TNCO Testing procedures

Chris Proctor

Pete Davis

British American Tobacco Group R&D UK

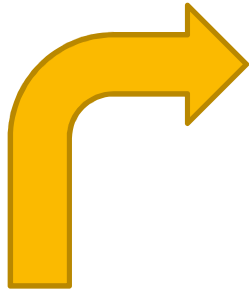# TNCO in Cigarette Smoke

A.   Describe the different extraction steps used when analysing cigarette smoke for TNCO.

Particulate phase (gravimetric)

Extraction in IPA (including internal standards)

Nicotine and water (GC FID & GC-TCD)

Vapour phase

Carbon monoxide (NDIR)

TNCO in Cigarette Smoke

A. Describe the different extraction steps used when analysing cigarette smoke for TNCO.

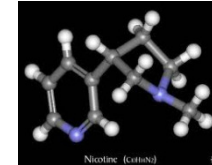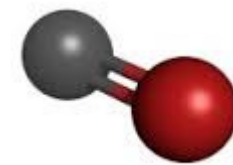**ISO 4387**   **ISO 10315**   **ISO 10362-1**

'Tar' = NFDPM = TPM − Nicotine − H_2O

ISO 3308

Gas Phase

ISO 4387   CO by **ISO 8454**

TNCO in Cigarette Smoke

B.   Provide the typical concentration ranges for TNCO and the potential method adjustments to accommodate different cigarette strengths and physical parameters

	TPM (mg/cig)	CO (mg/cig)	Nicotine (mg/cig)	Water (mg/cig)	NFDPM (mg/cig)
Product 1	12.5 ± 0.26	8.7 ± 0.29	0.97 ± 0.02	1.73 ± 0.10	9.8 ± 0.17
3R4F	9.8 ± 0.13	12.0 ± 0.18	0.77 ± 0.01	1.15 ± 0.08	7.9 ± 0.07
Product 2	4.9 ± 0.30	5.2 ± 0.23	0.37 ± 0.02	0.39 ± 0.06	4.1 ± 0.24
Product 3	1.6 ± 0.12	1.6 ± 0.15	0.13 ± 0.01	0.36 ± 0.80	1.1 ± 0.77

- 5 cigarettes smoked onto a 44mm CFP at ISO smoking regime
- Covers typical range analysed in BAT GR&D

TNCO in Cigarette Smoke

B.   Provide the typical concentration ranges for TNCO and the potential method adjustments to accommodate different cigarette strengths and physical parameters

Standard	Nicotine concentration (mg/mL)	Water concentration (mg/mL)
1	0.025	0.1
2	0.05	0.25
3	0.5	0.5
4	0.625	2.2

Calibration ranges for nicotine and water
- Some <1mg tar products fall below standard 1
- Number of cigarettes reduced to conform with ISO 4387 for high yield products
- Number of cigarettes reduced from 5 cigarettes per CFP for ISO to 3 for HCI
- Water calibration linear to 10.2mg/mL

Comparability of calibration curve parameters over time

Date of Calibration	Instrument ID	Nicotine		Water	
		Gradient	Intercept	Gradient	Intercept
25/10/2011	GC 15	2.90E+00	-8.42E-04	2.37E-01	-5.42E-01
10/02/2012	GC 15	3.07E+00	-5.13E-03	2.52E-01	2.67E-02
28/03/2012	GC 13	2.73E+00	2.52E-04	2.74E-01	4.34E-02
24/05/2012	GC 13	3.01E+00	7.73E-04	2.76E-01	5.85E-02
26/04/2012	GC 15	2.90E+00	1.16E-02	2.73E-01	6.19E-02
26/04/2012	GC 13	2.93E+00	4.56E-03	2.79E-01	6.15E-02
Mean		2.92E+00	1.87E-03	2.66E-01	-4.83E-02
SD		1.15E-01	5.69E-03	1.69E-02	2.42E-01

TNCO in Cigarette Smoke

C.   Discuss the optimal solvents, extraction solution, standards, and reference tobacco product(s) typically used when analysing TNCO.

- TPM extracted in IPA containing internal standards for both nicotine and water, in accordance with ISO 10315:2013
  - N-heptadecane used as internal standard for nicotine analysis
  - Ethanol used as internal standard for water analysis

- Internal standard in extracting solution ensures consistent delivery for every sample in fixed volume of extraction solvent, but does not replicate incurred nature of analyte in sample.

