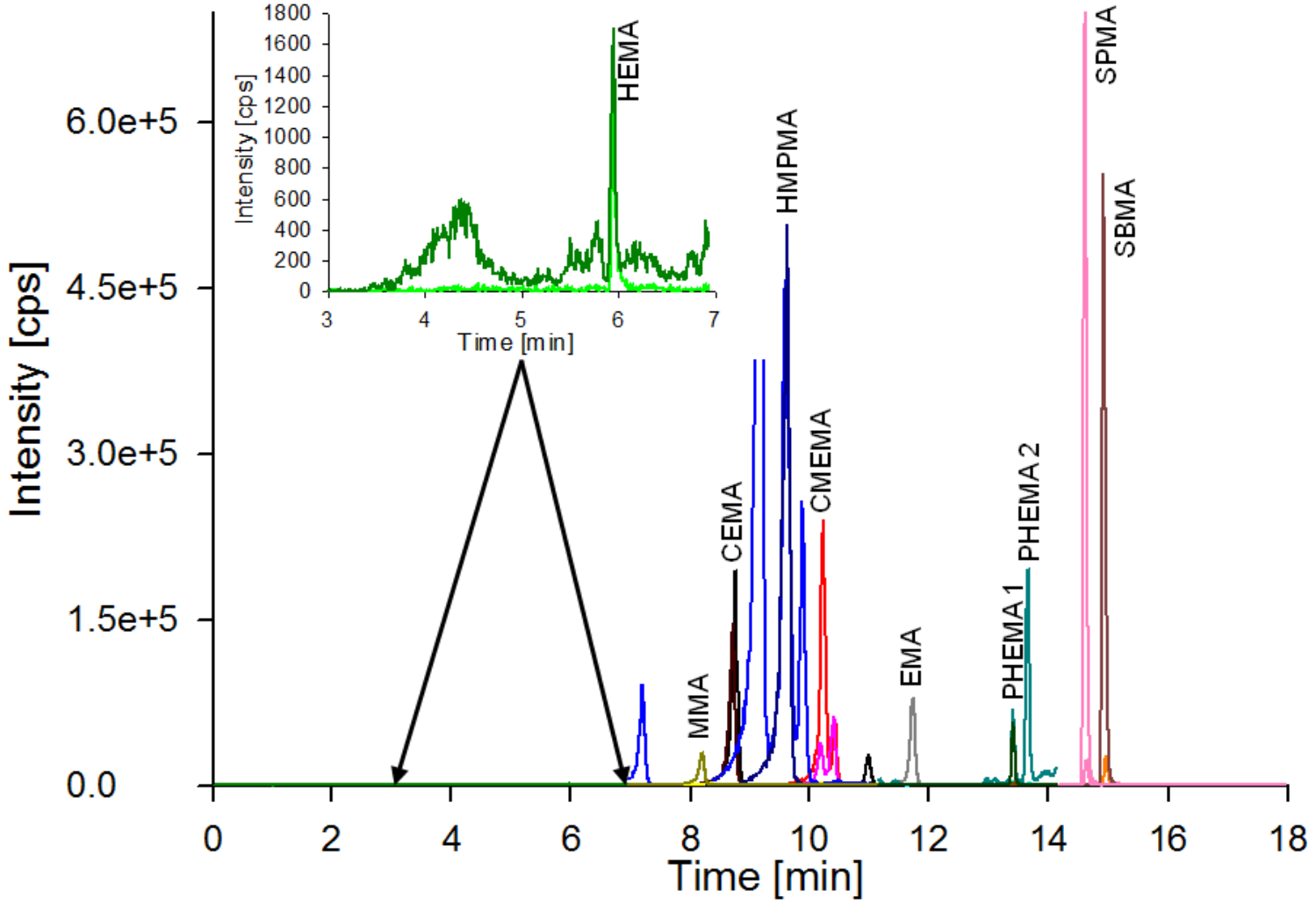
XIC of representative smoker´s urine



LC-MS/MS: Multi-method II

(AAMA, GAMA, 2-/3-HPMA, DHBMA, MHBMA, AMCC)

