APPLICATIONS OF GC TANDEM MASS SPECTROMETRY TO THE ANALYSIS OF CHEMICAL CONSTITUENTS IN MAINSTREAM CIGARETTE SMOKE

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Analysis of HPHCs

Instruments for high throughput laboratories

- Fewest types of instruments possible
- Common software platform across all instruments
- Appropriate level of technology for each target
- Robust instruments, low cost of ownership

Instrumentation Options

For analysis of Hoffmann/HPHC Analytes

- Ion Chromatography: Ammonia, Nitrate
- GC (FID & TCD): Nicotine and Water
- HPLC: Phenols and carbonyl compounds

What about everything else?

Is mass spectrometry the answer?

Instrumentation Options

Mass Spectrometry

LC-Single Quadrupole: Low cost, not suited for smoke analysis.

GC-Single Quadrupole: Low cost, industry standard for complex samples (SIM), long run times.

LC- Triple Quadrupole (Tandem): High cost, great for complex samples, limited to TSNAs.

GC- Triple Quadrupole (Tandem) Medium cost, great for complex samples, TSNAs, Volatile nitrosamines plus all standard GC-MS methods.

Advantages of GC-MS/MS

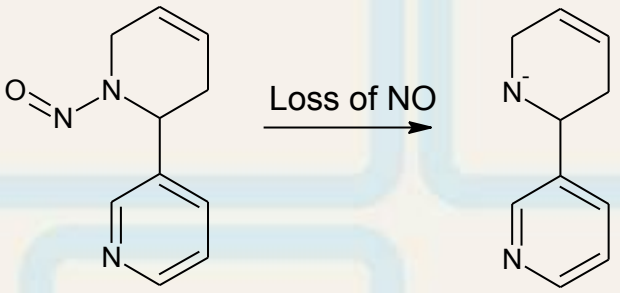
- Large number of compounds can be analyzed on a single instrument.
- Can be operated in single quadrupole mode for compatibility with SIM methods.

Compounds we analyze:

VOCs, Semi-volatiles, TSNAs, volatile nitrosamines, aromatic amines, benzo[a]pryrene, PAH compounds, over 50% of the FDA full HPHC list on a single instrument

Tandem Mass Spectrometry

- Improves both method detection limit and compound identification
- Commonly used for analysis of samples in difficult matrix, biological fluids, agricultural products and tobacco.
- Tandem mass spectrometry has not been widely used for the analysis of “Hoffmann” or HPHC analytes.



M/Z = 278

M/Z = 248

Compound is separated based on mass and then further fragmented in the instrument. After fragmentation, only compounds that lose a pre-determined amount are quantitated.

Tandem Mass Spectrometry

- Equipment is readily available from several vendors
- Newer instrumentation was designed for use with gas phase chromatography.
- GC-friendly instruments have heated quadrupoles, low mass ranges and are easily connected to existing gas chromatographs.
- Similar design to single quadrupole instruments, reduced operator training and common consumables.

Instrumentation

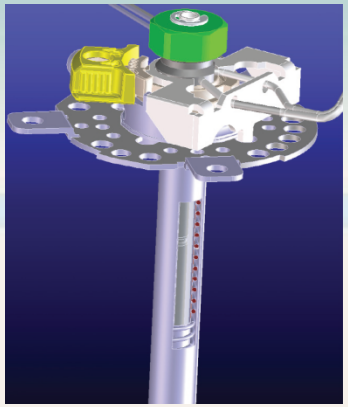


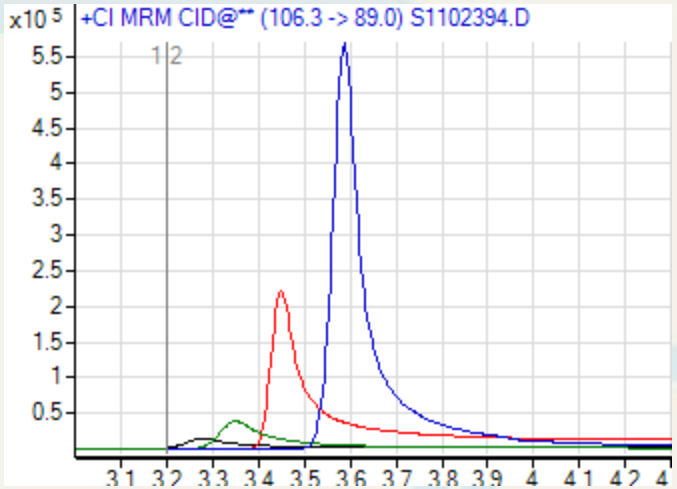
Image: agilent.com

Agilent 7890 GC and 7000 qqq
Mass range to 1000 m/z
Chemical and Electron Impact ionization
Fast GC oven, ramp speeds of >100°C per minute

