DETERMINATION OF NINE VOLATILE NITROSAMINES AND HYDROXY-NITROSAMINES IN CIGARETTE FILLER AND MAINSTREAM TOBACCO SMOKE

Mehran SHARIFI, Peter JOZA, Bill RICKERT

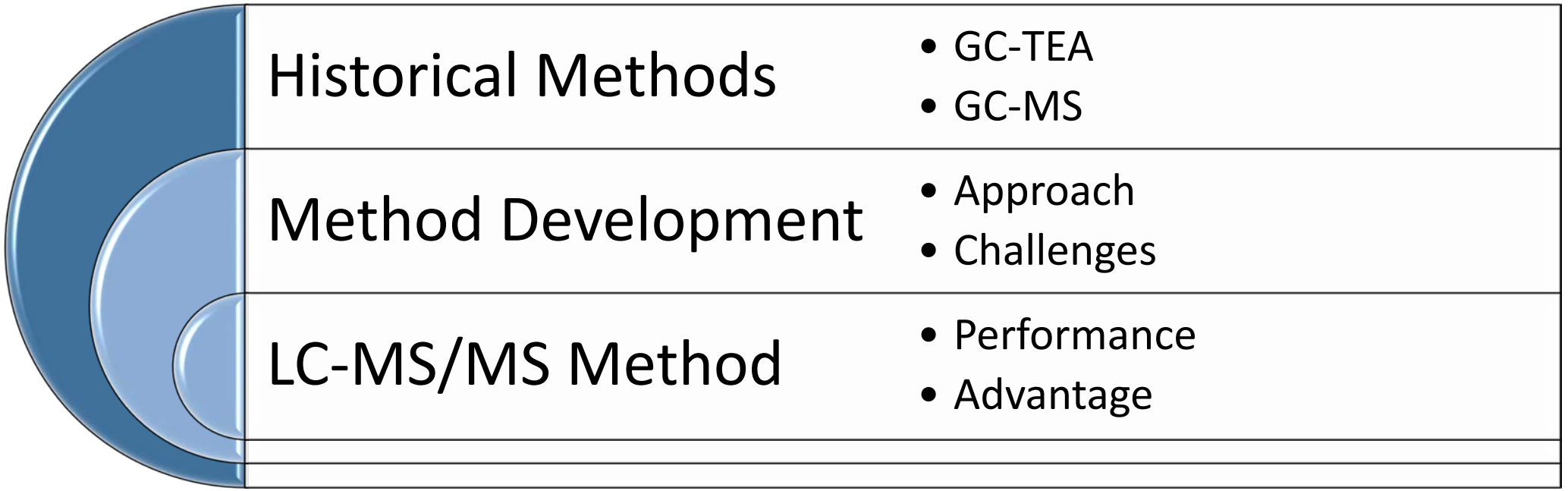
69th TOBACCO SCIENCE RESEARCH CONFERENCE
September 20-23, 2015
Naples, Florida, USA

LABSTAT INTERNATIONAL ULC.
262 Manitou Drive
Kitchener, Ontario, Canada N2C 1L3
Phone: (519) 748-5409 Fax: (519) 748-1654
www.labstat.com