Sample preparation

1. Add internal standards (D_x-authentic stds; $^{15}N^{13}C_3$-3-HPMA) to 100µL urine and evaporate
2. Resuspension in 100µL methanol; 20 min vortex
3. Centrifugation

LC-MS/MS

- LC: Agilent1200; MS/MS: API5000; ESI^-, scheduled MRM mode; Run time: 21 min
- Acquity UPLC BEH HILIC, 150x3mm, 1.7µ

Method characteristics

Analyte	AAMA	GAMA	2-HPMA	3-HPMA	DHBMA	MHBMA1	MHBMA2	AMCC
LOD [ng/mL]	8.7	0.36	1.29	12.6	4.63	0.09	0.029	0.93
Range [ng/mL]	10-2000	1.0-200	2.5-2000	25.0-10000	12.5-2000	0.12-9.66	0.13-10.3	2.5-2000

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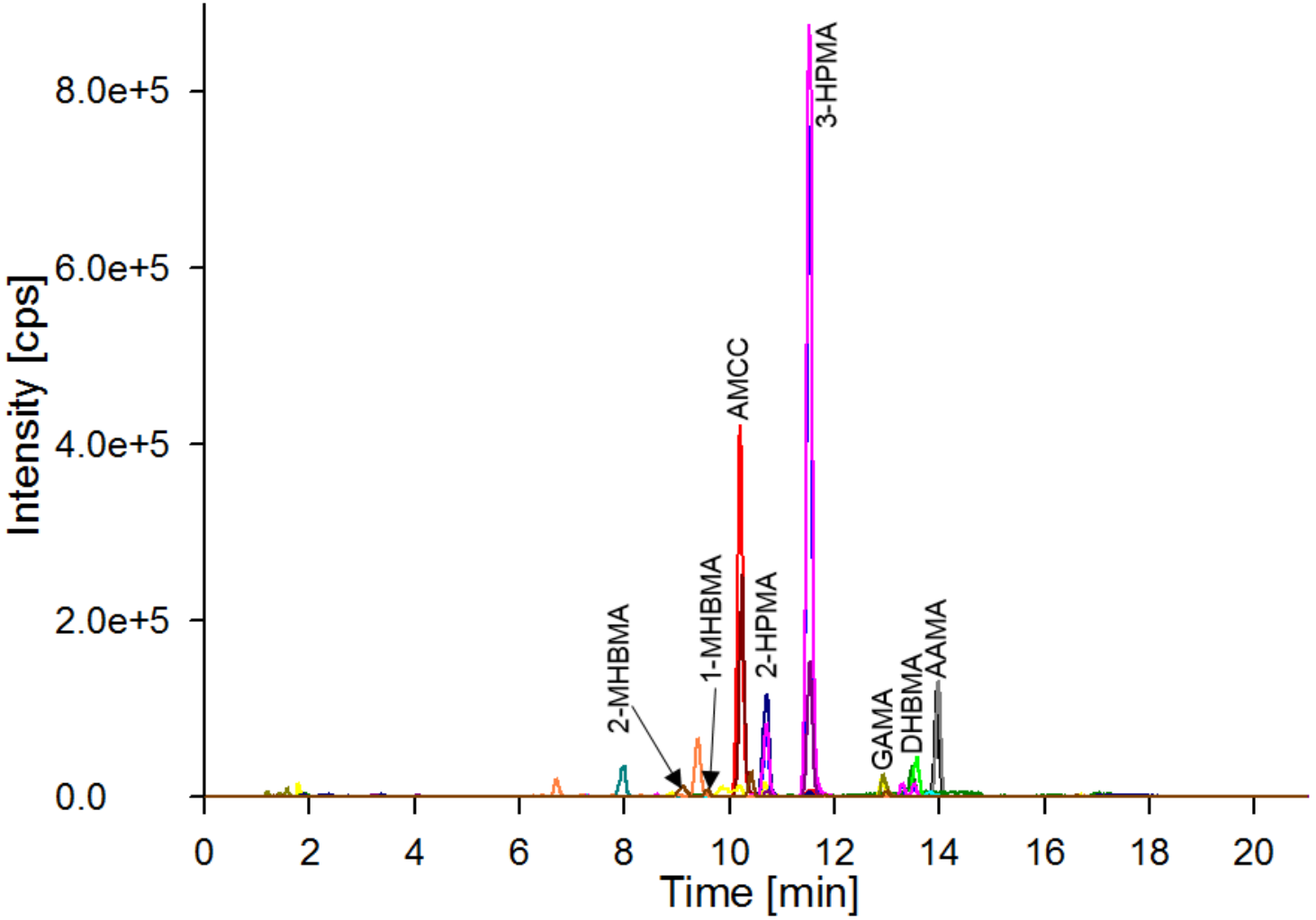
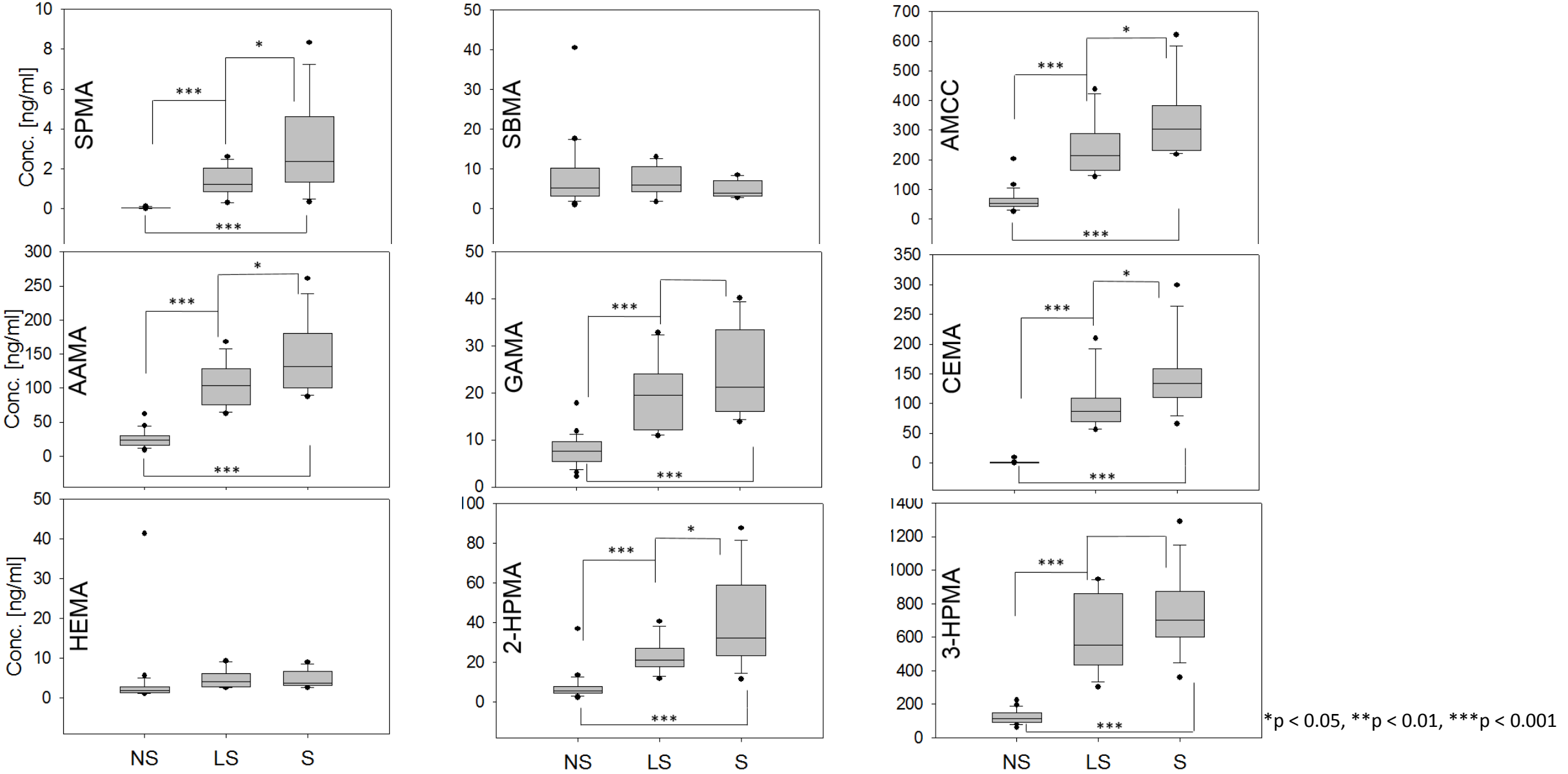