Multi-mode inlet (PTV) operated in solvent vent mode allows for the injection of large volume samples (5 to 50μL)

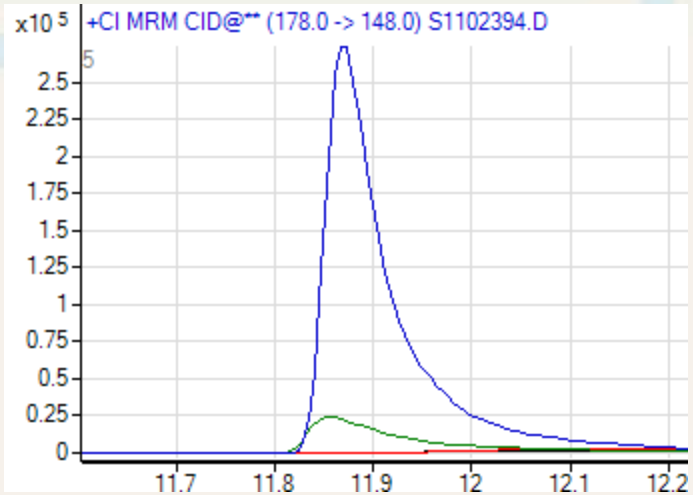
Instrumentation

Multi-mode inlet (PTV) operated in solvent vent mode greatly increases signal-to-noise



N-Nitrosomethylethylamine

>1000X increase in S/N over a split injection



NNK

black - 2:1 pulsed split at 275C, 5uL
green - PTV starting at -5C, 1uL
red - pulsed splitless at 275C, 5uL
blue - PTV starting at -5C, 5uL (TSNA method)

N-Nitrosamines

LC-MS/MS Method

- Samples extracted in aqueous solution containing internal standards.
- Linear calibration range of ~0.5ng/cigt to 200 ng/cigt.
- Deuterated compounds used for all target compounds.
- Limited to 4 TSNAs.

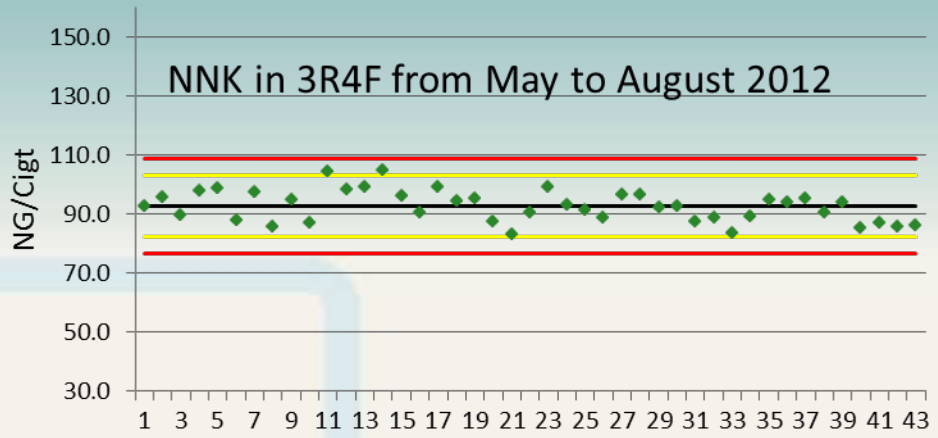
Enthalpy's GC-MS/MS

- Same extraction procedure as LC-MS/MS method.
- Same standards and MS/MS transitions as LC-MS/MS method.
- Samples and standards solvent exchanged to methylene chloride before analysis.
- Expanded compound list, 10 nitrosamines.

N-Nitrosamines

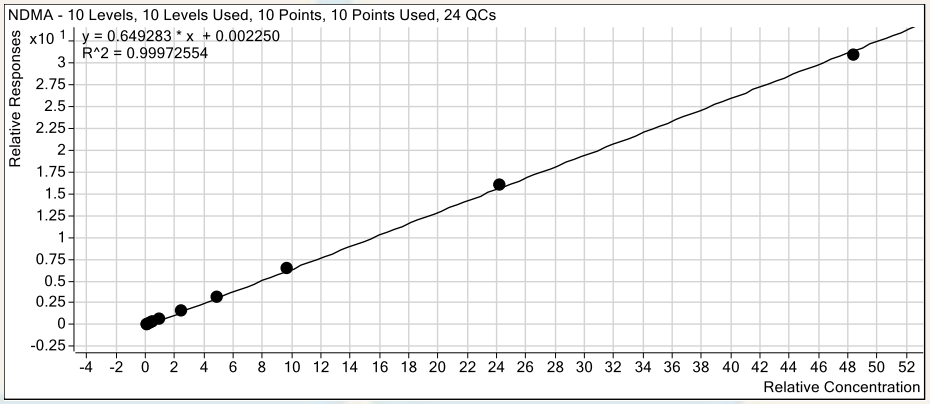
- Robust GC-MS/MS method for the routine analysis of nitrosamines in tobacco and tobacco smoke.
- Little or no matrix effects, same LOD for all samples.
- Our method allows for the quantitative determination of the 4 TSNA + 6 other N-nitrosamines in ~12 minutes.
- Semi-qualitative detection of N-nitrosodiethanolamine (NDELA) and N-nitrososarcosine (NSAR).

