Development of an LC-MS/MS Method for the Determination of Heterocyclic Aromatic Amines (HAAs) in Mainstream Smoke Using a Simple Extraction and Sample Preparation

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OBJECTIVES

- Improve extraction procedure for HAAs from cigarette smoke
- Reduce time and cost of extract clean-up and pre-concentration
- Improve chromatography

-*J. Agric. Food Chem*, **2005**, 53, 3248-3258.

-*Environmental Pollution*, **1990**, 64, 121-132.

-*Analytical Biochemistry*, **1996**, 235, 185-190.

-*Journal of Chromatography*, **1992**, 592, 271-278

-*Carcinogenesis*, **1991**, 12, 1945-1947

INTRODUCTION

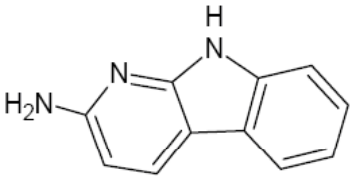
- HAAs are considered highly mutagenic, as much as 3000x more potent than B[a]P in Ames testing
- Generated in smoke at ~ng/cig quantities
- Clean-up and concentration techniques often difficult and time consuming and yield varying results
- LC-MS/MS is used due to superior selectivity and sensitivity

NOMENCLATURE OF HETEROCYCLIC AROMATIC AMINES (HAAS)

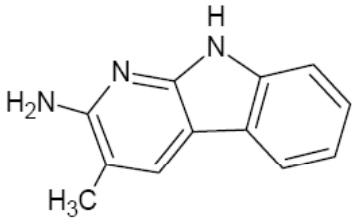
2-Amino-9H-pyrido[2,3-B]indole	**AαC**
2-amino-3-methyl-9H-pyrido[2,3-b]indole	**MeAαC**
3-amino-1,4-dimethyl-5H-pyrido [4,3-b]indole	**Trp-P-1**
3-amino-1-methyl-5H-pyrido[4,3-b]indole	**Trp-P-2**
2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole	**Glu-P-1**
2-aminodipyrido[1,2-a:3',2'-d]imidazole	**Glu-P-2**
2-Amino-3,4-dimethylimidazo-[4,5-f]quinoline	**MeIQ**
2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline	**MeIQx**
2-amino-3-methylimidazo[4,5-f]quinoline	**IQ**
2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine	**PhIP**

