TSNA Method Development

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FDA Center for Tobacco Products
Scientific Workshop – Tobacco Product Analysis

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TSNA Work Program

- **Propose practical and robust recommended methods for smoke analysis (CORESTA Recommended Methods, CRMs)**
- **Priority compounds identified as benzo[a]pyrene and four tobacco specific nitrosamines (TSNAs)**
  - NNN, NNK, NAT and NAB

CORESTA Special Analytes Sub-Group
Development of Recommended Methods

- **Approach used for the development of robust methods**
  - ✓ **Should involve a relatively large number of laboratories**
    - CORESTA well represents global expertise in this type of measurement
  - ✓ **Should involve a wide range of product and design styles**
    - Range of blend styles
    - Range of tar yields
    - Manufactured in various global regions
  - ✓ **Round-table discussions during development provide valuable insight into causes and ways to reduce inter-laboratory data variability**
  - ✓ **Consensual and unanimous decisions on "standardisation"**

Tobacco-Specific Nitrosamines (TSNAs) by GC-TEA between 1999 and 2005

- **Review of existing methods for cigarette smoke**
  - ✓ Two types of methods were identified (GC-TEA and LC-MSMS)
  - ✓ Insufficient laboratories for collaborative work using LC-MSMS
- **Joint experiments using GC-TEA method**
  - ✓ Several clean up procedures investigated and optimised
- **Investigation of GC-TEA method variability**
  - ✓ Collaborative study under ISO 3308 regime
  - ✓ 9 laboratories using 7 test cigarettes
  - ✓ Publication as CRM 63 in 2005

Cigarette (ng/cig)	*NNN Mean*	*r*	*R*	*R%*
2R4F	146	11	32	22
A	180	29	45	25
B	274	54	90	33
C	42	10	22	53
D	11	3	5	51
E	26	7	15	57
F	85	12	46	54
Cigarette (ng/cig)	*NNK Mean*	*r*	*R*	*R%*
2R4F	141	16	44	31
A	88	13	53	60
B	202	37	146	73
C	36	8	23	65
D	5	2	6	109
E	23	7	12	53
F	52	9	23	44

TSNA method development by LC-MS/MS between 2009 and 2012

- **Review of applied methods for TSNA in cigarette smoke**
  - **Many laboratories have switched to the LC-MS/MS technique.**
- **Joint experiments using LC-MS/MS**
  - **Investigation of various methodological parameters e.g. calibration standards**
- **Development of Recommended method**
  - **Through collaborative study involving 20 laboratories from 12 countries using statistical procedures outlined in ISO 5725-2**

TSNA method development by LC-MS/MS between 2009 and 2012

- Cigarettes are smoked on a standard smoking machine. Mainstream smoke is trapped on a glass fiber filter pad.
- After addition of an internal standard, the total particulate matter collected on the glass fiber filter pad is extracted into 20 mL of 0.1M ammonium acetate solution using a shaker for 60 minutes.
- The extract is syringe filtered through a 0.45 µm PTFE column directly into an auto sampler vial.
- The samples are subjected to reversed phase high performance liquid chromatography (HPLC) and quantified via tandem mass spectrometry (MS/MS).

