An Improved High Performance Ion Chromatography Method for the Determination of Ammonia in Tobacco and Tobacco Smoke Samples

Jingcun WU, Bill RICKERT, Andrew MASTERS, Peter JOZA

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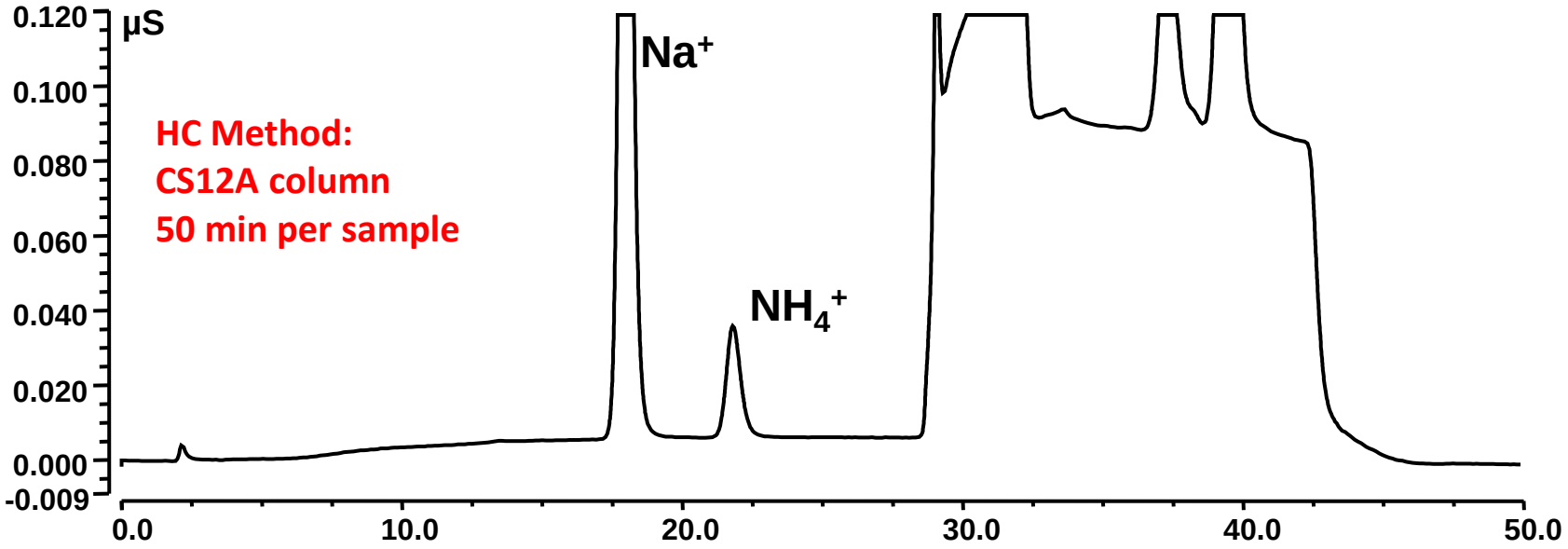
LABSTAT INTERNATIONAL ULC.
262 Manitou Drive
Kitchener, Ontario, Canada N2C 1L3
Phone: (519) 748-5409 Fax: (519) 748-1654 Web: www.labstat.com

Objective

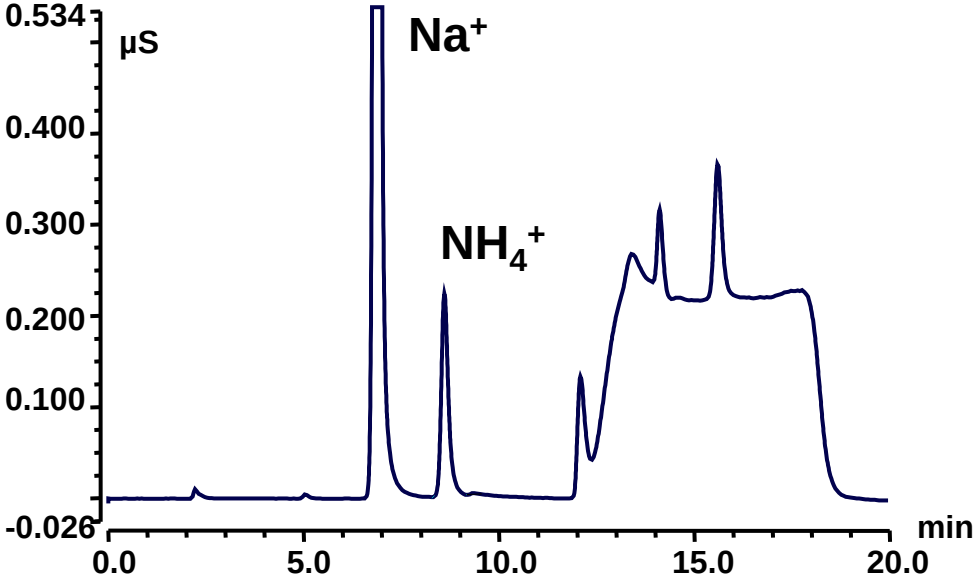
Method Improvement

- Short analysis time - High productivity
- Better separation efficiency or peak resolution
- Higher sensitivity and accuracy
- Simplified and more robust method