INTRODUCTION

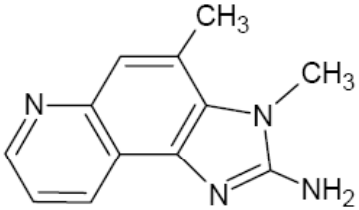
Structures of 10 target HAAs



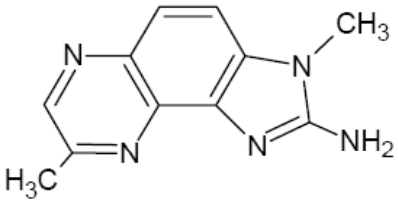
AαC



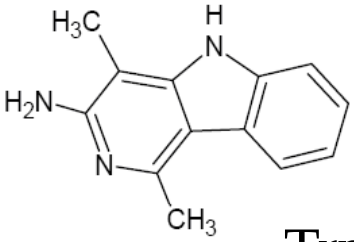
MeAαC



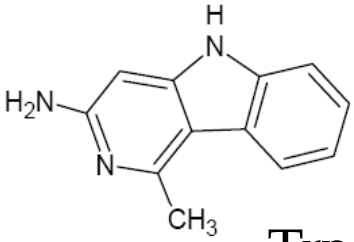
MeIQ



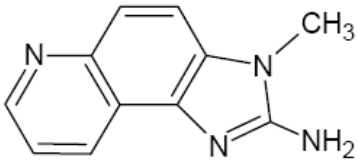
MeIQx



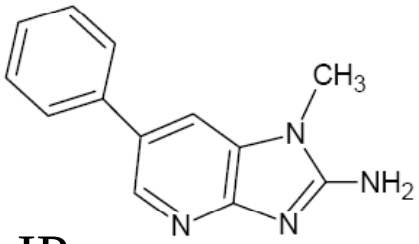
Trp-P-1



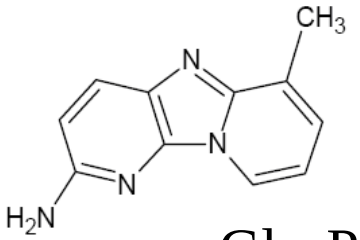
Trp-P-2



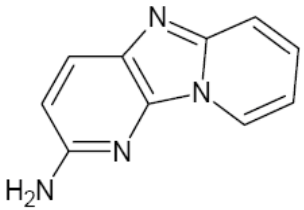
IQ



PhIP



Glu-P-1

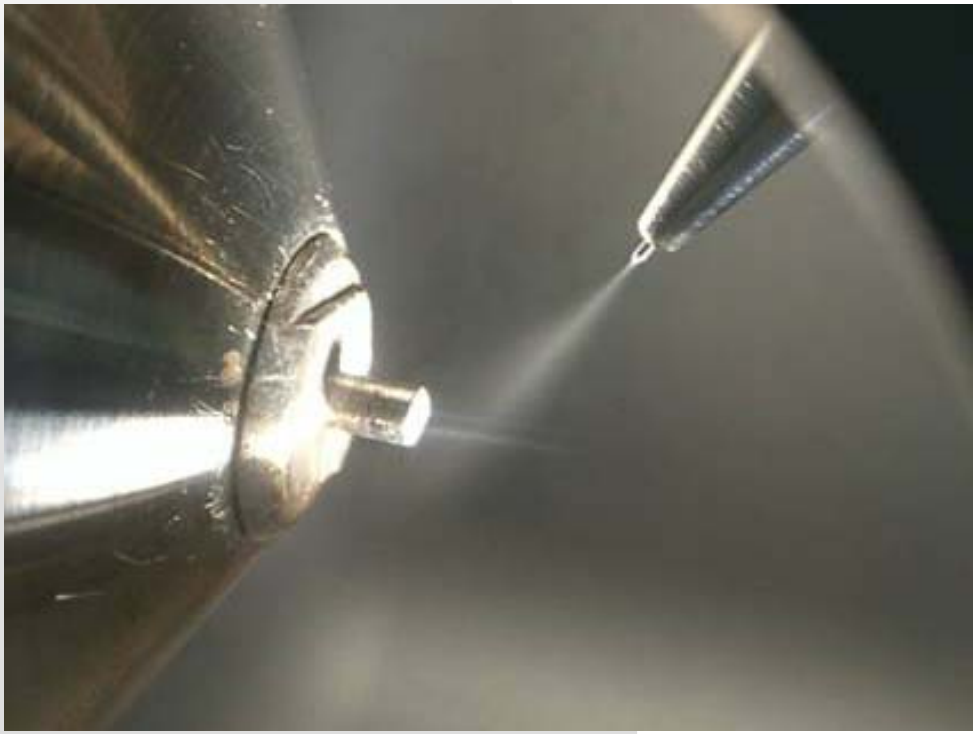


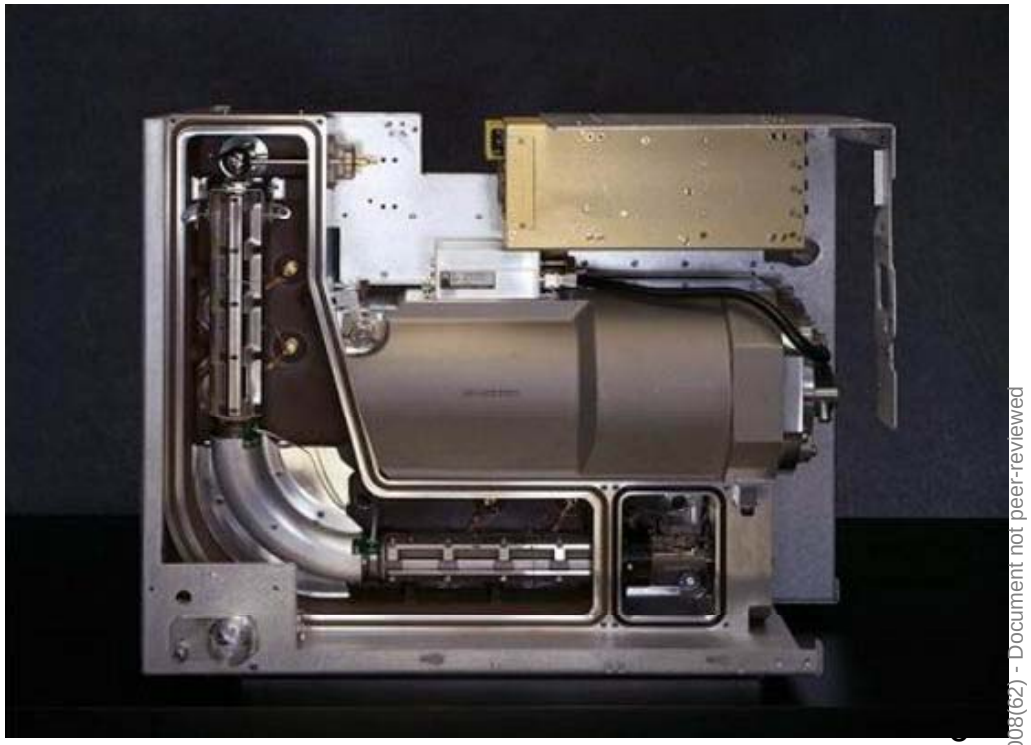
Glu-P-2

Use Extreme Caution When Handling These Compounds!

LC-MS/MS-HOW DOES IT WORK?

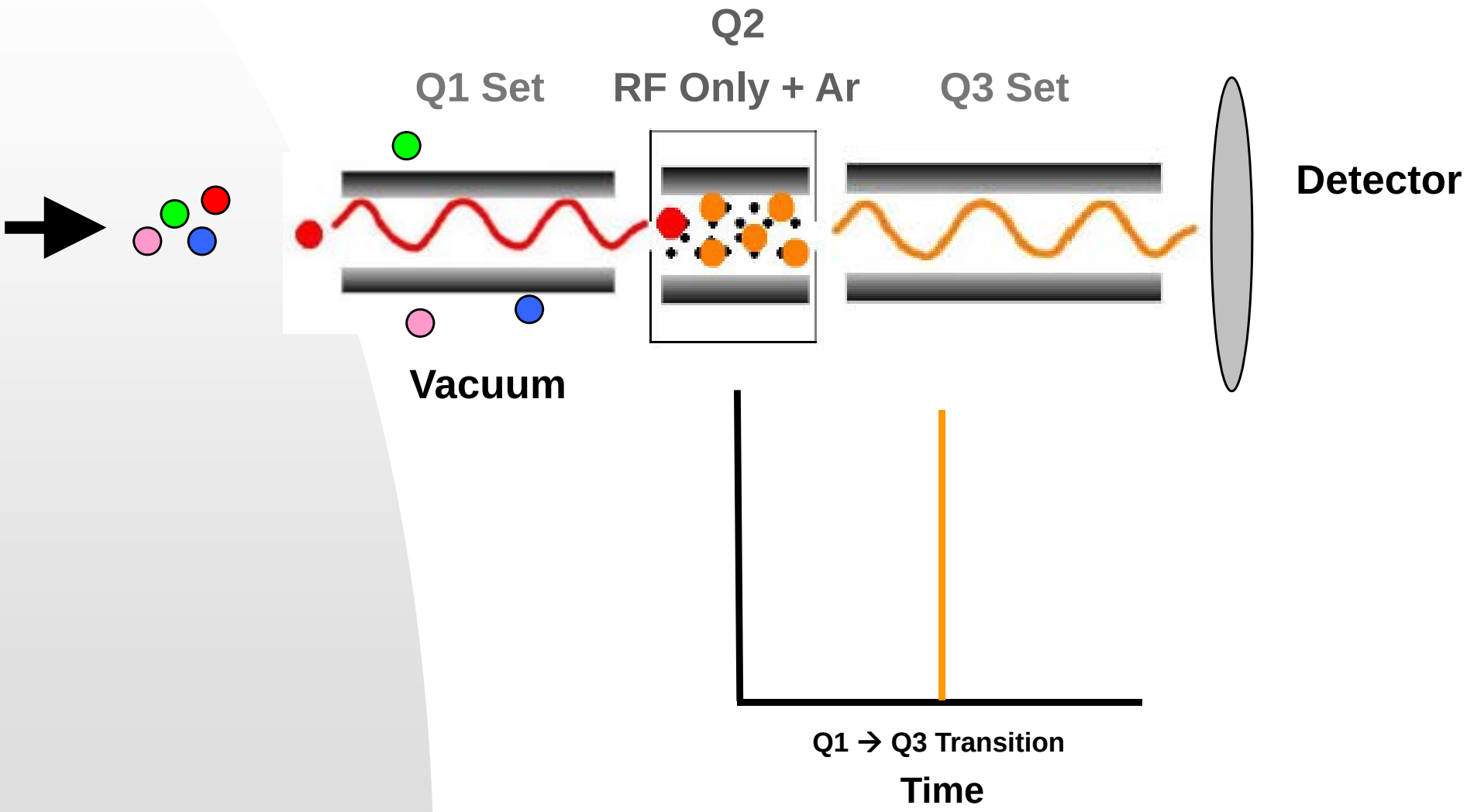
- Electrospray Ionization (ESI) is a soft ionization technique that introduces ions generated by solution chemistry (ex. acidic mobile phase) via electrically charged spray plume into the tandem mass spectrometer (MS-MS or triple-quad)
  - Basic Functional Groups (-NH2) → $[M+H]^+$





