

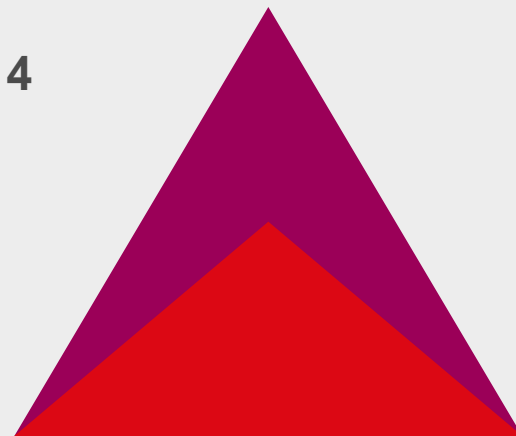


ESSENTRA

Determination of Acetamide and Acrylamide in Mainstream Cigarette Smoke Using Liquid Chromatography-Mass Spectrometry

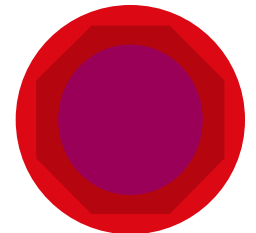
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68th Tobacco Science Research Conference
Charlottesville, Virginia, USA. 28th Sep – 1st Oct 2014

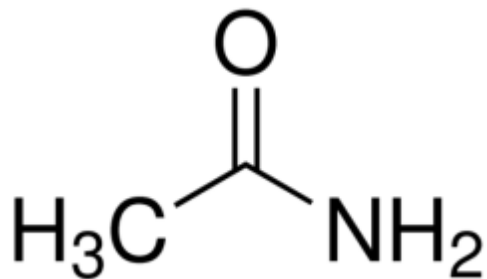


OUTLINE

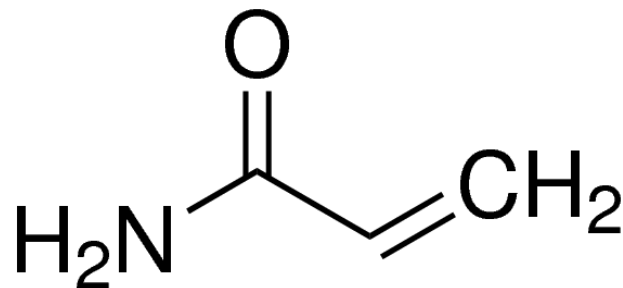
- Introduction
- Method Objectives
- Method Development
 - Effect of extraction solution on Acetamide stability
- Analytical Methodology
- Method Performance Characteristics
 - Mainstream smoke ISO and HCl
 - Chromatograms
- Summary
- Sidestream Analysis
- E-Cigarette Analysis



INTRODUCTION



Acetamide



Acrylamide

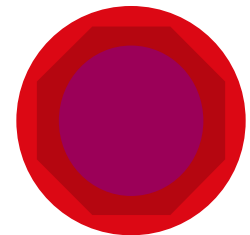
Analyte	IARC Classification ¹	FDA HPHC List ²
Acetamide	2B (<i>Possibly carcinogenic to humans</i>)	Carcinogen
Acrylamide	2A (<i>Probably carcinogenic to humans</i>)	Carcinogen

¹IARC Monographs Volume 7, Sup 7, 71 1999 and Volume 60 1994

²Federal Register / Vol. 77, No. 64 / Tuesday, April 3, 2012 / Notices

METHOD OBJECTIVES

- Develop and validate an accredited LC-MS/MS method for both compounds
- Simple extraction
 - Clean extracts
 - Clean Chromatograms
 - Use Deuterated Internal Standards
 - Use 3R4F as control cigarette
- Selective
- Sensitive
- Fast run time



PREVIOUS APPROACHES

Author	Journals	Method Details	Results (ISO regime)
Kim I. et al	J. Korean Society of Tobacco Science (2007) Vol.29	LC-MS/MS Extract in 0.1% Acetic acid	2R4F Acryl 1.7 µg/cig
Diekmann J. et al	J. Chromatogr. (2008) Vol.46	GC-MS Column-on-column injection Extract in Acetone	2R4F Acryl 2.3 µg/cig 2R4F Acet 4.7 µg/cig
Serban C. et al	J. Chromatogr. (2011) Vol.49	LC-MS/MS Extract in water/Methanol	3R4F Acryl 0.95 µg/cig
Roemer E. et al	Beiträge (2012) Vol.25	GC-MS Extract in Acetone	3R4F Acryl 1.30 µg/cig 3R4F Acet 4.46 µg/cig
Joza P. et al	CORESTA 2012 Congress	GC-MS Extract in Methanol	3R4F Acryl 1.37 µg/cig 3R4F Acet 3.02 µg/cig

