Method Modifications and Validation to Expand Scope of CRM No. 95 to Analyze Select Aromatic Amines in Heated Tobacco Products

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Abstract

Heated Tobacco Products (HTPs) are a growing product category, as evidenced by the increasing commercialization of products such as IQOS, glo, and PLOOM in markets worldwide. HTPs contain a tobacco substrate that is heated to low temperatures (<350°C), as opposed to burning it in a conventional cigarette. Heating tobacco in an HTP prevents high-temperature pyrolysis and combustion reactions and reduces the generation of harmful and potentially harmful constituents (HPHCs), such as aromatic amines (AAs), which include 1-Aminonapthalene (1-NA), 2-Aminonapthalene (2-NA) and 4-Aminobiphenyl (4-ABP).

Due to significantly lower concentrations of AAs in HTPs, the CORESTA recommended method (CRM No. 95)[1,2] was modified to be fit for the determination of these low-level AAs in HTPs. For this modified HTP method, several changes were made to processes in CRM No. 95[1,2], such as changing the solvent system from dichloromethane to toluene, implementing a modified derivatization procedure, and lowering the limit of quantification. The method was validated using a novel heated tobacco capsule (HTC) prototype, consisting of a hand-held battery-operated device (BVR 3.2) and a disposable tobacco-containing capsule which is inserted into the device.

The method validation data demonstrated conformance to acceptance criteria with percent recoveries for all analytes in the matrix being between 75% and 125% and all repeatability measurements having %RSD ≤20%. In addition, the concentrations of AAs in aerosols of the novel HTC prototype were significantly lower than those in cigarette smoke. Considering the absence of standardized methods for HTPs, the results of our validation study show the suitability and reliability of this modified method in measuring and reporting of AAs in HTPs, including the HTC format.

Experimental Details

- The method modifications to CORESTA recommended method (CRM No. 95)[1,2] included:
  - Reducing the scope to the three analytes 1-NA, 2-NA and 4-ABP to align with the FDA Established List of 93 HPHCs.
  - Reducing extraction volume from 50 mL to 10 mL, to increase the concentration of the analytes in the extract.
  - Changing the extraction solvent to Toluene instead of Dichloromethane, as it is less volatile.
  - Lowering the Limit of Quantification (LOQ) by approximately two orders of magnitude, as the low levels found in HTPs are significantly below the lowest calibration level of CRM No. 95
  - Using an SPE manifold at ambient pressure that had disposable liners, due to the observation of contamination from extraction to extraction.
- The method was validated using a novel Heated Tobacco Capsule (HTC) prototype, using both non-intense (ISO 3308)[3] and intense (ISO 20778)[4] puffing regimes. 10 capsules were used per sample replicate for each regime.
- Instrument: a single quadrupole Gas Chromatograph-Mass Spectrometer (GC-MS) equipped with a Chemical Ionization source in Selected Ion Monitoring mode. Accuracy, instrument precision, repeatability, intermediate precision, selectivity, LOQ, robustness, stability and system suitability were all assessed.

Results

- **All test method validation parameters met the acceptance criteria.** A matrix of the validation parameters, acceptance criteria, and a summary of their results is presented in **Table 1**. Select highlights of the test method validation results are described below:
  - **Calibration:** Calibration curve $R^2 \geq 0.999$ for all analytes across three days, %Deviation (%Dev) for Cal 1: -2.0% to 5.6% for all analytes across three days.
  - **Accuracy**: Average recovery in intense sample: 76.6% to 105.1%.
  - **Repeatability:** % Relative Standard Deviation (%RSD) for intense: 5.0% to 16.8% for 1-NA and 4-ABP, %RSD for non-intense: 7.4% to 13.1% for 1-NA and 4-ABP (2-NA BLOQ).
  - **Intermediate Precision:** %RSD for non-intense over 3 days: 12.9% to 13.6% for 1-NA and 4-ABP, %RSD for intense over 3 days 5.6% to 5.9% for 1-NA and 4-ABP (2-NA BLOQ).
  - **LOQ:** 0.00625 ng/mL and 0.00625 ng/unit for each analyte.
  - A summary comparing parameters for the Standard and Modified CRM No. 95 is presented in **Table 2**.

