Determination of Heterocyclic Aromatic Amines in Cigarette Smoke by UPLC-MS/MS

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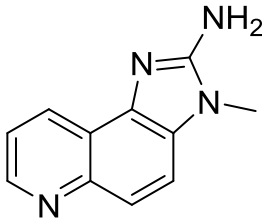
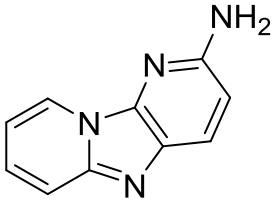
Introduction

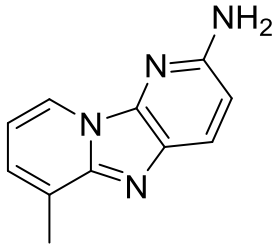
Eight Heterocyclic Aromatic Amines (HAAs) are included in FDA's "Established list of Harmful and Potentially Harmful Constituents (HPHCs) in Tobacco Products"[1]



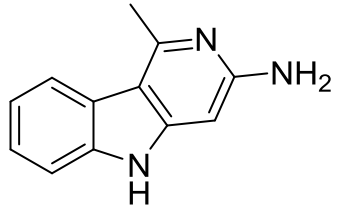
2-Amino-3-methyl-3H-imidazo[4,5-f]quinoline (**IQ**)



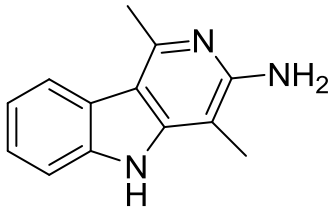
2-Aminodipyrido[1,2-α:3', 2-D]imidazole (**Glu-P-2**)



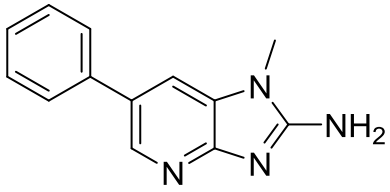
2-Amino-6-methyldipyrido[1,2-α: 3',2'-D]imidazole (**Glu-P-1**)



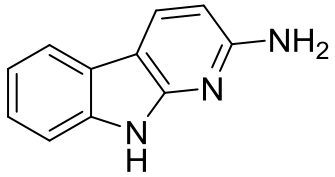
3-Amino-1-methyl-5H-pyrido [4,3-b]indole (**Trp-P-2**)



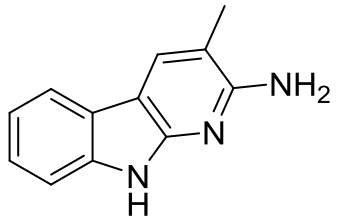
3-Amino-1,4-methyl-5H-pyrido[4,3-b]indole (**Trp-P-1**)



2-Amino-1-methyl-6-phenyl imidazo-[4,5-b]pyridine (**PhIP**)



2-Amino-9H-pyrido [2,3-b]indole (**AC**)



2-Amino-3-methyl-9H-pyrido [2,3-b]indole (**MeAC**)

[1] FDA 2012. Draft guidance for industry: reporting harmful and potentially harmful constituents in tobacco products and tobacco smoke under section 904(a)(3) of the Federal Food, Drug, and Cosmetic Act

Literature Review

- Limited published methods exist for the determination of HAAs in mainstream cigarette smoke[1-5]
- Methodologies have included gas chromatography with nitrogen-phosphorous (GC-NPD) or mass spectrometry (GC-MS) detection as well as liquid chromatography with fluorescence or multistage MS (LC-MS/MS) detection
- Methods typically requires multi-step extraction and sample clean up prior to analysis
- No method reports measurable yields for all the eight compounds in cigarette smoke

1. Zhang et al (2011) Nicotine and Tobacco Research, 13, 120-126
2. Zhao et al, (2014) Chromatographia, 77, 813-820
3. Ming et al (2012) Labstat presentation CORESTA 2012 , Sapporo, Japan
4. Wang H. et al, (2010) Tobacco Chemistry, 2, 28-34
5. Sasaki et al, (2001) Anal. Lett., 1749-1761

Data From Scientific Literature

Analyte	1R5F ISO [1-2,5] (ng/Cigarette)	3R4F ISO [1-4] (ng/Cigarette)
AC	18 to 33	40 to 95
MeAC	2 to 4.9	3 to 9
Trp-P-1	ND to 1	ND to 2
Trp-P-2	ND to 1.2	ND to 3
IQ	ND	ND to 2.8
PhIP	ND	ND
Glu-P-1	ND	ND
Glu-P-2	ND	ND