Analysis Time - Efficiency - Productivity

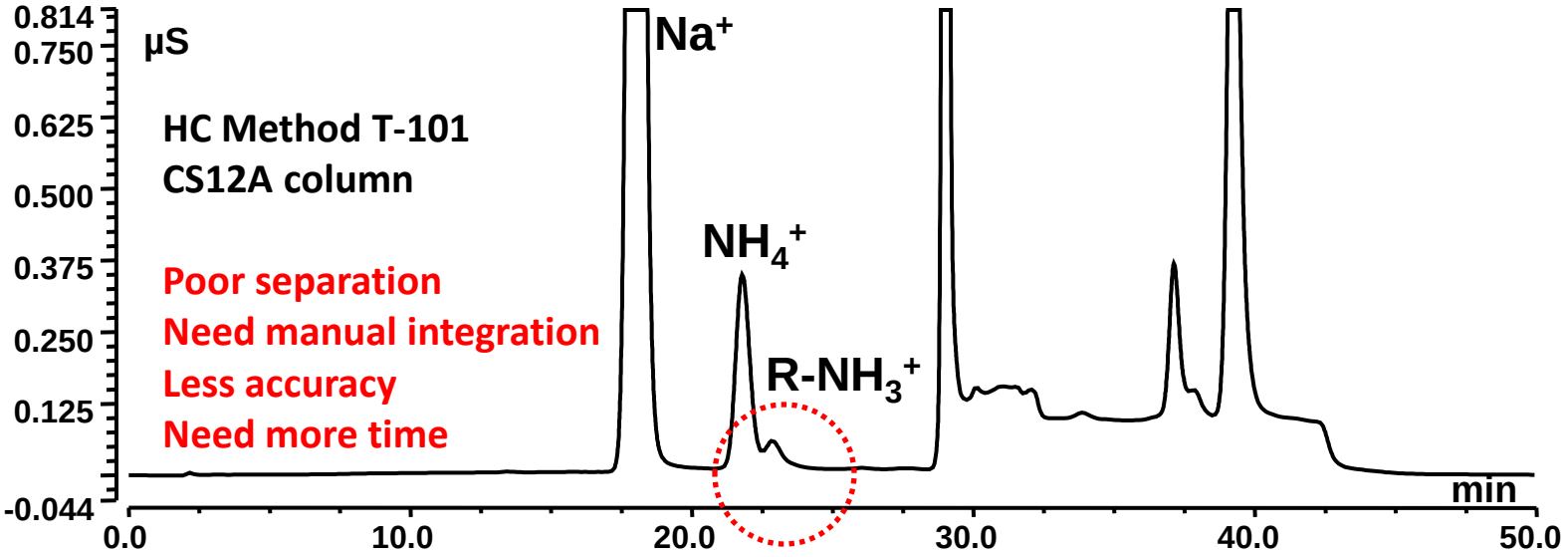


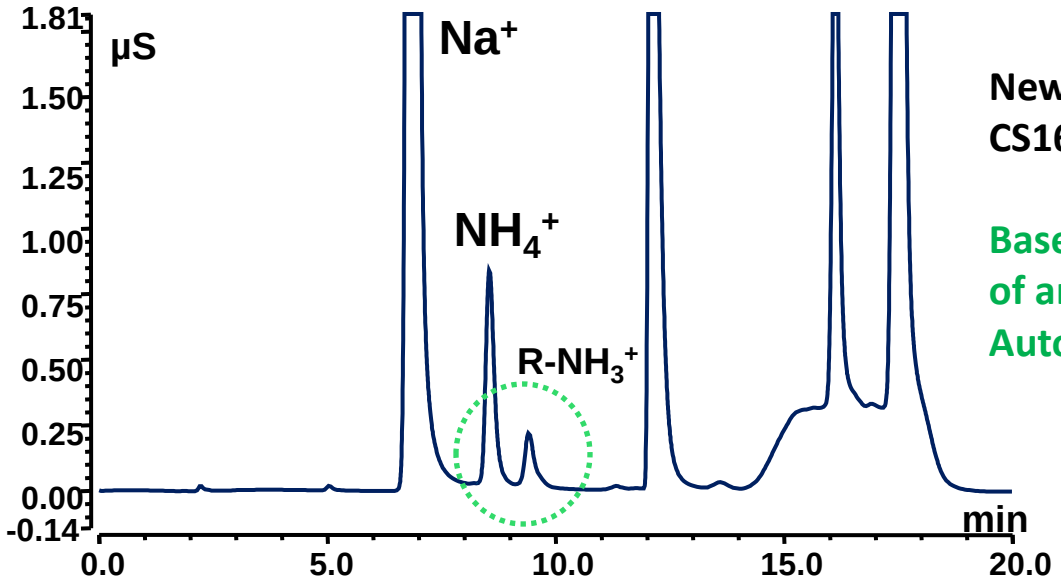
HC Method:
CS12A column
50 min per sample



