# Improvement of Analytical Method for Quantification of TSNA in Tobacco using UHPLC-MS/MS

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# Background



- Currently, TSNA analysis using LC-MS/MS is popular.
- In recent years, UHPLC-MS/MS which is faster than LC-MS/MS is being used for the analysis of various analytes.
- A method to analyze TSNA using UHPLC-MS/MS will probably be faster than using LC-MS/MS, with adequate accuracy and reproducibility.

# Objective



- **To investigate whether a new analytical method using UHPLC-MS/MS will shorten the runtime with an equal or better level of accuracy and reproducibility,**
  **without undue matrix effects,**
  **compared to the LC-MS/MS method which we have been using.**

# Instrument



## New method

**UHPLC - MS/MS**

**Waters LC : ACQUITY UPLC®**

**MS/MS : Quattro Premier™XE**

## Previous method

**LC - MS/MS**

**LC : Agilent 1100 Series**

**MS/MS : Applied Biosystems API 4000**

# Comparison



| | New method | Previous method |
|---|---|---|
| Column | ACQUITY UPLC HSS T3 1.8 µm 2.1 x 50 mm | Xterra MS C18 2.5 µm 2.1 x 50 mm |
| Guard column | ACQUITY UPLC HSS T3 Vanguard | - |
| Injection volume | 2.5 µL | 5 µL |
| Mobile phase A | 10mM acetic acid | Mili-Q water |
| Mobile phase B | 0.1% (v/v) acetic acid in methanol | 0.1% (v/v) acetic acid in methanol |
| Runtime | 7 min/inj | 20 min/inj |

# Liquid Chromatography



- **LC parameters**
  - **Column temperature : 60°C**
  - **Sample temperature : 5°C**
  - **Injection volume : 2.5 μL**
  - **Flow rate : 0.3 mL/min**
  - **Mobile phase A : 10mM acetic acid (pH 4.7±0.05)**
  - **Mobile phase B : 0.1% (v/v) acetic acid in methanol**

| LC gradient | | | | |
|---|---|---|---|---|
| TIME (min) | Flow rate (mL/min) | %A | %B | Gradient type |
| 0.0 | 0.30 | 98 | 2 | Initial |
| 0.2 | 0.30 | 98 | 2 | Linear |
| 3.5 | 0.30 | 5 | 95 | Linear |
| 4.5 | 0.30 | 5 | 95 | Linear |
| 4.6 | 0.30 | 98 | 2 | Linear |
| 7.0 | 0.30 | 98 | 2 | Linear |

