Determination of PON in tobacco and tobacco products using UPLC/MS/MS

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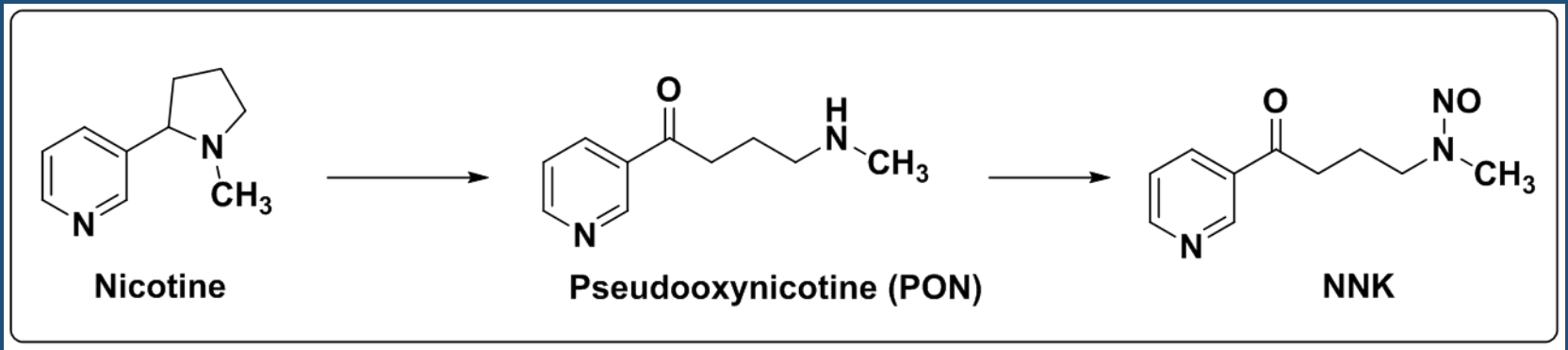
Pseudooxynicotine (PON)

- PON -- an oxidation product of nicotine
- PON -- precursor of NNK



Previous method for PON analysis

- **GC/MS method**

Tobacco extracted with buffer and CH_2Cl_2

⇩

Aqueous phase, pH adjusted to 10

⇩

10% NaCN and CH_2Cl_2

⇩

Organic phase (CH_2Cl_2), evaporated to dryness

⇩

Reconstituted and injected to GC/MS

Disadvantage of GC/MS method

GC/MS method:

- **NaCN used as derivatization solvent---toxic!**
- **Complicated & time consuming**

Objective

- To develop and validate a routine assay with LC/MS/MS to quantify PON in tobacco and tobacco products