LC-MS/MS-SELECTED REACTION MONITORING

Purpose: **Quantitation on a single product ion population**



Precursor (Parent) Ion → Product (Daughter) Ion Transitions

EXPERIMENTAL

Instrumentation

- Thermo Surveyor HPLC with Mass Spec low flow pump
- Column: Agilent C18 2.1 x 150 mm, 5 μm particle, at 30ºC
- Autosampler:
  - -Temperature: 4ºC
  - -Injection Volume: 10 μL
- Thermo TSQ Quantum Ultra triple quadrupole mass spectrometer with ESI probe
  - -Spray Voltage of 4.0kV and heated capillary at 350ºC

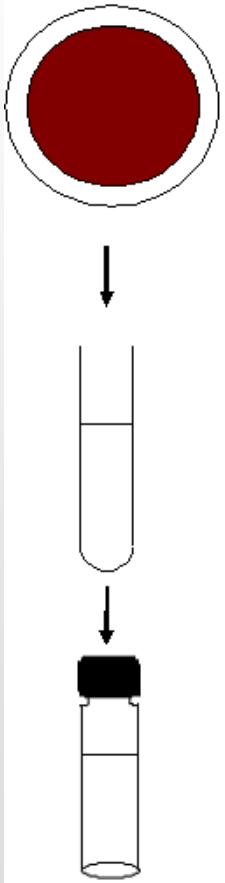
EXPERIMENTAL- Gradient Table

Time	LC Flow Divert Valve	A % 0.5% HAc in H_2O	B % Acetonitrile
0.00	Closed	100.0	0.0
3.80	Closed	100.0	0.0
14.80	Open	90.0	10.0
20.00	Open	86.0	14.0
20.01	Open	86.0	14.0
26.00	Open	75.0	25.0
28.00	Open	20.0	80.0
35.00	Closed	100.0	0.0
40.00	Closed	100.0	0.0

Flow Rate= 200 μL/min

Sample Preparation

- Mainstream smoke is collected on a 44 mm Cambridge filter pad (2-5 cigs.)
- Filter pad is extracted in a capped test tube with 5 mL of extraction solution, methanol containing internal standard (4,8-DiMeIQx), at 300 rpm for 30 minutes
- The extract is filtered through a 0.45 μm PTFE syringe filter
- The filtrate is then diluted 1:12 with extraction solution into an LC vial. Vials are capped, vortexed, and placed on LC-MS/MS system and analyzed