Nitrosamines-Method Performance



NNK in mainstream smoke

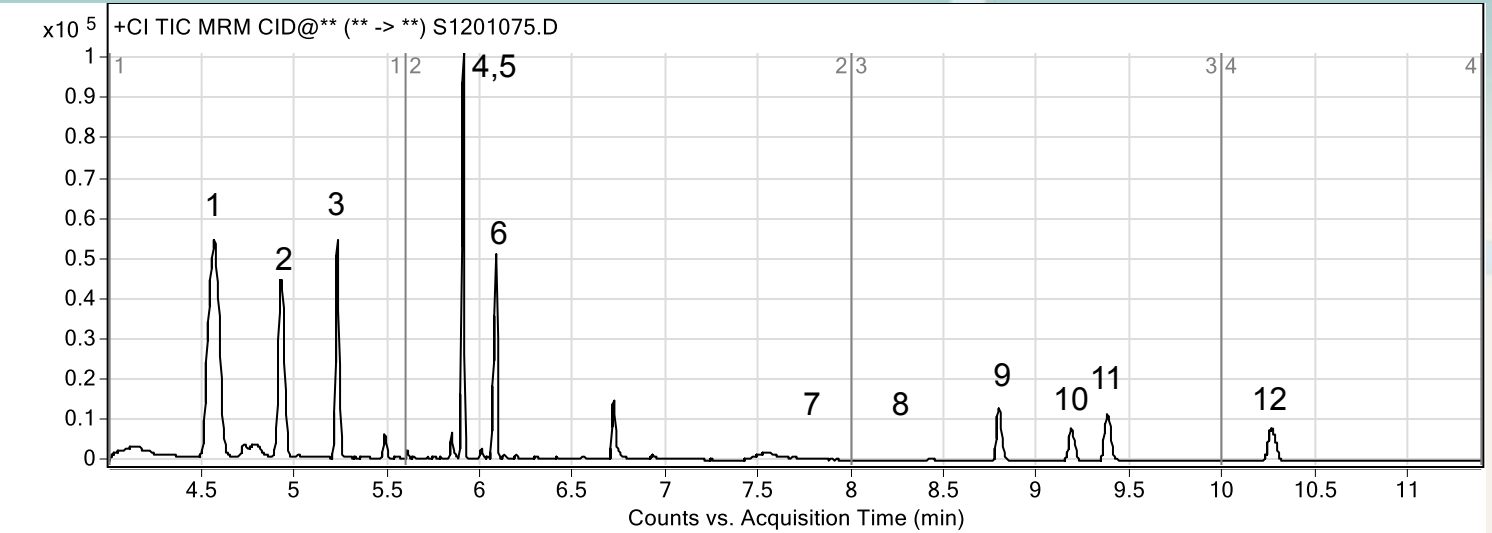
Compound Name	LOD ng/ml	LOD ng/gram	LOD ng/cigt.
NDMA	0.28	2.80	0.56
NMEA	0.11	1.10	0.22
NDEA	0.15	1.53	0.31
NMOR	0.16	1.59	0.32
NPYR	0.13	1.31	0.26
NPIP	0.17	1.68	0.34



Compound Name	LOD ng/ml	LOD ng/gram	LOD ng/cigt.
NNN	0.20	1.99	0.40
NAB	0.32	3.20	0.64
NAT	0.26	2.60	0.52
NNK	0.21	2.10	0.42

The method is linear for all compounds from ~1 ng/ml to 500 ng/ml.