- Adding internal standards to the sample with equilibration prior to extraction would provide a more robust assessment of extraction in the method.
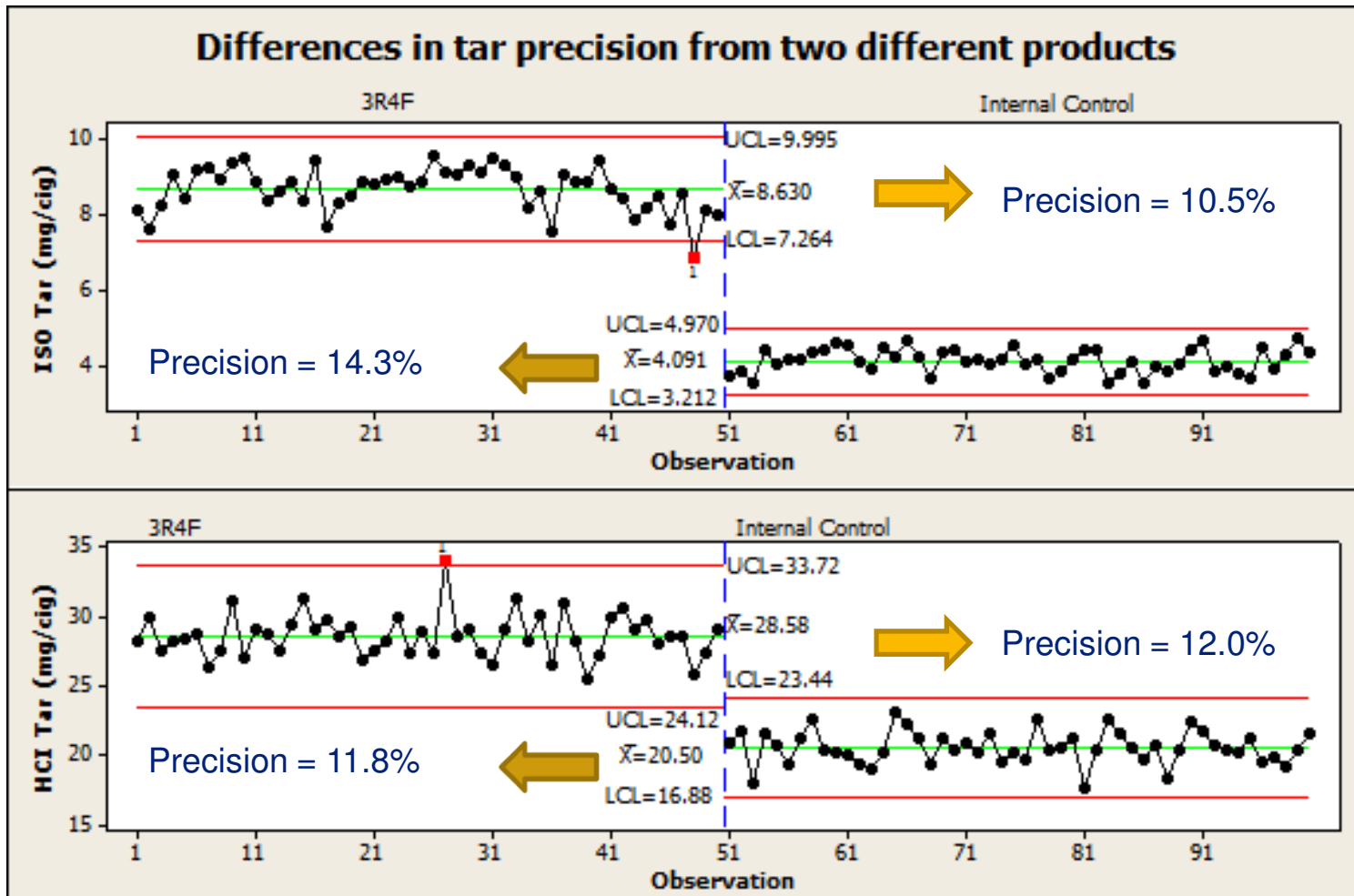
TNCO in Cigarette Smoke