ND → Not Detected

HAA yields in cigarette smoke range from not detected to low nanogram per cigarette

1. Zhang et al (2011) Nicotine and Tobacco Research, 13, 120-126
2. Zhao et al, (2014) Chromatographia, 77, 813-820
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HAA's Method Challenges

- Highly selective and sensitive detection is needed
- Significant interferences from sample matrix
- Analytes have a wide range of pKa's
- Sample cleanup and concentration required

Analytes	pKa's
AC	4.6
MeAC	4.9
Trp-P-1	8.6
Trp-P-2	8.5
IQ	3.8, 6.6
PhIP	5.7
Glu-P-1	6.0
Glu-P-2	5.8

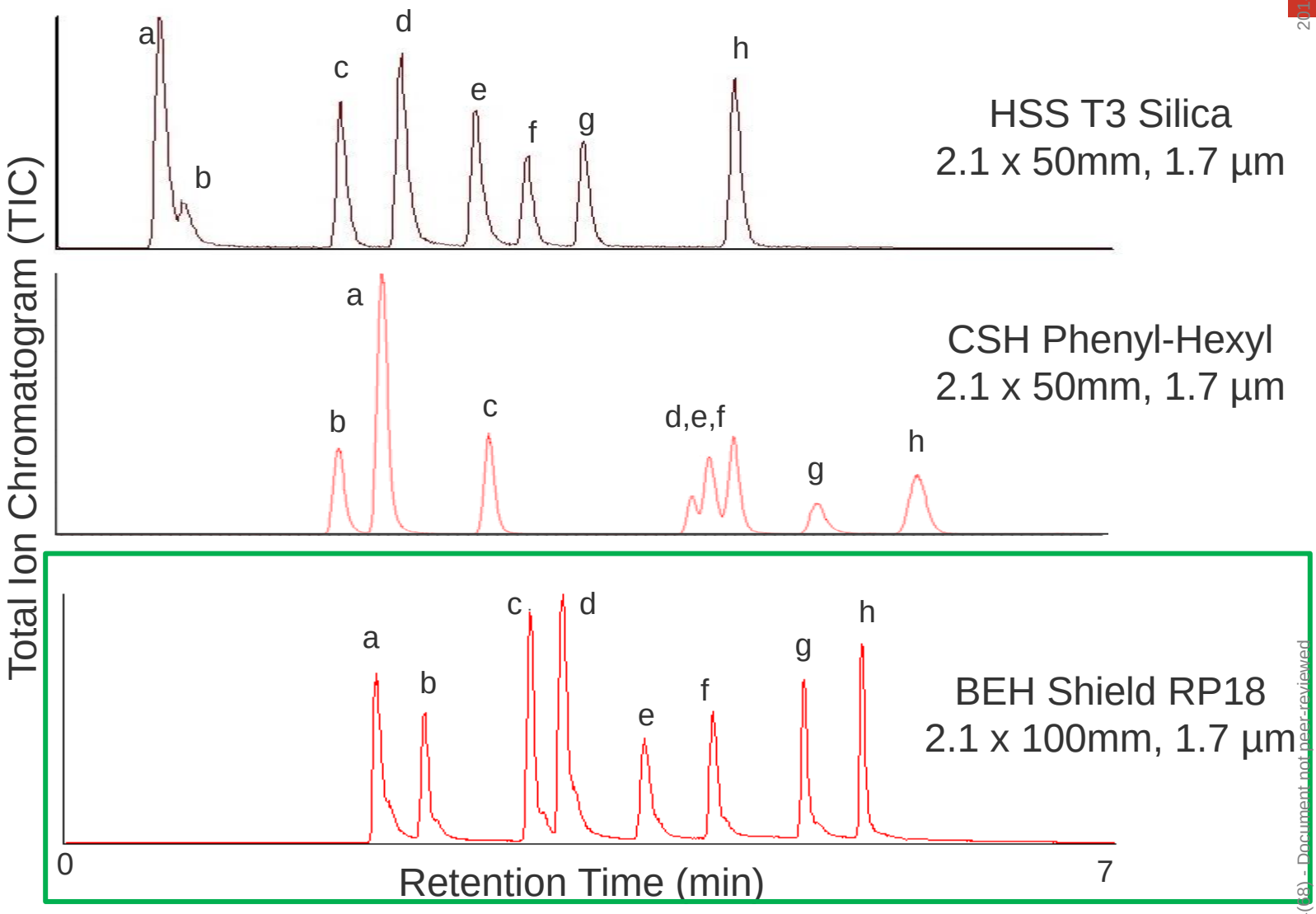
Objectives

- Develop a sensitive and selective method for quantitative analysis of eight HAAs in mainstream cigarette smoke by UPLC-MS/MS