METHOD DEVELOPMENT

Extraction Solution Selection

Initial results obtained from extracting a 44mm Cambridge filter pad containing the particulate matter from 5 3R4F cigarettes. ISO smoking. n = 4

Extraction Solvent	Acetamide µg/cig	Acrylamide µg/cig
Water	2.96	1.37
0.1% Formic Acid	3.20	1.39
0.1% Acetic Acid	2.94	1.28
90/10 0.1% Formic/MeOH	3.05	1.32
90/10 Water/MeOH	2.88	1.29

METHOD DEVELOPMENT

Acetamide stability – 2 day study

44mm Cambridge filter pad containing the particulate matter from 5 3R4F cigarettes. ISO smoking. n = 4. Samples refrigerated.

Extraction Solvent	Acetamide µg/cig Day 0	Acetamide µg/cig Day 1	Acetamide µg/cig Day 2	Overall increase %
90/10 0.1% Formic/MeOH	3.05	3.41	3.63	+18.8
90/10 Water/MeOH	2.88	3.01	3.22	+11.8

METHOD DEVELOPMENT

Acetamide stability – 6 day study

44mm Cambridge filter pad containing the particulate matter from 5 3R4F cigarettes. ISO smoking. n = 4. Samples refrigerated.

Extraction Solvent	Acetamide µg/cig Day 0	Acetamide µg/cig Day 4	Acetamide µg/cig Day 5	Acetamide µg/cig Day 6	Overall Increase %
Water	2.96	3.24	3.72	3.53	+19.3
0.1% Formic Acid	3.20	3.05	3.40	4.13	+29.3
0.1% Acetic Acid	2.94	2.88	3.01	3.85	+30.8

METHODOLOGY

Sample Preparation and Pad Extraction

Linear Smoking: ISO (5 cigs) INT (3 cigs)