Summary of Analytical Method

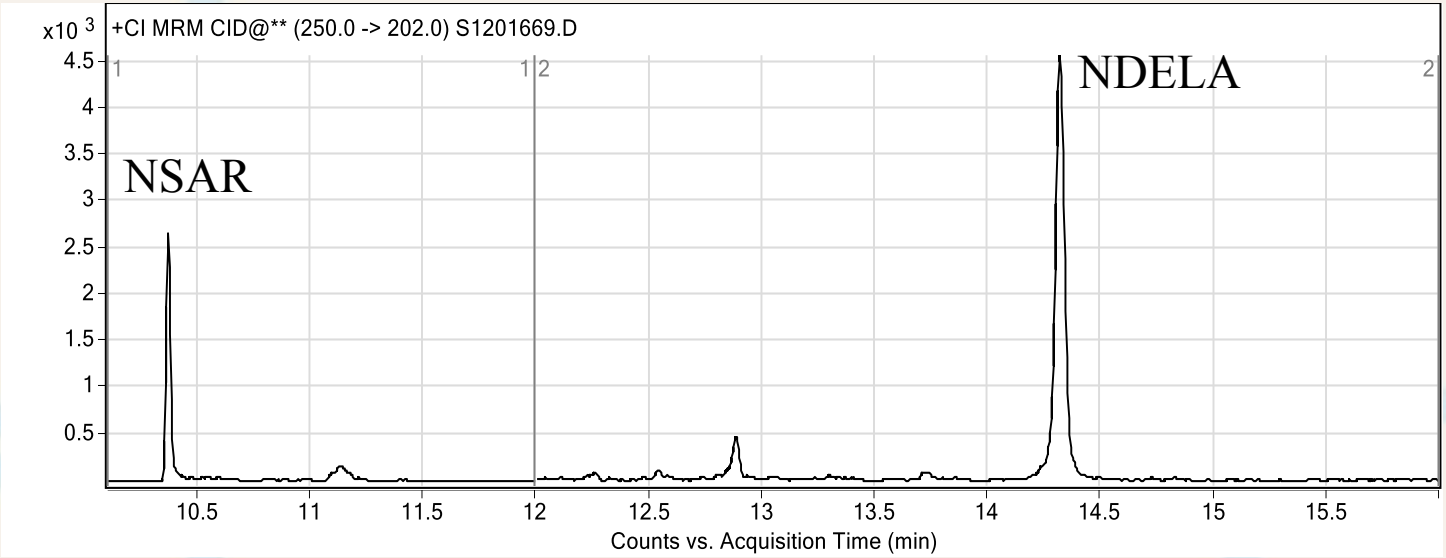


Peak #	Compound	Retention Time, min
1	NDMA	4.58
2	NMEA	4.93
3	NDEA	5.23
4	NPYR	5.91
5	NMOR	5.91
6	NPIP	6.09

Peak #	Compound	Retention Time, min
7	NSAR	ND
8	NDELA	ND
9	NNN	8.81
10	NAT	9.12
11	NAB	9.39
12	NNK	10.28

Nitrosamines-Derivatization

- General method for TSNAs and volatile nitrosamines did not produce suitable peaks for quantitation of NDELA and NSAR.
- Derivatization of sample extracts using BSTFA resulted in clearly-defined peaks.
- Standards for smokeless tobacco analysis were prepared in CRP-2 tobacco to negate matrix effects that precluded use of solvent standards.