Presentation no 28

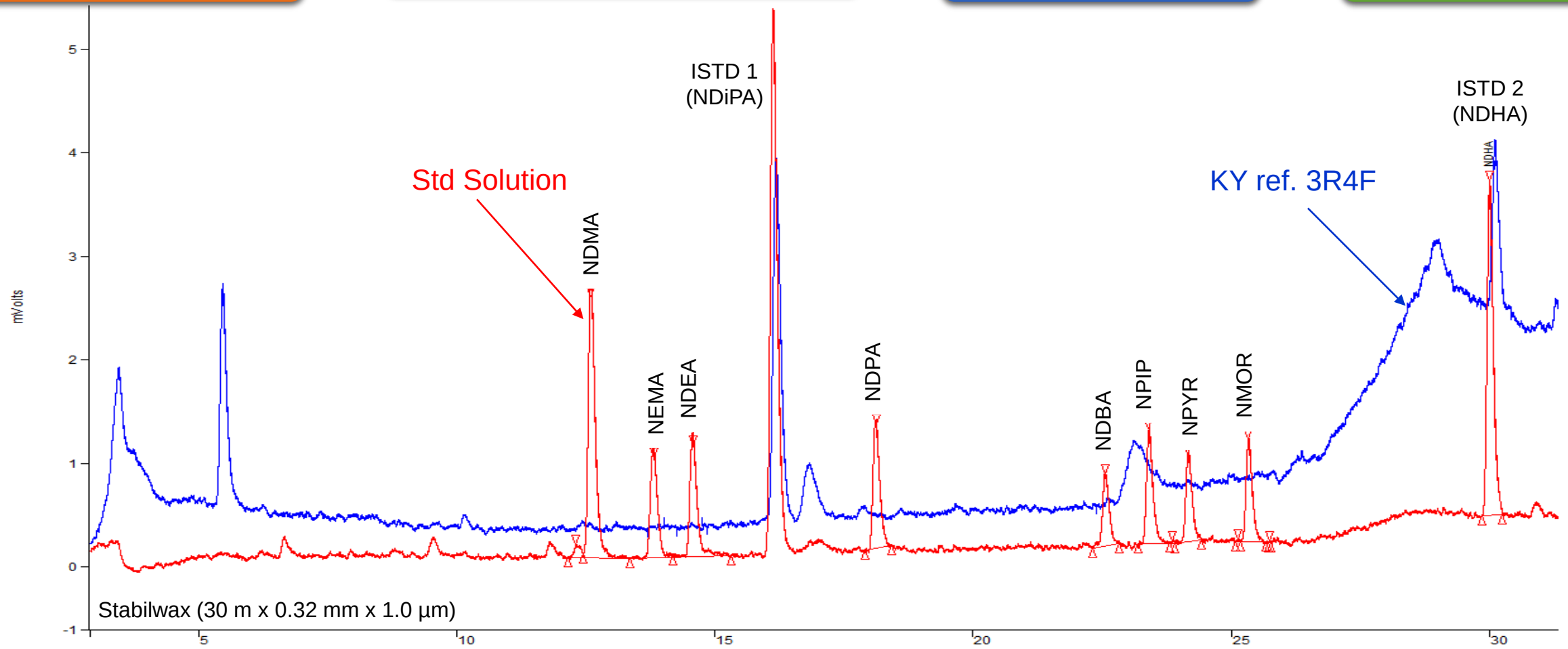
Objective

To develop a sensitive and specific method for quantitative analysis of volatiles and hydroxy-nitrosamines in cigarette filler and mainstream smoke.



Volatile Nitrosamines (Cigarette Filler)
Historical Approach - GC/TEA

3 g sample → Extract with Citrate-Phosphate Buffer + ISTD → LPE (Hydromatrix) → Concentrate



Volatile Nitrosamines (Mainstream) Historical Approach – GC/TEA

20 cigarettes (Citrate/Phosphate Buffer + Filter Pad)

Add ISTD to the Filter Pad

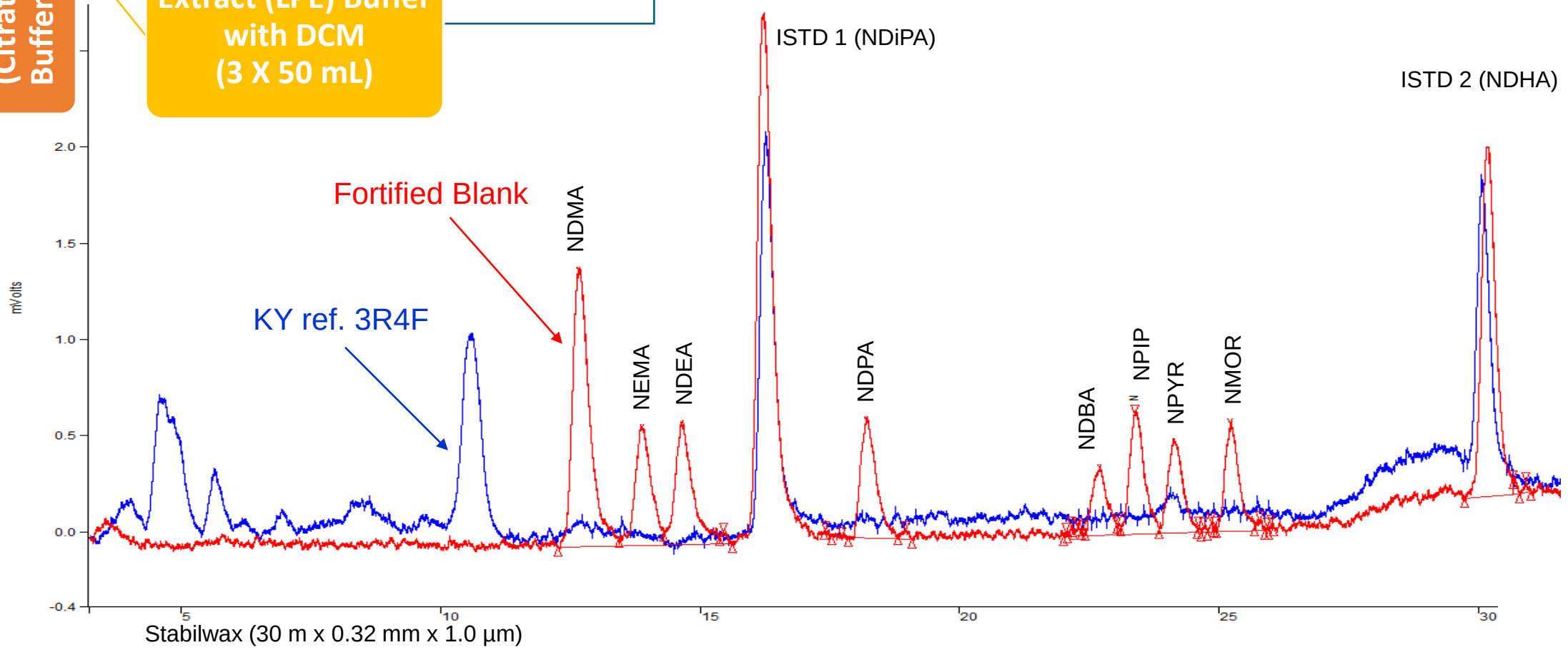
Extract (LPE) Buffer with DCM (3 X 50 mL)

Extract Filter Pad with DCM extract

- Filter
- Concentrate

Alumina Clean-up

Concentrate



Stabilwax (30 m x 0.32 mm x 1.0 µm)

N-Nitrosodialkanolamines
Recent Approach - GC/MS

Cigarette Filler

1 g sample
+ Internal Std (NDELA-d_8)

Extracted with 50 ml Water

Acidified with
$NH_4SO_3NH_2/H_2SO_4$

Mainstream Smoke

20 cigarettes
($NH_4SO_3NH_2/H_2SO_4$ Buffer)

Add Internal Standard
(NDELA-d_8)

What are the Drawbacks of Historical Methods?