# Sample Extraction

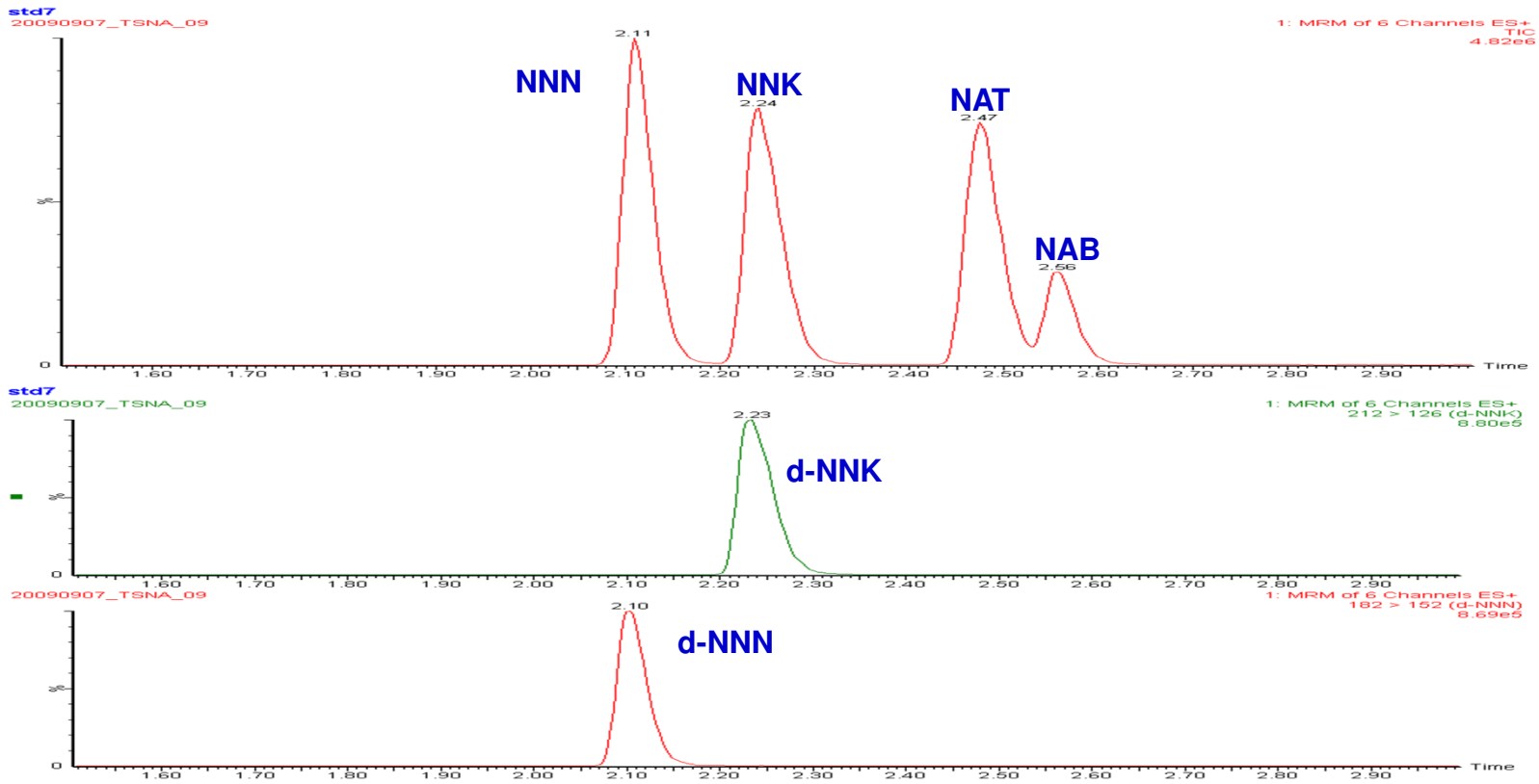


- Accurately weigh 0.5 g of ground tobacco into a 50mL sample tube
- Add 0.200 mL of a 5,000 ng/mL internal standard spiking solution
- Add 20 mL of 0.1 M ammonium acetate solution. Cap the sample tube
- Shake the sample(s) for 30 min. at 100 rpm on a horizontal shaker
- Dilute the extract tenfold with 0.1 M ammonium acetate solution
- Filter the diluted extract using Millipore® filter (0.20 μm) into a LC vial for analysis

