

Novel Trapping System for the Quantification of Two Tobacco-Specific Nitrosamines in Indoor Air

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1. Introduction and Objectives

Tobacco-specific nitrosamines (TSNA) are an important group of harmful and potentially harmful constituents (HPHC) in cigarette smoke and environmental tobacco smoke (ETS)-related contaminants associated with human health concerns [1]. To assess the impact of the Tobacco Heating System (THS) or electronic cigarette consumption on indoor air quality (IAQ) in comparison with cigarettes, it is important to quantify the most abundant TSNA, namely nicotine-derived nitrosamine ketone (NNK) and *N*-nitrosanonicotone (NNN) (Figure 1), in an accurate and reliable manner. Quantification of TSNA, such as NNK in ETS (reported to be lower than 30 ng/m³ [1]), requires long, laborious sample preparation. As the aerosols produced during the use of new-generation tobacco products (Figure 2) contain significantly lower levels of HPHCs than cigarette smoke due to the absence of combustion [2-4], it was necessary to develop a more sensitive method.

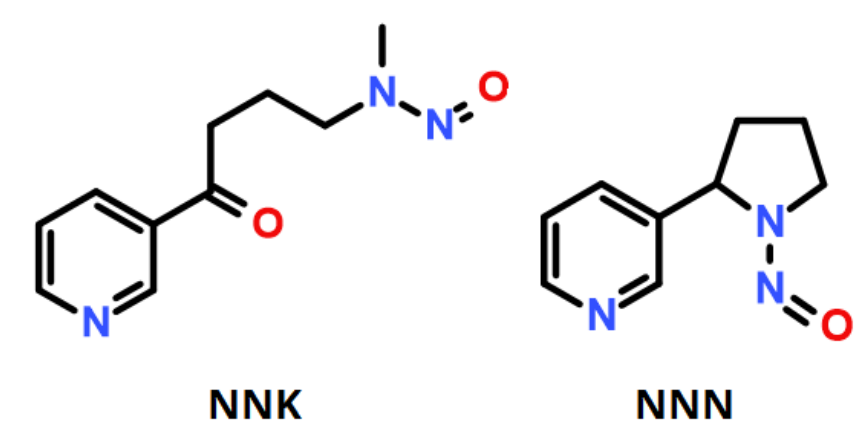


Figure 1: Chemical structure of the two TSNA target compounds.



Figure 2: Test items used for the generation of surrogate environmental aerosols of new generation products.



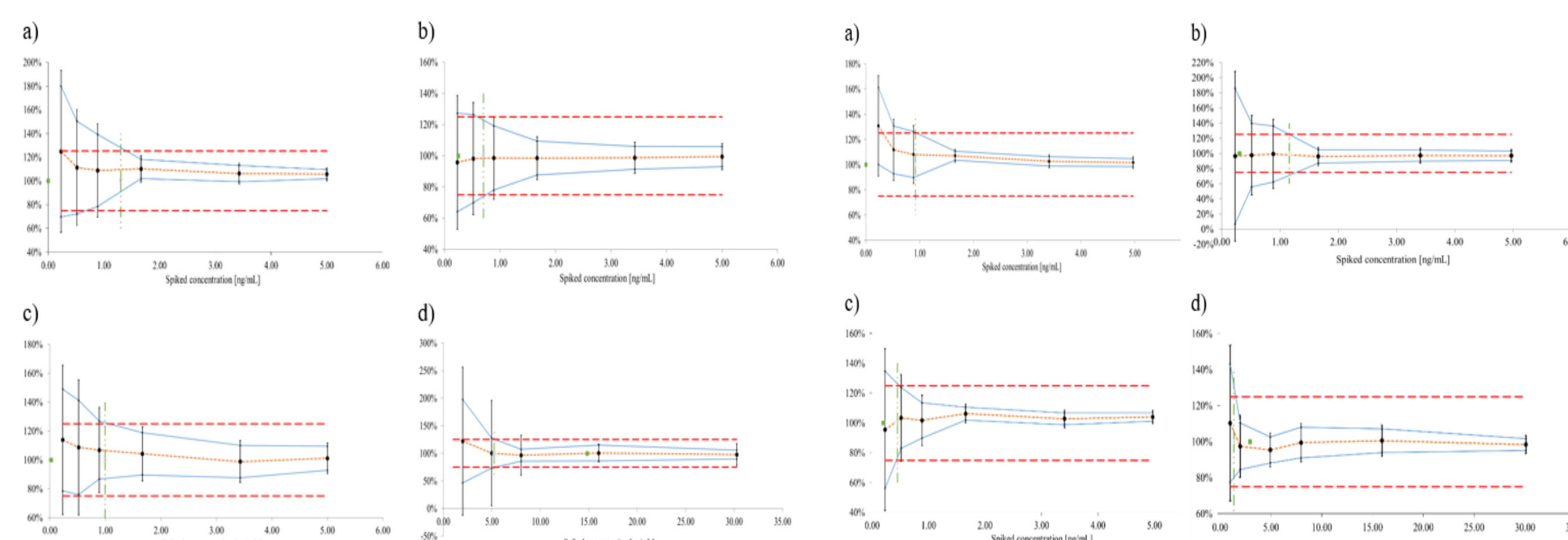
Figure 3: Graphical representation of the IAQ room (72.3 m³, 24.1 m²) during the TSNA validation.

