Comparison of WHO SOPs and CORESTA CRMs for tobacco product testing

CORESTA
Smoke Science & Product Technology
Joint Study Groups
Kitzbühel, Austria,
8-12 October 2017

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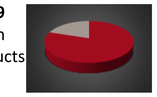
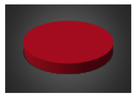
1. Introduction

The Conference of the Parties to the WHO FCTC, at their 3rd session in 2008, mandated the WHO Tobacco Laboratory Network (TobLabNet) to validate the analytical chemical methods for testing and measuring cigarette contents (nicotine, ammonia, humectants) and emissions (tar, nicotine and carbon monoxide (TNCO), aldehydes, benzo[a]pyrene (BaP), and volatile organic compounds (VOCs)). WHO has published the standard operating procedures (SOPs) for TNCO, BaP and TSNA in cigarette smoke and Nicotine, Humectants and Ammonia in cigarette tobacco filler, while validations of VOCs and Aldehydes are ongoing to date.

This poster compares the SOPs from TobLabNet with the existing CORESTA Recommended Methods (CRMs) to identify major differences and potential challenges that must be addressed before the new methods adopted implemented within regulatory testing programs. In any case, changes to methodology – regardless of its extend, would necessitate a full accreditation process, due to the ISO certification of most product testing laboratories.

2. Overall Method Comparison

SOP	CRM	Similarity	SOP	CRM	Similarity
SOP 01 Intense smoking of Cigarettes	The SOP is only referring to ISO standards		**SOP 05** B[a]P in Smoke	**CRM No.58** B[a]P in Smoke	
SOP 03 TSNA in Smoke	**CRM No.75** TSNA in Smoke		**SOP 06** Humectants in Tobacco Filler	**CRM No. 60** 1,2-Propylene Glycol and Glycerol in Tobacco Products	
SOP 04 Nicotine in Tobacco Filler	**CRM No. 62** Nicotine in Tobacco Products (GC)		**SOP 07** Ammonia in Tobacco Filler	**CRM No. 79** Ammonia in Tobacco Products	
SOP 04 Nicotine in Tobacco Filler	**CRM No. 35** Total alkaloids as Nicotine		**SOP 10** Nicotine and CO in Smoke	The SOP is only referring to ISO standards	

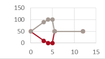
References

WHO: *Standard Operating Procedure For Intense Smoking of Cigarettes*
WHO: *Standard Operating Procedure For Determination of Tobacco-Specific Nitrosamines in Mainstream Cigarette Smoke Under ISO and Intense Smoking Conditions*
WHO: *Standard Operating Procedure For Determination of Nicotine in Cigarette Tobacco Filler*
WHO: *Standard Operating Procedure For Determination of Benzo[a]Pyrene in Mainstream Cigarette Smoke Under ISO and Intense Smoking Conditions*
WHO: *Standard Operating Procedure For Determination of Humectants in Cigarette Tobacco Filler*
WHO: *Standard Operating Procedure For Determination of Ammonia in Cigarette Tobacco Filler*
WHO: *Standard Operating Procedure For Determination of Nicotine and Carbon Monoxide in Mainstream Cigarette Smoke Under Intense Smoking Conditions*
CORESTA RECOMMENDED METHOD N° 75: *DETERMINATION OF TOBACCO SPECIFIC NITROSAMINES IN MAINSTREAM CIGARETTE SMOKE BY LC-MSMS*
CORESTA RECOMMENDED METHOD N° 62: *DETERMINATION OF NICOTINE IN TOBACCO AND TOBACCO PRODUCTS BY GAS CHROMATOGRAPHIC ANALYSIS*
CORESTA RECOMMENDED METHOD N° 58: *DETERMINATION OF BENZO(a)PYRENE IN MAINSTREAM CIGARETTE SMOKE BY GC-MS*
CORESTA RECOMMENDED METHOD N° 60: *DETERMINATION OF 1,2-PROPYLENE GLYCOL AND GLYCEROL IN TOBACCO AND TOBACCO PRODUCTS BY GAS CHROMATOGRAPHY*
CORESTA RECOMMENDED METHOD N° 79: *DETERMINATION OF AMMONIA IN TOBACCO AND TOBACCO PRODUCTS BY GAS CHROMATOGRAPHIC ANALYSIS*

3. Detailed Method Comparison

A detailed method comparison only for the SOPs having a difference to the existing CRMs was carried out in an overview below:

TSNA in smoke

SOP03 CRM No. 75	SOP 03 Standard operating procedure for determination of tobacco-specific nitrosamines in mainstream cigarette smoke under ISO and intense smoking conditions (2014)	CRM No 75 Determination of tobacco specific nitrosamines in mainstream cigarette smoke by LC-MS/MS	Comparison
Method	LC MS/MS	LC-MS/MS	✓
Smoking :	ISO4387 SOP 1	ISO4387 CI	✗
Preparation of Solution	100 mM ammonium acetate buffer	100 mM ammonium acetate buffer	✓
Mobile Phase	Water / 0.1% acetic acid in Methanol	Water / 0.1% acetic acid in Methanol	✓
Standard solutions concentration	0 – 200 ng/mL	0 – 80 ng/mL / 10 ng/mL for NAB-d4	✗
Internal standard solution concentrations	calculated	50 ng/mL for NNN, NNK and NAT	✗
Cigarette sampling	ISO 8243	ISO 8243 :2006	
Conditioning of cigarettes	ISO 3402	ISO3402 :1999	✓
Filter Pad extraction			
44 mm pad	200 µL internal standard 20 mL acetate buffer	200 µL internal standard 20 mL acetate buffer	
92 mm pad	500 µL internal standard 50 mL acetate buffer	400 µL internal standard 40 mL acetate buffer	✗
extraction	30 min shaker 250 rpm	60 min shaker 210 rpm	
filtration	0.45 µm membrane	0.45 µm membrane	
Important HPLC settings			
Column	Agilent Zorbax Eclipse XDB-C18 or equivalent	Reversed phase column	
Column temperature	40°C	65°C	✗
Injection volume	5 µL	5 µL	✗
Elution gradient			✗
Analysis time	12 min	20 min	✗
MS MS			
Ionisation	ESI	ESI	✓

B[a]P in smoke

SOP05 CRM No. 58	SOP 05 Standard operating procedure for determination of Benzo[a]pyrene in mainstream cigarette smoke under ISO and intense smoking conditions	CRM No 58 Determination of Benzo[a]pyrene in mainstream cigarette smoke by GC-MS	Comparison
Analytic principle	GC-MS	GC-MS	✓
Sample	Cigarettes	Cigarettes	✓
Standard procedure	TPM collected on CFP Extraction and spiking SPE (Solid Phase extraction) GC-MS	TPM collected on CFP Extraction and spiking Dilution with water SPE (Solid Phase extraction) GC-MS	✓
Extraction Solution	Cyclohexane	Methanol	✗
Internal standard	Benzo[a]pyrene-D12	Benzo[a]pyrene-D12	✓
SPE	Silica SPE cartridge	Cyclohexyl bonded silica SPE cartridge	✗
Important GC settings			
Column	Fused silica capillary column with a methylphenyl (5%) polysiloxane stationary phase 30 m, 0.25 mm internal diameter, 0.25 µm film thickness (DB-5ms Agilent or equivalent	Fused silica capillary column with a methylphenyl (5%) polysiloxane stationary phase 30 m, 0.25 mm internal diameter, 0.25 µm film thickness	✓
Column temperature			
Temperature gradient			✗
Flow rate	1.2 mL/min	0.9 mL/min	✗
Detector	MS : BaP : m/z=252 BaP-D12 : m/z=264	MS : BaP : m/z=252 BaP-D12 : m/z=264	✓
Analysis time	About 46 min per GC run	About 65 min per GC run	✗

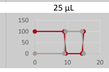
Nicotine in tobacco (GC)

SOP04 CRM No. 62	SOP 04 Standard operating procedure for determination of nicotine in cigarette tobacco filler	CRM No 62 Determination of nicotine in tobacco and tobacco products by gas chromatographic analysis	Comparison
Analytic principle	GC	GC	✓
Sample	Cigarettes	Cigarettes & tobacco products	✓
Extraction Solution		2 possible Versions	
Solution 1	n-hexane 20 mL water 10 mL NaOH 80 g/L solution 60 min extraction on shaker	n-hexane 20 mL water 10 mL 8 N NaOH solution 60 min extraction on shaker	✓
Internal standard	n-heptadecane or alternative	n-heptadecane or alternative	
Solution 2	-	MTBE (2-Methoxy-2-methylpropan)	✗
Internal standard	-	quinoline	
Important GC settings			
Column	Varian WCOT fused silica 25 m x 0,25 mm ID	Hexane method: typical 0.53 mm ID by 15 m MTBA method: typical 0,32 mm or 0.25 mm ID by 30 m	✗ ✗ ✓
Column temperature	170°C	Hexane method: 170°C MTBA method: 110°C-245°C	✗
Injection volume	1 µL	1 µL	✓
Split	1:10	Hexane 1:10 MTBA 40:1	✗
Carrier gas	helium	Helium, nitrogen or hydrogen	
Flow rate	1.5 mL/min	Hexane method 5-6 mL/min MTBA method : 1.7 mL/min	✓
Detector	FID	FID	
Analysis time	About 10 min	Hexane method: 10 min MTBA method: 28.5 min	✓ ✗