Method application to urine of smokers and non-smokers



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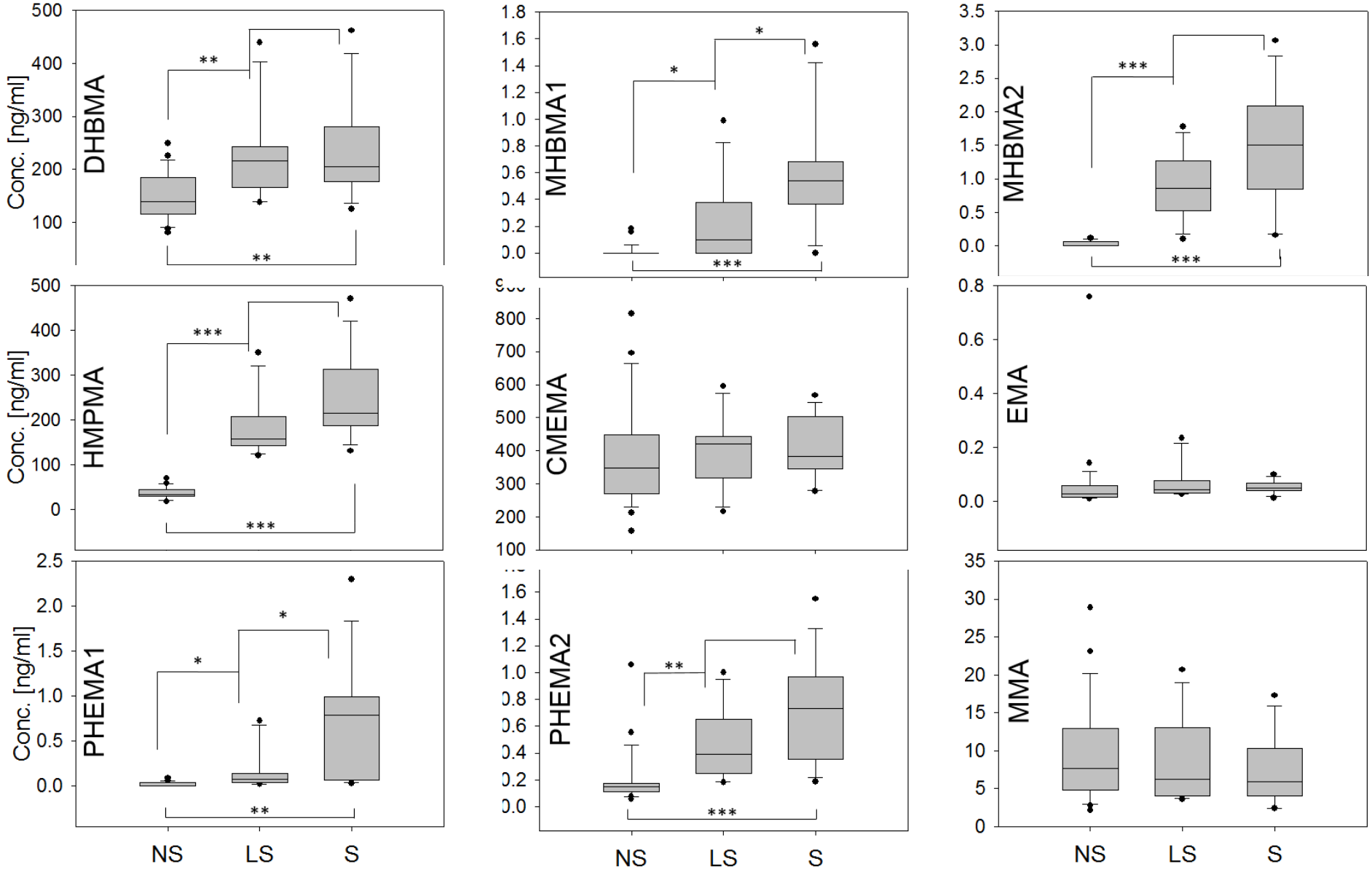