3. Method Validation and ISO 17025 Accreditation

For the validation, a matrix-based approach was selected applying the accuracy profile procedure. The evaluated matrices, generated with smoking machines in an environmentally controlled room (Figure 3), were background air samples, environmental aerosols of the THS 2.2 or the Solaris electronic cigarette (generated using a puffing machine and released directly in the room), and the side-stream smoke of a cigarette (*Marlboro Gold*), NNN-D₄ and NNK-D₄ were used as internal standard for the quantification. The performance of the method was compared for all four matrices under investigation. The parameters evaluated and applicability limits of the method determined during the validation were the following:

- Selectivity
- Response function
- Stability
- Limit of detection (LOD), lower limit of quantification (LLOQ), and upper limit of quantification (ULOQ)
- Instrumental repeatability
- Intermediate precision and critical difference
- Comparison with historical results
- Working range
- Robustness
- Uncertainty

The LODs of the method for NNN and NNK were 0.0108 ng/m³ and 0.0136 ng/m³, respectively. The calculated lower working range limit (LWRL) of the method for NNN was 0.126 ng/m³, and the LWRL for NNK was 0.195 ng/m³. The accuracy profiles for NNK and NNN are shown in Figure 6 and Figure 7, respectively. The average endogenous content per matrix type is presented in Table 4.



*Legend: average recovered concentration per spiking level (black circle), trueness expressed as recoveries (orange spots line), upper and lower β -expectation tolerance intervals (blue continuous lines), upper and lower acceptance limits set at 25% (red dashed lines), average NNK nominal concentration (green square), LWRL (green vertical dashed line).

Matrix		Average endogenous content in homogeneous matrix		Average endogenous content in non-homogeneous matrix		Average endogenous content per matrix type (all values)	
		NNN ng/m ³	NNK ng/m ³	NNN ng/m ³	NNK ng/m ³	NNN ng/m ³	NNK ng/m ³
BKG	Average	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	STDEV	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
EA of THS2.2 ¹	Average	0.0849	0.0683	0.0792	0.0593	0.0830	0.0653
	STDEV	0.0155	0.0136	0.0149	0.0125	0.0153	0.0138
EA of e-cig	Average	0.0557 ¹	0.0061 ²	0.0570 ¹	0.0046 ²	0.0561	0.0056
	STDEV	0.0319	0.0124	0.0252	0.0093	0.0296	0.0114
ETS	Average	0.819	4.121	0.811	4.140	0.816	4.127
	STDEV	0.108	0.941	0.114	1.253	0.109	1.042

Table 4: Average matrix endogenous content of NNN and NNK in ng/m³ calculated for homogeneous and non-homogeneous samples as well as their average.

¹ Average values are between the LLOQ and the LWRL of the method
² Average values under LOD

All the documents generated during the method development and the validation (Figure 8) were evaluated by quality assurance and scientific experts before becoming effective.