D.   Discuss the method variability and whether or not it is dependent upon different products in your portfolio.

Differences in tar precision from two different products

- ISO: 3R4F UCL=9.995, X̄=8.630, LCL=7.264; Precision = 14.3% (3R4F); Internal Control Precision = 10.5%; UCL=4.970, X̄=4.091, LCL=3.212
- HCI: 3R4F UCL=33.72, X̄=28.58, LCL=23.44; Precision = 11.8% (3R4F); Internal Control Precision = 12.0%; UCL=24.12, X̄=20.50, LCL=16.88

TNCO in Cigarette Smoke

D. Discuss the method variability and whether or not it is dependent upon different products in your portfolio.
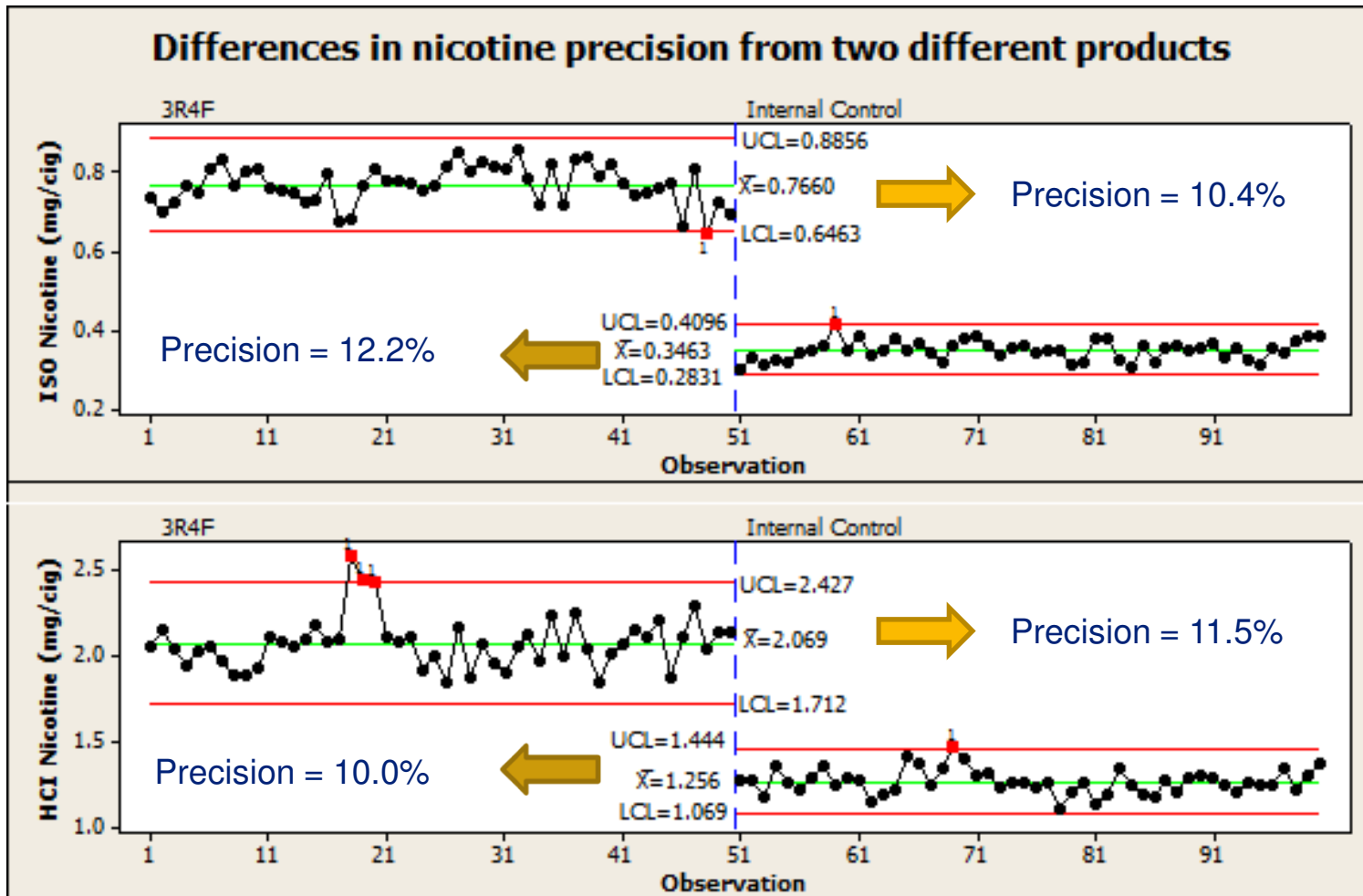