Protocol

Ground tobacco sample

⇩

Spike Internal Standard

⇩

Extract in pH =3.0 buffer

⇩

Shake 45 min

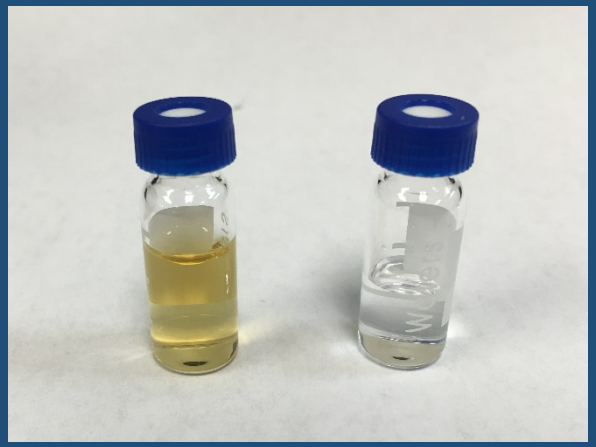
⇩

Filter with 0.22 µm PTFE membrane

⇩

Extract with CH_2Cl_2 to clean up

⇨ Inject to UPLC/MS/MS



Mobile Phase Selection

- **Option 1:**

  M.P. A: 0.1% acetic acid in water

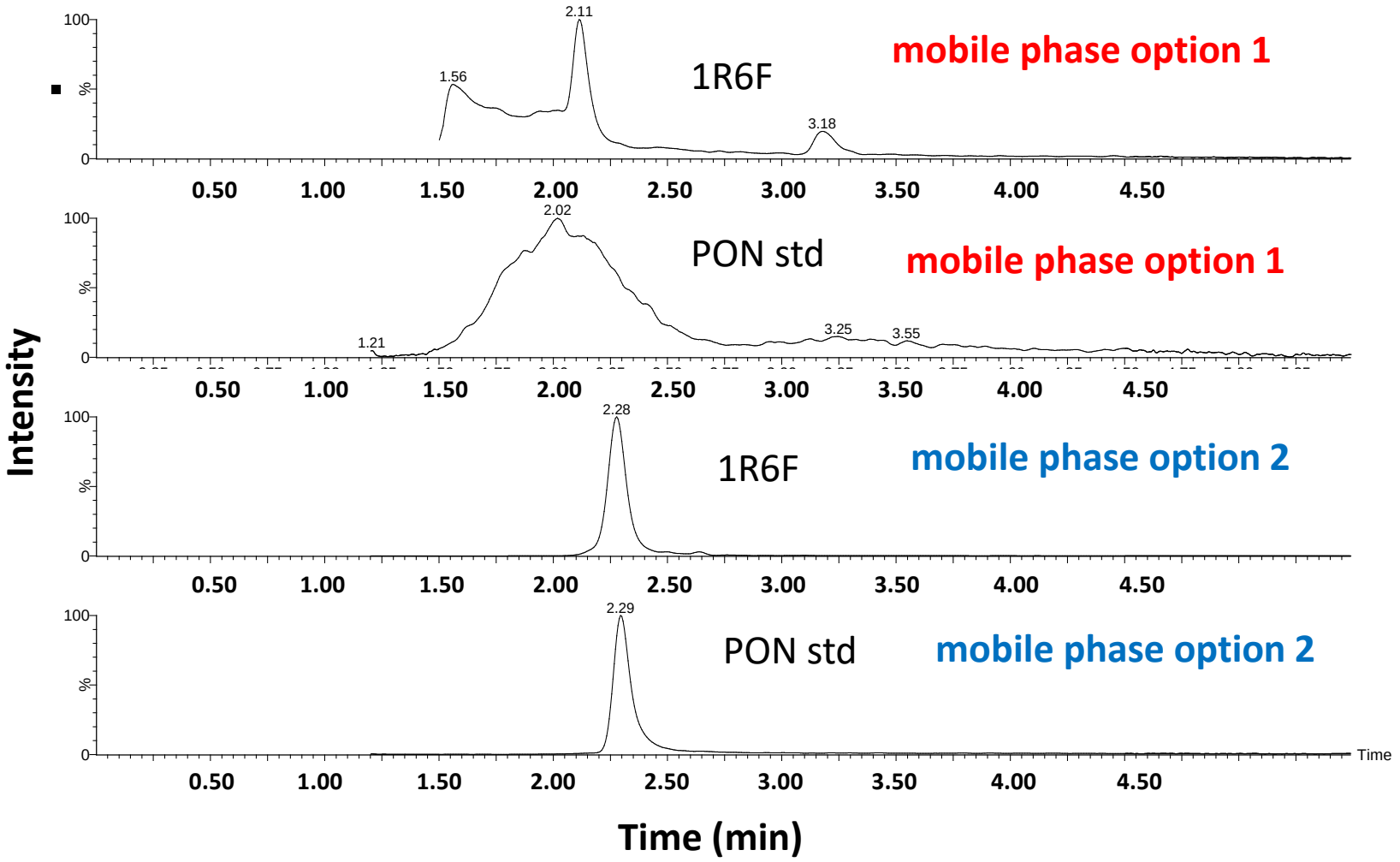
  M.P. B: 0.1% acetic acid in methanol

- **Option 2:**

  M.P. A: 10 mM ammonium acetate with 0.1% NH_4OH

  M.P. B: 100% acetonitrile

Mobile Phase Selection



Upper two with mobile phase option 1; lower two with option 2

UPLC/MS/MS parameters

- UPLC column: ACQUITY UPLC BEH C18 column(2.1 X 50mm, 1.7 µm particle size)
- Column temperature: 60 ˚C
- Injection volume: 2 µL
- Flow rate: 0.25 mL min^{-1}
- Mobile phase A: 10 mM ammonium acetate with 0.1% NH_4OH
- Mobile phase B: 100% acetonitrile

	Time	%A	%B	Curve
1	initial	98	2	6
2	3.50	10	90	6
3	3.60	0	100.0	6
4	4.60	0	100.0	6
5	4.70	98	2	6
6	8.00	98	2	6