The training of the operators, the storage of the generated documents, and the storage of raw data and results were tracked and recorded. The method was presented for internal audit.

The method was presented for ISO 17025 accreditation and accredited in 2017 (STS 0045, SAS, Bern, Switzerland).

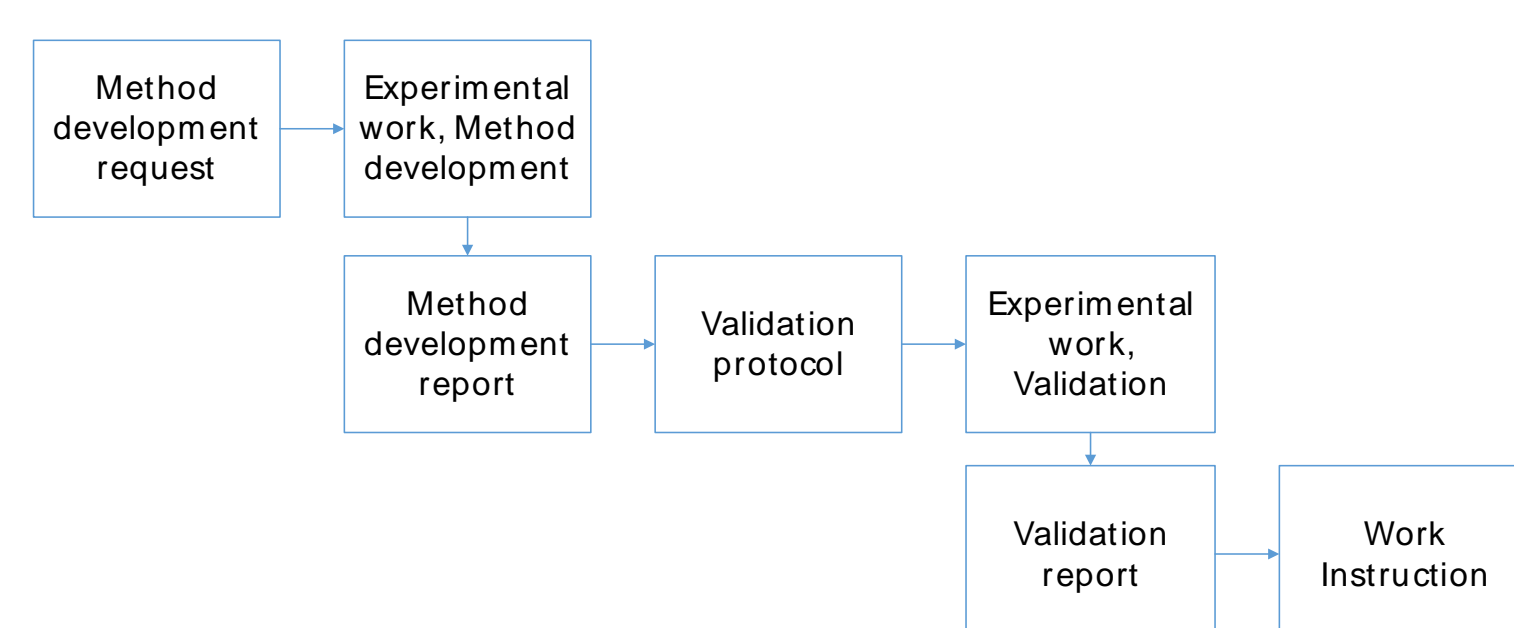


Figure 5: Steps for the analytical method validation.

2. Method Development

Reduction of the steps during sample preparation as well as an efficient concentration of the target compounds before the analysis was necessary. This was achieved through a novel approach for the collection phase using silica sorbent cartridges with simplified posterior sample preparation (extraction, concentration, and reconstitution) (Figure 4). This procedure improved recoveries during the sample preparation compared with the conventional trapping on Cambridge filters. The extracts were then analyzed by isotope dilution-liquid chromatography electrospray ionization tandem mass spectrometry - in positive mode (LC-ESI MS/MS pos). The specific parameters used for those analyses are shown in Table 1 to Table 3. A typical chromatogram for TSNA analysis obtained by the application of the new methodology is presented in Figure 5.