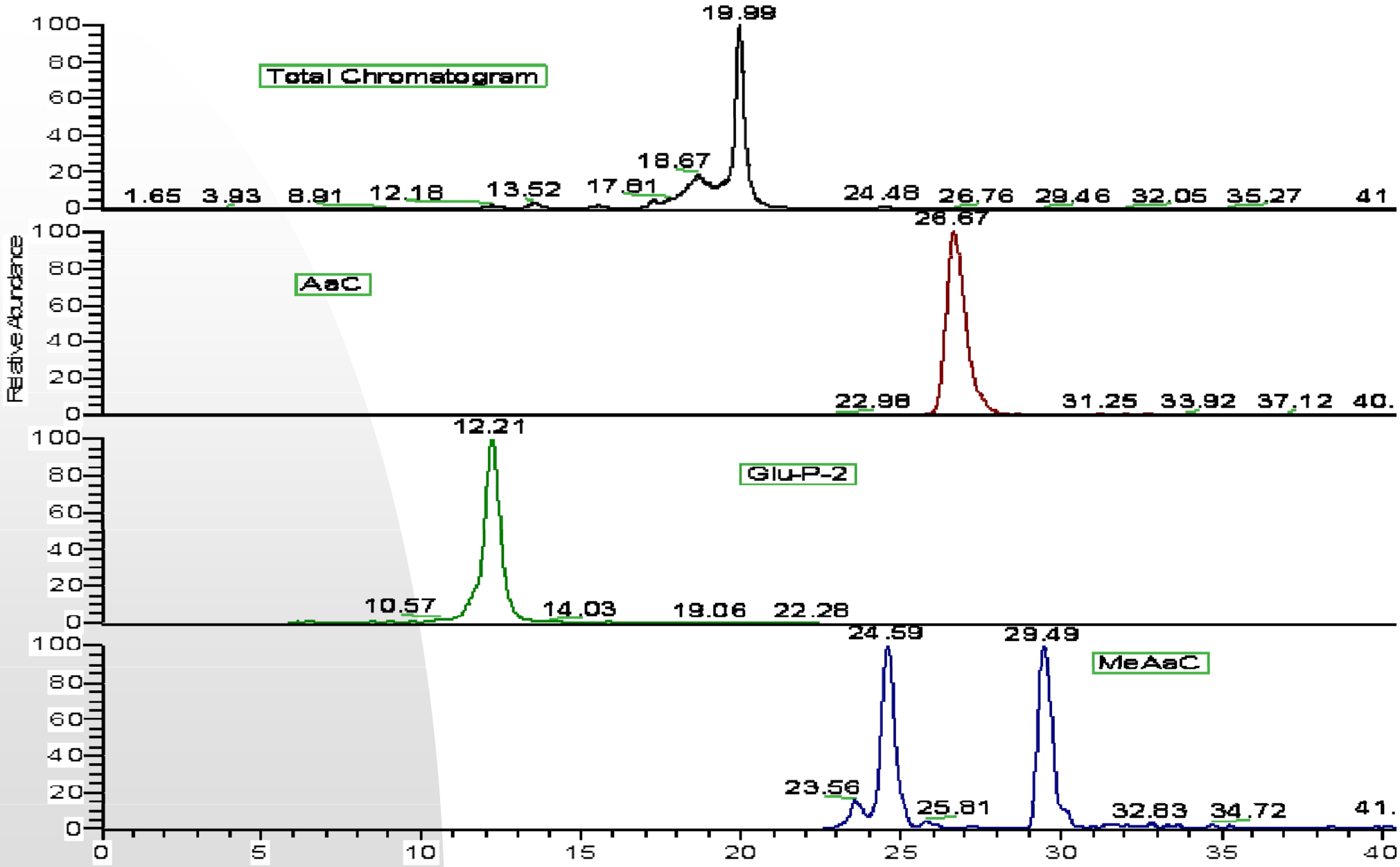


IDENTIFICATION OF COMPOUNDS

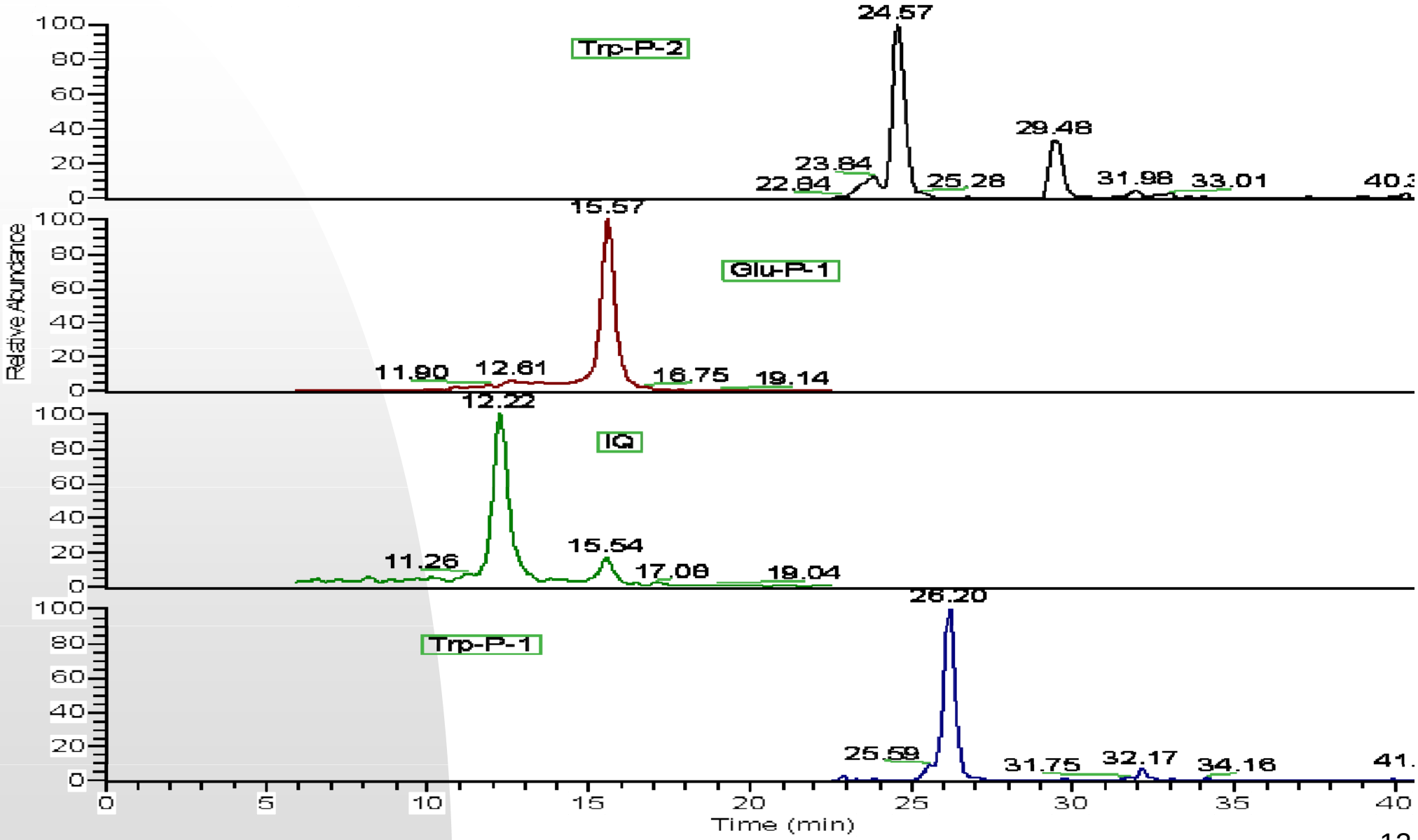
HAA	Retention Time (min)	Precursor Ion *m/z*	Product Ion 1 *m/z*	Product Ion 2 *m/z*	Product Ion 3 *m/z*
AaC	26.67	184.1	140.0		
*MeAaC**	29.36	*198.1*	181.0	128.1	129.1
*Glu-P-1**	15.64	*199.1*	92.1	119.0	
Glu-P-2	12.29	185.1	168.0		
*IQ**	12.39	*199.1*	79.1	156.0	
MeIQ	13.69	213.1	198.1		
MeIQx*	17.30	214.1	173.1	199.1	
PhIP	24.32	225.1	210.0		
Trp-P-1*	26.20	212.1	167.0	115.1	
*Trp-P-2**	24.59	*198.1*	154.1	181.0	
4,8-DiMeIQx (ISD)*	19.97	228.1	212.1	213.1	

***In several instances, multiple reactions are used. In this case, the sum of all products from each precursor seen in the table is used in the reproduced chromatogram of mass spectra.**