GC-TEA (VNA)	Detector	Not as specific as Mass Spectrometric techniques
	Solvent (CH_2Cl_2)	Health risk, low vapour pressure Chlorinated waste disposal (environment) Inconvenient for GC polar column (frequent maintenance)

Method Development Approach / Challenges

Method Development - LC
Mobile Phase and Ionization Efficiency

Acetonitrile : Formic Acid (0.01%)

Acetonitrile : Ammonium Acetate (2mM)

Methanol : Ammonium Acetate (2mM)

Methanol : Formic Acid (0.01%)

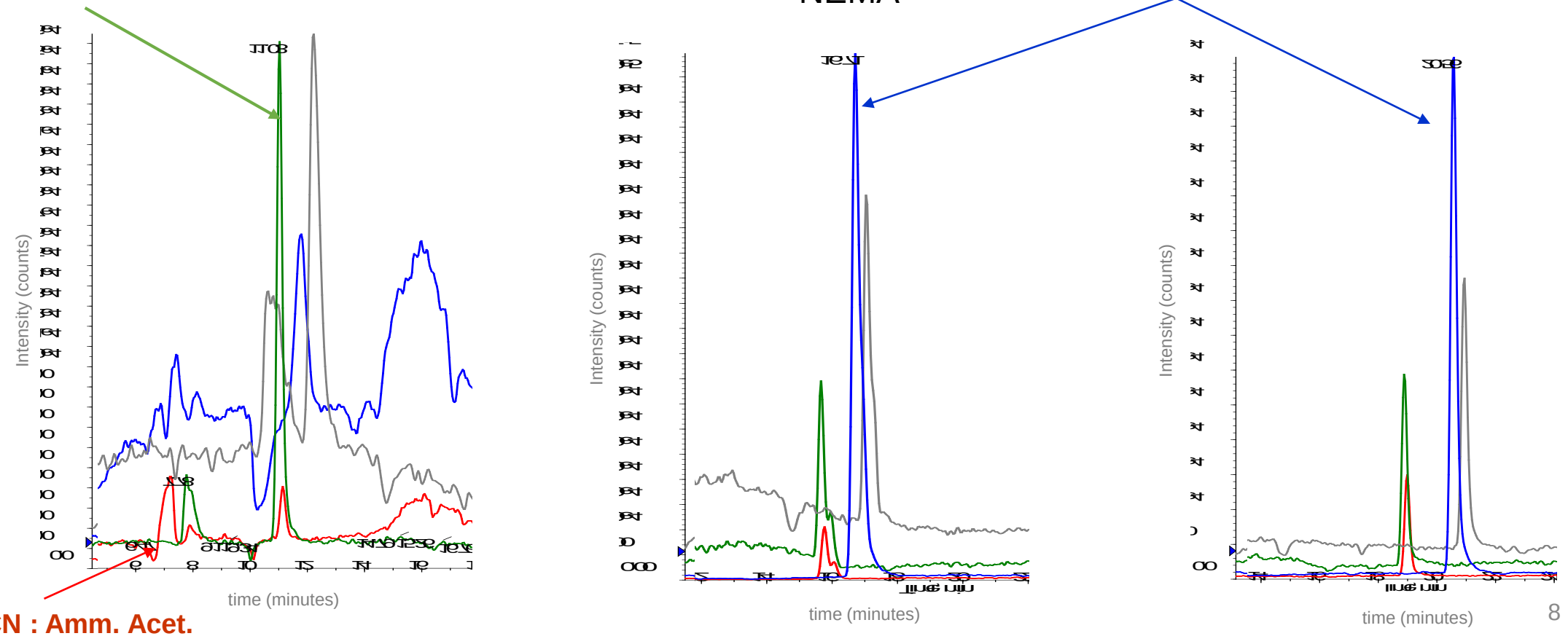
Acetonitrile : Formic Acid

NDMA

NEMA

Methanol : Ammonium Acetate

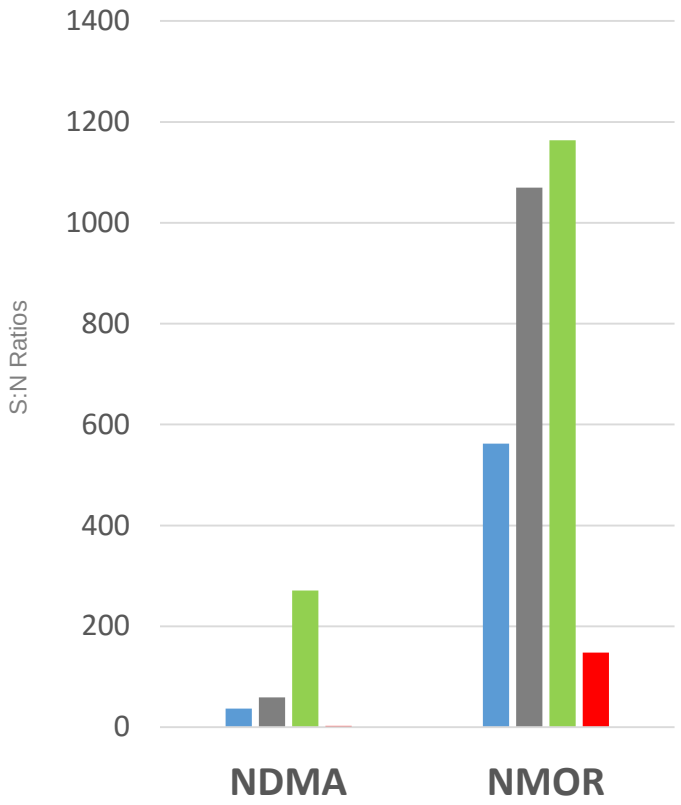
NDEA



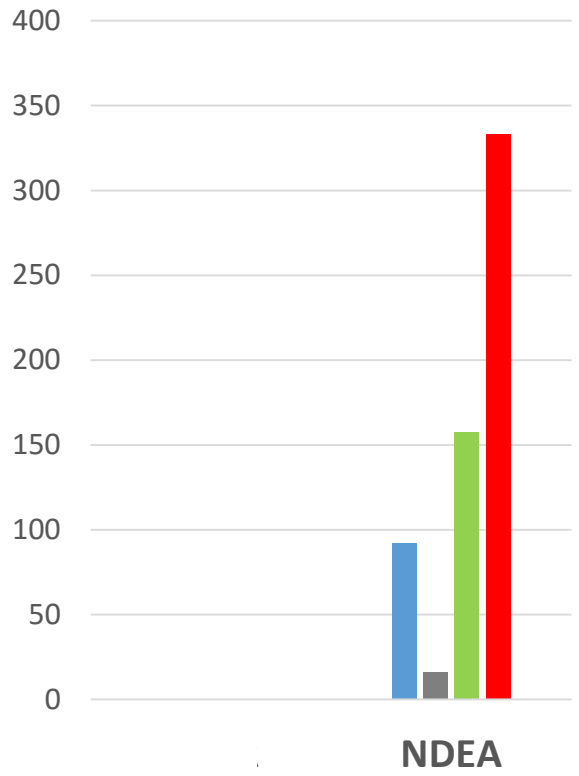
CH_3CN : Amm. Acet.

Method Development - LC
Mobile Phase and Ionization Efficiency

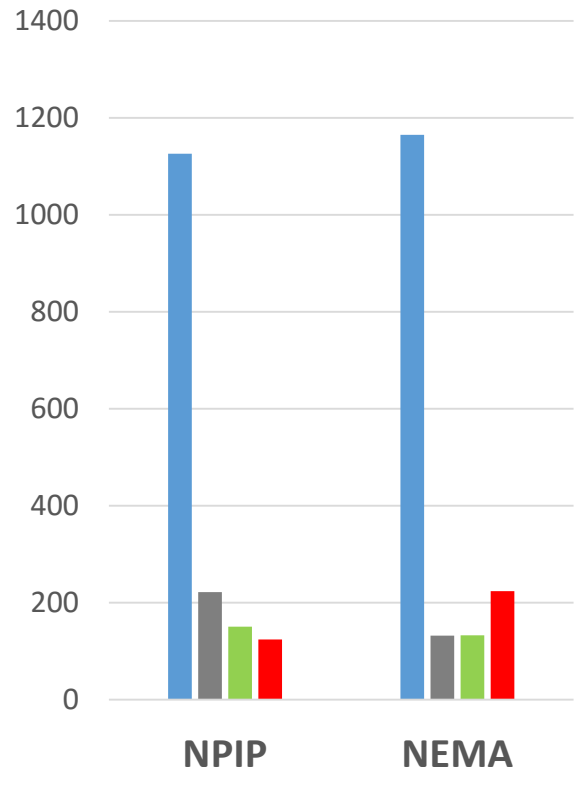
Response Factor = *fct* (mobile phase composition)