Differences in nicotine precision from two different products

ISO

HCI

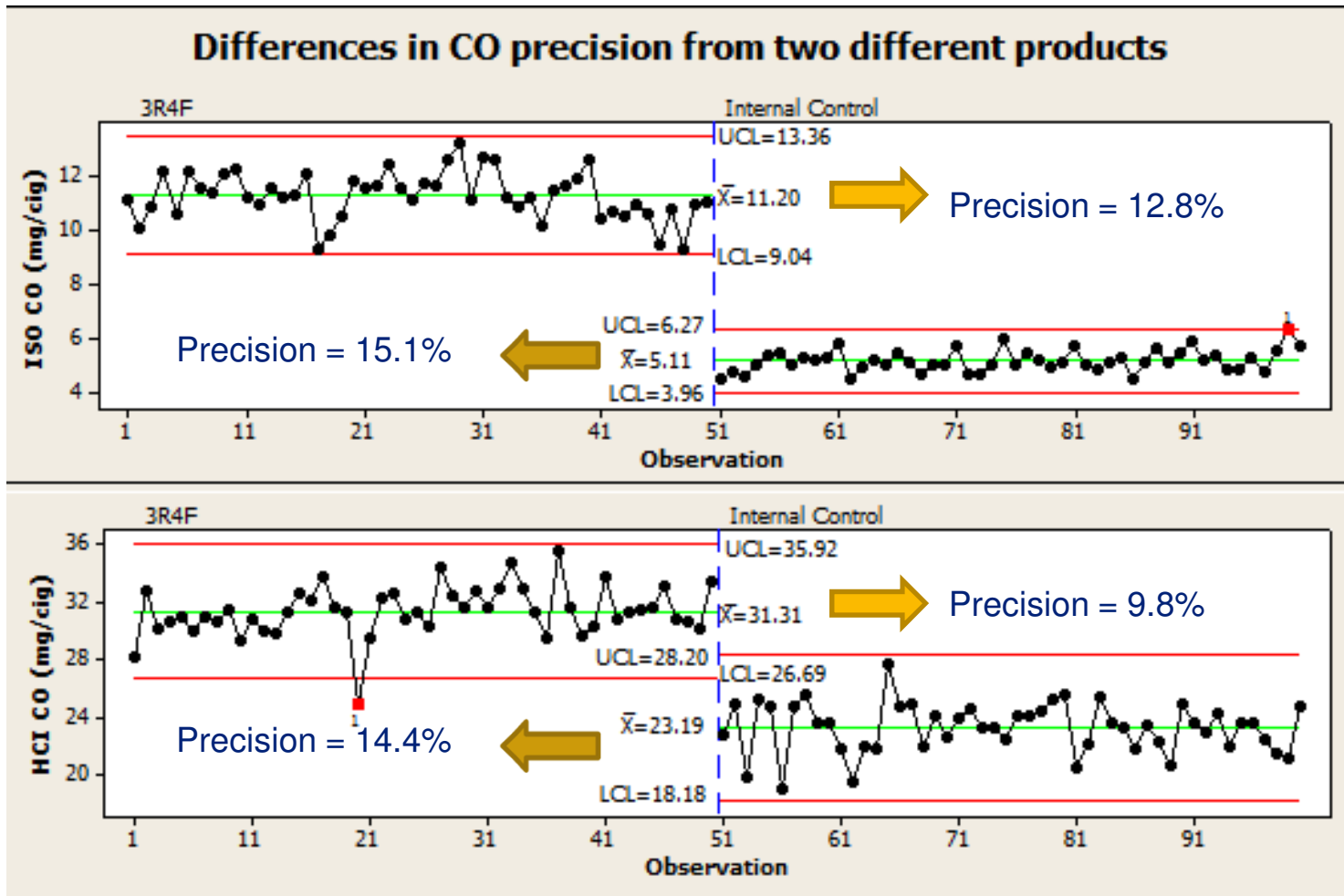# TNCO in Cigarette Smoke

D. Discuss the method variability and whether or not it is dependent upon different products in your portfolio.

Differences in CO precision from two different products

ISO

HCI

TNCO in Cigarette Smoke

E.   Discuss specific method challenges and limitations when testing TNCO, such as environmental moisture, water measurement variability, and extraction efficiency