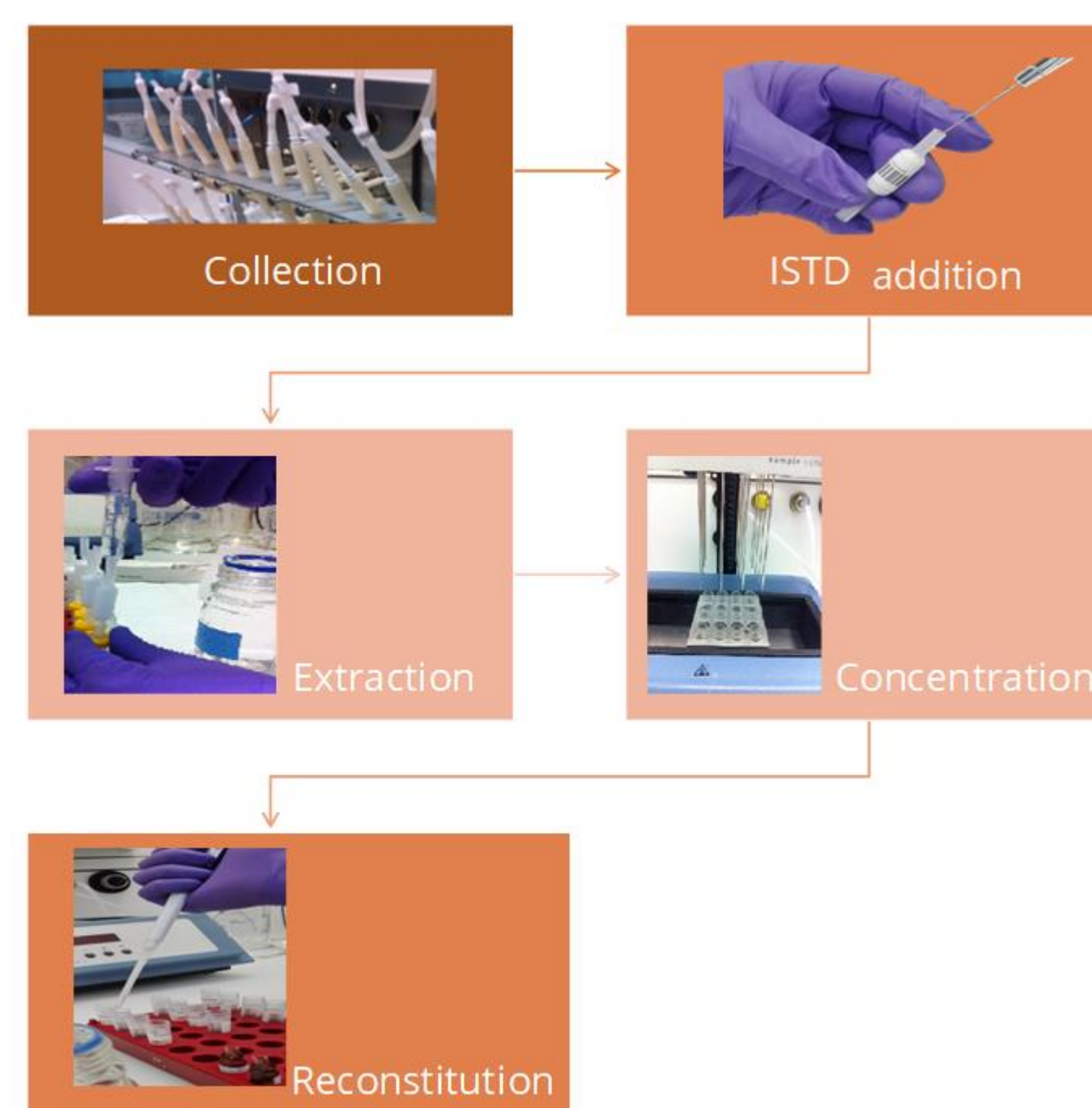


Figure 4: TSNA collection and sample preparation.