CH_3CN : HCOOH



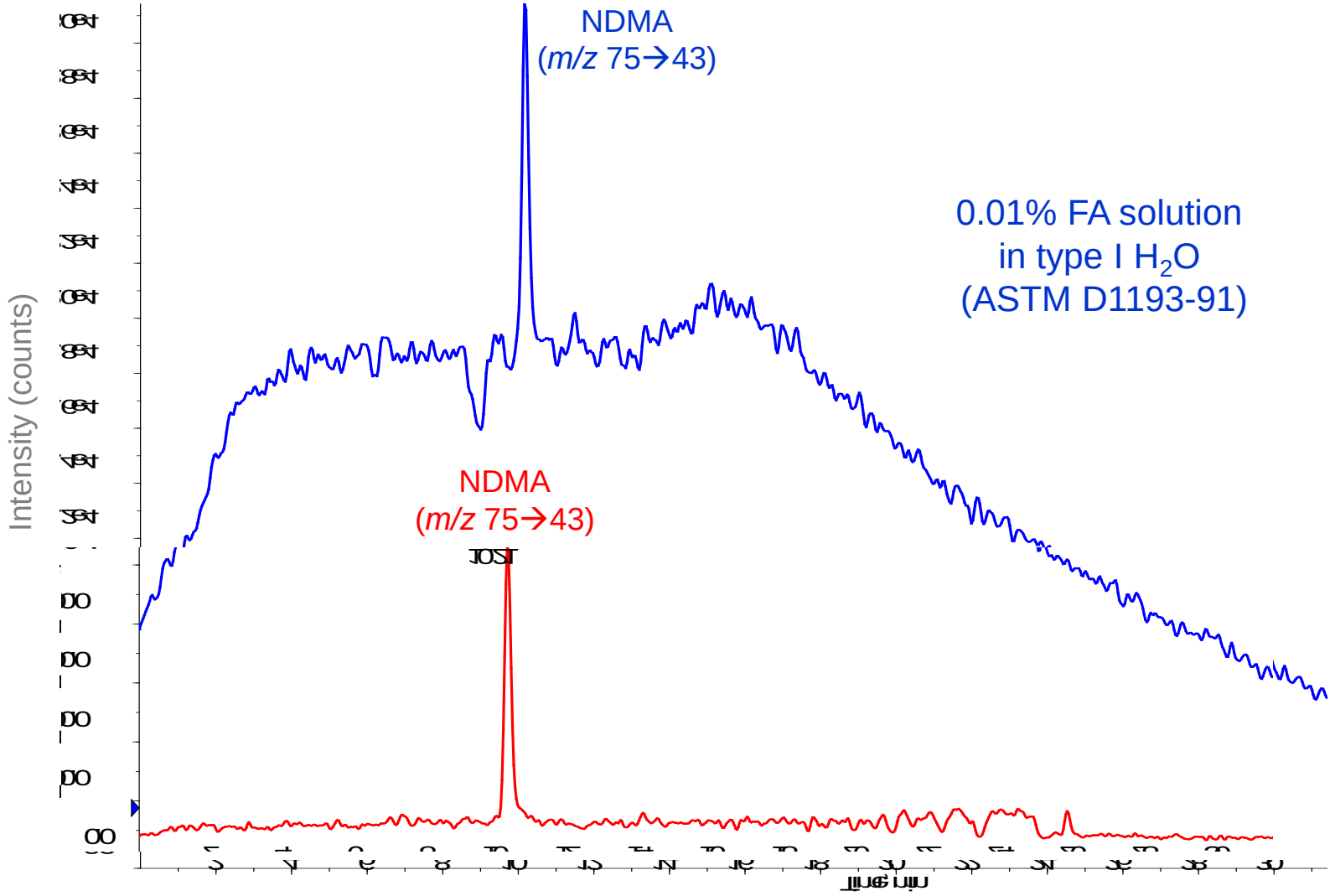
CH_3CN : CH_3COO^-,NH_4^+



CH_3OH : CH_3COO^-,NH_4^+

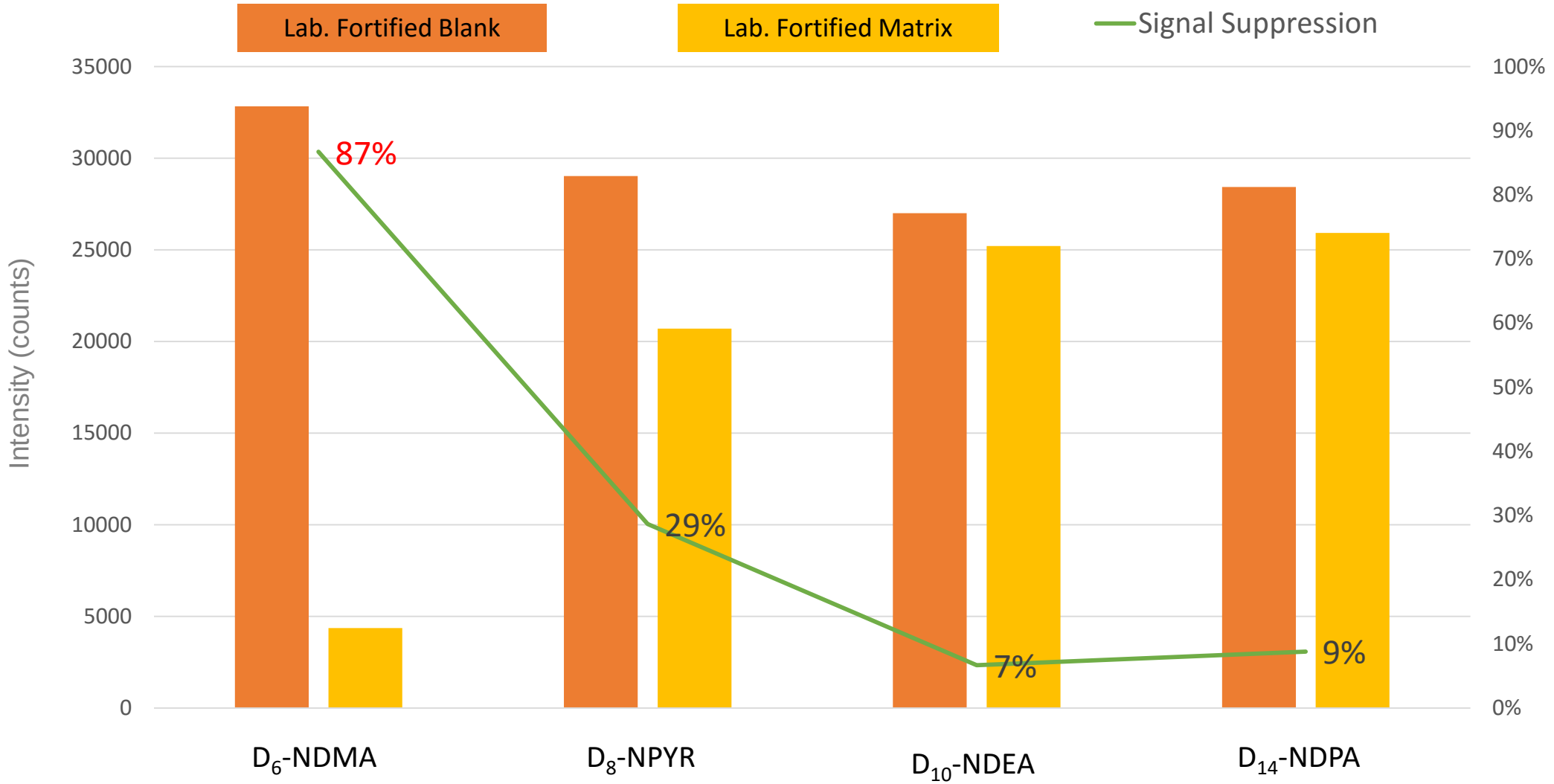
CH_3CN : HCOOH combination appeared to be the best compromise for all analytes.

