A Versatile Method for the Analysis of TSNAs in Tobacco Products and Cigarette Smoke by LC-MS-MS

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ABSTRACT

A versatile method for the analysis of TSNAs has been applied to different tobacco sample matrices such as tobacco filler, smokeless (snus, moist snuff etc.) and other tobacco materials, as well as cigarette smoke. The method used an HPLC separation on a Phenomenex Gemini C18 column with 3 micron particle size, and gradient mobile phase with aqueous ammonium acetate buffer/acetonitrile and acetic acid/acetonitrile as mobile phases A and B. The detection was performed by MS/MS with multiple reaction monitoring (MRM) in positive mode using specific transitions from precursor ion to product ion, specific for each TSNA compound. The instrumentation used for the analysis was a 1290 Infinity from Agilent Technologies and API 4000 from AB Sciex. The method provides good selectivity with no interference from the sample matrix. For this reason, after the sample extraction, no clean up procedure was necessary regardless of the sample type. The method has a wide calibration range (1-600 ng/mL for NNN, NAT and NNK, 0.25-150 ng/mL for NAB) that allows the analysis to be performed on all tobacco and smoke samples without modification. The method is rapid, highly reliable, and shows excellent repeatability and robustness. The procedure has been applied successfully in the laboratory for a number of years and on a variety of samples.

INTRODUCTION

TSNAs are extracted from tobacco products and cigarette smoke pads with 100 mM aqueous ammonium acetate solution containing internal standards (deuterated compound for each of the four TSNA analytes). The filtered extracts are analyzed as is with no clean-up on an Agilent 1290 HPLC system coupled with API 4000 triple-quadruple tandem mass spectrometer with positive ESI source for ionization.

LC Conditions

Column:	Phenomenex Gemini 2.0 x 150 mm 3 micron particle size
Guard Column:	No
Column Temperature:	70 ºC
Mobile Phase A:	95 % 10mM ammonium acetate in DI water at pH 6.75 5% Acetonitrile
Mobile Phase B:	0.1% acetic acid in Acetonitrile
Flow Rate:	0.6 mL/min
LC Ramp:	Gradient method over 8 minutes
Injection Volume:	2 – 3 µL

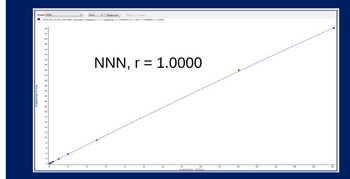
Mass Spectrometry Parameters

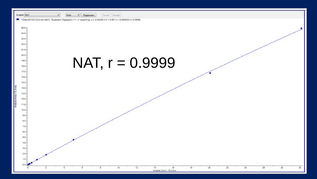
Ion Source Parameters	
Collision Gas (CAD)	4 mL/min
Curtain Gas (CUR)	20 mL/min
Ion Source Gas 1(GS1)	80 mL/min
Ion Source Gas 2 (GS2)	40 mL/min
Ion Spray Voltage (IS)	3800 V
Temperature (TEM)	520 C
Interface Heater	on

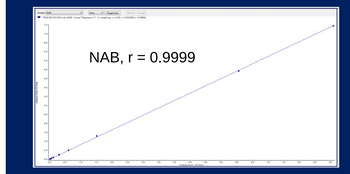
Compound Parameters		**Declustering**	**Entrance**	**Collision**	**Collision Cell**
Compound	**Ion Transition**	**Potential (DP)**	**Potential (EP)**	**Energy (CE)**	**Exit potential (CXP)**
		(V)	**(V)**	**(V)**	**(V)**
NNN	178.1 → 148.1	41.3	8.9	15.1	9.0
NNN-d4	182.1 → 152.1	37.8	6.7	14.5	9.9
NAT	190.1 → 160.1	41.8	6.7	16.9	10.3
NAT-d4	194.1 → 164.1	39.4	6	16.6	7.0
NAB	192.1 → 162.1	36.4	9.6	15.4	9.2
NAB-d4	196.1 → 166.1	40.2	7.9	15.1	10.2
NNK	208.1 → 122.1	39.3	7.6	18.5	10.2
NNK-d4	212.1 → 126.1	38.3	6.4	17.5	7.1