Humectants in tobacco

SOP06 CRM No. 60	SOP 06 Standard operating procedure for determination of humectants in cigarette tobacco filler	CRM No 60 Determination of 1,2-propylene glycol and glycerol in tobacco and tobacco products by gas chromatographic analysis	Comparison
Analytic principle	GC	GC	✓
Sample	Cigarettes	Cigarettes and tobacco products including smokeless tobacco	✓
Extraction Solution	Methanol/1,3-butandiol	Methanol	✗
Internal standard	1,3-butandiol used as internal standard	1,4-butandiol	✗
Important GC settings			
Column	DB-WAX fused silica 30 m x 0.32 mm ID 25.0 µm coating	Not limited but suitable: 0.25 mm ID with 35% diphenyl – 65% dimethyl polysiloxane 1 µm coating	✗
Temperature Gradient	120°C for 3 min 10°C/min to 180°C hold for 11 min	110°C 1 min 10°C/min to 150°C 3min 30°C/min to 220°C 5 min	✗
Injection volume	1 µL	2 µL	✗
Split	25:1	50:1	✗
Carrier gas	Helium	Helium	✓
Flow rate	1.8 mL/min	Velocity : 20 – 30 cm/s at 220°C	✗
Detector	FID / or MS	FID	✓
Analysis time	20 min	Not stated	✗

Ammonia in tobacco

SOP07 CRM No. 79	SOP 07 Standard operating procedure for determination of ammonia in cigarette tobacco filler	CRM No 79 Determination of ammonia in tobacco and tobacco products by ion chromatographic analysis	Comparison
Method	Ion Chromatography	Ion Chromatography	
Sample	Cigarettes	Cigarettes and tobacco products	
Preparation of extraction solution	0.0125 mol/L H_2SO_4	0.0125 mol/L H_2SO_4	✗
Mobile Phase 1	20 mM MSA in water	10 mM MSA in water	✓
Mobile Phase 2	-	40 mM MSA in water	
Working standard ammonia solutions concentration	0.1 – 10 µg/mL 5 points	0.1 – 10 µg/mL 7 points	✗
Cigarette/tobacco sampling	Mill tobacco sample to mesh size < 1 mm	Cigarettes ISO 8243 :2006 At least 7 g tobacco NO Milling	✗ ✗
Important Ion chromatography settings			
Column	Not mentioned	Not mentioned	
Detector	Suppressed conductivity detector	Suppressed conductivity detector	✓
Column temperature	30°C	30°C	
Injection volume	25 µL	25 µL	✓
Elution gradient			✗
Analysis time	12 min	15 min	✗

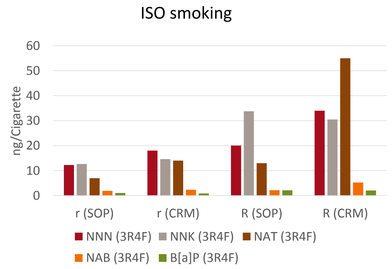
Nicotine in tobacco (CF)

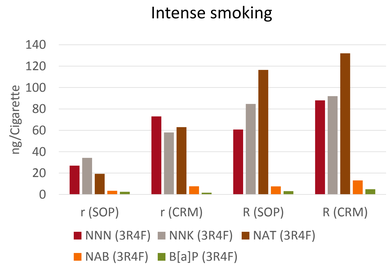
CRM No. 35 describing Continuous Flow Analysis for total alkaloids as nicotine is a total different, well established analytical principle leading to comparable results. ✗

✓ Similar
✗ Slight different
✗ Different

4. R and r comparison for smoke

Repeatability and reproducibility of smoke analytes for reference product 3R4F are displayed in the figures below. It can be observed, that the SOP methods show slightly lower repeatability and reproducibility values than the CRMs. The total number of participating laboratories for the r and R investigations are lower for the SOPs.





5. Conclusions

- Method comparison of the Standard Operating Procedures by TobLabNet and the CORESTA Recommended Methods for the same analytes shows no major differences in terms of analytical principles for the detection of TSNA and B[a]P in smoke as well as humectants, ammonia and nicotine (by GC) in tobacco.
- There is no Continuous Flow method from TobLabNet compared to the CRM No. 35 to analyse total alkaloids as nicotine.
- The robust and comprehensive framework used to develop CRMs has developed methods, suitable for evaluation and adoption through ISO processes. CRM's are widely used in different laboratories and are further strengthened and maintained by international collaborative studies with a greater number of participants compared to the SOPs.
- Current and future publications of WHO/SOP might require systematic investigations and technical reports by CORESTA working groups before further initiatives are taken at ISO of potential standard revision or publication.
- Overall it can be stated, that every method change, slightly or not, would lead to a full accreditation process due to the ISO certification of most laboratories.

SSPT2017 - Document not peer-reviewed by CORESTA