

Electronic supplementary information (ESI) for

Establishment of a two-dimensional liquid chromatography-tandem mass spectrometry system for detection of four tobacco specific N-nitrosamines

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Materials and methods:

One piece of Cambridge filter was put into a conical flask, then 20 mL NH₄Ac solution with deuterated internal standard (5 ng/mL d₄-NNN, d₄-NNK, d₄-NAT and 1 ng/mL d₄-NAB) was added. The TSNAs were fully extracted under ultrasonication vibration for 40 min. After 5 minutes standing, all the extract solution was transferred to a centrifuge tube and centrifuged for 5 min, 10000 rpm. 1 mL supernatant was taken out and filtered by the 0.22 μm filter membrane. The final solution was ready for LC-MS analysis.

ESI was performed in the positive ion mode (ion spray voltage 5500 V) with nitrogen as nebulizing gas (483 kPa), heater gas (483 kPa), curtain gas (172 kPa), and collision gas (41 kPa). The turbo ion spray temperature was set at 550 °C. The dwell time was set at 50 ms.

Figures and tables:

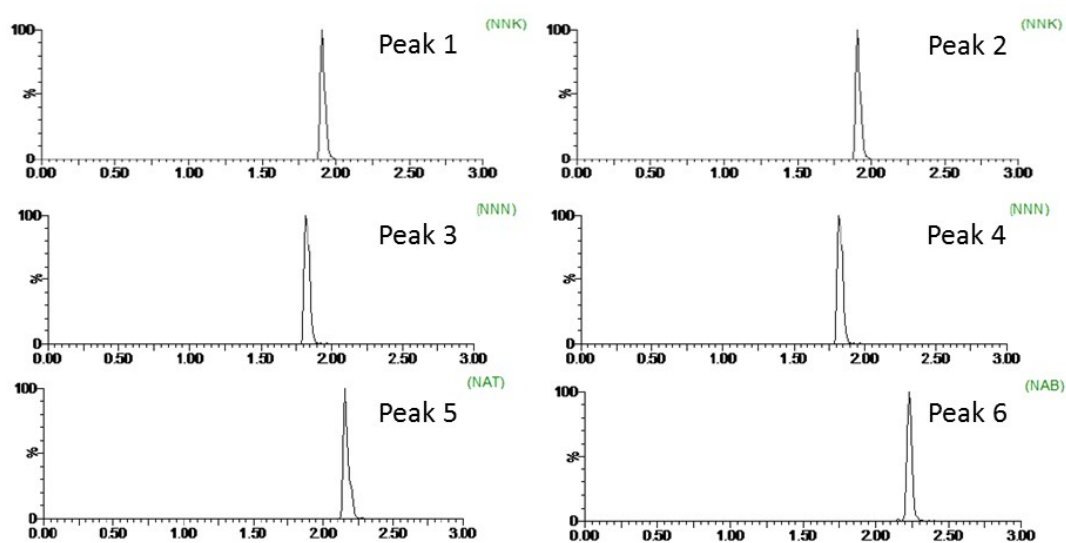
Table S1 The formulas of 7 standard work solutions. All of them contained deuterated internal standard (5 ng/mL d₄-NNN, d₄-NNK, d₄-NAT and 1 ng/mL d₄-NAB).

Number	NNN, NNK, NAT (ng/mL)	NAB (ng/mL)
1	0.5	0.1
2	1.25	0.25
3	2.5	0.5
4	5	1
5	10	2
6	25	5
7	50	10

Table S2 Parameters of multiple reaction monitoring analysis of the four TSNAs and their internal standards.

Analyte	Precursor ion (m/z)	Declustering potential (V)	Product ion (m/z)	Collision energy (eV)
NNN	178.1 ^a	41	148.1	16
	178.1 ^b	41	120.1	16
NNK	208.1 ^a	41	122.1	12
	208.1 ^b	41	148.1	12
NAT	190.1 ^a	41	160.0	30
	190.1 ^b	41	106.1	21
NAB	192.1 ^a	41	162.1	13
	192.1 ^b	41	133.1	13
d ₄ -NNN	182.1	43	152.1	14
d ₄ -NNK	212.1	42	126.1	15
d ₄ -NAT	194.1	37	164.0	20
d ₄ -NAB	196.1	42	166.0	13

^a Quantitation ion; ^b Confirmation ion.

**Fig. S1** Chromatograms of six peaks of Fig 2(D) in MRM mode.