Conclusion

- We present a fit-for-purpose validated method, modified based on CRM No. 95[1,2], for quantifying the aromatic amines 1-NA, 2-NA and 4-ABP in the aerosol generated by HTP.
- As shown in **Table 3**, levels of the three aromatic amines in aerosols of the HTC prototype and a comparator HTP that uses heated tobacco sticks (HTS), were found to be significantly lower (>99.7%) than those found in the 1R6F Reference Cigarette.

References

1. Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) Recommended Method (CRM) No. 95, *Determination of Aromatic Amines in Mainstream Cigarette Smoke by Gas Chromatography Mass Spectrometry with Negative Chemical Ionisation (GM/MS(NCI))*, Version 1, January 2021.
2. Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA), *2016 Collaborative Study on Aromatic Amines in Mainstream Cigarette Smoke*, March 2019.
3. ISO 3308:2012, "Routine analytical cigarette-smoking machine — Definitions and standard conditions", October 2012.
4. ISO 20778:2018, "Cigarettes — Routine analytical cigarette smoking machine — Definitions and standard conditions with an intense smoking regime", October 2018.

We demonstrate that the modified CRM No. 95 is fit for the purpose of analyzing select Aromatic Amines in HTPs

Figure 1: Representative Chromatograms for 1-Aminonaphthalene (1-NA), 2-Aminonaphthalene (2-NA) and 4-Aminobiphenyl (4-ABP) in a Heated Tobacco Capsule (HTC) prototype