Management of environmental moisture

- **All samples and Cambridge filter pads conditioned**
  - The manner in which pads are stored whilst in the conditioning room is significant
  - Bespoke racks manufactured to store pads vertically and separated to ensure suitable airflow around each pad to ensure equilibrium is reached
- **Extraction solution dispenser fitted with desiccator cap (contains a drying agent to prevent ingress of moisture)**
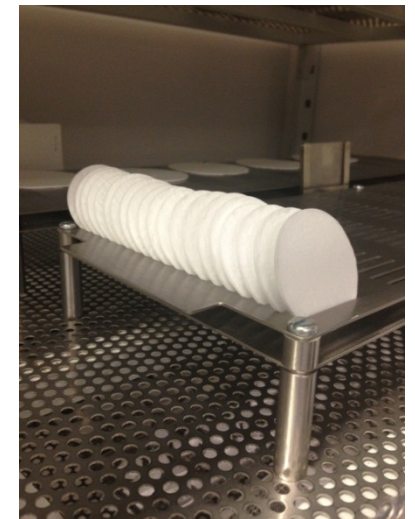
TNCO in Cigarette Smoke

E.   Discuss specific method challenges and limitations when testing TNCO, such as environmental moisture, water measurement variability, and extraction efficiency

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Management of environmental moisture

- All glassware must be dried
  - Minimum of 1 hour in 'wet' oven at 105°C
  - Transferred to a 'dry' oven at 105° for another 1 hour minimum
  - Once dry, allowed to cool in desiccator and then used

- Maximum of 4 sample vials taken from each QC flask to minimise adsorption of water
  - Each vial injected once
- 2 blank pads per smoke run (one at start and one at end)
  - Average blank value subtracted from samples
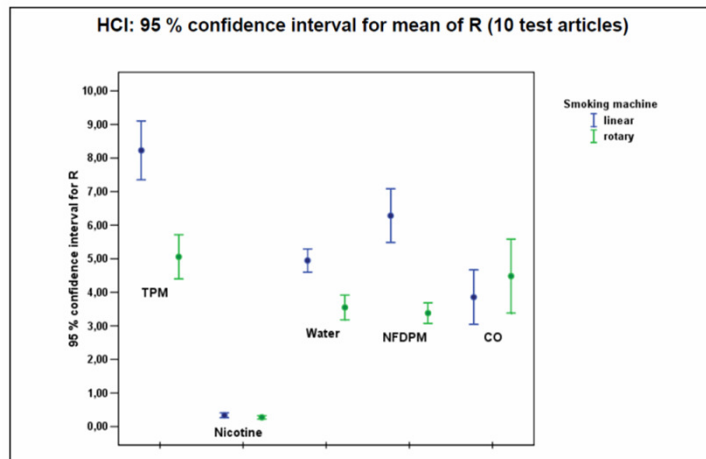 - System suitability includes maximum value of water blank

TNCO in Cigarette Smoke

F.   Provide the major sources of variability (e.g., smoking machine or regimen, sample preparation, separation, and detection).

ISO: 95 % confidence interval for mean of R (10 test articles)

HCI: 95 % confidence interval for mean of R (10 test articles)

- WG10 (formed by ISO TC126)