# Validation 1/9



- **Selectivity - standard**

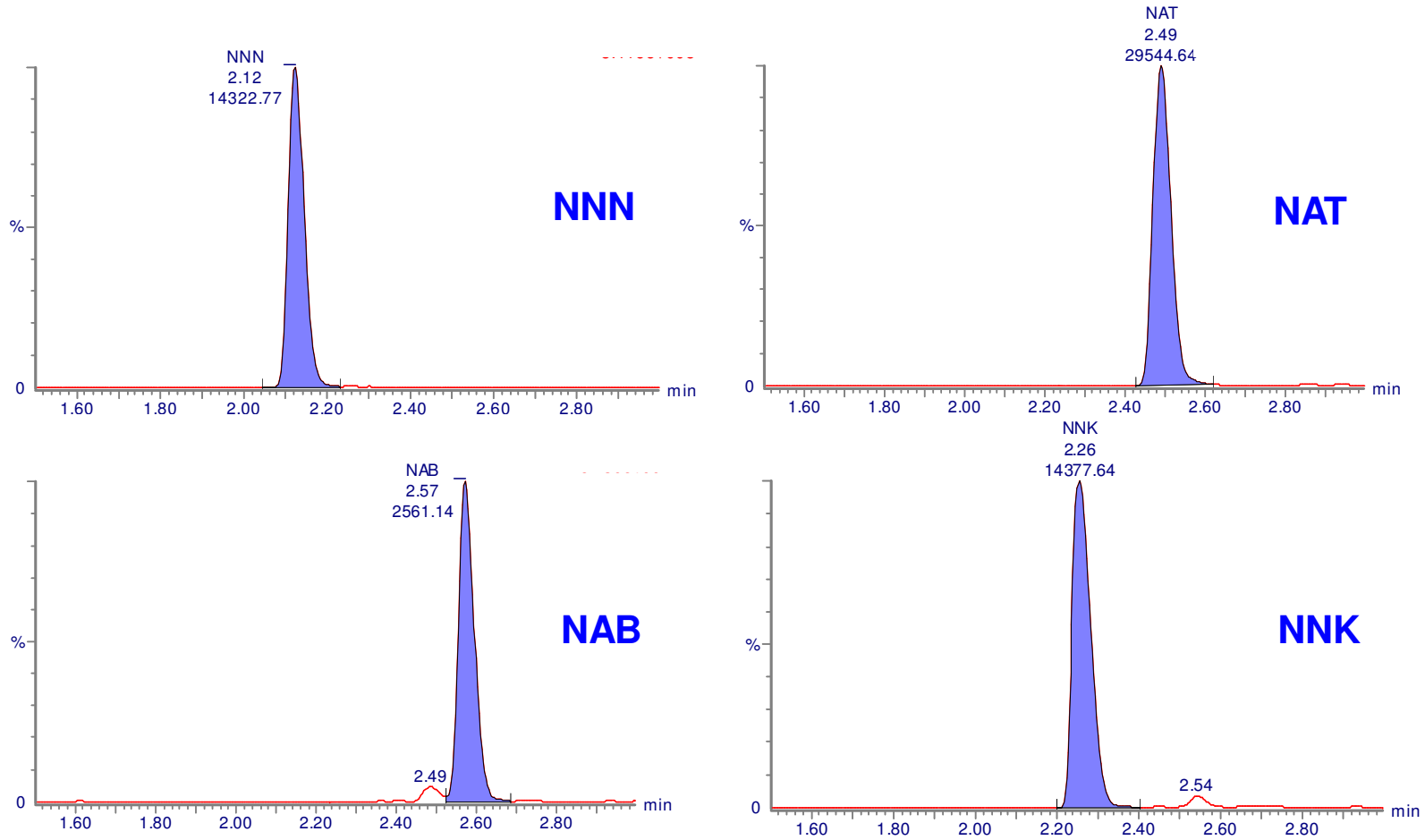



**Chromatograms of standard solution showed that the four common TSNAs, d-NNK and d-NNN were clearly separated.**

# Validation 2/9



- **Selectivity - sample**