Collect smoke condensate on 44mm pad



Add Deuterated (d_3) Isotopes to pad



Shake Pad with 20ml DCM

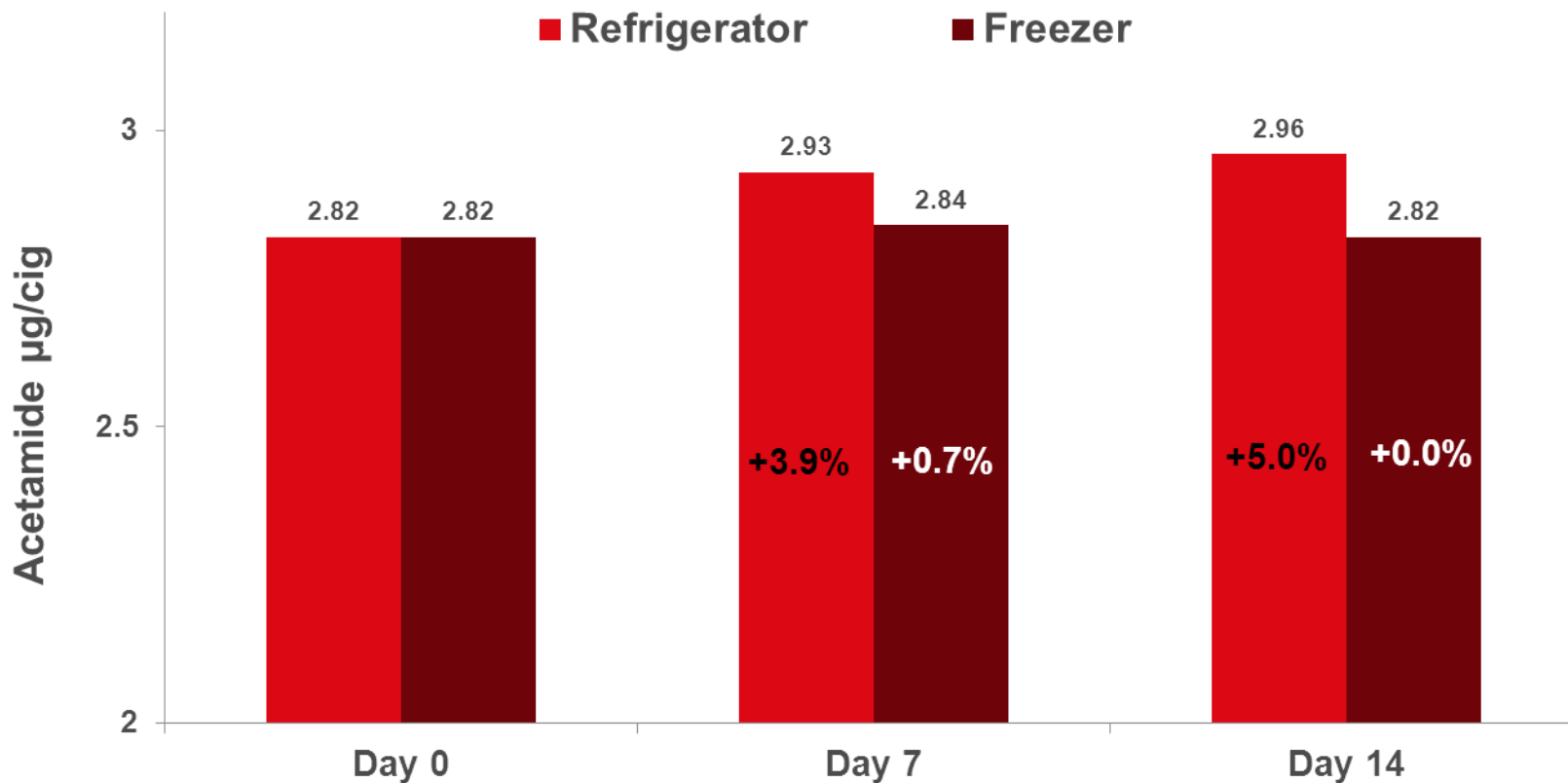


Shake DCM with 20ml water (1 min)



METHOD DEVELOPMENT

Acetamide stability – water/DCM extraction
14 day study graphic



No significant generation of Acetamide over 14 days

Chart source:

METHODOLOGY

Instrumentation: LC

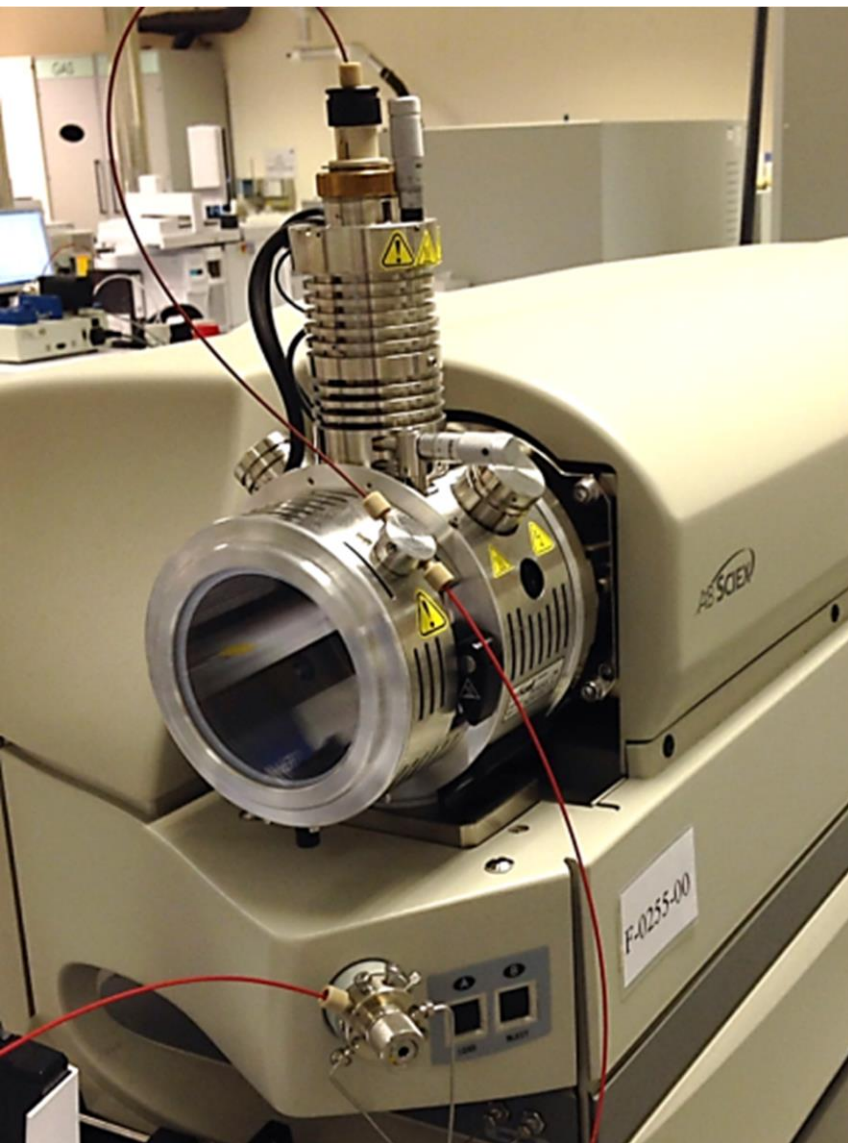


Agilent 1200 HPLC

Column	SIELC Primesep 200 150 x 2mm
Eluent	0.05% Formic Isocratic
Flow	250μlmin⁻¹
Column temp	25°C
Injection vol.	10μl

METHODOLOGY

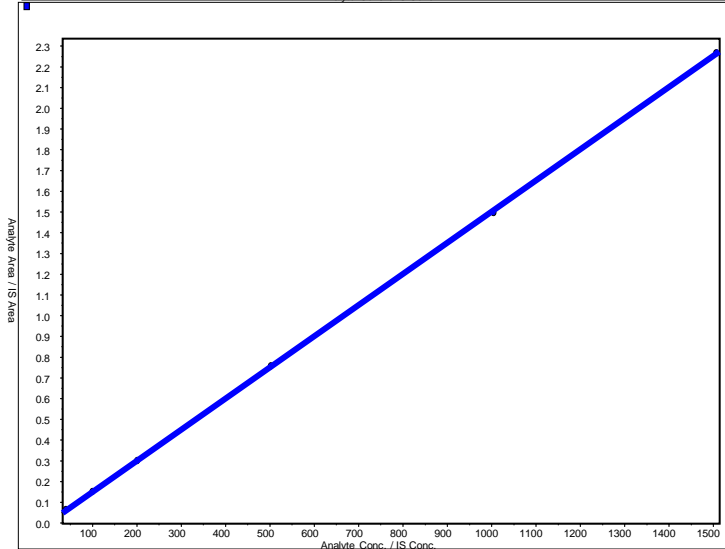
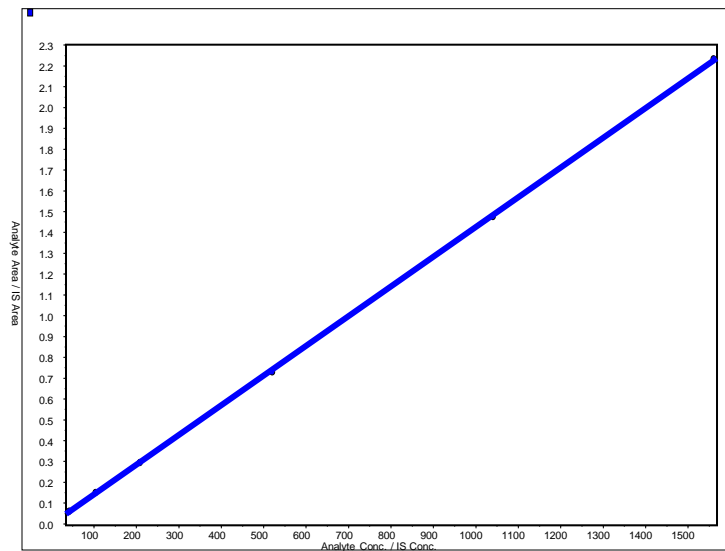
Instrumentation: MS



AB Sciex API 3200 MS/MS		
Ionisation mode	Positive ESI	
Spray Temp	400°C	
Analyte	Parent ion (m/z)	Daughter ion (m/z)
Acetamide	60.0	43.0
Acrylamide	72.0	55.0
Acetamide d₃	63.0	46.0
Acrylamide d₃	75.1	58.1

METHOD PERFORMANCE CHARACTERISTICS

Range and Linearity



Acetamide	
Linear range (ng/ml)	0 - 1500
Linear range ISO (µg/cig)	0.17 – 6.00
Linear range HCl (µg/cig)	0.33 – 6.00
R²	1.0000

Acrylamide	
Linear range (ng/ml)	0 - 1500
Linear range ISO (µg/cig)	0.15 – 6.00
Linear range HCl (µg/cig)	0.27 – 6.00
R²	1.0000

METHOD PERFORMANCE CHARACTERISTICS

Recovery

Analyte	Units	Mainstream (ISO)		Mainstream (HCI)	
		Level 1 (ng added)	Level 2 (ng added)	Level 1 (ng added)	Level 2 (ng added)
	ng	8	16	16	32
Acetamide	%	92.5	98.6	91.2	99.1
Acrylamide	%	103.1	105.0	96.1	100.2

