Validation of TNCO, TSNAs, and PAHs

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General Method Testing for TNCO, TSNAs and PAHs

A. Discuss the solution stability for prepared solutions and procedures to ensure their integrity.

TNCO

Calibration solutions are demonstrably stable for 3 months when stored under refrigeration. Calibration graphs are compared for consistency of slope and intercept.

Extraction solutions are demonstrably stable for 3 weeks but are prepared weekly.

PAHs

Native and labelled PAHs are stable indefinitely (stability >3 years at -20℃ & dark)

Audit trail (by mass) to assess solvent evaporation during storage.

Calibrations are compared to assess stability over time.

TSNAs

Native and labelled TSNA standard solutions are demonstrably stable for 12 months under refrigeration, but are replaced more frequently due to volume of use.

Replacement standards are analysed to assess isotopic purity (*e.g.* possible presence of native TSNAs or di-deuterated analogues).

Calibrations are compared to assess stability over time.

Standards are obtained with appropriate certification and traceability from ISO Guide 34 suppliers (*e.g.* LGC standards) where practicable

General Method Testing for TNCO, TSNAs and PAHs

B. Describe the typical storage conditions and shelf life (i.e., expiration dates) for tobacco product standards and samples.

Tobacco product standards

3R4F cigarettes and tobacco are stored and handled in accordance with storage information provided by the University of Kentucky.

CORESTA Smokeless Tobacco Reference Products are stored and handled in accordance with information provided by the North Carolina State University.

CORESTA Monitor products are stored and handled in accordance with ISO 16055.

For CORESTA CM7, practical experience from previous monitor test pieces has shown the smoke yields to be stable for at least 4 years under the condition that the test pieces are stored unopened and below +4 °C.

Samples

Test samples are stored and conditioned in accordance with ISO 3402.

Shelf life is normally 12 months from manufacture for unopened cartons stored under appropriate conditions, can be extended by frozen storage and is reduced by storage at increased temperature / humidity.

General Method Testing for TNCO, TSNAs and PAHs

C. Describe the standard, reference, or known sample solutions used as blanks or for quality control (QC), working, and check standards when testing TNCO, TSNAs, and PAHs.

TNCO

Standards – Nicotine ERM AC802b (LGC Standards), for calibration and calibration check, certified purity 99.7 mass%

Blanks – Conditioned 44mm Cambridge Filter Pad, extracted as for samples

QC – 3R4F cigarette, internal control product (RAL1)

PAHs

Standards – PAH-CVS-A, PAH-ISS-A & PAH-LCS-A, ex Wellington Laboratories

Blanks – Conditioned 92mm Cambridge Filter Pad, extracted as for samples

QC – 3R4F cigarette, internal control product (RAL1)

TSNAs

Standards – Kinesis S-163406 (IS 40-80µg/ml); LGC CUS-12395 (native TSNAs10-20 µg/ml)

Blanks - Conditioned 44mm Cambridge Filter Pad, extracted as for samples

QC – 3R4F cigarette, internal control product (RAL1)

General Method Testing for TNCO, TSNAs and PAHs

D. Discuss the system suitability and acceptance criteria for each test method. The discussion may include calibration, QC, working, bracketing, and verification standards, confirmation ion ratio for mass spectrometry, chromatographic parameters (i.e., retention times, tailing factor, or peak resolution), injector precision, and blanks.

TNCO

System suitability - as ISO 3308:2012, ISO 4387:2000, ISO 10315:2013, ISO 10362-1:1999; Environmental conditions; smoke machine calibration; balance calibration checks

Nicotine/water – peak tail factor and GC calibration (slope, intercept, R^2)

PAHs

System - as ISO 22634:2008; calibration (slope, intercept, R^2), chromatographic separation (D_{12}-benzo(b)fluoranthene/D_{12}-benzo(k)fluoranthene), signal/noise for lowest native calibrants

TSNAs

System – calibration (slope, intercept, R^2), chromatographic separation (D_4-NAB/D_4-NAT), signal/noise for lowest native calibrants

For all methods, data acceptance criteria – blank value < limit; calibration check within specification; smoking QC results (puff number, TPM, nicotine, water, CO) within control limits, calibration check acceptable; QC results within limits.
For TSNAs and PAHs, inspection of RT for native substance relative to IS and isotopic abundance / MRM response ratios.