*p < 0.05, **p < 0.01, ***p < 0.001

Suitable biomarkers of exposure to tobacco smoke

Biomarker (Mercapturic acid)	Mean ± SD [ng/mL] Smoker (N=13)	Light Smoker (N=12)	Nonsmoker (N=25)	Exposure to
CEMA	144 ± 58***	99 ± 44***	1.2 ± 1.7	Acrylonitrile
HEMA	4.8 ± 2.1	4.9 ± 2.3	3.8 ± 7.9	Acrylonitrile; Ethylene oxide, Vinyl chloride
SPMA	3.1 ± 2.3***	1.3 ± 0.7***	0.04 ± 0.03	Benzene
HMPMA	247 ± 92***	182 ± 65***	37 ± 13	Crotonaldehyde
CMEMA	409 ± 93	398 ± 106	394 ± 162	Crotonaldehyde
PHEMA 1	0.67 ± 0.65**	0.17 ± 0.23*	0.02 ± 0.03	Styrene
PHEMA 2	0.70 ± 0.38***	0.46 ± 0.27**	0.20 ± 0.20	Styrene
SBMA	4.9 ± 2.1	7.0 ± 3.8	7.9 ± 8.2	Toluene
MMA	7.3 ± 4.5	8.5 ± 5.5	9.6 ± 6.6	Methylating agents
EMA	0.05 ± 0.02	0.07 ± 0.07	0.07 ± 0.15	Ethylating agents
AAMA	142 ± 52***	106 ± 31***	25 ± 12	Acrylamide
GAMA	25 ± 9***	19 ± 8***	7.7 ± 3.2	Acrylamide
2-HPMA	41 ± 23***	23 ± 8***	7.6 ± 6.7	Propylene oxide
3-HPMA	758 ± 226***	613 ± 218***	123 ± 41	Acrolein
DHBMA	233 ± 92**	225 ± 241**	148 ± 46	1,3-Butadiene
MHBMA 1	0.59 ± 0.41***	0.21 ± 0.30*	0.01 ± 0.05	1,3-Butadiene
MHBMA 2	1.5 ± 0.9***	0.93 ± 0.49***	0.03 ± 0.04	1,3-Butadiene
AMCC	331 ± 123***	241 ± 93***	64 ± 37	N,N-dimethylformamide

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Summary / Conclusion

- Aim: “Building on experience to shape the future!”
  Multiplexing assays for mercapturic acids relevant as biomarkers of exposure to tobacco smoke starting from various assays at ABF

- Successful development of two highly sensitive, robust LC-MS/MS assays for 18 mercapturic acids
  - ✓ validated according to FDA guidelines
  - ✓ large linear range
  - ✓ LODs in low ng/mL or even pg/mL range
  - ✓ simple, straight-forward sample clean up

  ➡ High sensitivity + high throughput

- Application to urine samples from smokers and nonsmokers
  - ✓ Significant differences in numerous MAs S vs. NS
  - ✓ Dose-dependence