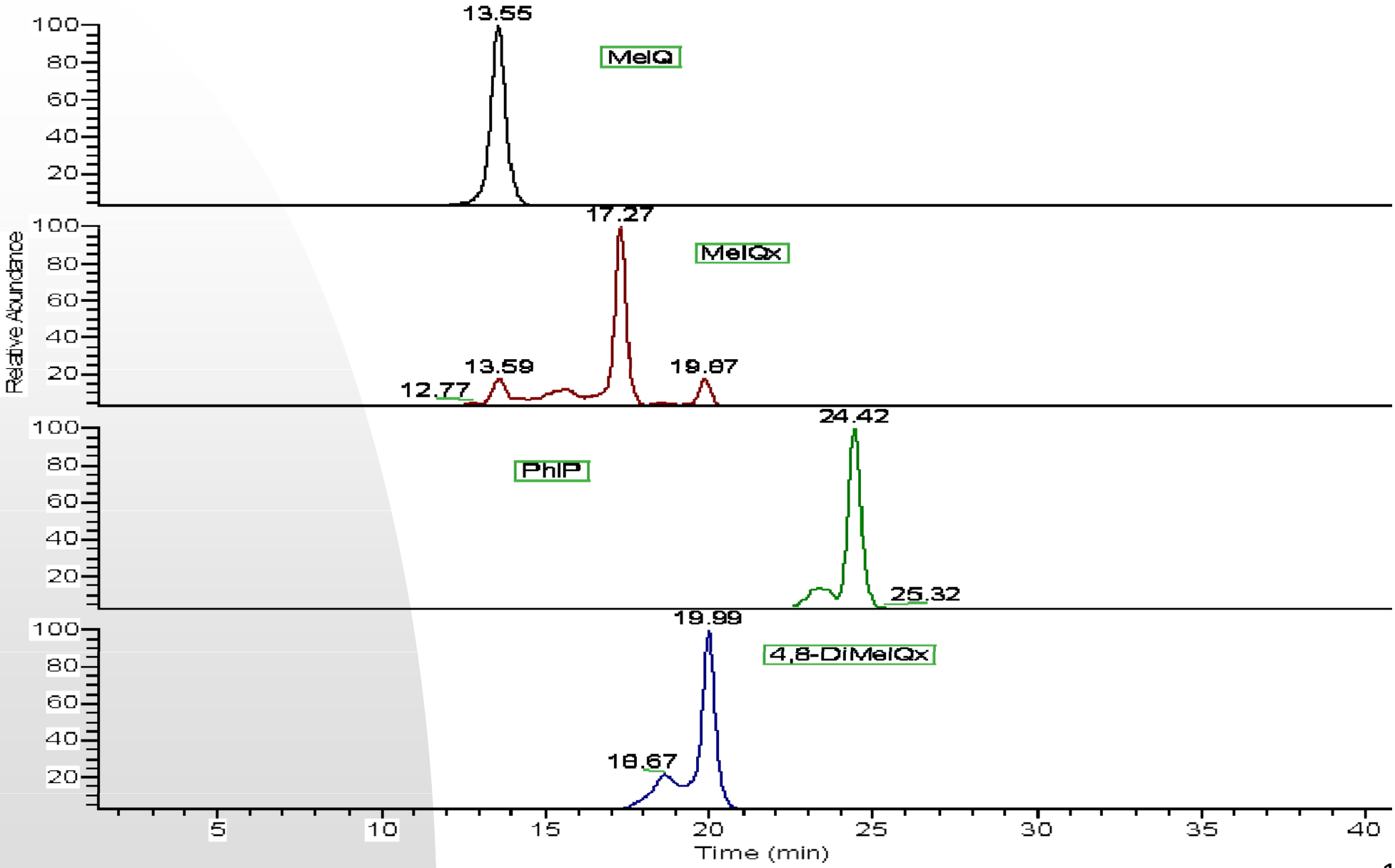
CHROMATOGRAMS OF HAA STANDARD



CHROMATOGRAMS OF HAA STANDARD



CHROMATOGRAMS OF HAA STANDARD



LINEARITY OF STANDARDS

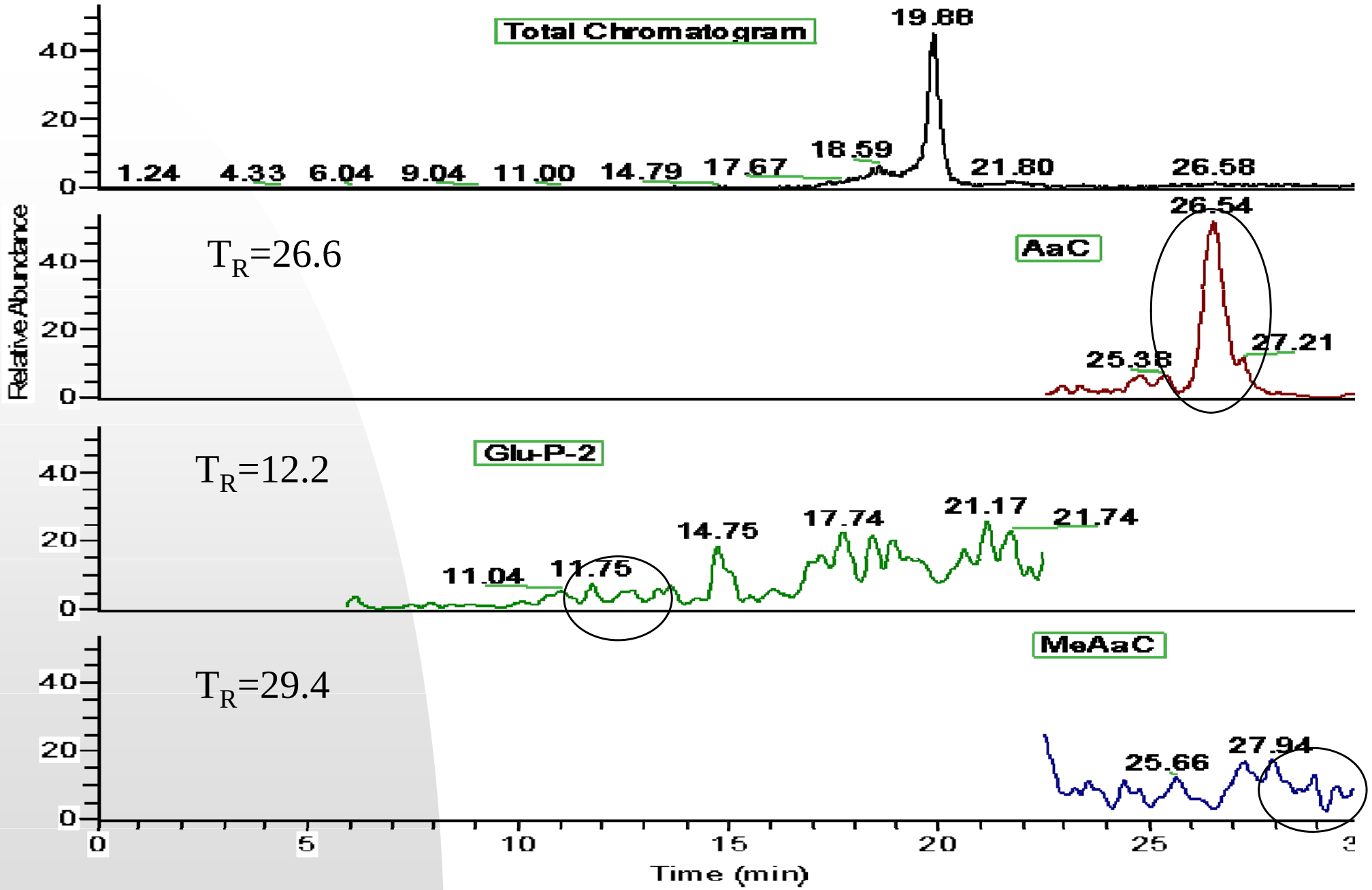
HAA	Linear Regression Coefficient R^2	Dynamic Range of Standard Concentrations (ng/mL)
AαC	0.999	0.1 - 20.0
MeAαC	0.995	0.1 - 20.0
Glu-P-1	0.999	1.0 - 200.0
Glu-P-2	0.999	1.0 - 200.0
IQ	0.999	0.6 - 120.0
MeIQ	0.999	0.6 - 120.0
MeIQx	0.999	0.6 - 120.0
PhIP	0.997	0.1 - 20.0
Trp-P-1	0.996	0.1 - 20.0
Trp-P-2	0.998	0.1 - 20.0