Calculated from spiked 3R4F extracts, n= 10

Level 1 equivalent to 50% concentration of analytes found in 3R4F

Level 2 equivalent to 100% concentration of analytes found in 3R4F

METHOD PERFORMANCE CHARACTERISTICS

Mean and Sensitivity: Kentucky reference 3R4F

Analyte	Units	Mainstream (ISO)			Mainstream (HCI)		
		Mean	LOQ $\mu\text{g}/\text{cig}$	n	Mean	LOQ $\mu\text{g}/\text{cig}$	n
Acetamide	$\mu\text{g}/\text{cig}$	2.95	0.172	39	12.09	0.323	39
Acrylamide	$\mu\text{g}/\text{cig}$	1.34	0.150	39	4.39	0.267	39

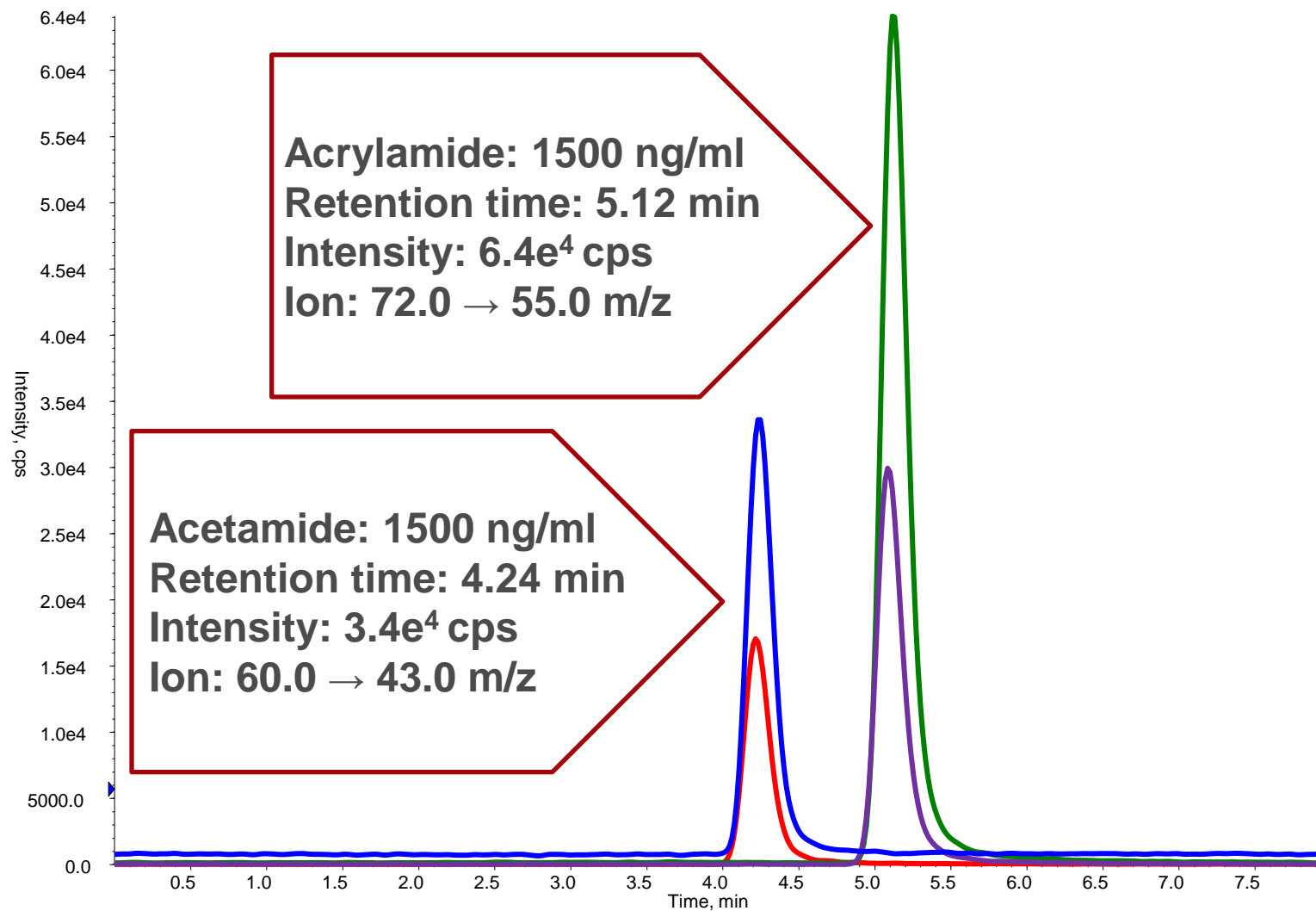
3R4F RESULTS COMPARISON

MAINSTREAM SMOKING

Author	Journal	Technique	Acetamide µg/cig		Acrylamide µg/cig	
			ISO	HCI	ISO	HCI
Roemer E. et al	Beiträge (2012) Vol.25	GC-MS	4.46	15.2	1.37	
Joza P. et al	CORESTA 2012 Congress	GC-MS	3.02	12.9	1.30	3.88
Essentra	TSRC 2014	LC-MS/MS	2.95	12.1	1.34	4.39

CHROMATOGRAMS

CALIBRATION STANDARDS

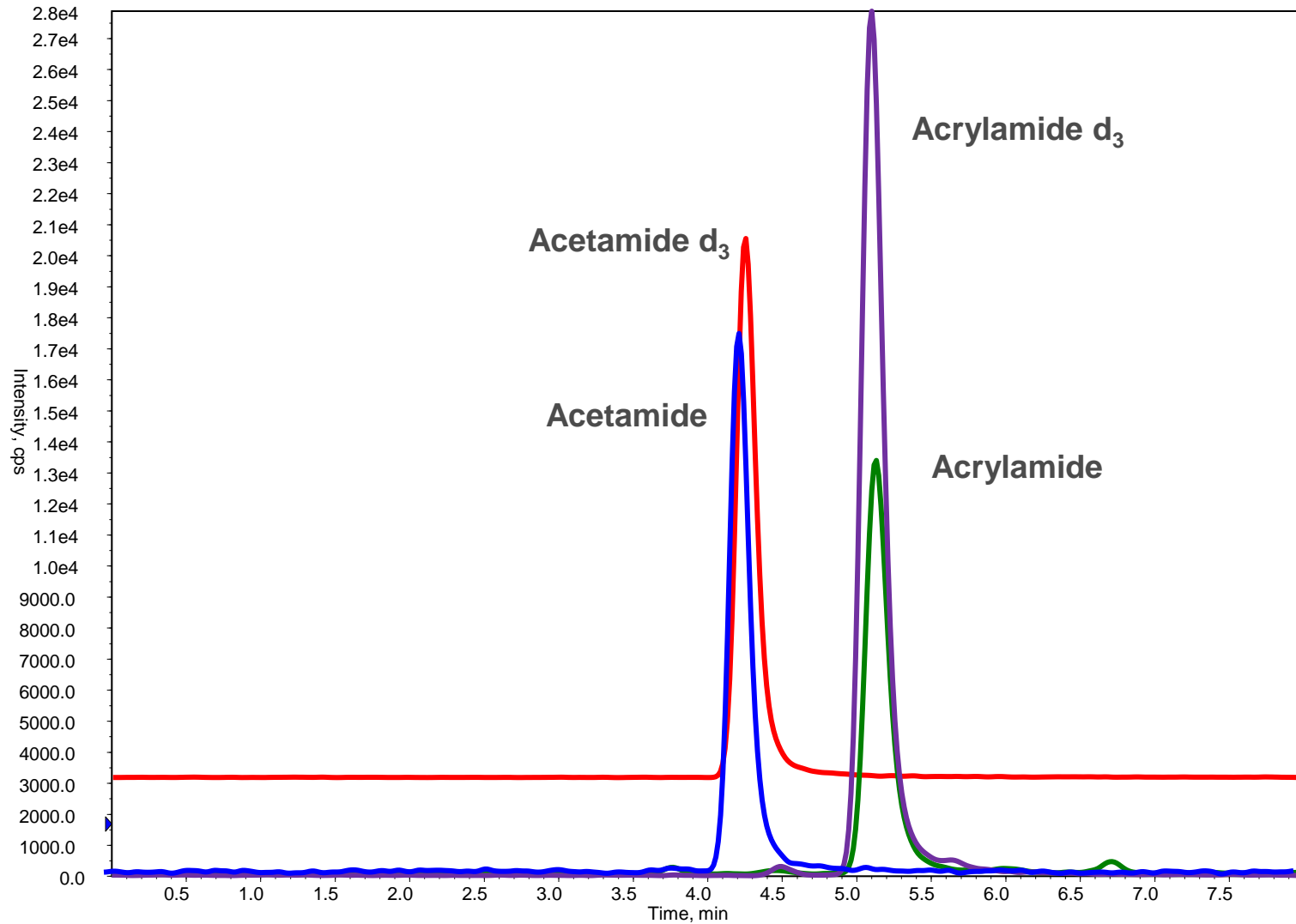


Acrylamide: 1500 ng/ml
Retention time: 5.12 min
Intensity: 6.4e⁴ cps
Ion: 72.0 → 55.0 m/z