Method Development
Reagent Water Purity – Background Level

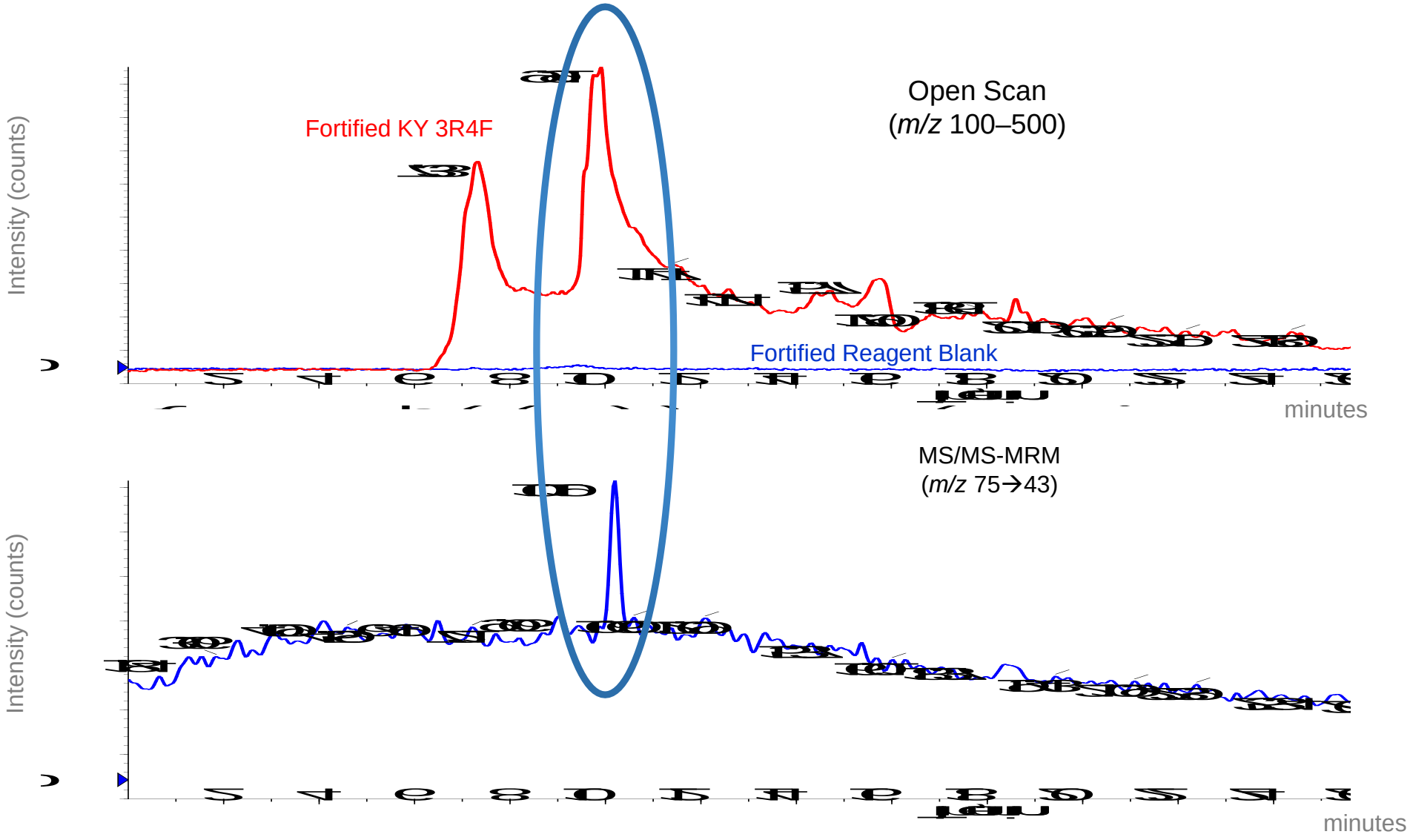


0.01% FA solution
in distilled H_2O

Method Development
Matrix Effect & Signal Suppression



Method Development
Signal Suppression - NDMA

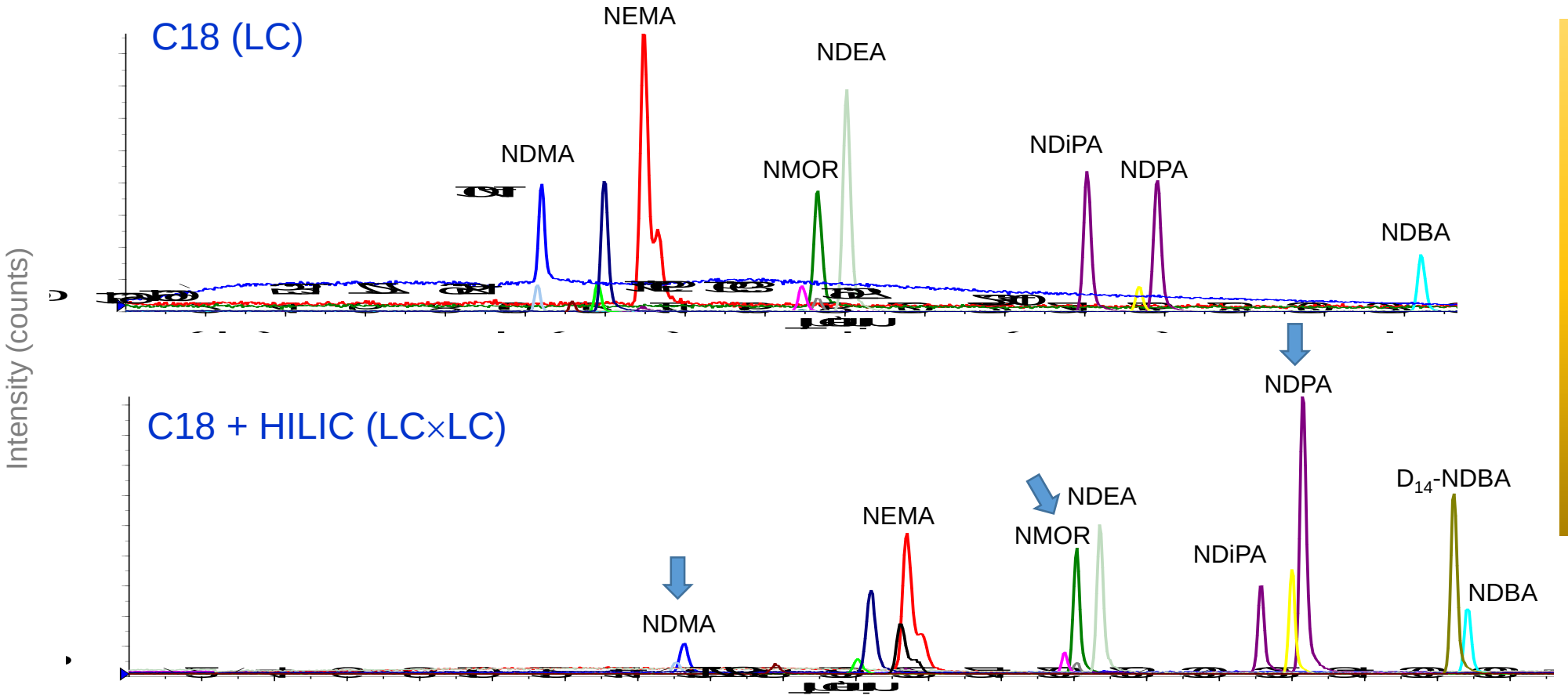


Further clean-up

or

better LC separation?

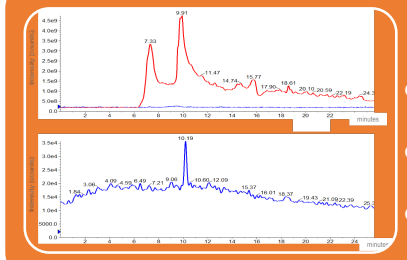
Method Development
Reducing Signal Suppression - LC × LC



Improved separation of polar compounds

Reduced signal suppression

Findings from Method Development



Background Levels

- NDMA content in deionized water
- NPYR found in Ethylformate (removal of NDMA from ethylfomate)
- NMOR/NDBA in Hydromatrix (removal of NDMA from ethylfomate)

Detection (APCI$^+$-MS/MS-MRM)