LIMIT OF QUANTITATION

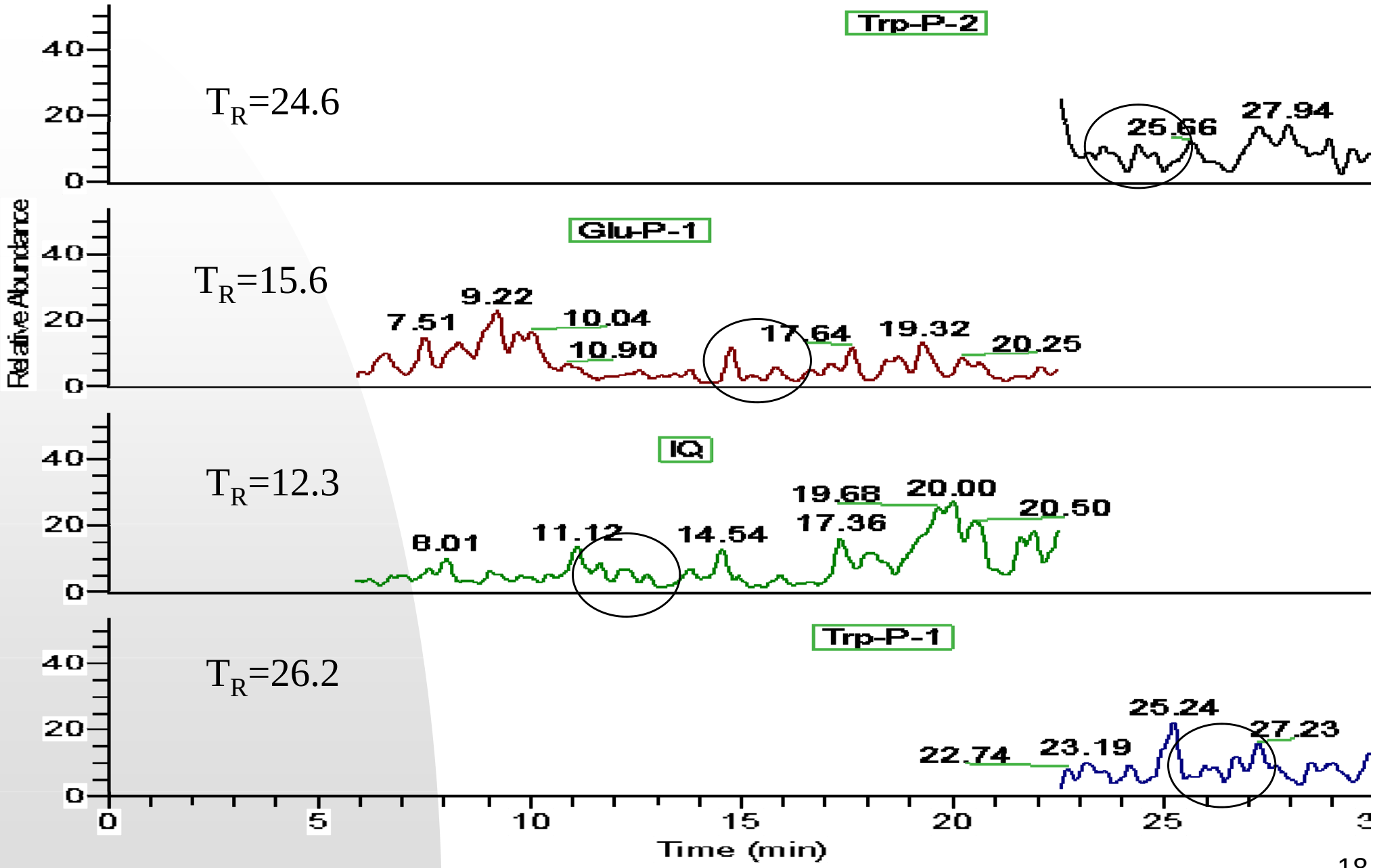
HAAs	LOQ in Smoke Extract (ng/cig)
AαC	0.7
MeAαC	7.4
Trp-P-1	7.0
Trp-P-2	1.5
Glu-P-1	7.0
Glu-P-2	8.7
MeIQ	4.1
MeIQx	4.2
IQ	4.1
PHIP	8.2

-By Standard Addition Method (S/N $\geq$10)