General Method Testing for TNCO, TSNAs and PAHs

E. Discuss the critical system suitability parameters that are critical when testing TNCO, TSNAs, and PAHs.

Critical system suitability parameters

1. Demonstration of chromatographic selectivity
– peak separation; peak purity

2. Demonstration of adequate detectability
- signal/noise ratio; impact of recovery, artefacts, suppression

3. Stability of calibration
- drift check throughout analytical sequence;
- short and long-term repeatability of calibration

4. Minimisation of blank contribution
- materials specification; washing / conditioning of media; blank checks

5. Precision of analysis
- batch to batch and over time; between analysts and instruments;
Shewhart charts are used to monitor precision over time

General Method Testing for TNCO, TSNAs and PAHs

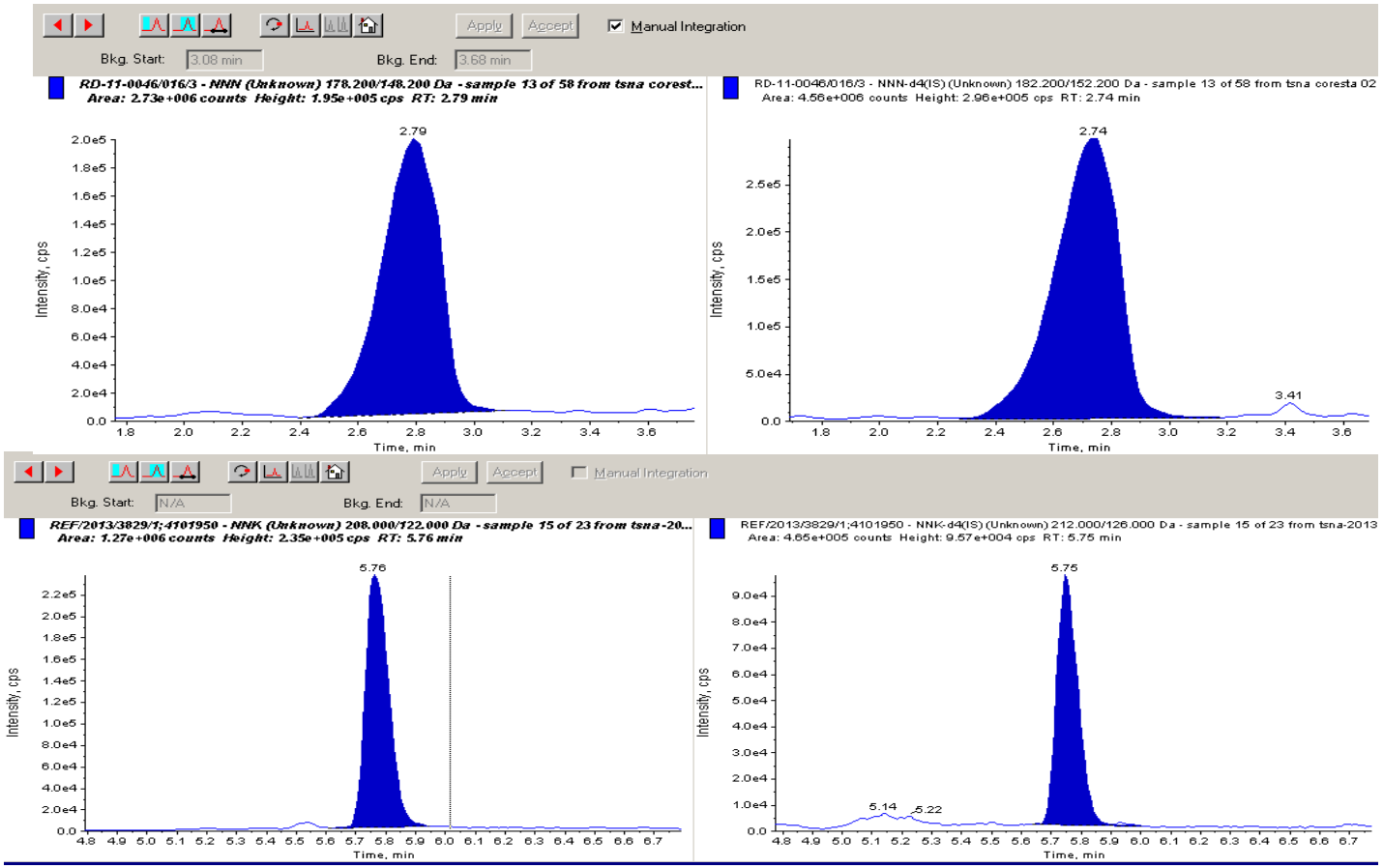
F. Discuss the major sources of method variability, e.g., include sources from the smoking machine or regimen, sample preparation, separation, and detection of different tobacco product types and strengths.

