Determination of TSNAs in mainstream cigarette smoke by using heart–cutting 2DLC–MS/MS

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Summary:
A novel heart-cutting two dimensional liquid chromatography–tandem mass spectrometry (2DLC-MS/MS) method has been developed for analyzing the low levels of TSNAs in virginia type cigarette smoke. The method offers simple sample preparation involving only extraction and filtration, with almost no matrix interference and low quantitative limit as compared with traditional methodologies. A strong cation exchange (SCX) column is utilized for first dimensional separation, which effectively removes acidic and neutral components in smoke. To reserve TSNAs on the trap column, compensate pump is applied for on-line dilution and pH adjustment during the period of TSNAs fraction transfer and enrichment. Then C18 column was employed for 2nd dimensional separation and coupled to tandem mass spectrometry under multiple reaction monitoring (MRM) mode. 2DLC-MS/MS method with isotope deuterated internal standards is applied to detect the TSNAs levels in the mainstream cigarette smoke, which show fairly high selectivity, sensitivity, good reproducibility and accuracy. Detection results of Kentucky reference cigarettes (3R4F, 1R5F) agree well with CORESTA joint experiments.

Objective:
Because of the health implications and progresses been made in reducing the TSNAs content in tobacco products especially low TSNA content in virginia-type cigarettes, it is necessary to develop a simple, fast, sensitive and selective method which can determine very low level of TSNA in cigarette smoke.

Materials and Methods:
Analyses were carried out using a 6-Way Valve and two liquid chromatographic instruments(Agilent 1260, Santa Clara, United States) interfaced with triple quadrupole mass spectrometer(AB SCIEX API 4000, Massachusetts ,United States) equipped with electron spray Ionization source(ESI) operated in positive mode. SCX column: Spherisorb S5 SCX 5μm; Trap column: Thermo scientific Acclaim PolarAdvantageII C18(4.6*50mm ,3μm); RPLC column: Shiseido Capcell PAK MGII C18(4.6*150mm ,3μm); detection wavelength 230nm。

Results and Disscussion:
Main problem of traditional LC-MS/MS method is matrix effect. And two-dimensional HPLC has recently widely used recently due to its higher peak capacity compared to one-dimensional as well as a good technique for analysis of mixtures containing thousands of components. (34-36) By heart-cutting the TSNA elution to the 2nd dimension, most of the matrix interference is removed. During the analytical method development of 2DLC, conditional matrix elimination are predicted for fully optimized 1DLC and practical 2DLC separations for the cutting elution constituents which are able to coupled with ESI-MS/MS. Ion exchange combined with reverse phase liquid phase system is commonly used in proteomics studies, advantages of the system are orthogonal separation mode, compatible mobile phase, convenient connected to MS.

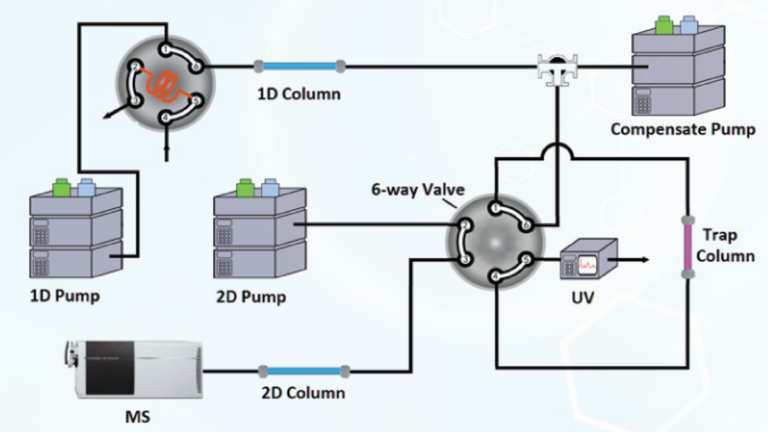

Fig.1. Schematic diagram of heart-cutting 2DLC-MS/MS system for analysis of TSNAs in mainstream cigarette smoke

1st Dimensional Separation.
To reserve TSNAs on the column and remove acidic and neutral interference compounds, we employed a strong cation exchange column in the 1st dimensional with acidic mobile phase because of the alkalinity of TSNAs which are cationic state under acidic conditions. What's more, this system is aqueous phase which are able to couple with reversed-phase liquid chromatography for further separation.