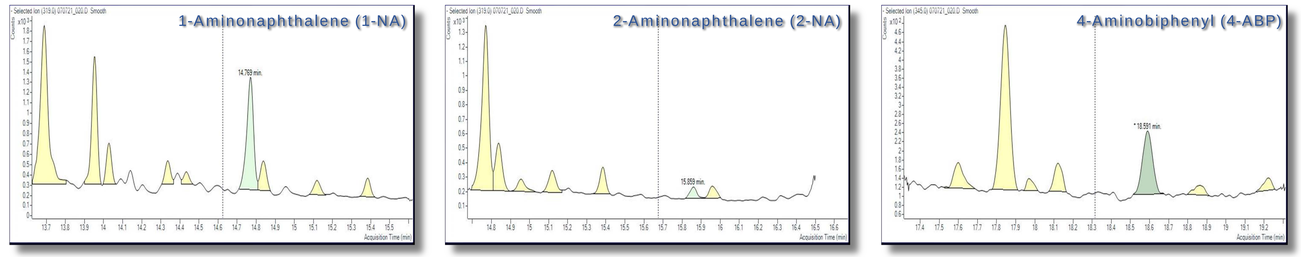

Table 1: Summary of ALCS Method Validation Parameter Results

Validation Parameter	Acceptance Criteria	Validation Results
Calibration	Coefficients of determination (R^2): $R^2 \geq 0.990$ for each day Percent Deviation (%Dev): ≤ ± 20% for the lowest calibration level (Cal Std 1) and ≤ ± 15% for the remaining calibration levels and calibration check standard (CCS)	Calibration curve $R^2 \geq 0.999$ for all analytes across three days %Dev for Cal 1: -2.0% to 5.6% for all analytes across three days %Dev for Cal 2 thru 6 and CCS: -6.8% to 5.2% for all analytes across three days
Accuracy	Average percent recovery (n=3) must be between 75% and 125%	Average recovery in LFB: 96.6% to 100.5% Average recovery in Intense sample: 76.6% to 105.1%
Instrument Precision	Calibration Standard 1: %RSD ≤ 20% Calibration Standard 4: %RSD ≤ 15% Intense Sample: %RSD ≤ 15%	%RSD for Cal 1: 4.3% to 5.5% for all analytes %RSD for Cal 4: 1.4% to 2.1% for all analytes %RSD for Intense sample: 2.0% to 3.7% for all analytes
Repeatability	Repeatability for each day (non-intense, n=6): % RSD ≤ 20% Repeatability for each day (intense, n=6): % RSD ≤ 20%	%RSD for Non-Intense: 7.4% to 13.1% for 1-NA and 4-ABP (2-NA BLOQ) %RSD for Intense: 5.0% to 16.8% for 1-NA and 4-ABP (2-NA BLOQ)
Intermediate Precision	Intermediate Precision over 3 days (non-intense, n=18): % RSD ≤ 20% Intermediate Precision over 3 days (intense, n=18): % RSD ≤ 20%	%RSD for Non-Intense over 3 days: 12.9% to 13.6% for 1-NA and 4-ABP (2-NA BLOQ) %RSD for Intense over 3 days 5.6% to 5.9% for 1-NA and 4-ABP (2-NA BLOQ)
Selectivity	Background contribution in the Reagent Blank with Internal Standard shall be ≤ 40% of the AA concentration in the lowest calibration standard. A representative chromatogram with labeled peaks and other appropriate information will be provided for both non-intense and intense samples.	No significant matrix interferences were observed
LOQ	The 6 replicate injections of calibration standard 1 must have an average S:N ≥10:1 and ≤ 40:1, %Dev ≤ ±20%, and %RSD ≤ 20%. The 6 replicate injections of the serially diluted intense sample should have an average S:N ≥10:1 and ≤ 40:1, %Dev ≤ ±20% and %RSD ≤ 20	0.00625 ng/mL and 0.00625 ng/unit for each analyte (set based on instrument sensitivity)

Table 2: Summary of Method Modifications made to CRM No. 95[1,2]

Method Parameter	Standard CRM No. 95	Modified CRM No. 95
Extraction Volume	50 mL	10 mL
Extraction Solvent	Dichloromethane	Toluene
SPE Cartridge	Florisil 12 cc, 2g Sorbent	Florisil 12 cc, 2g Sorbent
SPE Elution Solvent	Dichloromethane	Dichloromethane
SPE Elution Volume	8.5 mL	8.5 mL
Analytes In Scope	Seven	Three
1-Aminonaphthalene LOQ	0.40 ng/mL	0.00625 ng/mL
2-Aminonaphthalene LOQ	0.40 ng/mL	0.00625 ng/mL
4-Aminobiphenyl LOQ	0.07 ng/mL	0.00625 ng/mL

Table 3: Summary of data obtained for 1R6F*, an HTP Comparator Product**, and the HTC Prototype using the validated Modified CRM No. 95 method**

Analyte	Average Concentration in Aerosol (ng/unit)			% Reductions compared to 1R6F (per unit basis)	
	1R6F Cigarette*	HTC Prototype**	HTP Comparator**	HTC Prototype**	HTP Comparator**
1-Aminonaphthalene	20.2	0.0605	0.0226	99.7%	99.9%
2-Aminonaphthalene	16.8	0.00628***	0.00688***	100.0%	100.0%
4-Aminobiphenyl	2.64	0.00622***	0.00669***	99.8%	99.7%

*Data was measured by Labstat International Inc. at the request of ALCS, using a method that is harmonized to CRM No. 95.
**Data was generated using the modified method described in this poster, after method validation was completed. The HTP Comparator was an HTP that uses heated tobacco sticks (HTS).
***Results were determined to be below the limit of quantitation, and instead the instrument reported value for calibration standard 1 is listed and used in subsequent calculations.
Note: Results are presented in ng/unit. A unit is defined as one cigarette, HTC or HTS.

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