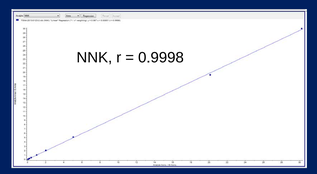
RESULTS

Calibration Curves









S/N at Standard Level 1*

	NNN	NAT	NAB	NNK
S/N	63	92	82	51

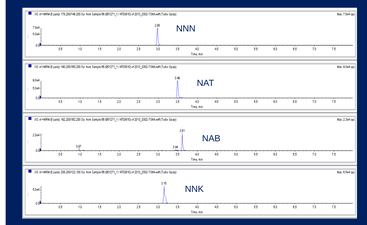
*: Average S/N in 18 runs on the same column.
Each run had 100-140 tobacco, smoke and TSNA standards samples.

Limit of Quantitation

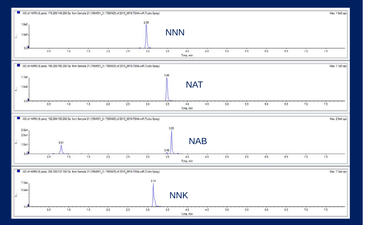
Sample Type	NNN	NAT	NAB	NNN
Tobacco	30 ng/g	30 ng/g	7.5 ng/g	30 ng/g
Smoke	6 ng/cig	6 ng/cig	1.5 ng/cig	6 ng/cig

Tobacco and Smoke Samples (w/o SPE) Chromatograms by ELL/RJRT Method

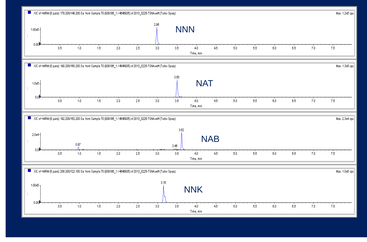
Smoke 3R4F ISO



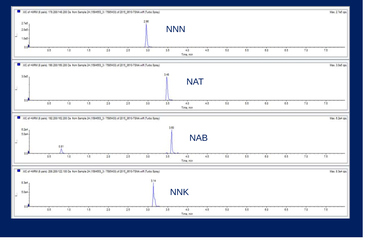
CRP1 (Snus)



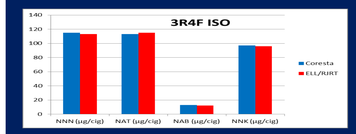
Smoke 3R4F HCA

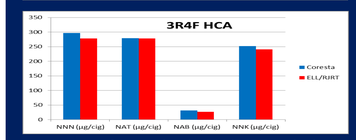


CRP2 (Moist Snuff)



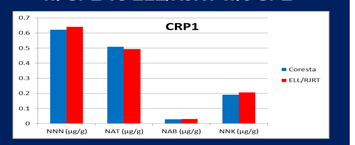
Smoke: Coresta CRM N° 75 vs ELL/RJRT*

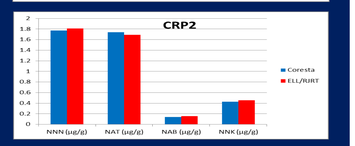




*: Average of 1 year data by ELL/RJRT method.

Smokeless: Coresta CRM N° 72 w/ SPE vs ELL/RJRT w/o SPE**





**: Average of 3 replicates.

CONCLUSIONS

The LC-MS-MS method developed in our laboratories for the quantitation of TSNAs in tobacco products and cigarette smoke requires no SPE cleaning for any samples. The method has been utilized for multiple years and proved to be very robust. The results regarding the levels of TSNAs in tobacco and smoke samples are in good agreement between our method and Coresta methods CRM N° 72 & 75.

Future Application: Preliminary work indicates that up to a 20 fold decrease in LOQ's may be achieved using the same chromatography conditions on a Phenomenex Kinetex EVO 1.7 µm column on an Agilent 1290/AB Sciex 6500 system. This will be applicable for the determination of TSNAs at substantially lower levels of interest.