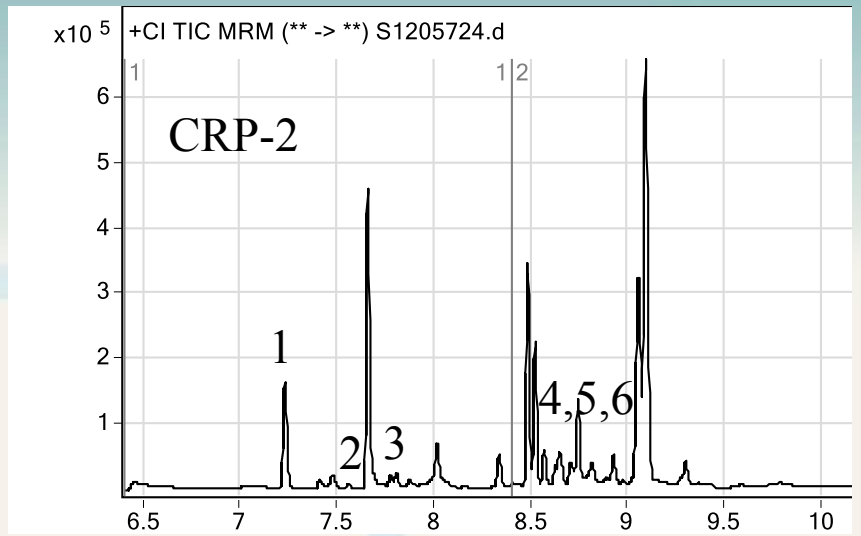
Standard in CRP-2 tobacco at 10 ng/mL

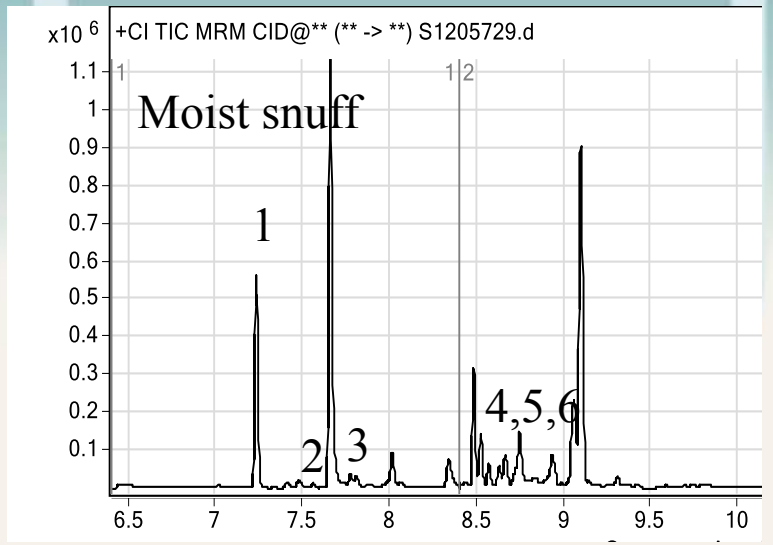
Volatile N-Nitrosamines

- Lower limits of detection are possible for volatile nitrosamines.
- Multi-mode inlet (PTV) operated in solvent vent mode allows for the injection of 25µL.
- Compounds are separated on thick film 624 column which gives superior separation for volatile nitrosamines.
- This method allows for confirmation of "hits" in samples.

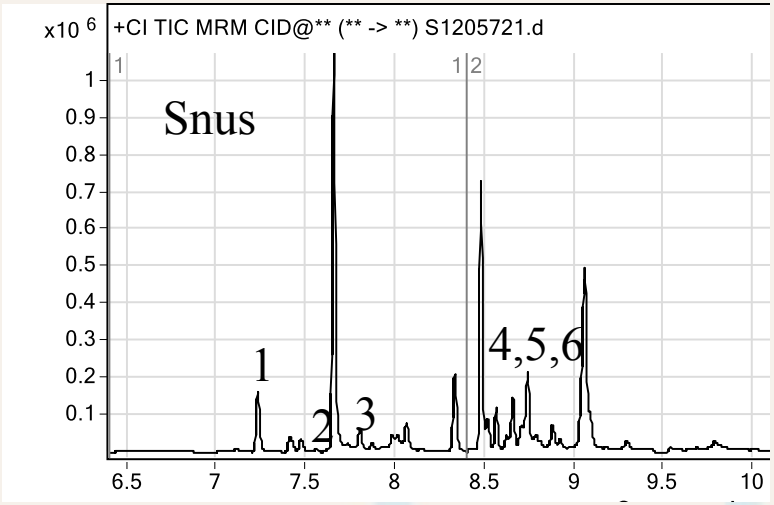
Compound	LOQ, ng/mL	LOD, ng/g Tobacco	LOD, ng/cig ISO
NDMA	0.0213	0.213	0.043
DMEA	0.0212	0.212	0.042
NDEA	0.0211	0.211	0.042
NMOR	0.0592	0.592	0.118
NPYR	0.105	1.05	0.210
NPIP	0.211	2.11	0.421

Volatile N-Nitrosamines





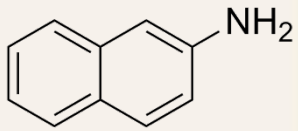
Peak #	Compound	RT, min	CRP-2	Snuff	Snus
1	NDMA	7.24	1.1	1.7	<0.2
2	NMEA	7.61	<0.2	<0.2	<0.2
3	NDEA	7.89	12	12	16
4	NMOR	8.58	<0.2	<0.2	<0.2
5	NPYR	8.63	14	19	5
6	NPIP	8.76	37	26	20



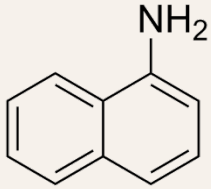
Results in ng/gram as received

Aromatic Amines

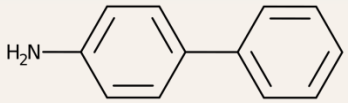
Analysis of primary and secondary amines after derivatization with heptafluorobutyric anhydride