UPLC/MS/MS instrumentation

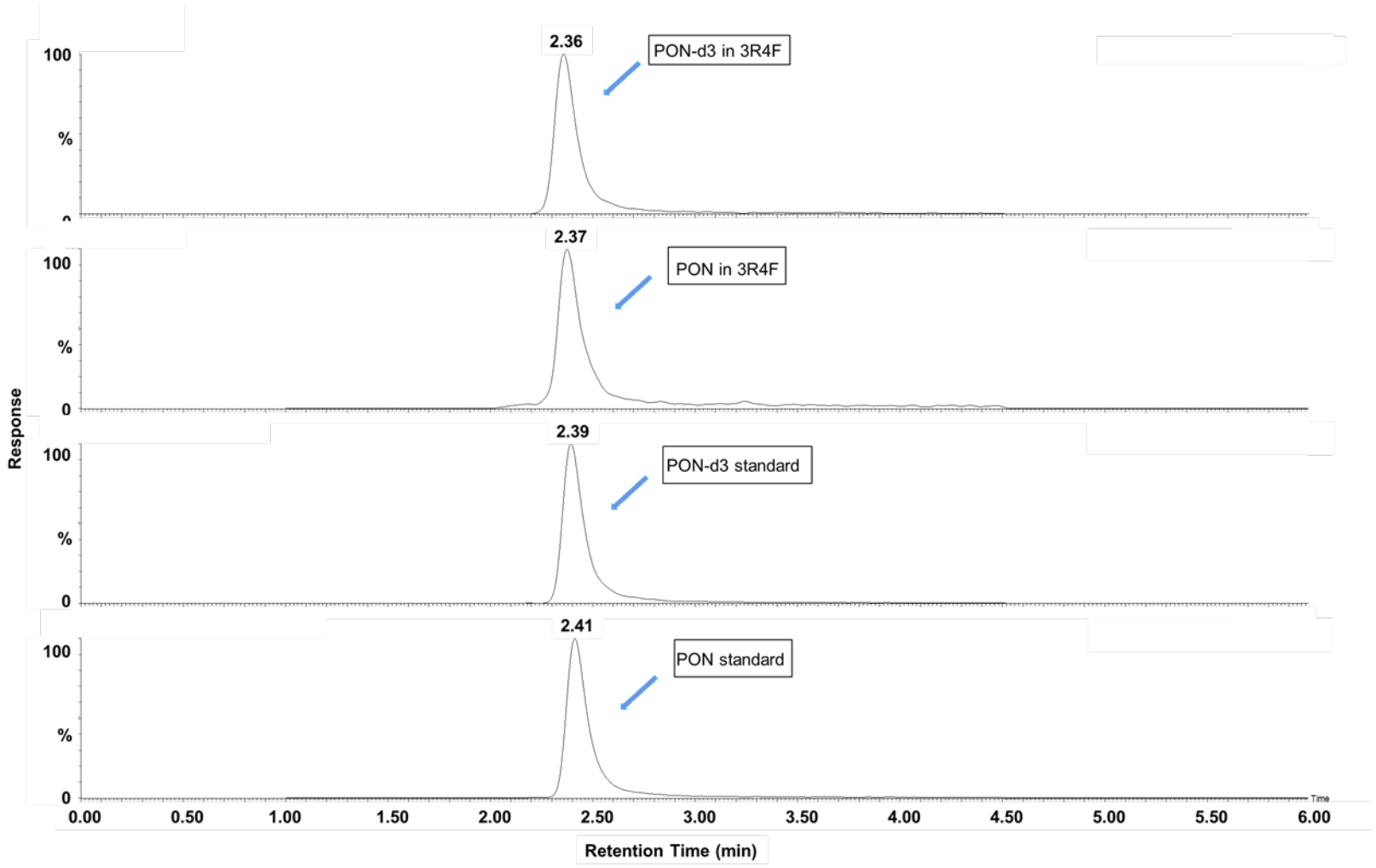
MS conditions:

- Mass spectrometer: Waters Xevo TQD
- Ionization source: electrospray ionization
- Source Temp: 150 ˚C
- Desolvation Temp: 500 ˚C; desolvation gas: 800 L/hr
- Capillary voltage: 0.75 kV

Analyte	Precursor (m/z)	Product (m/z)	CE (eV)
PON	179	106	20.0
		148	12.0
PON-d3	182	106	20.0
		148	12.0

Chromatography

PON in 3R4F filler



Method Validation

Parameters		Results
Calibration	Linearity range (ng mL^{-1}) Coefficient of determination	20-2000 0.999
Recovery (%)	50% spiked level 100% spiked level 150% spiked level	95 ~ 104 102 ~ 106 99 ~ 108
Precision (%)	Intra-day (%RSD); n=3 Inter-day (%RSD); 3days, n=9	4.1 6.3
LOQ	ng mL^{-1}	20
LOD	ng mL^{-1}	7

Compare results:
LC/MS/MS vs GC/MS

	PON ($\mu g\ g^{-1}$)	
Method	**GC/MS**	**LC/MS/MS**
Sample 1	**42.0 ± 5.8**	**43.8 ± 2.3**
Sample 2	**441.9 ± 81.6**	**445.6 ± 19.0**

PON in smokeless tobacco and tobacco products

Samples	PON (μg g^{-1})
CRP1.1	80.2
CRP1.2	72.4
CRP1.3	54.7
CRP1.4	61.6
3R4F	54.0
Burley tobacco	92.3

Summary

- Determination of PON by LC/MS/MS is simpler, faster, more selective and robust compared with GC/MS method.

- This method has been fully validated and can be applied to PON analysis in different tobacco sample matrices.

Acknowledgement

- **Funding support from**

Kentucky Tobacco Research and Development Center