- Approach to method development:
  - Optimize chromatographic separation and MS/MS acquisition
  - Evaluate smoke collection and extraction of HAAs from Cambridge filter pad (CFP)
  - Develop sample cleanup and concentration procedure
  - Determine accuracy from fortification experiments
  - Measure levels of HAAs in cigarette smoke of reference tobacco products (1R5F and 3R4F)

UPLC Column Selection

(a) IQ
(b) Glu-P-2
(c) Glu-P-1
(d) Trp-P-2
(e) Trp-P-1
(f) PhIP
(g) AC
(h) MeAC



Analytical Method - UPLC

Waters ACQUITY UPLC Xevo TQD

UPLC Parameters	
UPLC Column	Waters ACQUITY UPLC BEH ShieldRP18, 2.1x100 mm, 1.7 µm
Guard Column	Waters ACQUITY UPLC VanGaurd BEH ShieldRP18, 2.1x5 mm, 1.7 µm
Column Temperature	30°C
Injection Volume	3 µL
Flow Rate	0.250 mL/min
Mobile Phase A	10 mM Ammonium Acetate
Mobile Phase B	0.1% Acetic acid in Methanol
Collision Gas Flow	0.2 mL/min
Run Time	10 min

Analytical Method – MS/MS

MS/MS Parameters	
Ionization mode	Positive Electrospray
Capillary Voltage	0.3 kV
Source Temperature	150 ^{o}C
Desolvation Temperature	600 ^{o}C
Desolvation Gas Flow	800 L/Hr
Collision Gas Flow	0.2 mL/min
Data Acquisition	MRM mode

MS/MS Data Acquisition

MRM Transitions			
Analyte	**Precursor Ion**	**Product Ion**	**CE* (eV)**
AC	184.10	140.10	30
MeAC	198.27	181.10	26
Trp-P-1	212.15	195.20	23
Trp-P-2	198.08	181.0	32
IQ	199.10	183.99	30
PhIP	225.15	210.15	30
Glu-P-1	199.06	92.05	35
Glu-P-2	185.15	77.96	34

* CE → Collision Energy

Calibration Data

Analyte	Calibration Curve R^2	Linearity Range (ng/mL)
AC	0.999	0.4-140
MeAC	0.999	0.4-145
Trp-P-1	0.999	0.4-143
Trp-P-2	0.999	0.4-140
IQ	0.996	0.4-100
PhIP	0.999	0.4-148
Glu-P-1	0.999	0.4-140
Glu-P-2	0.997	0.4-140

Extraction Solvent Optimization

Extraction with 0.1 N HCl

Analyte	AC	MeAC	Trp-P-1	PhIP	Trp-P-2	Glu-P-1	Glu-P-2	IQ
Avg. Recovery	99.2	98.6	96.2	98.1	78.4	80.6	77.6	95.7
% RSD (n=3)	4.4	4.1	3.4	2.2	8.8	9.1	6.8	5.1

Extraction with 0.1 N HCl/25 mM Acetic acid

Analyte	AC	MeAC	Trp-P-1	PhIP	Trp-P-2	Glu-P-1	Glu-P-2	IQ
Avg. Recovery	95.2	101.9	92.2	95.1	92.6	93.6	89.6	91.2
% RSD (n=3)	2.6	3.3	7.0	2.4	7.2	6.8	8.0	1.0