New Method:
CS16 column
≤ 20 min per sample

Separation Efficiency - Mainstream Smoke

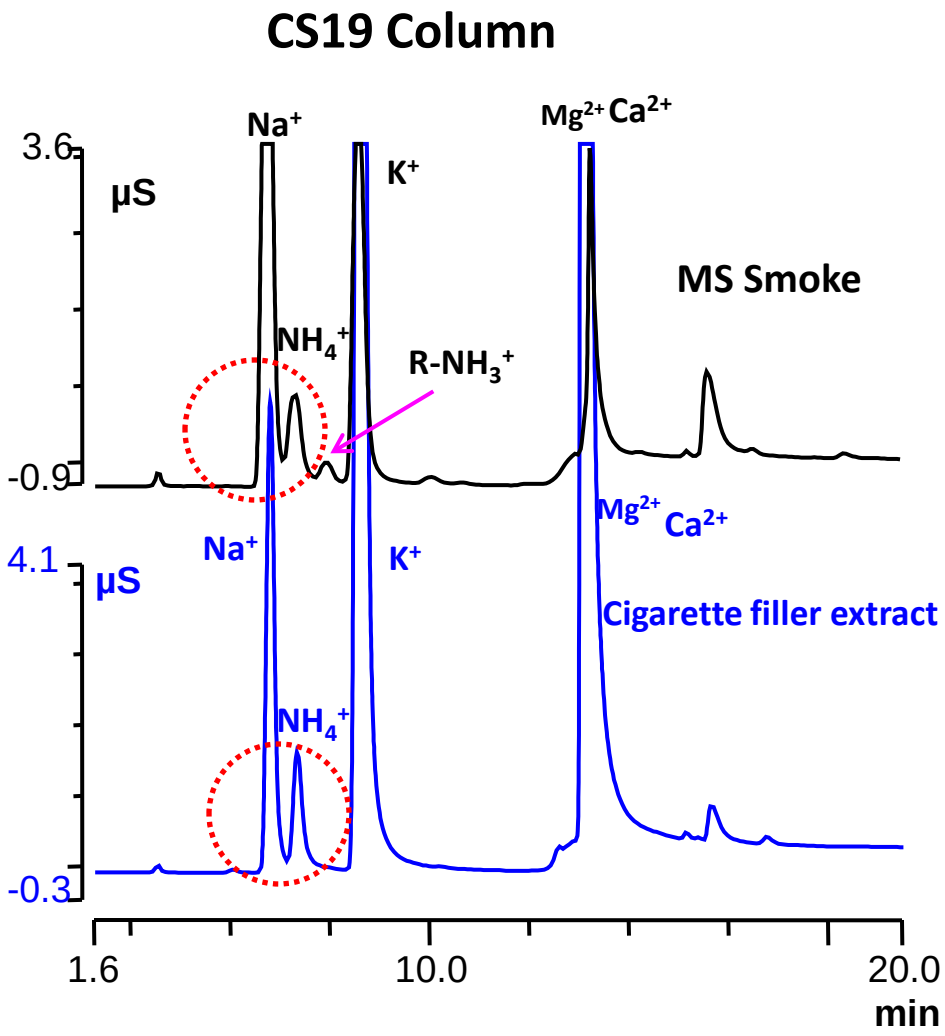


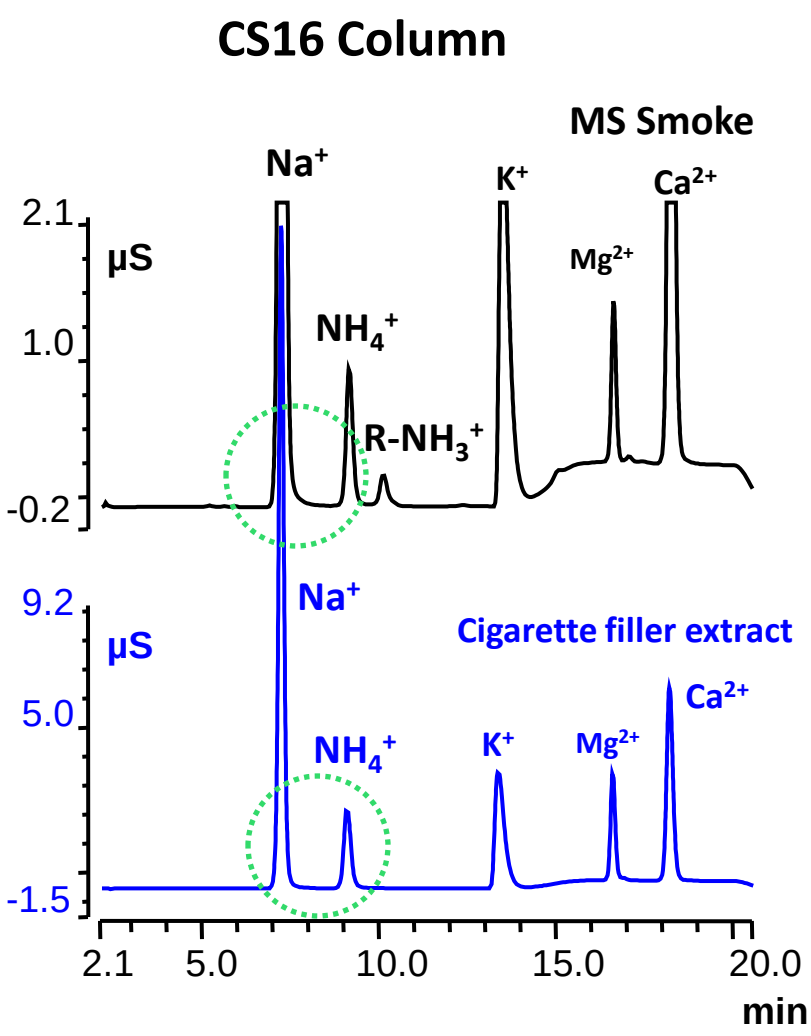


New Method:
CS16 column

Baseline separation
of ammonium and amine peaks
Automatic integration

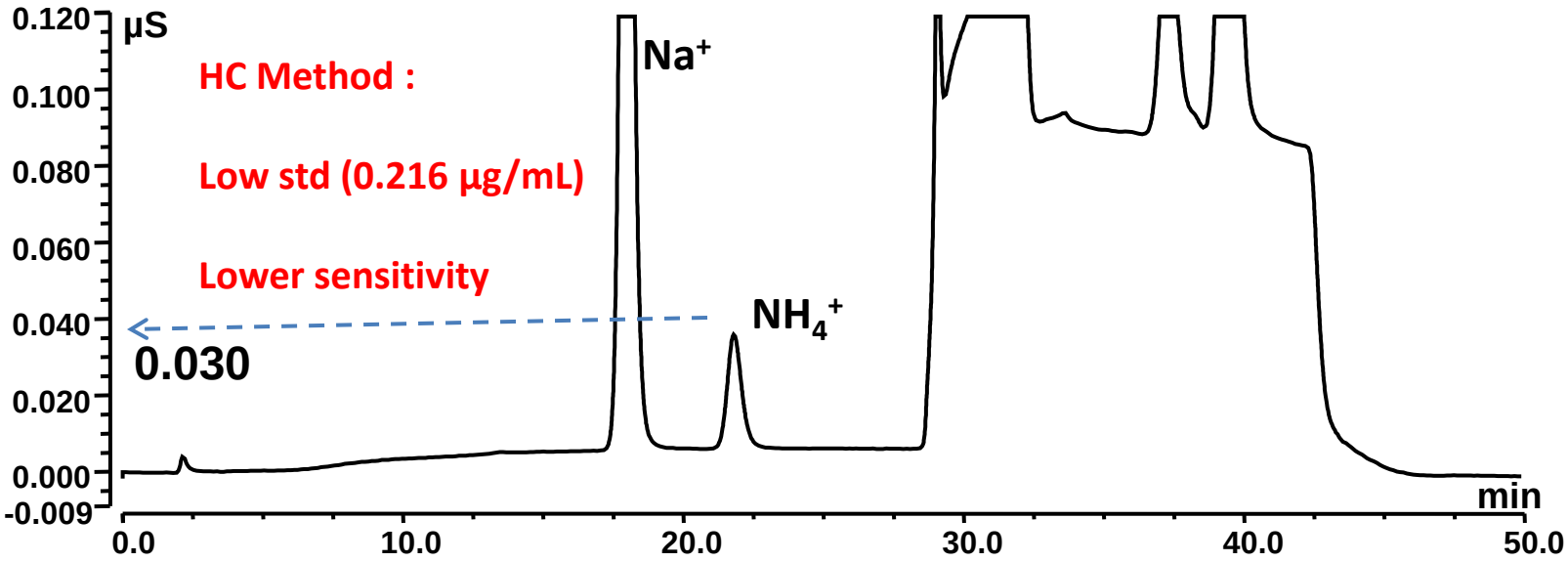
Separation Efficiency

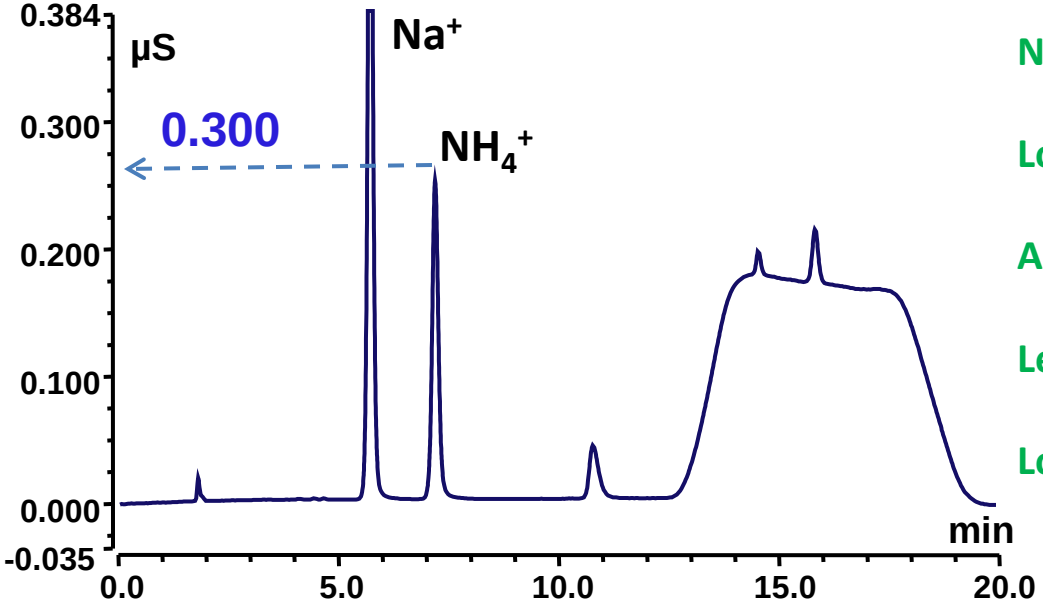