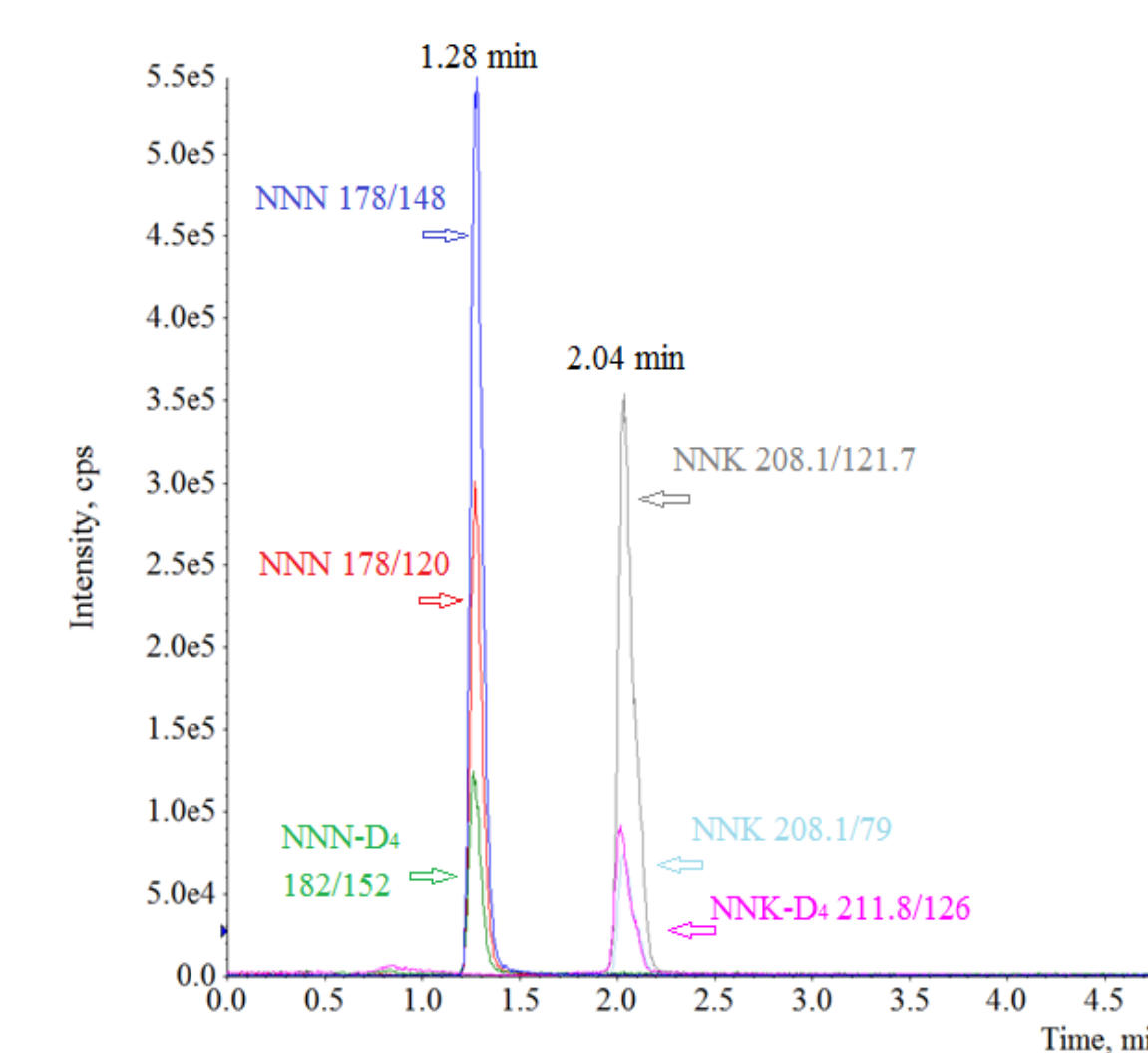


Figure 5: Typical chromatogram for NNN and NNK.