Acetamide: 1500 ng/ml
Retention time: 4.24 min
Intensity: 3.4e⁴ cps
Ion: 60.0 → 43.0 m/z

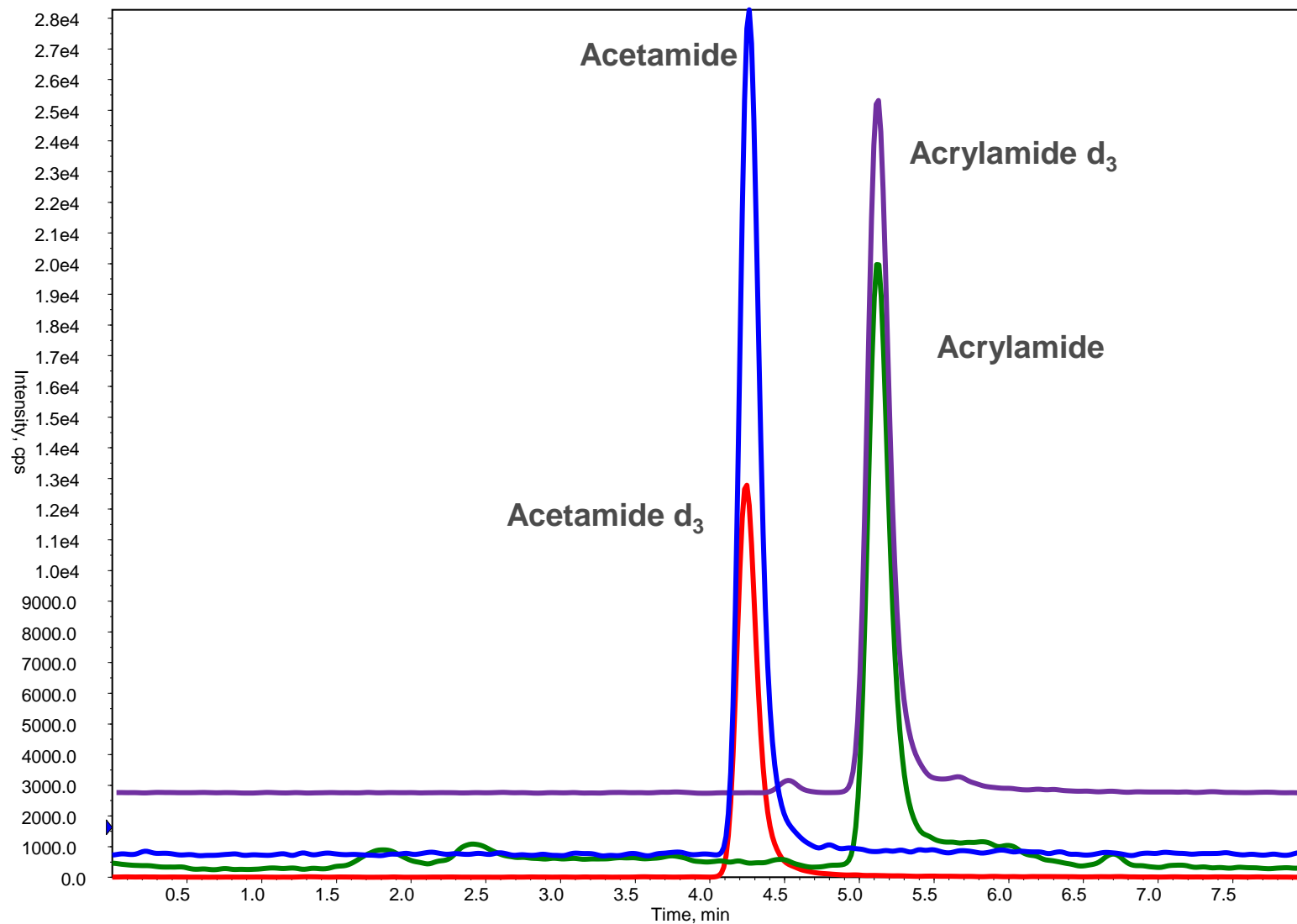
CHROMATOGRAMS

KENTUCKY 3R4F ISO SMOKING








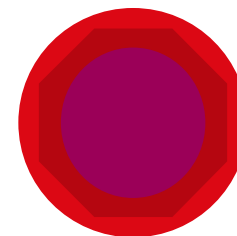
CHROMATOGRAMS

KENTUCKY 3R4F INT SMOKING



METHOD OBJECTIVES

- Develop and validate an LC-MS/MS method for both compounds 
ISO17025 accredited by UKAS
- Simple extraction (no impingers or SPE) 
Smoke/Spike/Shake
- Selective 
MS/MS; No interfering peaks in smoke chromatograms
- Sensitive 
<0.2µg/cig
- Fast run time 
8 min run time