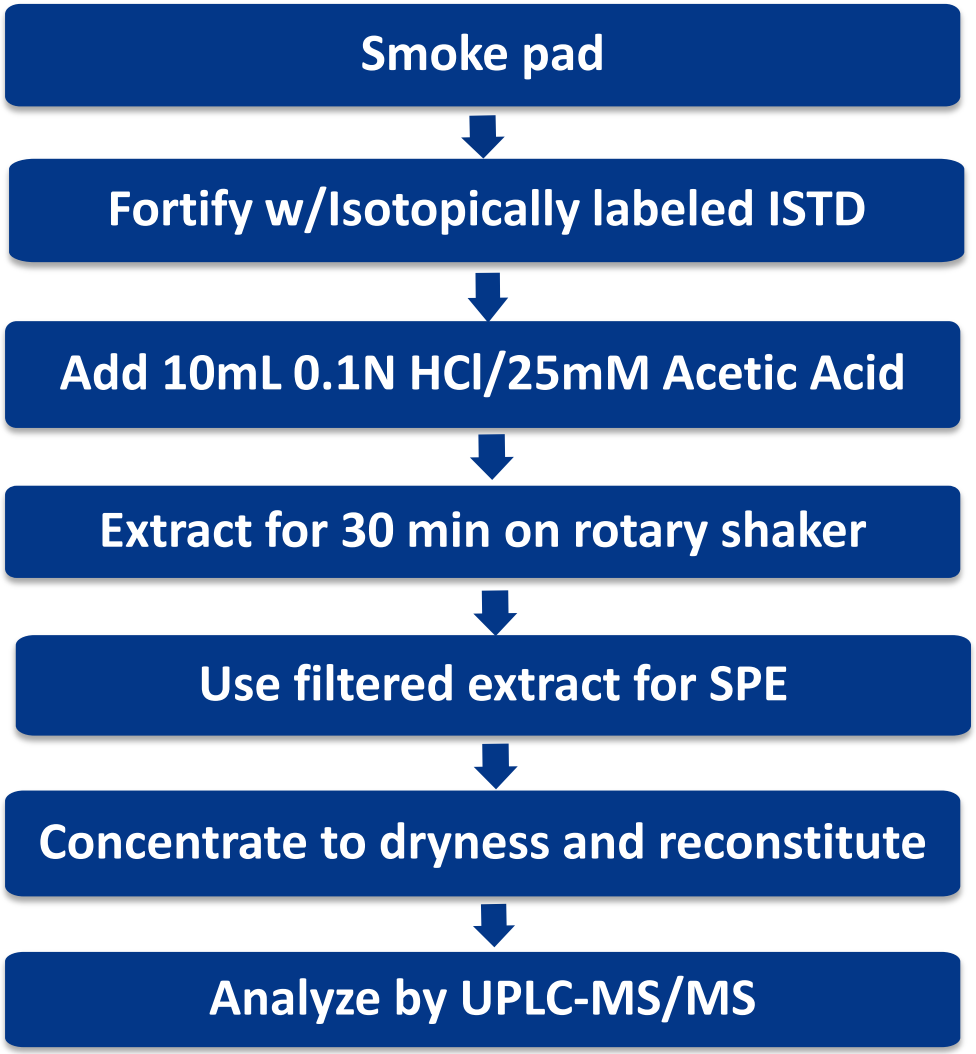
0.1 N HCl/25mM Acetic Acid was selected for extraction of HAAs from Cambridge filter pad

SPE Procedure

SPE Step	Cation Exchange (MCX, 150 mg, 6 mL)	Reverse Phase C18 (Strata C18-U, 200 mg, 3 mL)
Condition	Methanol, 0.1 N HCl	Methanol, Water
Load	Sample Extract	Neutralized sample extract
Wash 1	0.1 N HCl	10 mM NH_4AC in 20% Methanol
Wash 2	Methanol	N/A
Wash 3	2% NH_4OH in 20% Methanol	N/A
Elution	5% NH_4OH in Methanol	10 mM NH_4AC in Methanol

SPE using cation exchange cartridge eliminates neutral and polar interferences and is compatible with the extraction solvent