- Mobile Phase choice
- LC X LC separation

Scope

- Direct/simultaneous VNA/HO-NA analysis in Mainstream Emission
- HO-NA in Cigarette Filler requires additional clean-up step

Resulting Methodology (Mainstream Emission)

Sample Generation

- 10 Cigarette smoked
  (Sulfamate buffer traps + glass fiber filter disc)

Sample Extraction

- Extract pad with trap solutions (shake for 30 minutes).
- Acidify a 12 mL-aliquot of the extract with ammonium sulphamate/H_2SO_4
- Saturate the extract with $(NH_4)_2SO_4$

Sample Clean-up

- Clean-up on hydromatrix cartridge (20g)
- Elute with 70 mL ethyl formate – ethanol (98:2, v/v)
- Evaporate to 1 mL (Rotavap)

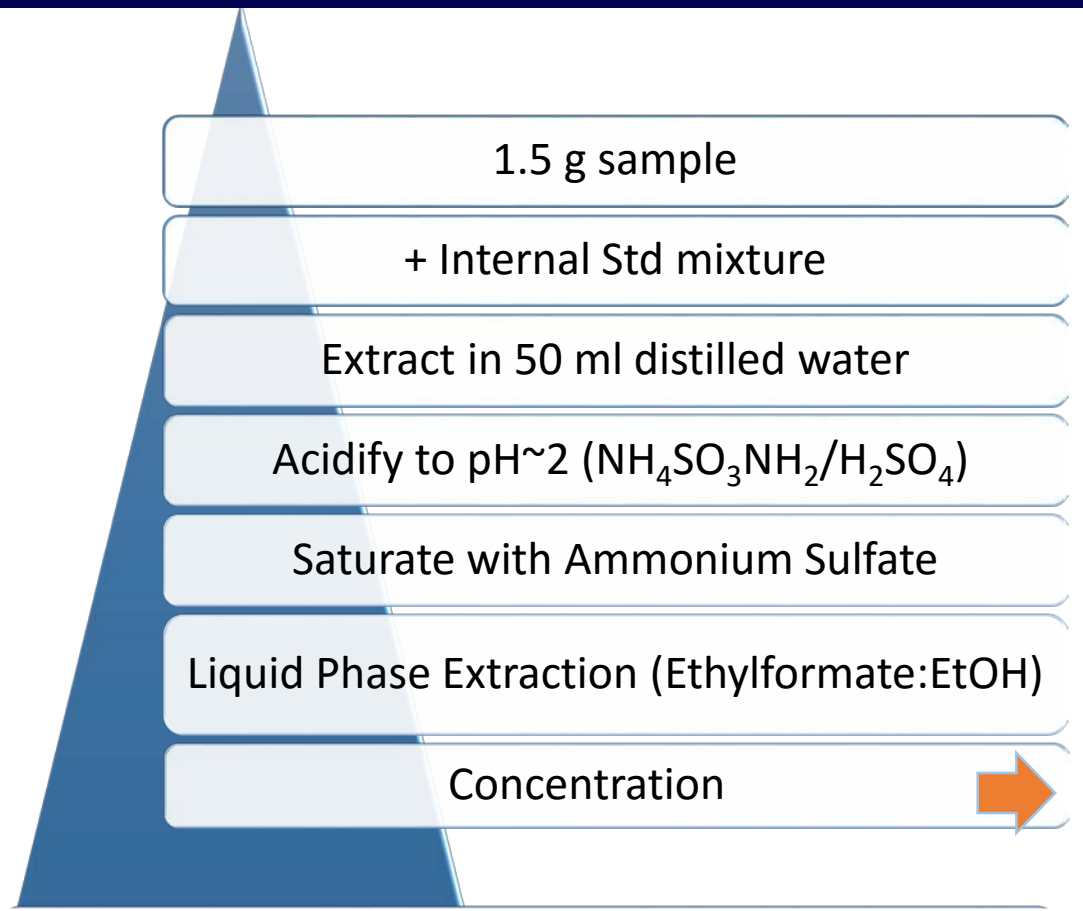
Sample Analysis

- LC-APCI$^+$-MS/MS-MRM (40 minutes)