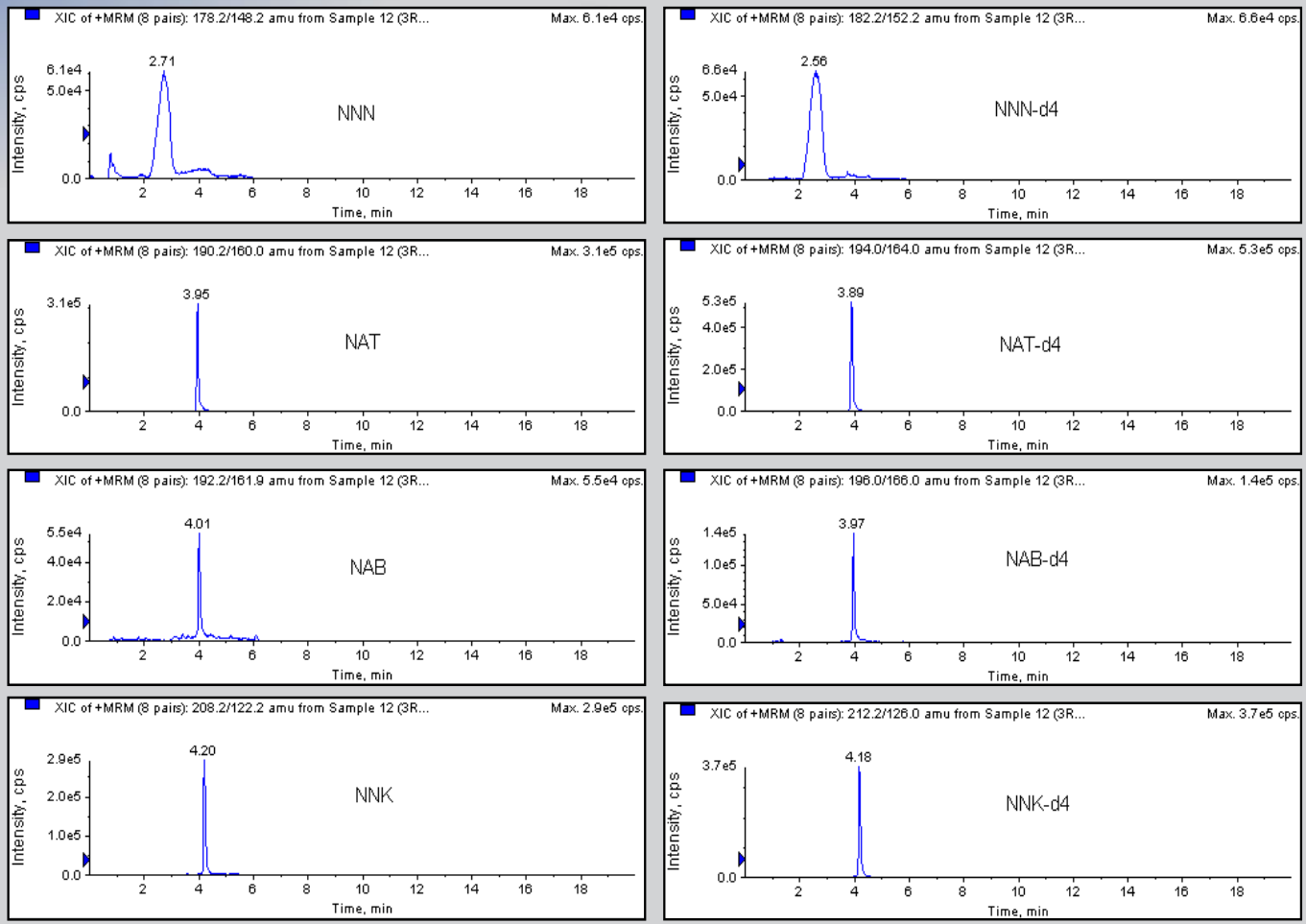
TSNA method development by LC-MS/MS between 2009 and 2012

Compounds	Precursor ion (m/z)	Quantifier (m/z)	Qualifier (m/z)	DP* (V)	CE* (V)	CXP* (V)	Dwell time (m sec)
NNN	178	148	120	41	15	10	150
NAT	190	160	106	41	15	10	150
NAB	192	162	133	36	17	10	150
NNK	208	122	79	41	17	8	150
NNN-d4	182	152	124	41	15	8	150
NAT-d4	194	164	110	41	15	10	150
NAB-d4	196	166	137	36	17	10	150
NNK-d4	212	126	83	41	17	8	150

*** DP: Declustering potential, CE: Collision energy, CXP: Collision cell exit potential**

TSNA method development by LC-MS/MS between 2009 and 2012

Figure 2: Chromatograms of TSNAs in mainstream cigarette smoke extract (KR 3R4F)