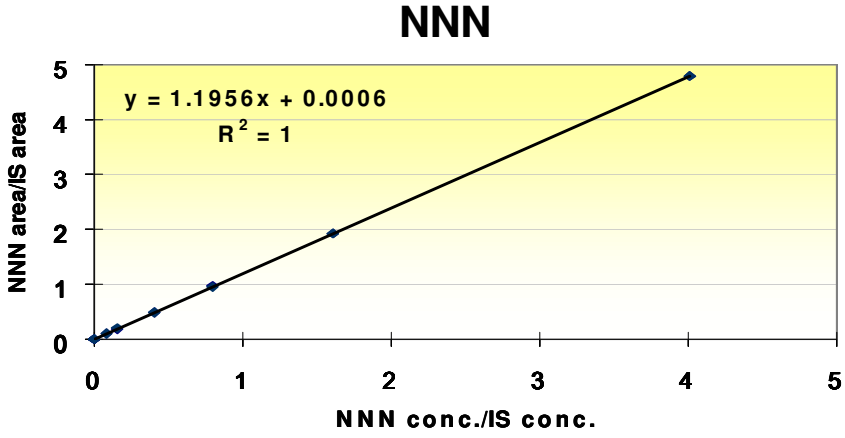


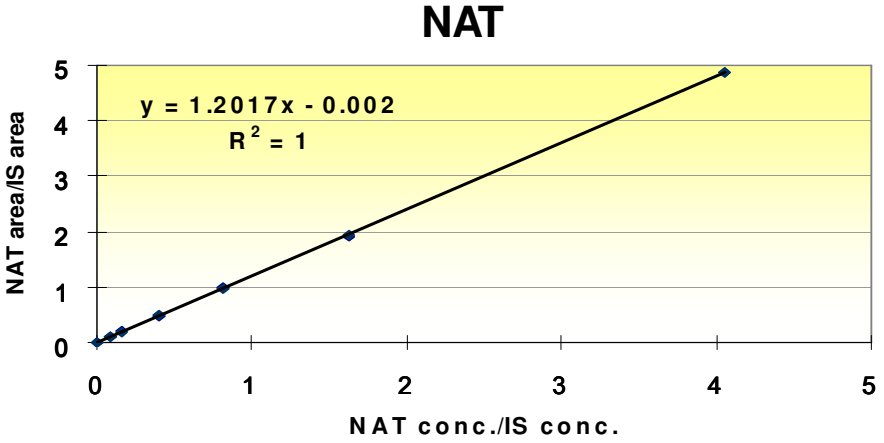

**Chromatograms showed that the analytes were determined without interference by the other components in a complex mixture.**