Resulting Methodology (Cigarette Filler)

N-Nitrosodialkanolamines

Volatile Nitrosamines

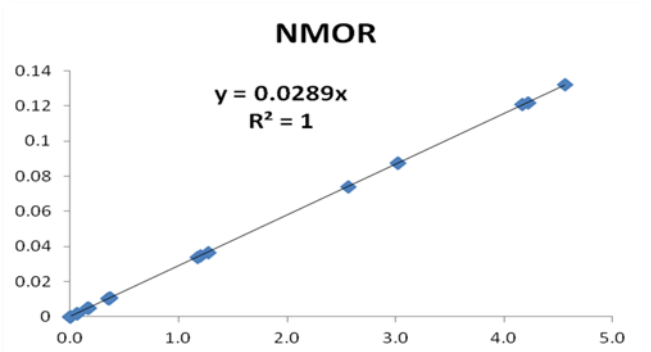


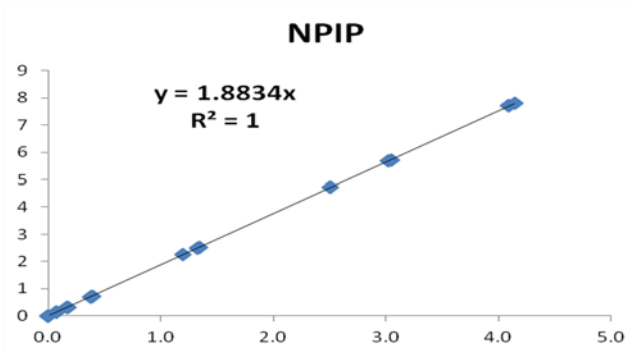
Analytical Performance Validation Data

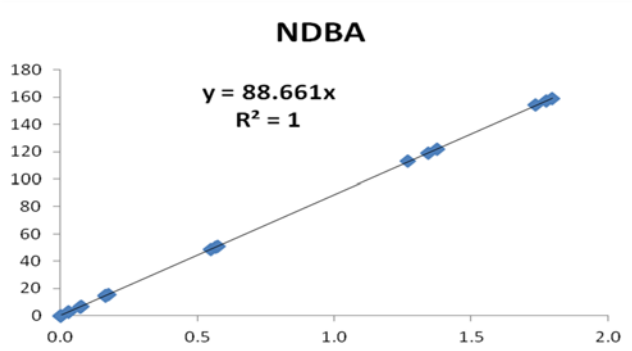
Analytical Performance
Method Linearity

Analytical Calibration range:

- Mainstream Smoke : 0.5 – 200 ng/cig
- Cigarette Filler: 5.0 – 1000 ng/g







Analytical Performances (Cigarette Filler)

Method Recovery & Precision

	Recovery (%)	Precision (%)
NDMA	103±9	8.6
NEMA	95±8	8.5
NDEA	102±9	8.7
NDiPA	95±4	4.6
NDPA	100±6	5.6
NPYR	102±5	4.8
NMOR	96±7	7.0
NPIP	103±8	7.8
NDBA	102±7	6.7
NDELA	103±10	10.2
NDiPLA	97±15	15.2

Method Sensitivity: LOQ Values

	LC-MS (ng/g)	GC-MS (ng/g)	GC-TEA (ng/g)
NDMA	2.9	-	3.9
NEMA	4.3	-	4.5
NDEA	4.5	-	4.7
NDiPA	1.2	-	1.9
NDPA	3.2	-	5.1
NPYR	4.7	-	5.5
NMOR	1.5	-	1.8
NPIP	2.9	-	7.6
NDBA	5.1	-	7.0
NDELA	2.0	2.6	-
NDiPLA	2.5	2.1	-

Analytical Performances (Mainstream Emission)

Method Recovery & Precision

	Recovery (%)	Precision (%)
NDMA	100±6	5.9
NEMA	103±3	3.1
NDEA	102±5	5.0
NDiPA	98 ±7	6.9
NDPA	111±6	5.5
NPYR	102±5	4.7
NMOR	100±14	14.4
NPIP	107±6	5.9
NDBA	110±16	14.2
NDELA	102±5	4.6

Method Sensitivity: LOQ Values

	LC-MS (ng/cig)	GC-MS (ng/cig)	GC-TEA (ng/cig)
NDMA	0.59	-	0.72
NEMA	0.85	-	0.68
NDEA	1.03	-	0.70
NDiPA	0.91	-	(ISTD)
NDPA	0.25	-	0.76
NPYR	0.66	-	0.83
NMOR	0.92	-	-
NPIP	0.29	-	1.15
NDBA	0.55	-	1.06
NDELA	0.14	0.11	-

Advantages of Current LC-MS/MS over Historical Methods

- ✓ Unequivocal Mass Spectral Identification (higher accuracy)

The LC-MS/MS technique is a quantitative, sensitive, and reliable method for analysis of volatiles and hydroxy-nitrosamines in tobacco, cigarette smoke and new generation of tobacco products .

- ✓ Higher Recovery (“absolute”)
- ✓ Higher Precision (use of isotopically-labelled analogues as ISTD)
- ✓ Higher Sensitivity (higher S:N ratios in samples)
- ✓ Significantly “cleaner chromatograms”:
  - ➢ providing ease of peak integration
  - ➢ minimizing the risk of peak misidentification.
- ✓ Safer Lab./personnel Safety (~~CH_2Cl_2~~)

Acknowledgment

Thank you for Your Attention!