- For tobacco filler and STPs, sample homogeneity and extraction efficiency require attention to avoid impact on quality of results.
- Matrix artefacts can bias results for substances that do not have an exact match IS.
- TPM delivery differs between products but is stable for individual products.
- For TPM, cleanup of the extract (PAHs, TSNAs) significantly reduces the effect of matrix artefacts on analysis but can reduce recoveries.
- Evaporative and adsorptive losses should be minimised during sample workup to avoid effects on LoQ, but are corrected for by SID.
- Naphthalene is not completely retained in TPM and may be further lost during sample handling.

General Method Testing for TNCO, TSNAs and PAHs

G. Discuss specific method challenges and limitations when testing NNN and NNK.

- **Matrix suppression is greater for NNN and NNK than NAT and NAB**
- **Clean up step in the method would be beneficial**
- **Poor peak shape is obtained for NNN when filler is extracted with aqueous buffer**



Validation or Method Performance

A. Discuss the specific details when evaluating each validation parameter.
B. Discuss how each criterion is determined for each validation parameter.

In accordance with ICH Q2B Methodology, IUPAC Harmonized guidelines for single-laboratory validation of methods of analysis and Commission Decision 2002/657/EC.

Linearity of response – dynamic range, correlation coefficient, residuals;

Calibration assessment – matrix (standard addition) vs solvent;

Extraction efficiency – incurred residue from matrix; compare techniques (*e.g.* shake, macerate, ASE) and solvents;

Recovery of native and internal standard – each stage of method, whole method;

Specificity – chromatographic peak purity (GC/HRTOF or LC/HRTOF) – informs optimisation of chromatography and matrix reduction;

Quantification limit – lowest concentration in sample eliciting RSD < 20%;

Accuracy (trueness) – use certified reference material where available, otherwise a reference product or a proficiency sample;

Repeatability – vary analyst, instrument, batch/lot of calibrants, matrix type, incurred or fortified level, time (days/weeks), consumables (*e.g.* column)

Validation or Method Performance

C. Describe the steps taken when validation parameter criteria are not met.

C. Review raw data and procedures followed.
Identify root cause of failure to meet criterion.
Evaluate options for technical improvement, *e.g.* additional calibration point, include IS, introduce clean-up step.
Implement technical improvement and evaluate method performance.
If no improvement achieved, evaluate method scope and purpose.

D. Discuss validation parameters that are performed with reference tobacco products or standards.

D. The following parameters are investigated using reference tobacco products –
linearity of response; calibration; extraction efficiency; recovery; specificity; quantification limit; repeatability / intermediate precision

Additionally, parameters investigated using reference and commercial tobacco products are –
linearity of response; calibration; extraction efficiency; specificity; quantification limit; intermediate precision

Validation or Method Performance

E. Discuss the types and strengths of tobacco product samples used during validation and method development.

Tobacco filler and smokeless tobacco products

- CORESTA STRPs; Swedish-style snus, US-style moist snuff, US-style dry snuff & loose leaf tobacco

- **University of Kentucky Reference Cigarettes; 3R4F blend**
- **Commercial products (*e.g.* chewing tobacco, other blends)**

Cigarettes

- University of Kentucky Reference Cigarettes; 3R4F

- Products tested comply with the Tobacco Products Directive (2001/37/EC) [10mg 'tar', 1mg nicotine, 10mg CO maximum], therefore high yield monitor products (*e.g.* CM7) are used only for research purposes

- Commercial products also used as internal control samples, generally <7mg 'tar'.

Validation or Method Performance

F. Discuss the process taken to revalidate a test method when changes to the method (i.e., solvent, extraction method, or column) are made.

Review of performance parameters that could be affected
- *e.g.* for column change, selectivity, peak shape, impact of matrix artefacts
Design of experiments to evaluate impact of change
- re-assess the performance parameter, compare to original method performance if within specification, *e.g.* no increase in intermediate precision.

G. Discuss the validation process when using a rotary and linear smoking machine with a nonintense and intense smoking regimen.