Method Workflow



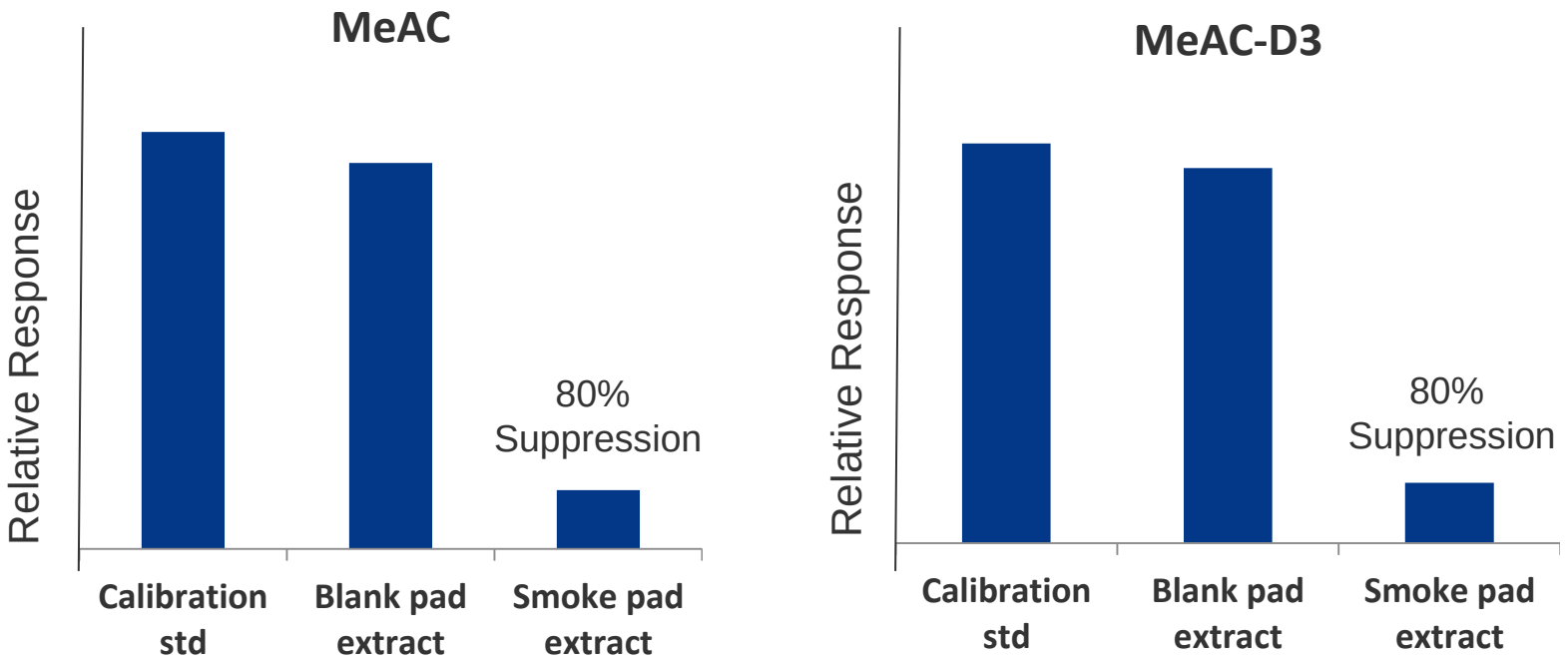
Method Accuracy

Analyte	% Recovery (Blank pad)	% RSD	% Recovery (Cigarette Smoke)	% RSD
AC	97.2	5.6	98.5	2.3
MeAC	106.8	7.2	101.2	1.2
Trp-P-1	108.6	6.0	109.2	9.0
Trp-P-2	97.0	2.7	87.2	12.8
IQ	103.6	3.0	105.3	5.4
PhIP	97.8	5.2	97.7	2.2
Glu-P-1	109.0	3.7	105.4	4.5
Glu-P-2	97.6	6.6	120.5	12.8