- Collaborative study in 2010

- Consistent deliveries between smoke machine types at ISO

- Statistically significant differences between linear and rotary smoking machines at HCI
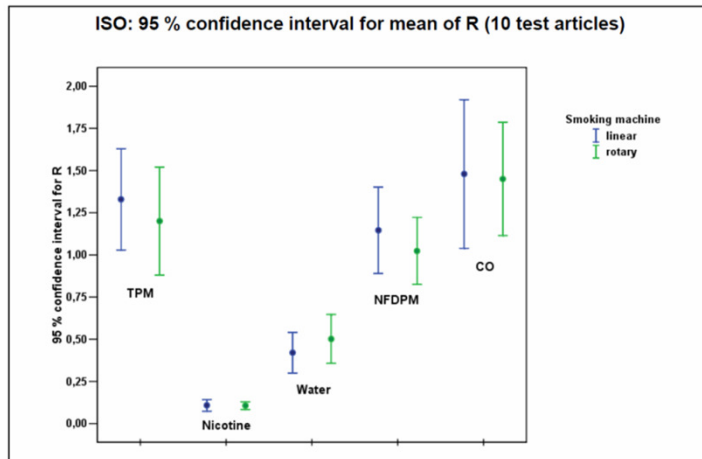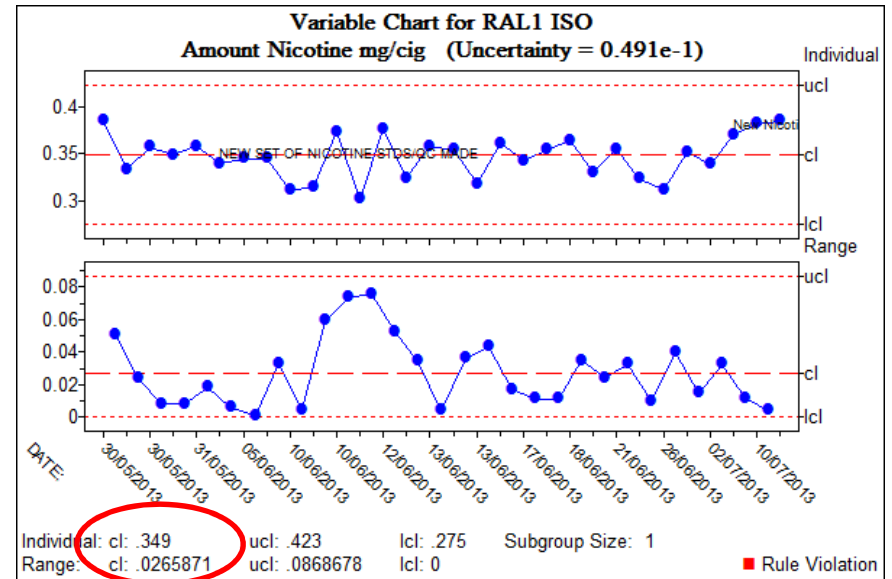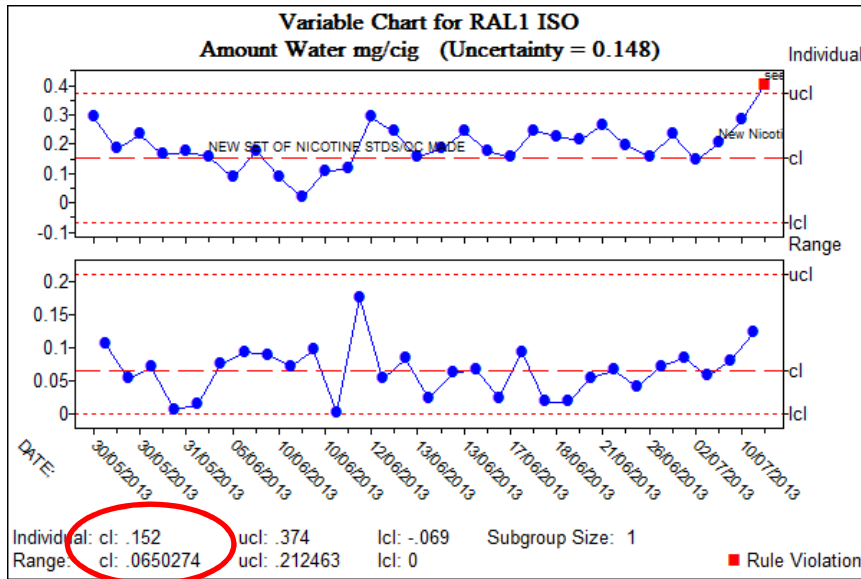 - TPM
  - Water
  - NFDPM

REFERENCE

TNCO in Cigarette Smoke

F.   Provide the major sources of variability (e.g., smoking machine or regimen, sample preparation, separation, and detection).
Comparison of method performance against repeatability data cited in ISO standards

Variable Chart for RAL1 ISO — Amount Water mg/cig (Uncertainty = 0.148)

Individual: cl: .152   ucl: .374   lcl: -.069   Subgroup Size: 1
Range: cl: .0650274   ucl: .212463   lcl: 0

Variable Chart for RAL1 ISO — Amount Nicotine mg/cig (Uncertainty = 0.491e-1)