Thermo Scientific: ***Application Note 1054*** **(2013)**

Sensitivity





New Method:

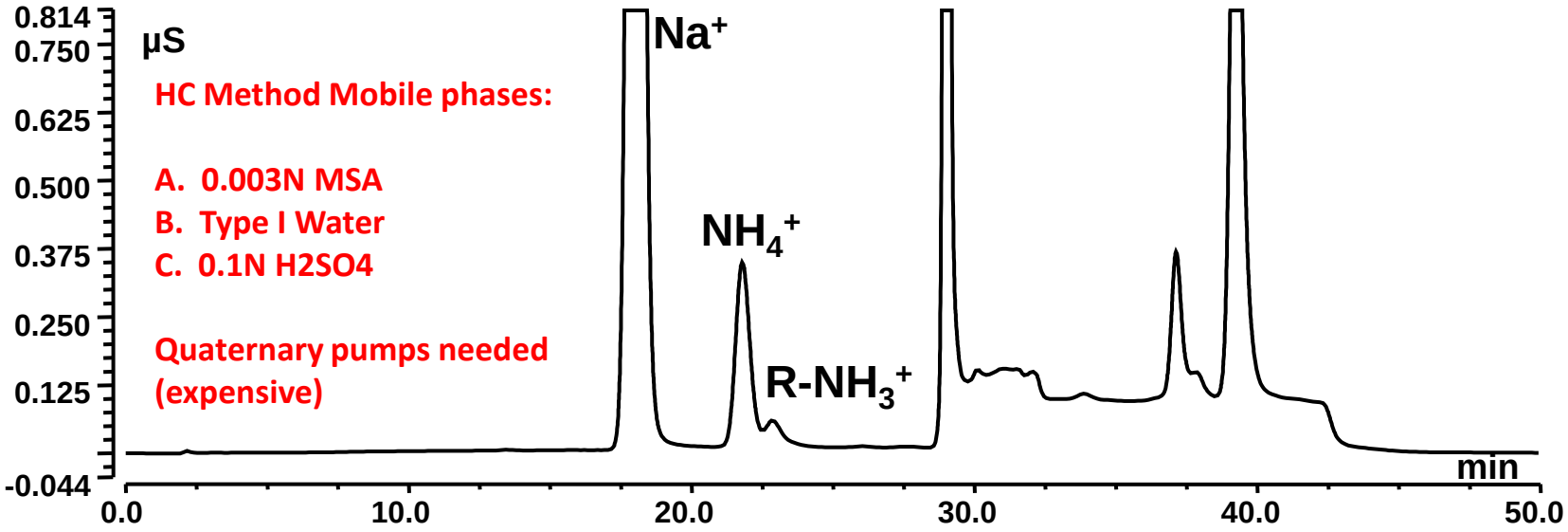
Low std (0.216 µg/mL)

About 10 times increase in response

Less sample or more diluted sample

Long life time for column+ suppressor

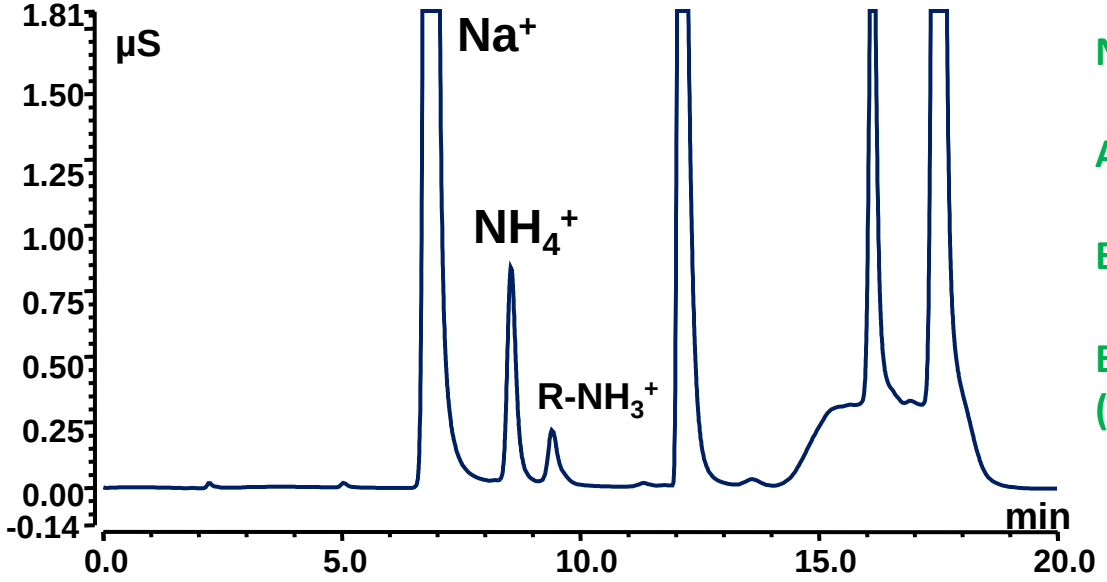
Simple, Cost Effective and Robust Method