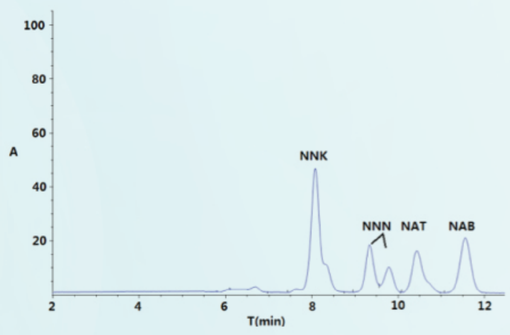

Fig.2. 1D Chromatographic spectrum of standards

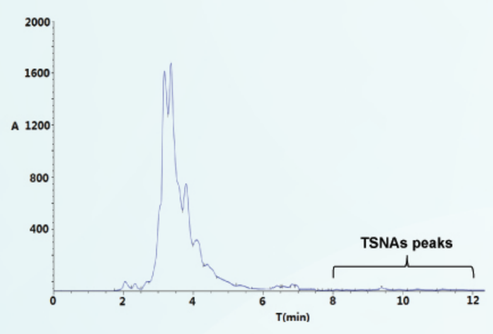

Fig.3. 1D Chromatographic spectrum of sample

Trap condition and 2nd Dimensional Separation.
One of the most important problems for heart-cutting of TSNAs fraction transformation and desalination, pH of the mobile phase was 2.8 containing 15% acetonitrile, and four kinds of TSNAs polar differences, are all retained a certain degree of difficulty. In this study, different lengths C18 column examines the direct transfer, transfer after dilution, after capturing method dilution and transfer the results showed that: NNN is easy outflow, the use of trapping methods and after dilution to fully capture four kinds of transfer TSNAs, the organic phase was diluted as compensation for passage through an aqueous solution containing K_2HPO_4 and adjust the mobile phase pH to 6.5.

Table 1. 1st dimensional elution program

T (min)	A (%)	B (%)	Flow rate (mL/min)
0	100	0	0.4
3	90	10	0.4
3.1	0	100	0.4
12.6	0	100	0.4
13.0	0	100	1.5
16	0	100	1.5
16.2	100	0	1.1
30	100	0	1.1

Table 2. 2nd dimensional elution program

T (min)	A (%)	B (%)	Flow rate (mL/min)
0	95	5	1.2
19.5	95	5	1.2
20	95	5	0.6
24	5	95	0.6
28	5	95	0.6
29.5	5	95	1.2

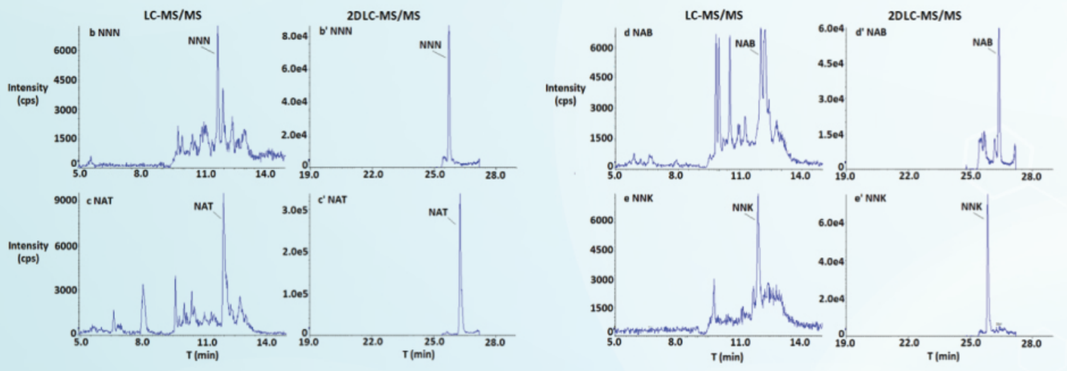

Fig. 4. Comparison of 2DLC-MS/MS and LC-MS/MS method for Virginia type cigarette smoke.

Table 3. Recovery studies of 2DLC-MS/MS method

Analyte	Intra-day RSD%	Inter-day RSD%	Spiked amount (ng/mL)	Calculated value* (ng/mL)	Recovery (%)
NNN	3.9	6.0	5	5.3	106.0
			10	9.4	94.0
			25	24.2	96.8
NAT	2.1	6.4	10	9.7	97.0
			25	25.2	100.8
			50	54.3	108.6
NAB	2.1	3.3	1.25	1.3	104.0
			2.5	2.5	100.0
			5	5.1	102.0
NNK	0.9	2.2	5	5.2	104.0
			10	10.4	104.0
			25	23.4	93.6

** Calculated value = Detect value after spiked - Spiked amount*

Table 4. Comparison of the results with those reported

	NNN (ng/cig)		NNK (ng/cig)		NAT (ng/cig)		NAB (ng/cig)	
	1R5F	3R4F	1R5F	3R4F	1R5F	3R4F	1R5F	3R4F
2DLC-MS/MS	47.7	106.5	23	92	48.7	113	6.6	13.8
*CORESTA	44.4	115	21.8	97.1	45.8	113	6.5	13.0
RSD%	5.1	5.4	3.8	3.8	4.3	0.0	1.1	4.2
r	8.6	18	3.9	14.6	9.2	14	1.5	2.3
R	16.7	34	8.0	30.5	23.4	55	2.9	5.2

**Mean of CORESTA joint experiments, r: repeatability, R: reproducibility.*

Conclusions:
In this study, the first 2DLC-MS/MS method for the determination of low levels of TSNAs analysis in mainstream cigarette smoke has been developed. This method takes good advantage of two dimensional separation power of chromatography, which makes it possible to use a simple sample preparation involving only extraction and minimize matrix interference without extra sample cleanup steps. When 2DLC-MS/MS method is applied to detect the TSNAs levels in a wide range in the mainstream cigarette smoke, it has been shown to produce fairly high selectivity, sensitivity, good reproducibility and accuracy. The limits of detection of NNN、NAT、NAB、NNK are 0.023、0.028、0.019 and 0.028 ng/cig, spiked recoveries of NNN、NAT、NAB、NNK ranged from 93.6%~ 108.6% , daily reproducibility within 5.4%.

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