Spiked at 10 ng/mL

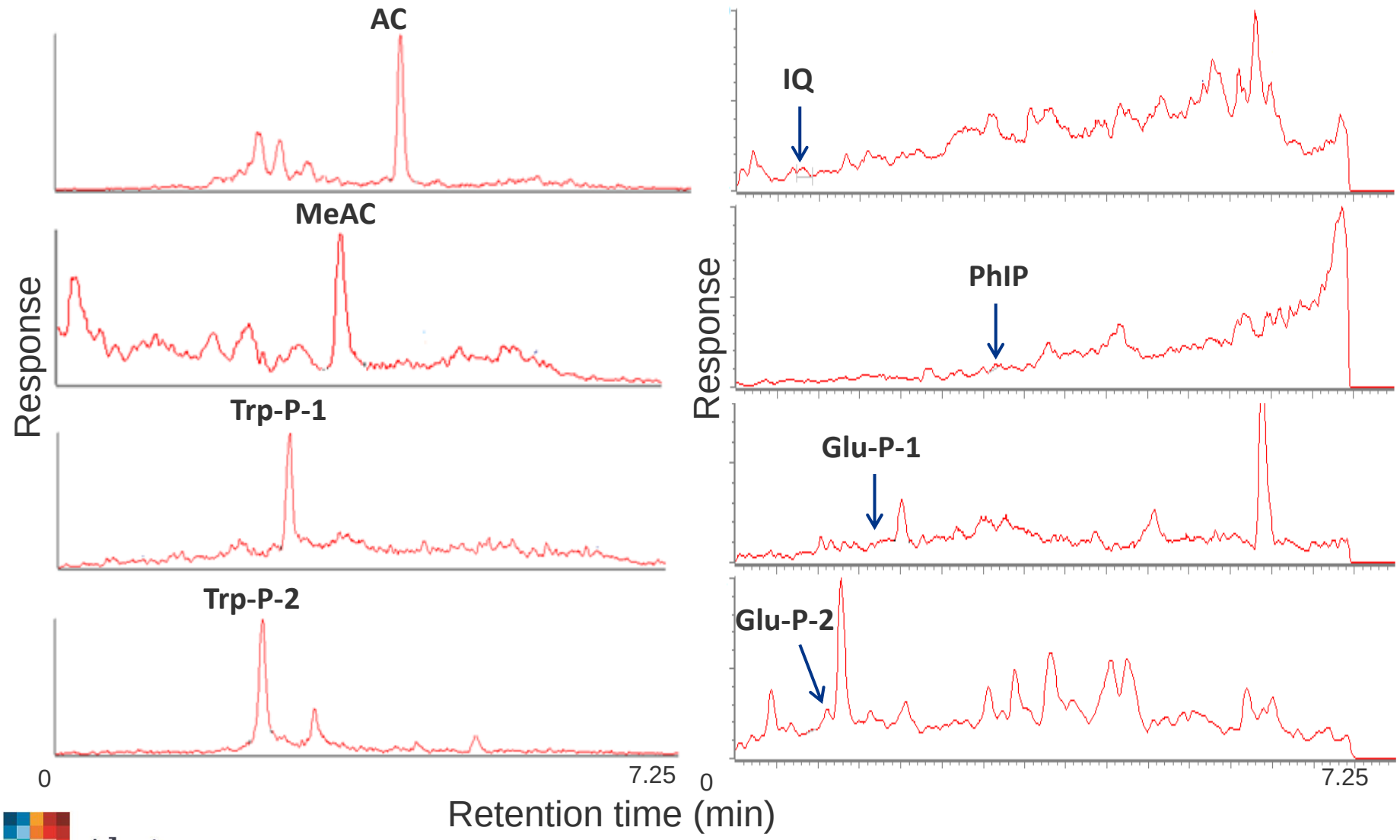
High recoveries for HAAs from blank pad and cigarette smoke pad

Matrix Interference



All analyte and internal standard responses are highly suppressed (50-80%) in presence of cigarette smoke matrix

Chromatogram of HAA's in 3R4F ISO Cigarette Smoke Extract



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Results – Reference Cigarette Products

Analyte	1R5F ISO (ng/Cigarette)	3R4F ISO (ng/Cigarette)
AC	17.7	50.8
MeAC	BLOQ	6.7
Trp-P-1	BLOQ	1.4
Trp-P-2	BLOQ	2.8
IQ	ND	ND
PhIP	ND	ND
Glu-P-1	ND	ND
Glu-P-2	ND	ND

BLOQ → Below Limit of Quantification

Results – Reference Cigarette Products

Analyte	1R5F ISO (ng/Cigarette)	3R4F ISO (ng/Cigarette)
AC	17.7 *(18 to 33)*	50.8 *(40 to 95)*
MeAC	BLOQ *(2 to 4.9)*	6.7 *(3 to 9)*
Trp-P-1	BLOQ *(ND to 1)*	1.4 *(ND to 2)*
Trp-P-2	BLOQ *(ND to 1.2)*	2.8 *(ND to 3)*
IQ	ND *(ND)*	ND *(ND to 2.8)*
PhIP	ND *(ND)*	ND *(ND)*
Glu-P-1	ND *(ND)*	ND *(ND)*
Glu-P-2	ND *(ND)*	ND *(ND)*

Data from Scientific Literature

Summary

- Method development demonstrates the challenges of accurately and reproducibly measuring low levels of HAAs in cigarette smoke.

- In the current study AC, MeAC, Trp-P-1, and Trp-P-2 were detected at low levels in 3R4F (ISO) while Glu-P-1, Glu-P-2, PhIP and IQ were not detectable. In 1R5F, except for AC, all other HAAs were either BLOQ or not detected.

- Some of the HAAs included in FDA's "Established list" of HPHCs in Tobacco Products[1] may not be detectable in cigarette smoke.

[1] FDA 2012. Draft guidance for industry: reporting harmful and potentially harmful constituents in tobacco products and tobacco smoke under section 904(a)(3) of the Federal Food, Drug, and Cosmetic Act