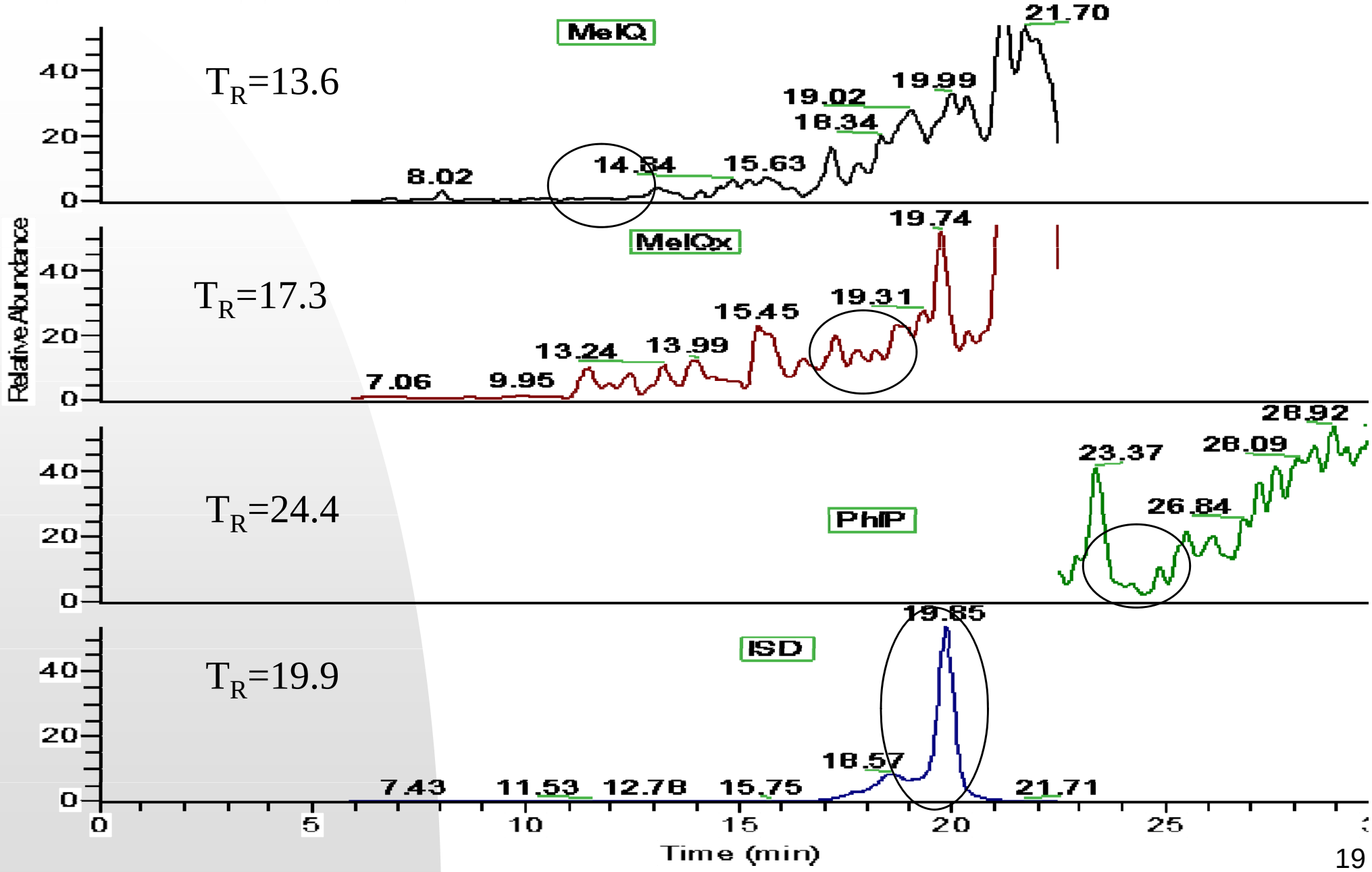
CHROMATOGRAMS OF HAA SMOKE SAMPLE



CHROMATOGRAMS OF HAA SMOKE SAMPLE

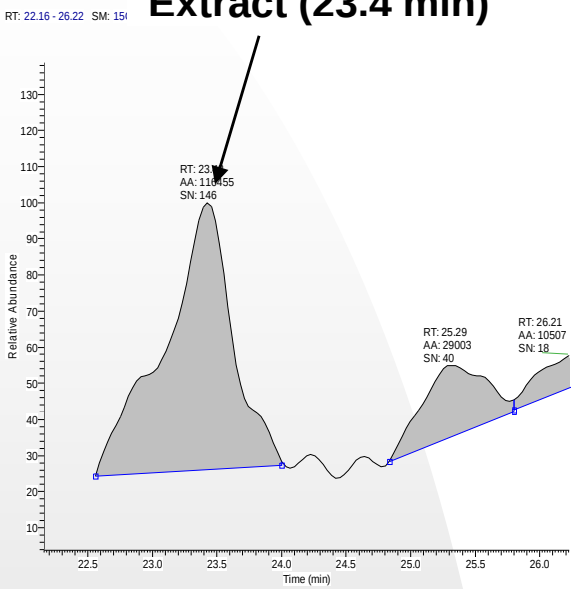


CHROMATOGRAMS OF HAA SMOKE SAMPLE



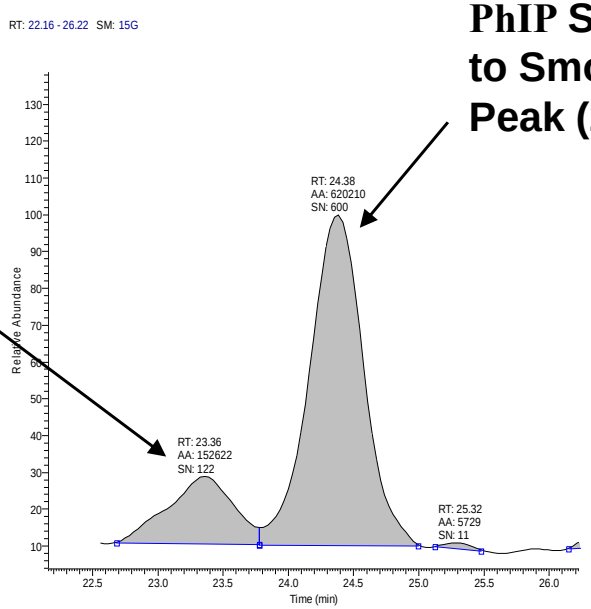
INTERFERENCES- PhIP

Suspect PhIP Peak in Smoke Extract (23.4 min)



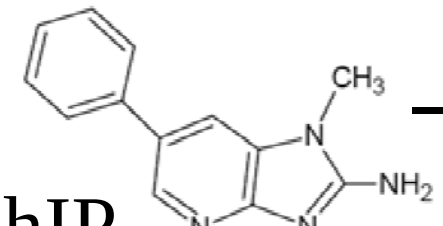
Suspect PhIP Peak (23.4 min)

PhIP Standard Added to Smoke Extract- Peak (24.4 min)



-The peak at 23.4 minutes has the correct PhIP product ion transition, but the retention time shows false ID.