Conclusion

Multiplex LC-MS/MS assays as powerful tools for the fast and sensitive analysis of urinary MAs as BoE to 9 relevant toxicants in tobacco smoke: benzene, styrene, N,N-dimethylformamide, acrylonitrile, acrylamide, acrolein, 1,3-butadiene, crotonaldehyde, propylene oxide

Pluym, N., et al., in preparation, **2014**

Acknowledgment

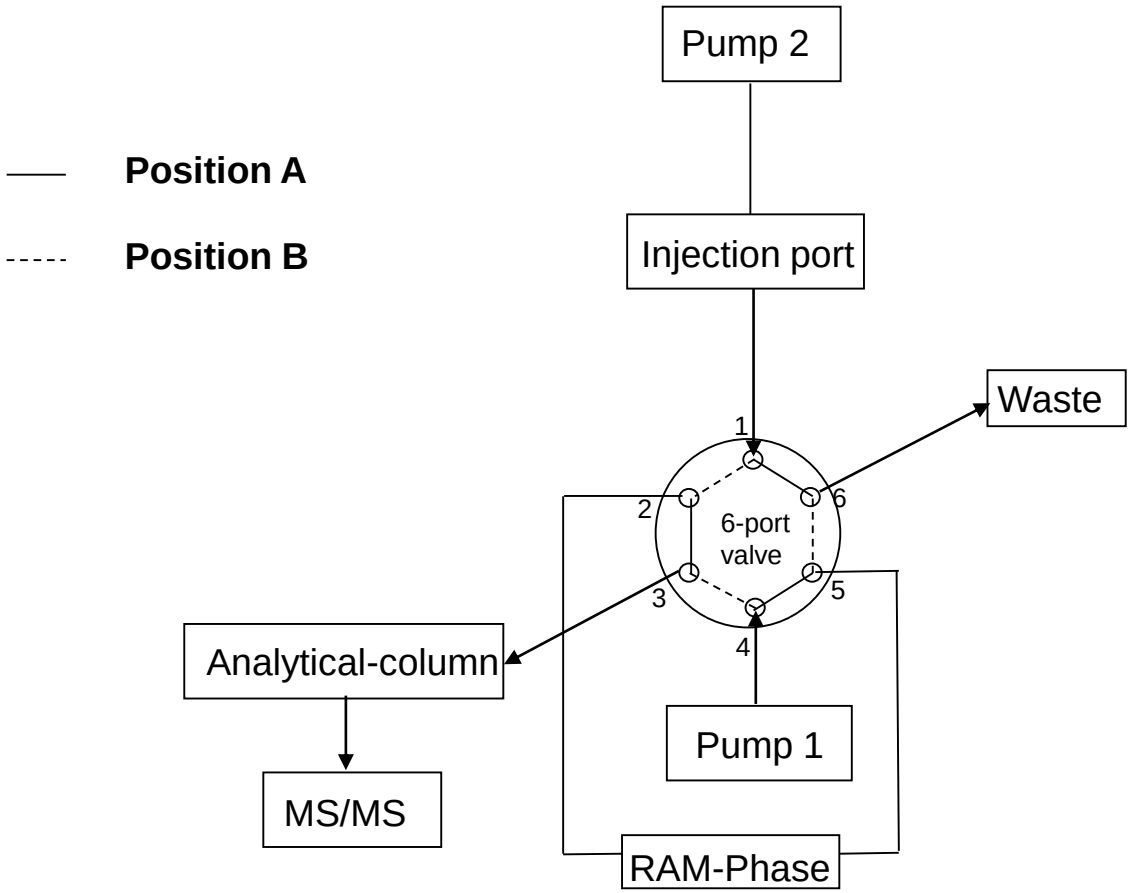
ABF

- Gerhard Gilch
- Katharina Sterz
- Dr. Max Scherer
- Prof. Gerhard Scherer

Thank you for your attention

Visit **www.abf-lab.com**

Backup


Position A
Position B
Pump 2
Injection port
Waste
1
2
3
4
5
6
6-port
valve
Analytical-column
MS/MS
Pump 1
RAM-Phase