HC Method Mobile phases:

A. 0.003N MSA
B. Type I Water
C. 0.1N H2SO4

Quaternary pumps needed (expensive)



New Method Mobile phases:

A. 50mM MSA

B. Type I Water

Binary pumps needed (less expensive)

HPIC Method Comparison

Analytical Method	Health Canada HPIC	Improved HPIC
Analytical Column	CS12A	CS16
Mobile Phases	A: MSA; B: H2SO4; C: Water	A: MSA; B: Water
Pumping System	Quaternary (Expensive)	Binary (Cheaper)
Time (min/sample)	50 to 60	20 or shorter
Flow rate (mL/min)	1.0 or 1.5	0.5
Injection Volume (µL)	20	5 or 10
Resolution/Separation efficiency	Poor (overlapped shoulder peaks)	High (baseline separation)
Sensitivity	Fine	Higher

Fundamentals

- Resolution Equation in Chromatography

Resolution (Rs) Plates (N) Selectivity (α) Retention (k')

$$Rs = \frac{\sqrt{N}}{4} \times \frac{\alpha - 1}{\alpha} \times \frac{k'}{k' + 1}$$

$$N \propto \frac{L}{dp}$$

L: Column Length

d: Column Diameter

p: Particle Size

Column (Cation Ex)	ParticleSize (μm)	Capacity (μeq)
CS12A (4x250mm)	8.0	2800
CS19 (2x250mm)	5.5	600
CS16 (3x250mm)	5.0	3000

Ion-Exchange Groups: Grafted carboxylic acid (+ phosphonic acid for CS12A)

Surface Characteristics: Medium hydrophobic

Instrumentation

Thermo Scientific /Dionex ICS-5000 IC System

Agilent Biocompatible HPLC/Dionex CD Detector

Dionex IonPac CS16 Analytical Column (3 x 250mm)

Dionex IonPac CG16 Guard Column (3 x 50mm); CTC Cation Trap Column (4 x 35mm)

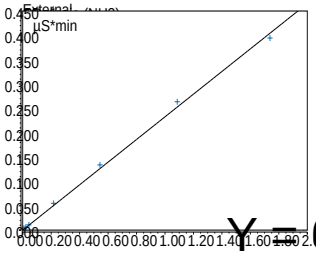
Dionex CSRS-Ultra 300 Cation Self-Regenerating Suppressor (2mm)

Sample Preparation

- HC Method T-101 for Mainstream Smoke
- HC Method T-201 for Sidestream Smoke
- HC Method T-302 for Tobacco & Smokeless Tobacco Extracts

Linear Concentration Range

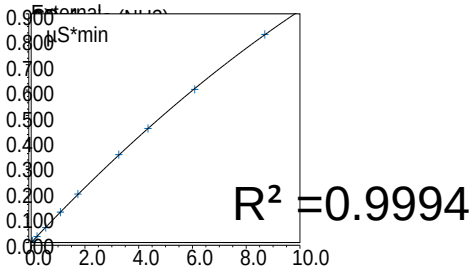
Std level	Ammonium (µg/mL)
1	2.0
2	1.0
3	**0.50**
4	0.20
5	0.04
6	0.02
7	0.01
0	0



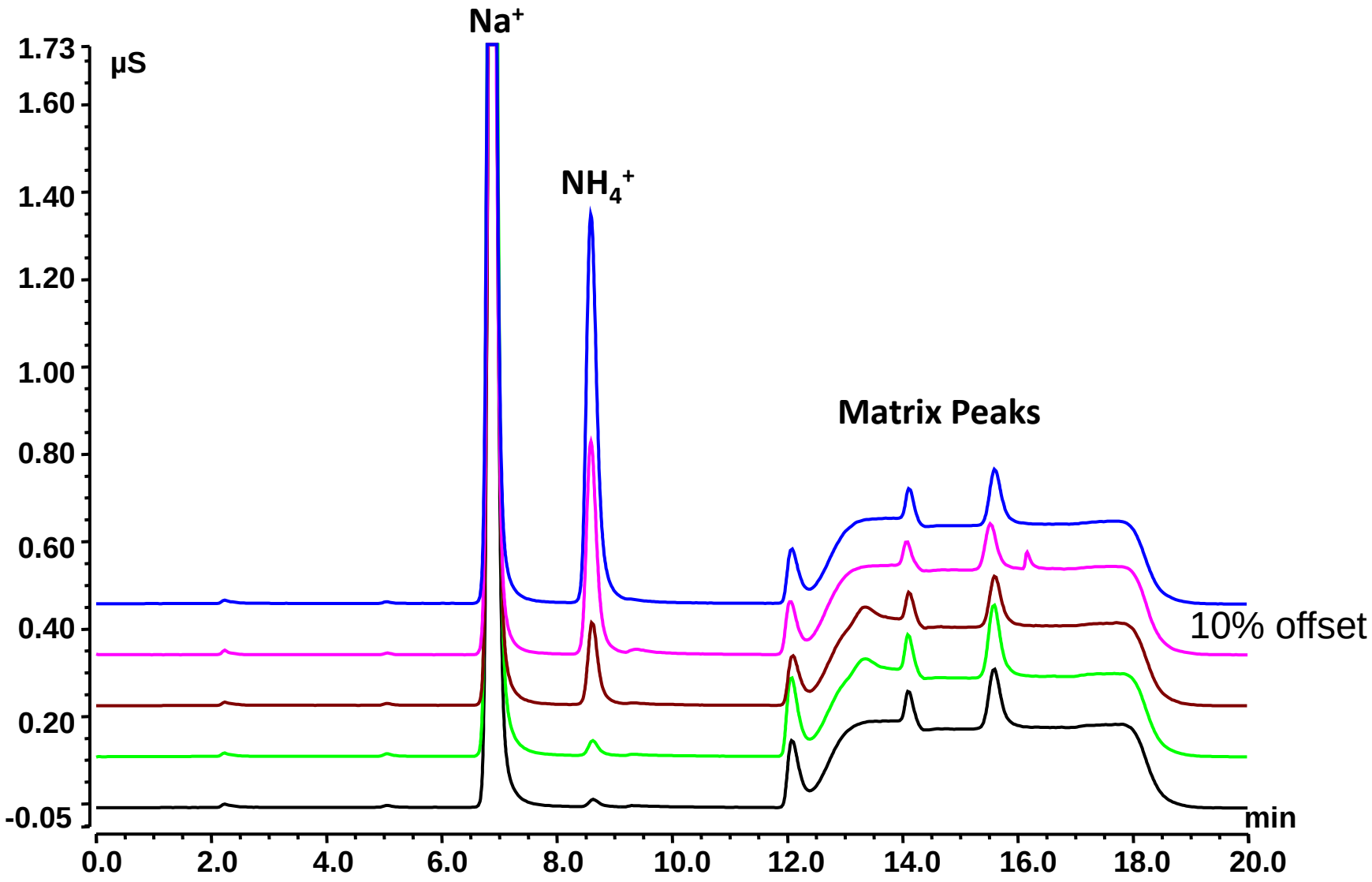
Y = 0.222 (R^2 =0.9986)