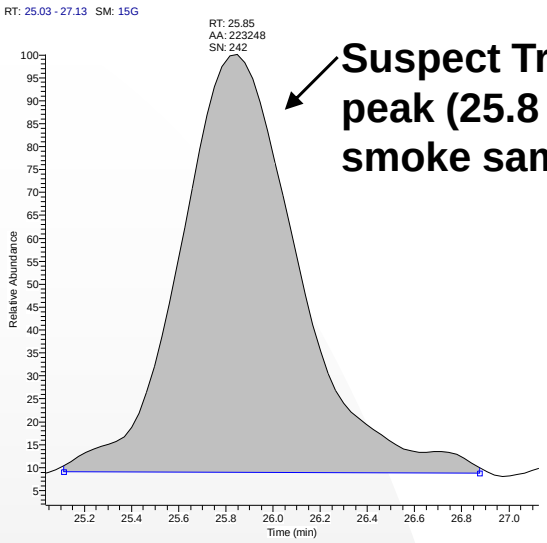
PhIP



Best Guess:

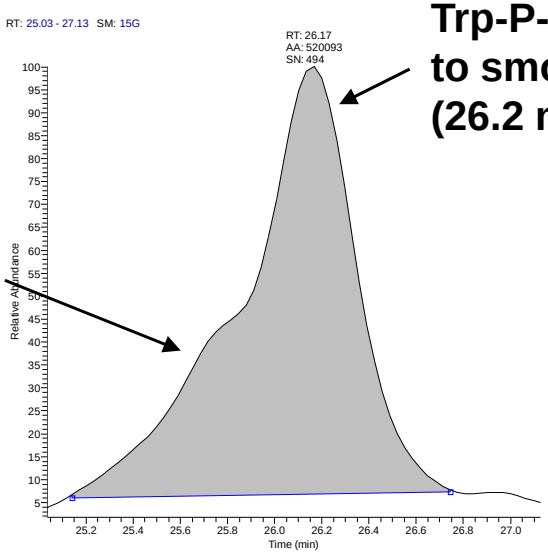
PhIP Analogue

INTERFERENCES- Trp-P-1



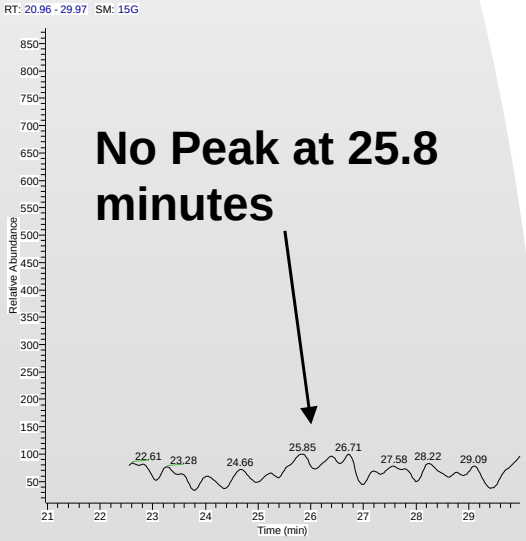
Suspect Trp-P-1 peak (25.8 min) in smoke sample

Suspect Trp-P-1 peak (25.8 min)



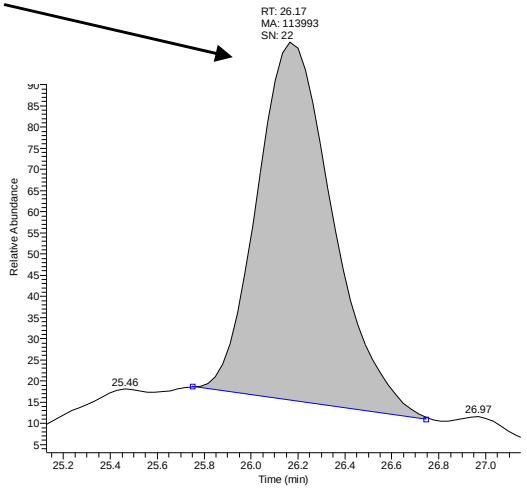
Trp-P-1 Standard added to smoke sample- Peak (26.2 min)

Used Extracted Ion Chromatogram of Product m/z 115.1



No Peak at 25.8 minutes

Standard Added-Peak at 26.2 min without fronting shoulder



CHROMATOGRAPHIC PRECISION (n=6)

AαC ng/cig	2R4F Rep 1	2R4F Rep 2	2R4F Rep 3	2R4F Rep 4	2R4F Rep 5	2R4F Rep 6
Injection 1	58.2	56.4	57.0	57.2	64.7	60.6
Injection 2	55.4	55.8	56.6	57.6	64.9	64.7
Injection 3	57.9	55.5	57.2	55.9	61.0	63.0
Injection 4	57.5	58.4	61.5	59.9	64.2	60.0
Injection 5	55.9	54.8	53.2	59.4	62.4	63.7
Injection 6	59.1	55.0	52.4	59.8	58.5	63.3
Average	*57.3*	*56.0*	*56.3*	*58.3*	*62.6*	*62.6*
Std Dev	*1.42*	*1.33*	*3.24*	*1.64*	*2.53*	*1.85*
% RSD	*2.5%*	*2.4%*	*5.8%*	*2.8%*	*4.0%*	*3.0%*

Overall Statistics (n=36)

Average	58.9
Std Dev	3.41
% RSD	5.8%

RECOVERY OF HAAs

HAA	*Average % Recovery
AaC	103%
MeAaC	99%
Trp-P-1	119%
Trp-P-2	116%
Glu-P-1	117%
Glu-P-2	107%
IQ	106%
MeIQ	103%
MeIQx	111%
PHIP	89%

*Average of Low, Mid, and High Tar Reference Cigarettes

DATA COMPARISON

			Amount (ng/cig)							
	Trp-P-1	**Trp-P-2**	**AaC**	**MeAaC**	**Glu-P-1**	**Glu-P-2**	**IQ**	**MeIQ**	**MeIQx**	**PhIP**
Literature Values (various cigs./regimes)	**0.02-.0.74**	**<0.05-1.54**	**1.96-80.5**	**0.37-37.0**	**<0.05-0.89**	**0.25-0.88**	**0.16-0.49**	**<0.03-0.45**	**N/A**	**<0.01-20.0**
Cigarette		**This Study (FTC)**								
1R5F	**ND**	**ND**	**30.6**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
1R4F	**ND**	**ND**	**69.9**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
2R4F	**ND**	**ND**	**58.9**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
IM 17	**ND**	**ND**	**90.3**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**

ND=none detected/below quant. limits.

N/A=no data available

Felton, J.; Jagerstad, M.; Knize, M.; Skog, K.; and Wakabayashi, K. (2000). *Food Borne Carcinogens*, 31-71.

CONCLUSIONS

- A method has been developed and validated for the quantitative determination of 10 target HAAs in mainstream tobacco smoke by LC-MS/MS
- Recovery for all analytes was 100±19%, with several at 100±10%
- The sample preparation is relatively simple and cost effective as compared to previously reported methods.
- Only AαC was consistently found in 2R4F cigarette smoke (~60ng/cig)

AKNOWLEDGEMENTS

- Nancy Qian, RJRT
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