Device	Parameter	Value
Autosampler	Loop size	50 μ L
	Injection volume	2 μ L
	Rinsing speed	35 μ L/sec
	Filling speed	1 μ L/sec
	Rinsing mode	Before and After Aspiration
Pumps	Rack Temperature	15 °C
	Mode	Binary flow
Oven	Flow	0.28 mL/min
	Temperature	60 °C
Mobile Phase	Mobile phase A	0.1 % formic acid in water
	Mobile phase B	1mL formic acid into 90% Methanol LC-MS grade/10% Tetrahydrofuran
Column	Type	Kinetex Pentafluorophenyl 100A
	Dimensions & particle size	50 x 2.1 mm, 1.9mm
	Supplier	Brechbuhler
Gradient run	Supplier nr.	00B-4476-AN
	Time (min)	B (%)
	0.09	13
	2.68	50
	3.58	70
	4.03	98
	5.37	98
	5.82	13
	10.74	13

Table 1: Parameters of the high-performance liquid chromatography method used for the method for NNN and NNK quantification.

Analyte	Ion type	Q1 [m/z]	Q3 [m/z]	DP [V]	EP [V]	CE [V]	CXP [V]
NNN	Quantifier	178	148	46	10	15	10
NNN	Qualifier	178	120	46	10	25	10
NNN-D ₄	Quantifier	182	152.1	36	10	15	10
NNK	Quantifier	208.11	121.7	40	10	55	10
NNK	Qualifier	208.11	79	86	10	55	10
NNK-D ₄	Quantifier	211.84	126	46	10	17	8

Table 2: Analyte-dependent instrumental acquisition MS/MS parameters used for the method for NNN and NNK quantification.

Parameter	Value
Source	ESI (positive mode)
Acquisition type	MRM
Curtain gas	30 psig
Collision gas	5 psig
Nebulizer current	5500 V
Temperature	600 °C
Ion Source Gas (GS1)	50 psig
Ion Source Gas (GS2)	50 psig
Target Scan time	0.27 s
EP (V)	10

Table 3: Source parameters for MS used for the method for NNN and NNK quantification.

4. Conclusions

A novel collection methodology as well as an LC-MS/MS method for the analysis of two TSNA (NNN and NNK) in indoor air was developed and validated in four different matrices (background, environmental aerosol of THS 2.2, environmental aerosol of e-cigarettes, and ETS). For the validation, samples were generated in an environmentally controlled room by puffing machines. Using puffing machines allows for a much better repeatability and is therefore ideally suited for method development and validation, but the results do not represent what would happen if people use the product. To that end, studies with panelists should be performed. The accuracy profile procedure allowed assessment of the method performance as a function of the matrices. The working ranges of the method allowed for the quantification of the target analytes with an accuracy of $\pm 25\%$ in the matrices under investigation. The validation results demonstrated the fitness-for-purpose of the method for prospective comparative assessments of the environmental aerosol of THS 2.2 and e-cigarettes as well as the ETS of cigarettes. The method for NNN and NNK measurement will be applied in future studies to evaluate the impact of the use of THS 2.2 and e-cigarettes on IAQ in simulated and real-life settings.

The paper "Development and validation of a method for quantification of two tobacco-specific nitrosamines in indoor air" has been submitted for publication.

Abbreviations

ESI, Electrospray Ionization; ETS, Environmental Tobacco Smoke; HPHC, Harmful and Potentially Harmful Constituent; IAQ, Indoor Air Quality; ISO, International Organization for Standardization; LC, Liquid Chromatography; LOD, Limit of Detection; LLOQ, Lower Limit of Quantification; LWRL, Lower Working Range Limit; MS/MS, Tandem Mass Spectrometry; NNK, 4-(*N*-methyl-*N*-Nitrosamine)-1-(3-Pyridyl)-1-butanone; NNN, 1-Nitroso-2-(3-pyridyl)pyrrolidine; THS, Tobacco Heating System; TSNA, Tobacco-Specific Nitrosamines; ULOQ, Upper Limit of Quantification; UWRL, Upper Working Range Limit

References

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