Wider Concentration Range - Quadratic

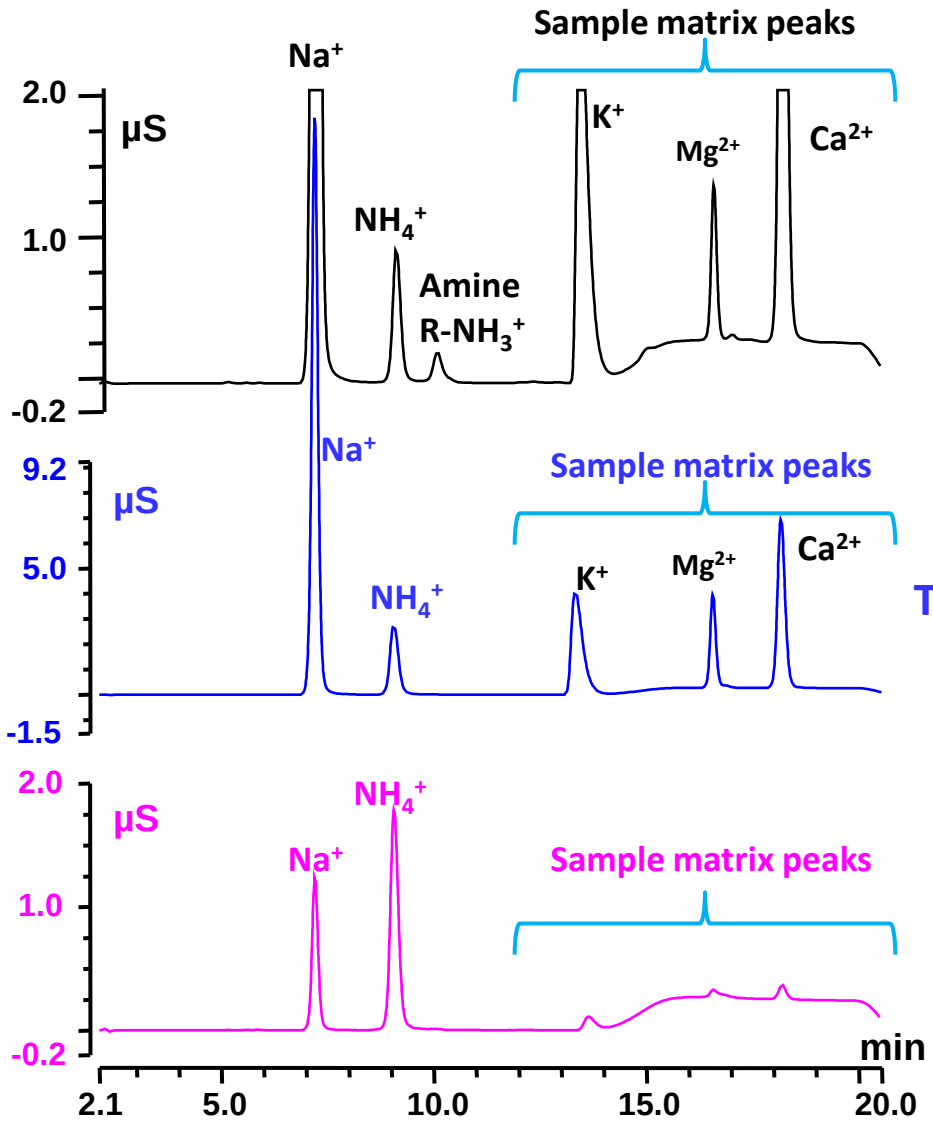
Std level	Ammonium (µg/mL)
1	10.0
2	8.0
3	**5.0**
4	2.0
5	1.0
6	0.5
7	0.2
8	0.01