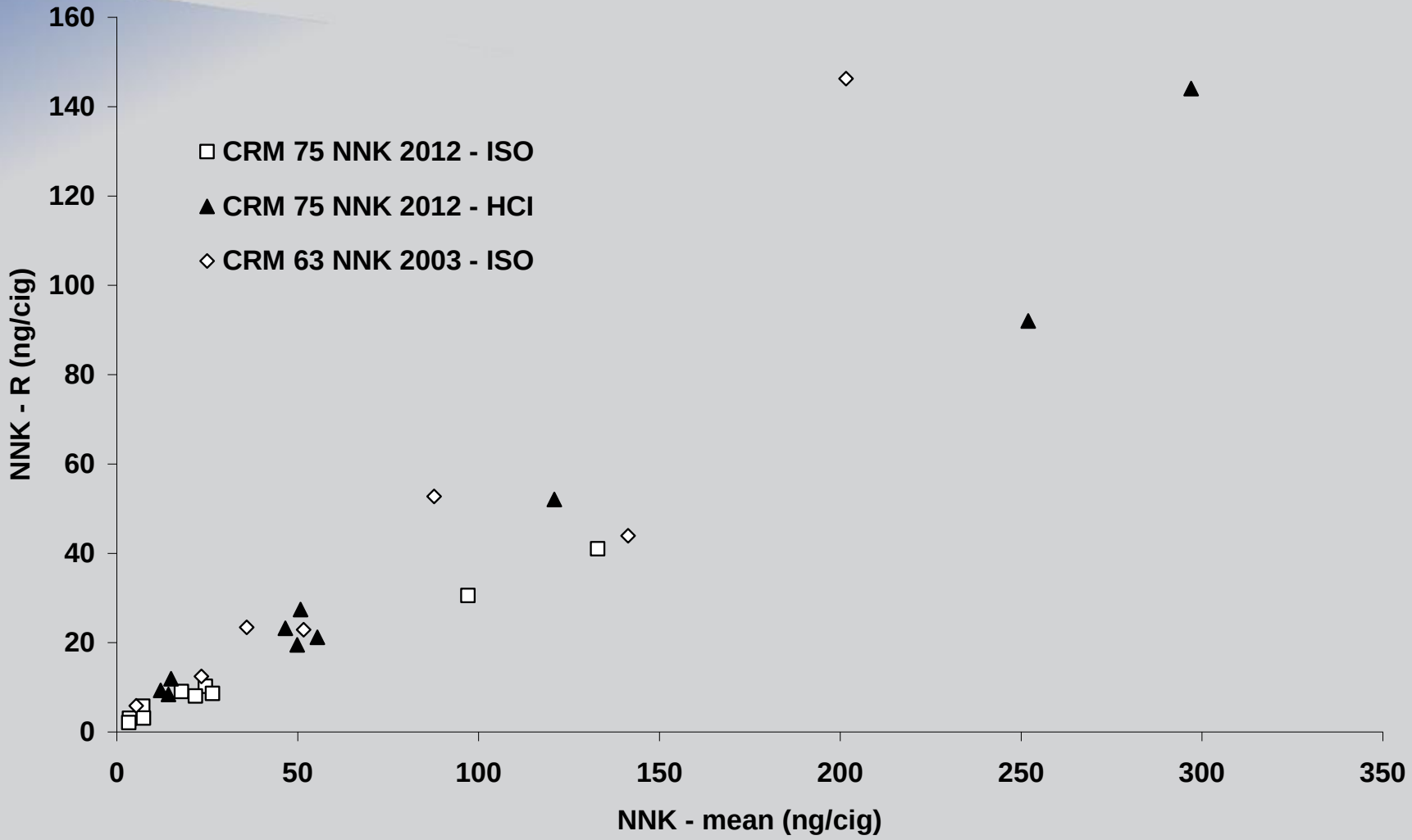
LC Column:
XTerra MS C18, 2.5 µm, 2.1 x 50 mm or equivalent

Mobile phase (Example):
A: 0.1 % Acetic acid in water
B: 0.1 % Acetic acid in methanol