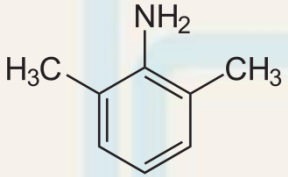
2-Aminonaphthalene



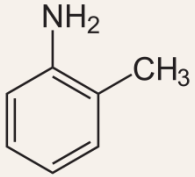
1-Aminonaphthalene



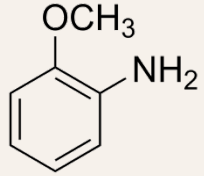
4-Aminobiphenyl



2,6-Dimethylaniline



o-Toluidine

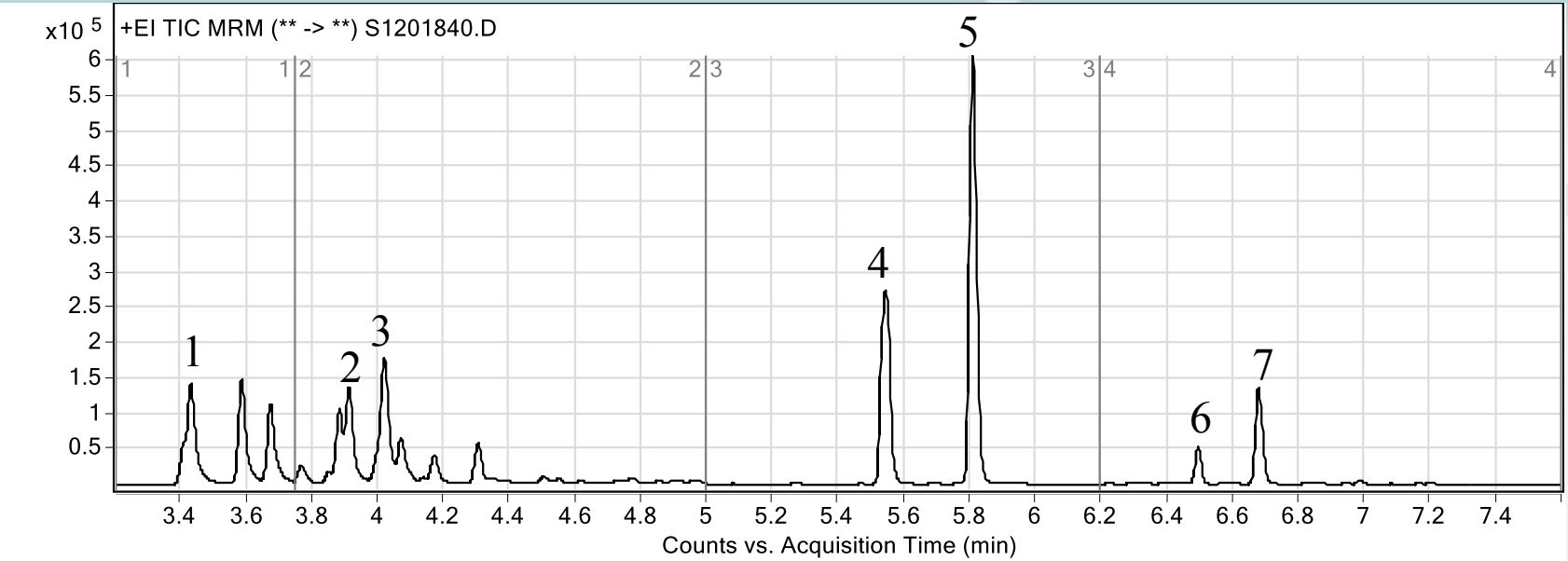


o-Anisidine

Tandem mass spectrometry allowed us to add three compounds to the target list without increasing run time.

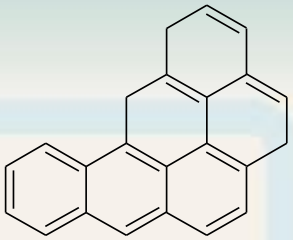
British American Tobacco Group Research & Development Method: "Determination of aromatic amines in mainstream cigarette smoke." March 31, 2008.

Aromatic Amines

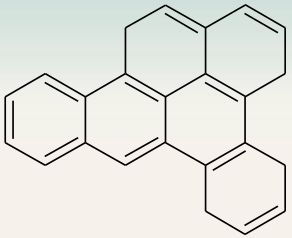


Peak #	Compound	3R4F-ISO ng/cig	LOD, ng/cig
1	*o* -Toluidine	45.2	0.036
2	2,6-Dimethylaniline	19.6	0.043
3	*o* -Anisidine	3.22	0.020
4	1-Aminonaphthalene	8.86	0.020
5	2-Aminonaphthalene	5.41	0.0076
6	3-Aminobiphenyl	1.44	0.0059
7	4-Aminobiphenyl	0.943	0.0047

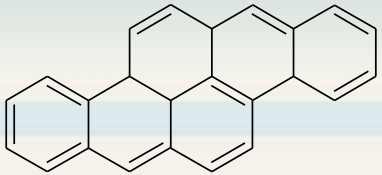
Large Ring PAHs



Dibenzo[a,l]pyrene

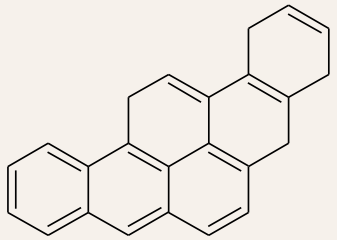


Dibenzo[a,e]pyrene



Dibenzo[a,h]pyrene

Poly-aromatic compounds are known to be present in cigarette smoke. Concentration of each compound is very low (<1ng/cigt.) under Canadian Intense smoking. These compounds are of regulatory concern since [a,h], [a,i] and [a,l] each have toxic equivalents 10X benzo[a]pyrene.