Individual: cl: .349   ucl: .423   lcl: .275   Subgroup Size: 1
Range: cl: .0265871   ucl: .0868678   lcl: 0

ISO r Estimates for Water Analysis	
Mean Value (mg/cig)	Repeatability, r (mg/cig)
0.083	0.154
0.153	0.228
0.338	0.272
0.962	0.407
1.595	0.561
3.187	0.908

ISO r Estimates for Nicotine Analysis	
Mean Value (mg/cig)	Repeatability, r (mg/cig)
0.091	0.040
0.179	0.046
0.326	0.050
0.673	0.077
0.832	0.079
1.412	0.107

Method performance is consistent with ISO repeatability specifications

TNCO in Cigarette Smoke

F.   Provide the major sources of variability (e.g., smoking machine or regimen, sample preparation, separation, and detection).

Comparison of BAT method performance against r and R data cited in ISO standards

BAT Global Cross Check r and R for Nicotine Analysis		
Mean Value (mg/cig)	Repeatability, r (mg/cig)	Reproducibility, R (mg/cig)
0.11	0.02	0.06
0.39	0.04	0.12
0.59	0.05	0.15
0.77	0.06	0.14

ISO r and R Estimates for Nicotine Analysis		
Mean Value (mg/cig)	Repeatability, r (mg/cig)	Reproducability, R (mg/cig)
0.091	0.040	0.069
0.179	0.046	0.069
0.326	0.050	0.076
0.673	0.077	0.109
0.832	0.079	0.142

BAT Global Cross Check r and R for Water Analysis		
Mean Value (mg/cig)	Repeatability, r (mg/cig)	Reproducibility, R (mg/cig)
0.08	0.10	0.18
0.39	0.19	0.48
0.69	0.27	0.67
0.71	0.24	0.66

ISO r and R Estimates for Water Analysis		
Mean Value (mg/cig)	Repeatability, r (mg/cig)	Reproducability, R (mg/cig)
0.083	0.154	0.241
0.153	0.228	0.353
0.338	0.272	0.381
0.962	0.407	0.734

- In all instances, repeatability is consistent with estimates in both ISO 10315 and ISO 10362-1
- In all instances, water reproducibility is consistent with estimates in ISO 10362-1
- In the majority of instances, nicotine reproducibility is higher than estimates in ISO10315

TNCO in Cigarette Smoke

F.   Provide the major sources of variability (e.g., smoking machine or regimen, sample preparation, separation, and detection).

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Comparison of method performance against repeatability data cited in ISO standards

WG10 collaborative study at ISO regime		
Mean Nicotine (mg/cig)	r	R
0,108	0,025	0,046
0,389	0,040	0,087
0,677	0,054	0,126
0,816	0,072	0,130
0,663	0,076	0,120
0,752	0,058	0,126
0,830	0,055	0,126
0,665	0,051	0,123
0,154	0,016	0,057
1,366	0,088	0,164

WG10 collaborative study at HCI regime		
Mean Nicotine (mg/cig)	r	R
1,267	0,079	0,199
1,342	0,092	0,218
1,789	0,116	0,348
2,106	0,151	0,401
1,407	0,139	0,359
2,069	0,120	0,366
2,086	0,151	0,338
1,681	0,116	0,301
0,994	0,084	0,249
2,683	0,160	0,490

ISO r and R Estimates for Nicotine Analysis		
Mean Value (mg/cig)	Repeatability, r (mg/cig)	Reproducibility, R (mg/cig)
0.091	0.040	0.069
0.179	0.046	0.069
0.326	0.050	0.076
0.673	0.077	0.109
0.832	0.079	0.142
1.412	0.107	0.195

- Comparison between laboratories:
- WG10 collaborative study data compared to ISO r and R estimates
  - ISO data consistent with estimates in ISO 10315
  - HCI data are not consistent with estimates in ISO 10315
    - Particularly reproducibility
- Cannot make similar comparisons with water data as HCI yields are significantly higher than means quoted in ISO 10362-1

TNCO in Cigarette Smoke

Main observations

- Methods are aligned with relevant ISO standards

- The laboratory uses linear smoke machines for TNCO

- Long-term precision of data is consistent with estimates in ISO standards

- Controlling environmental moisture is critical to precision

- Linear and rotary smoke machine yields of NFDPM and water are different at the HCI smoking regime

- Reproducibility at HCI is not consistent with ISO estimates