OTHER APPLICATIONS



SIDESTREAM SMOKING

ratio to mainstream smoking?



E-CIGARETTE EMISSIONS

rate of analyte transfer?

puffing e-cig to exhaustion?

SIDESTREAM SMOKING

Sample Preparation and Pad Extraction

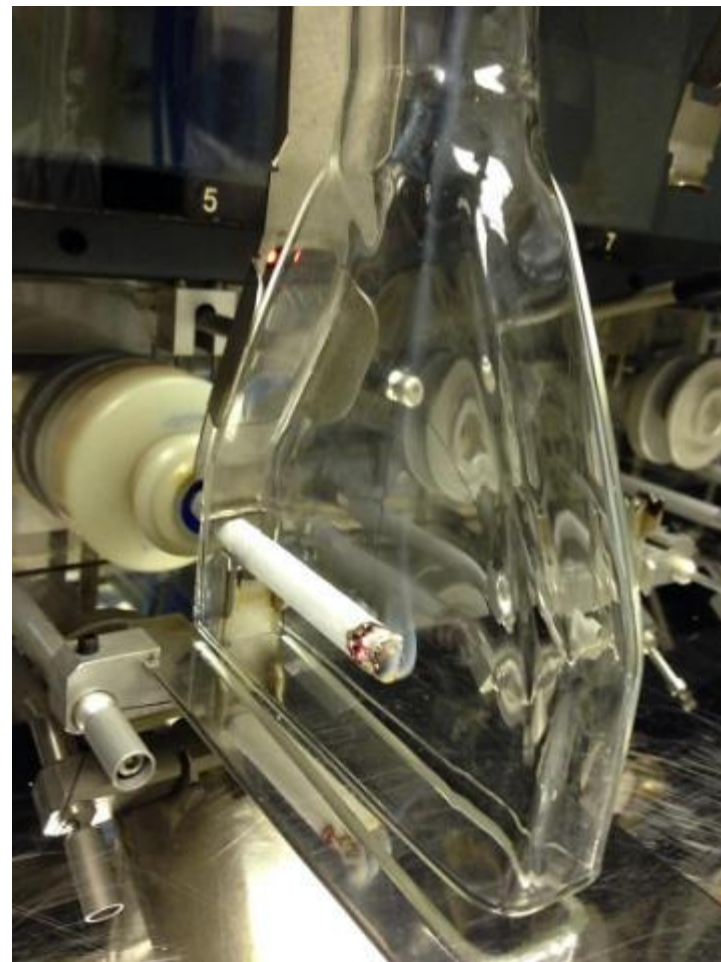
Sidestream Smoking: 3 cigs



Rinse chimney with 40ml DCM, add pad
Shake for 30 min



Shake DCM extract with 40ml water
Dilute aqueous phase



SIDESTREAM SMOKING

Kentucky reference 3R4F: Small study

Analyte	Units	Sidestream			
		Mean	SD (CoV %)	n	Ratio SS : MS
Acetamide	µg/cig	119.8	4.5 (3.8)	18	40:1
Acrylamide	µg/cig	25.0	1.3 (5.2)	18	19:1

E-CIGARETTE AEROSOL

Sample Preparation and Pad Extraction

Spiked E-liquid added to cartomizer



55ml puff volume, 3s duration, 2 puffs per min, square wave



50 puff blocks - to exhaustion



Collect condensate on 44mm pad



Prepare samples as for mainstream smoking



E-CIGARETTE

SPIKED CARTOMIZER STUDY n = 4

Puff Block	% Transferred		
	Acetamide	Acrylamide	TPM
0-50	27.8	27.0	27.4
51-100	23.4	24.0	24.4
101-150	22.2	23.2	23.5
150-200	11.0	11.2	11.8
201-250	3.5	3.7	4.1
251-300	0.8	0.6	0.8
301-350	0.1	0.3	0.2
Total	88.8	90.0	92.2



ESSENTRA

THANK YOU

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