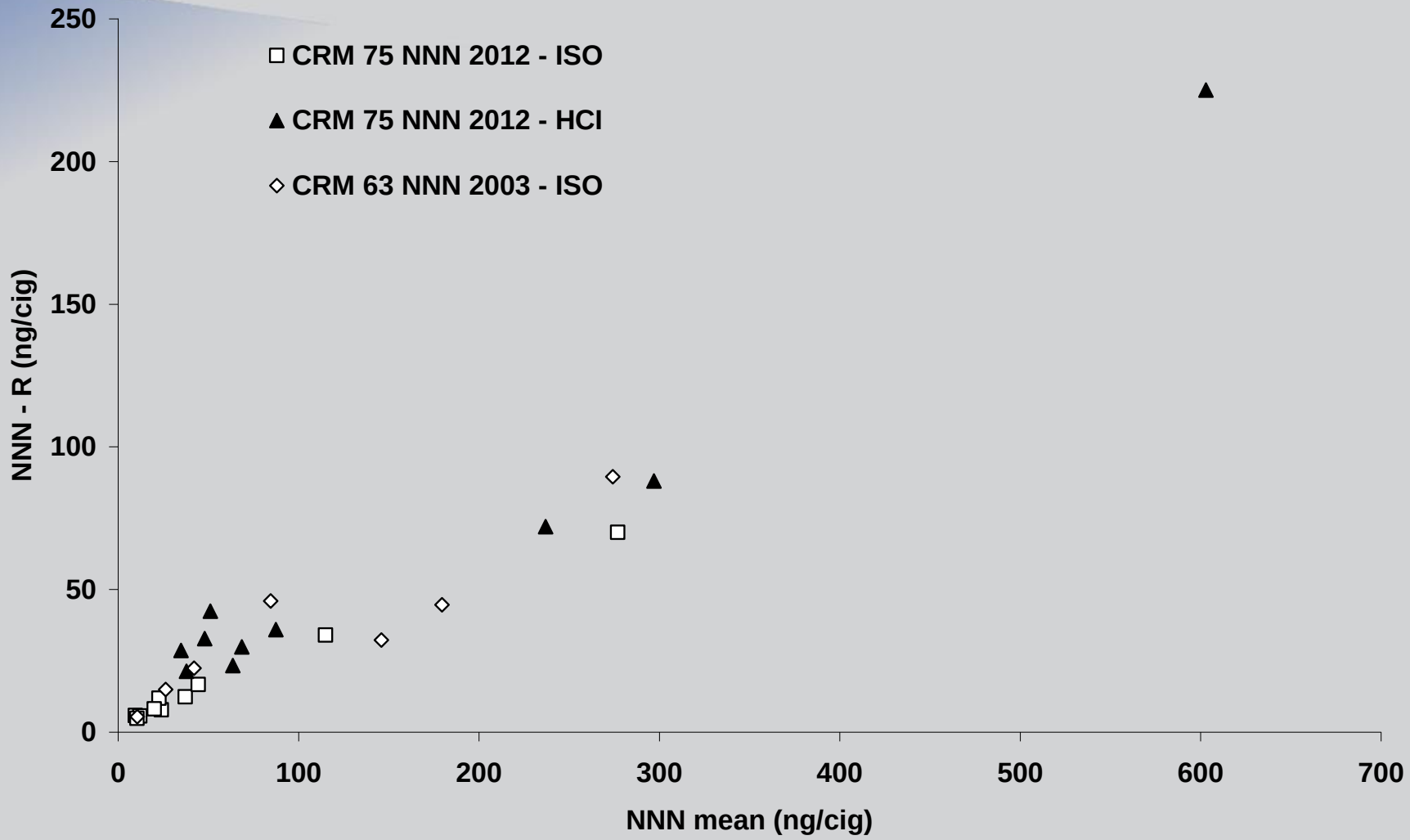
TSNA method development by LC-MS/MS between 2009 and 2012

Sample	*ISO Smoking*				*Health Canada Smoking*			
	NNN ng/cig		NNK ng/cig		NNN ng/cig		NNK ng/cig	
	Mean	R%	Mean	R%	Mean	R%	Mean	R%
1	277	25	133	31	603	37	295	48
2	37.3	33	24.5	42	87.5	41	55.5	38
3	24.0	33	17.9	50	68.6	44	49.9	39
4	9.6	60	3.6	85	34.9	82	12.1	77
5	12.1	46	3.3	62	51.2	83	15.0	79
6	22.7	52	7.2	79	48.0	68	14.3	59
7	10.5	46	7.4	41	63.6	36	46.6	50
CM 6	20.0	41	26.5	32	37.9	56	50.8	54
KR1R5F	44.4	38	21.8	37	237	30	121	43
KR3R4F	115	30	97.1	31	297	30	252	37

Tobacco-Specific Nitrosamines - NNK
R versus Mean yield from CRMs 63 & 75



Tobacco-Specific Nitrosamines - NNN
R versus Mean yield from CRMs 63 & 75



Tobacco-Specific Nitrosamines
Summary

- Collaborative Studies were conducted using GC-TEA and LC-MS/MS methodology.
- Both methods gave comparable results for variability but higher than for TNCO.
- Advantage of LC-MS/MS method is the significantly higher throughput.
- NNN-d4 and NNK-d4 are recommended for quantification to adjust for matrix effects.
- CRM 63 for GC-TEA method was published in 2005.
- CRM 75 for LC-MS/MS was published in 2012.
- Reproducibility under ISO and Canadian Intense regime was similar
- There were no significant differences between data obtained using either linear or rotary smoking engines
- The study from 2011 was published in Beiträge 2012.
- CRM 75 will be raised by CORESTA as a new work item within ISO (ISO 3308 smoking regime only)

Conclusions & Achievements

❖ Special Analytes Sub-Group

- Two CRMs have been developed for TSNAs.
- Experiences in method development were shared within the Sub-Group and made publicly available in a Beiträge publication.
- Round-table open discussions have provided valuable insight in method performance issues and technical limitations

Appendix - Publications

- **Intorp M., Purkis, S.W., Wagstaff, W.,**
  Determination of tobacco specific nitrosamines in cigarette mainstream smoke: the CORESTA 2011 collaborative study;
  Beitr. TabakForsch. Int. 2012; 25(4) 507- 519.

Questions?