Calibration Standard IC Profiles



Tobacco Sample IC Profiles

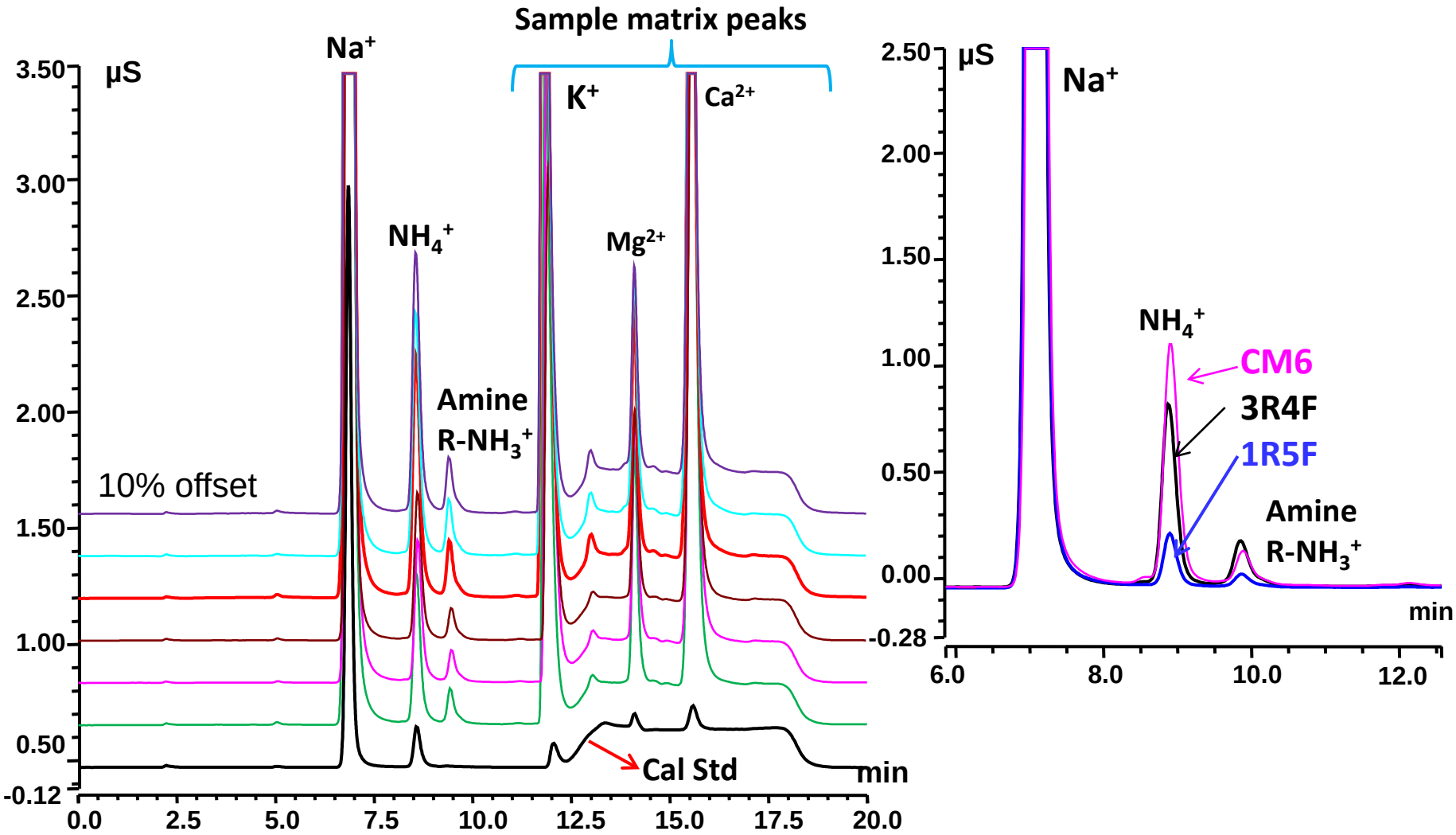


Mainstream Smoke Samples

Tobacco /Smokeless Tobacco Extracts

Sidestream Smoke Samples

Mainstream Smoke Samples



Method Validation: Tobacco Smoke Analysis

Precision

Sample:	3R4F (MS ISO)
Number of observations:	44
Ammonia found (µg/cig):	10.5
Standard deviation (SD):	0.98
RSD (%):	9.3

Recovery (Accuracy)

Laboratory Reagent Blank (LRB) (µg/cig):	0
Laboratory Fortified Blank (LFB) (%):	92 -108
Laboratory Fortified Matrix (LFM) (%):	88 - 113

Limit of Detection (LOD)

Instrument LOD (µg/mL):	0.004
Method LOD (µg/cig):	0.045

Limit of Quantification (LOQ)

Instrument LOQ (µg/mL):	0.013
Method LOQ (µg/cig):	0.151

Validation: Tobacco and Smokeless Tobacco

Precision

Sample:	3R4F (CRP-3)
Number of observations:	7 (7)
Ammonia found (µg/g):	1318 (4905)
Standard deviation (SD):	58 (22)
RSD (%):	4.4 (3.6)

Recovery (Accuracy)

Laboratory Reagent Blank (LRB) (µg/g):	0
Laboratory Fortified Blank (LFB) (%):	96 -106
Laboratory Fortified Matrix (LFM) (%):	86 - 112

Limit of Detection (LOD)

Instrument LOD (µg/mL):	0.004
Method LOD (µg/g):	2.27

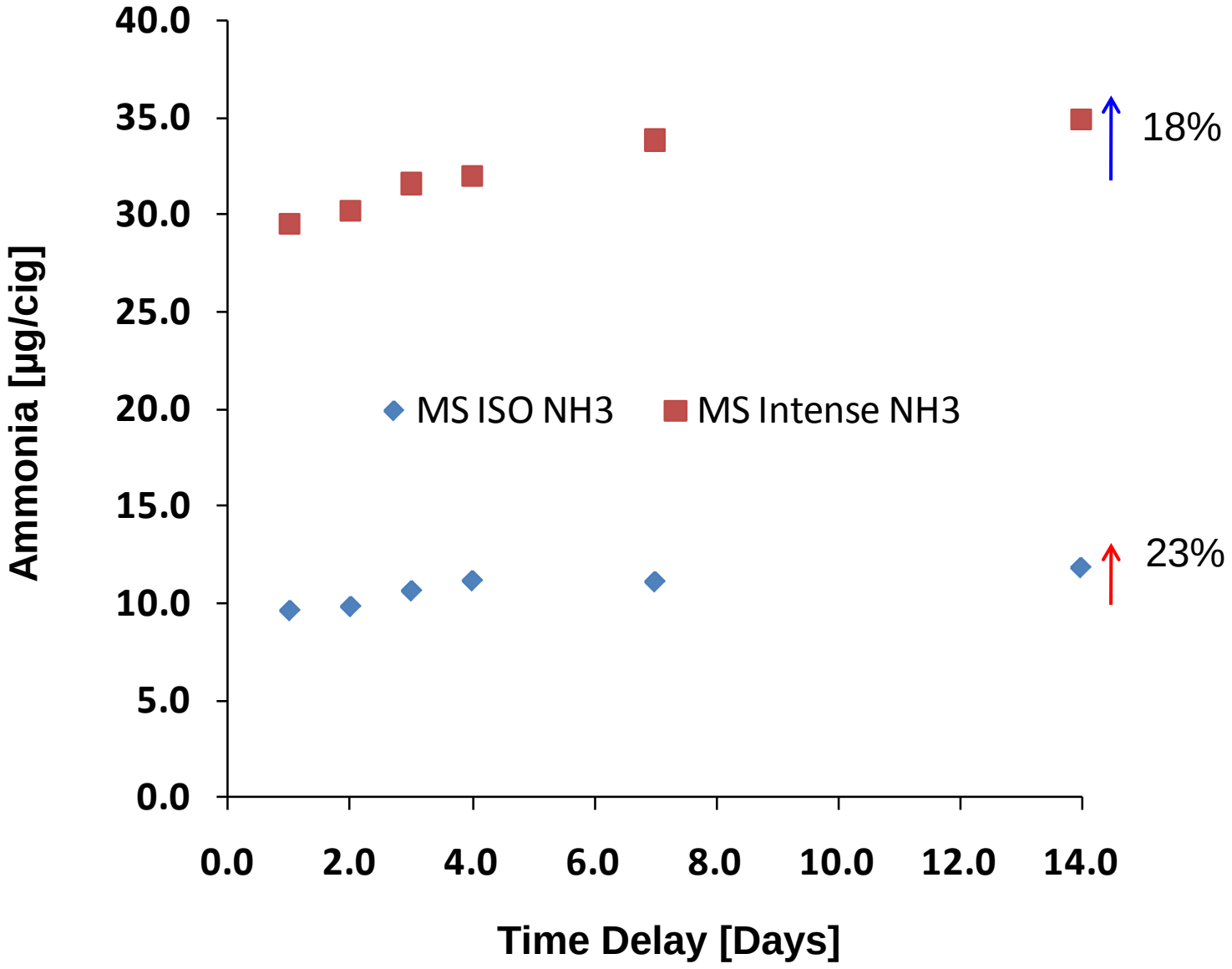
Limit of Quantification (LOQ)