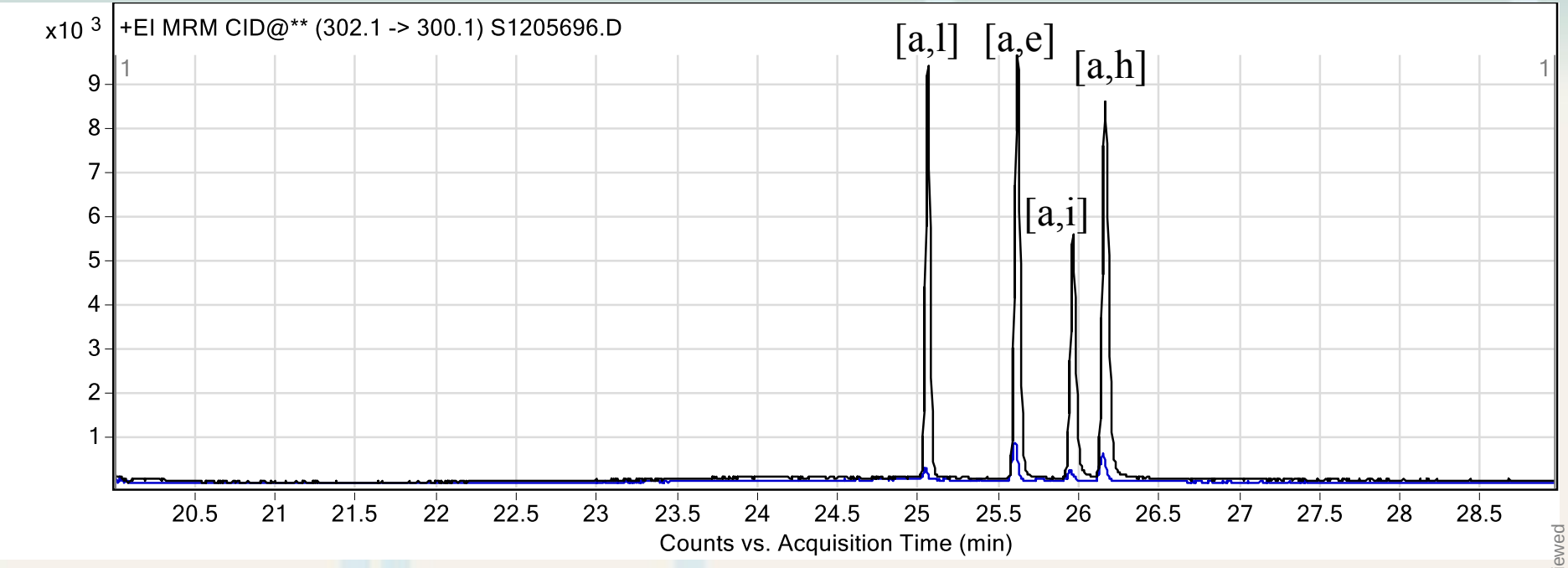
Dibenzo[a,i]pyrene

Large Ring PAHs

Method Summary

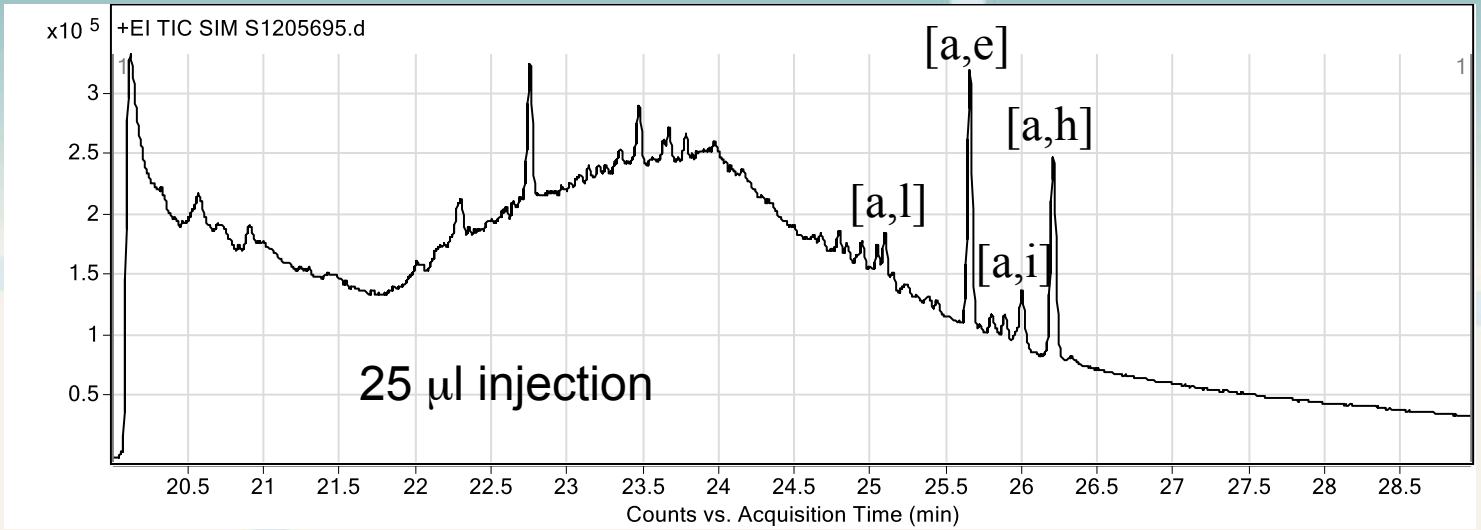
Extract pad in 20.0 mL of methanol.
Remove a 10mL aliquot of extract and add 15mL water
Add the sample to a conditioned C-18 SPE tube
Rinse the tubes with 10 mL 60/40% water/methanol solution and pull dry.
Elute with 15.0 mL of cyclohexane
Reduce cyclohexane with nitrogen evaporator to 1 mL final volume.

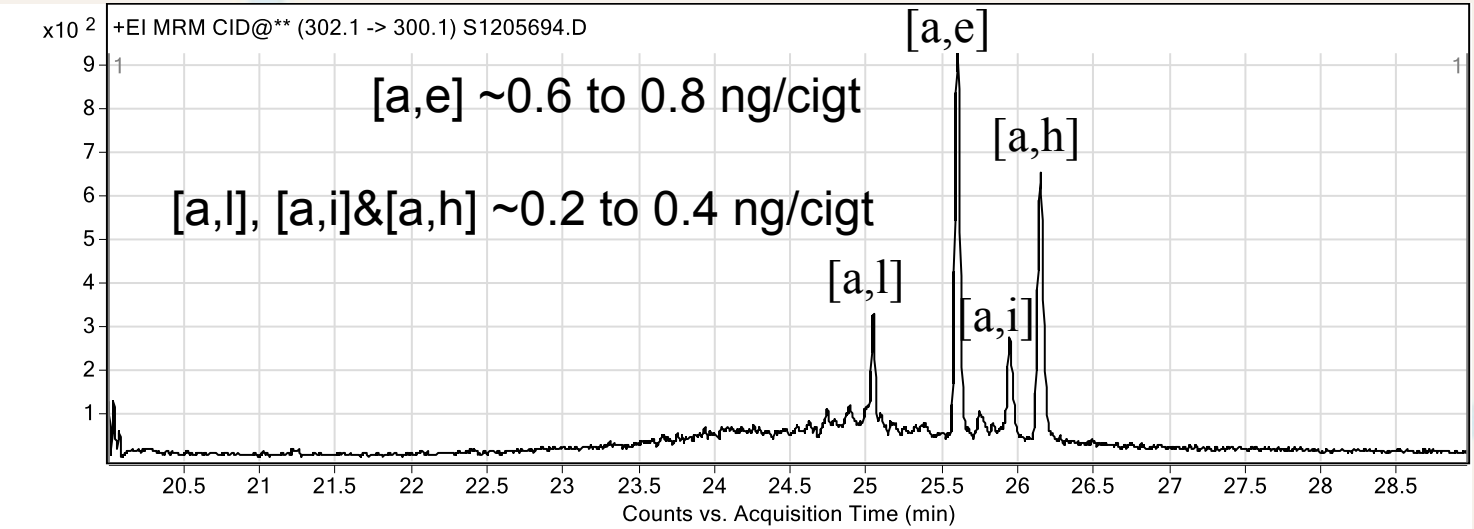
Large Ring PAHs



MRM chromatogram of 10 ng/mL standard (black) and 3R4F-Intense sample extract (blue)

Large Ring PAHs





SIM (m/z 302, top) and MRM (m/z 302→300, bottom) chromatograms of a 3R4F-Intense sample extract

Tandem Mass Spectrometry

- Analysis time can be greatly reduced when compared to SIM mass spectrometry.
- Secondary ions can be used to confirm compound identification.
- Matrix effects are reduced to an extent that allows for either faster analysis or less sample clean-up.
- Fewer instruments in the laboratory.
- Instrumentation is suitable for routine analysis.

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