For TNCO determination, linear smoke machines are used.
TSNA analysis utilises linear smoke machines.
PAH analysis utilises rotary smoke machines.

Validation includes ISO and Canadian intense regimes.

Validation or Method Performance

H. Describe the robustness or ruggedness tests that are conducted for extraction efficiency, solution stability, and small changes in instrument parameters.

Extraction efficiency
- Comparison of solvents with reference to product and analyte chemistry, peer published methods and related matrices (*e.g.* food, environmental)
- Comparison with peer reference methods, *e.g.* Soxhlet extraction, ASE or multiple partitioning extraction

Solution stability
- Comparison of concentration between solutions stored at room temperature/daylight; room temperature dark; 4°C dark; -20°C dark
- All comparisons against freshly prepared control solution

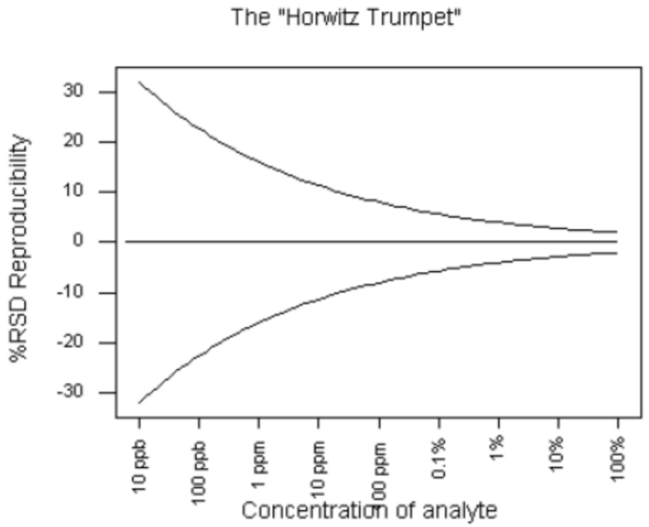
Instrument parameters
- Intermediate precision requires >1 analyst, >1 instrument, which usually covers small instrument variations:
- Also includes performance close to limits of system suitability

Validation or Method Performance

Concluding comments

Fitness for purpose criteria should include within- and between laboratory precision.
For example, target Reproducibility RSD_R can be calculated from the Horwitz function.

$$PRSDR\ (\%) = 2\ C^{-0.1505}$$



Reproduced from AMC technical Brief 17, Royal Society of Chemistry 2004.

Table 6. Comparison of published and predicted reproducibility for measurement of tobacco-specific *N*-nitrosamines in mainstream cigarette smoke.

Smoking conditions	Tobacco-specific *N*-nitrosamine							
	NNN		NAT		NAB		NNK	
ISO	High	Low	High	Low	High	Low	High	Low
Mean, µg/cig	277	9.6	145	11	20	1.5	122	3.3
R	70	5.8	74	6.2	9	0.9	41	2.1
RSD_R, %	25.3	60.4	51	56.4	45	60	33.6	63.6
Mass fraction	2.77×10^{-07}	9.6×10^{-09}	1.45×10^{-07}	1.1×10^{-08}	2×10^{-08}	1.5×10^{-09}	1.22×10^{-07}	3.3×10^{-09}
$PRSD_R$, %	19.4	32.2	21.4	31.5	28.8	42.6	22	37.8
HorRat	**1.3**	**1.9**	**2.4**	**1.8**	**1.6**	**1.4**	**1.5**	**1.7**
Intense								
Mean, µg/cig	603	34.9	322	39.4	42.9	5.5	297	12.1
R	225	28.6	214	25.2	21.4	6.2	144	9.3
RSD_R, %	37.3	81.9	66.5	64	49.9	112.7	48.5	76.9
Mass fraction	6.03×10^{-07}	3.49×10^{-08}	3.22×10^{-07}	3.94×10^{-08}	4.29×10^{-08}	5.5×10^{-09}	2.97×10^{-07}	1.21×10^{-08}
$PRSD_R$, %	17.3	26.5	19	26	25.7	35	19.2	31.1
HorRat	**2.2**	**3.1**	**3.5**	**2.5**	**1.9**	**3.2**	**2.5**	**2.5**

Concentration data from CORESTA Recommended Method 75, Determination of TSNAs in mainstream smoke by LC-MS-MS

from Wright 2013 *in preparation*