Instrument LOQ (µg/mL):	0.013
Method LOQ (µg/g):	7.56

Method Robustness

- Multiple Injections / Different Days
- Columns
- Suppressors
- Instruments
- Analytical Chemists

Sample Stability Study - 3R4F



Applications

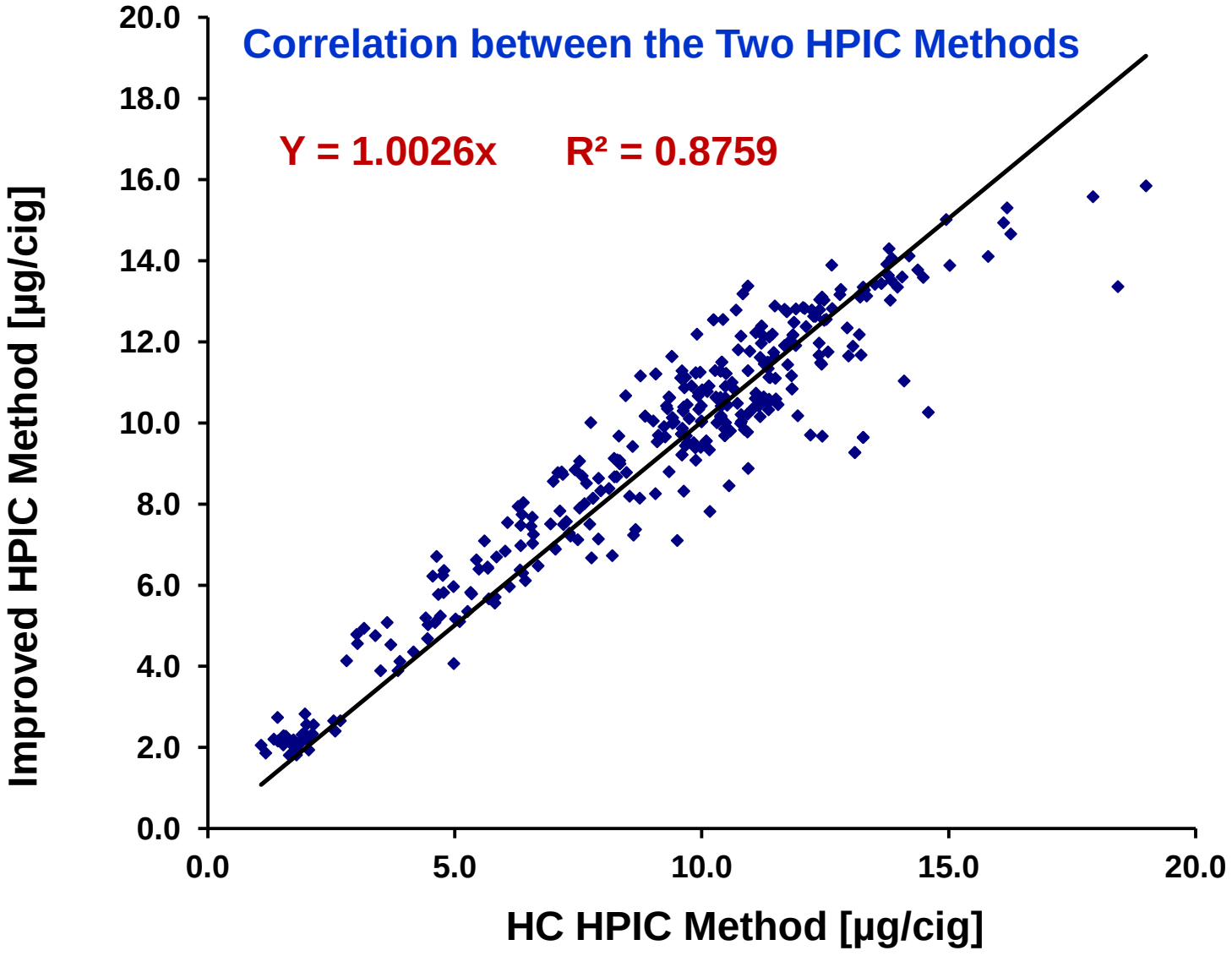
- Reference tobacco products: 1R5F, 3R4F, CM6, CRP-3
- Tobacco smoke analysis
- Cigarette filler and tobacco leafs
- Smokeless tobacco analysis
- E-cigarette emission analysis

Ammonia in Reference Tobacco Products

Sample ID	Sample Matrix	Replicates [n]	Mean [µg/cig] [µg/g]*	Std. Dev. [µg/cig] [µg/g]*	RSD [%]
3R4F	MS ISO	44	10.5 (10.4)**	0.98 (1.12)	9.3 (10.8)
3R4F	MS Intense	16	31.2 (30.5)	1.96 (2.85)	6.3 (9.3)
3R4F	SS ISO	12	6946 (7227)	290 (366)	4.2 (5.1)
1R5F	MS ISO	11	2.68 (2.59)	0.41 (0.34)	15 (13)
1R5F	SS ISO	7	7045 (7164)	285 (422)	4.0 (5.9)
CAN-M8	MS ISO	7	10.8 (11.0)	0.49 (1.21)	4.6(11.0)
CM6	MS ISO	7	16.4 (18.3)	0.51 (0.77)	3.1 (4.2)
CM6	SS ISO	7	5428 (5387)	251 (325)	4.6 (6.0)
3R4F	Cig Filler*	7	1318 (1326)	58 (48)	4.4 (3.6)
CRP-3	Smokeless*	7	4905 (4555)	22 (27)	0.5 (0.6)

**Values in parentheses were obtained by HC HPIC Methods (T-101, T-201, T302)

Mainstream ISO Smoke Ammonia Yields



Summary

- An improved HPIC method has been developed for ammonia analysis, which is simpler, faster, more sensitive, more selective and robust and cost effective compared with the current HPIC methods.
- This method has been fully validated and can be applied to ammonia analysis in different tobacco sample matrices.

Acknowledgements

- Labstat Sample Preparation Technicians and Analytical Team (Mr. Zlatko Lejic and Ms. Yanwei Zhan)
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- Your attention