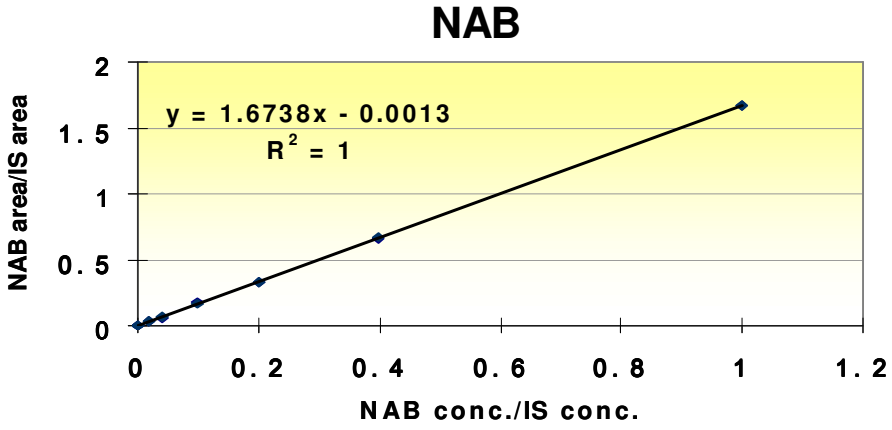
# Validation 3/9

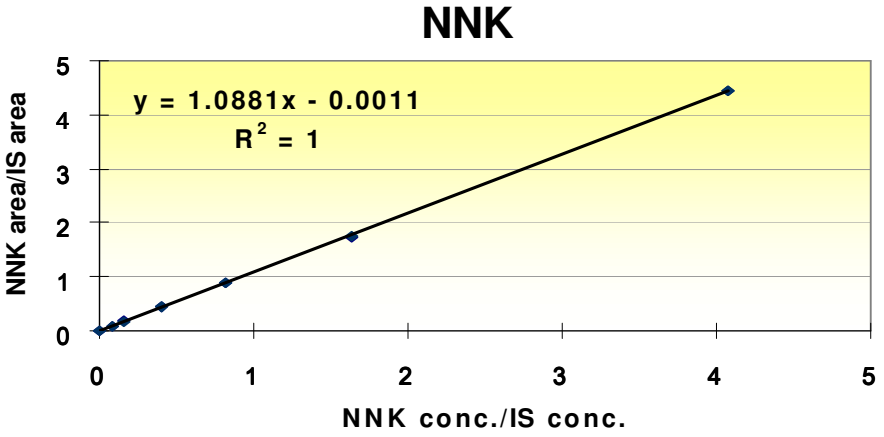


- **Linearity**



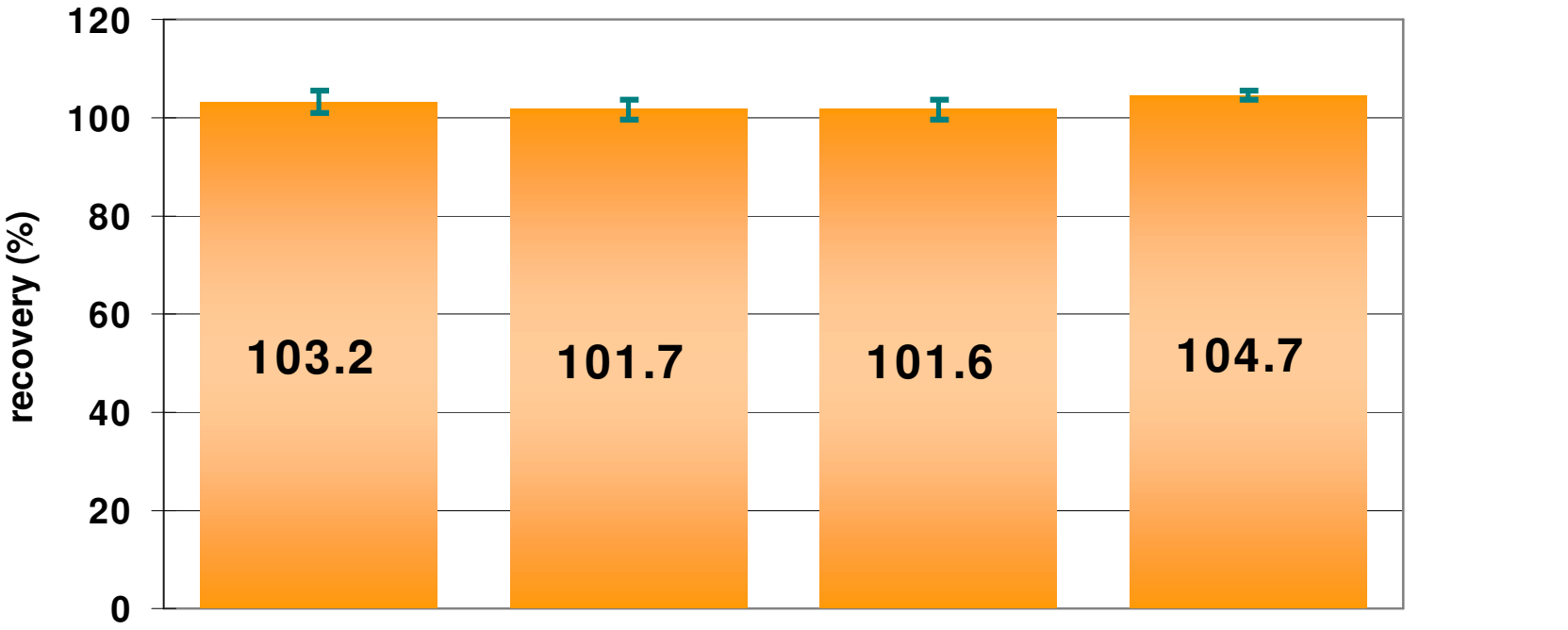




**The calibration curve of each analyte with eight standard solutions showed good linearity. ($R^2$ = 1.000)**

# Validation 4/9



- **LFB (n=6)**




*additive amount

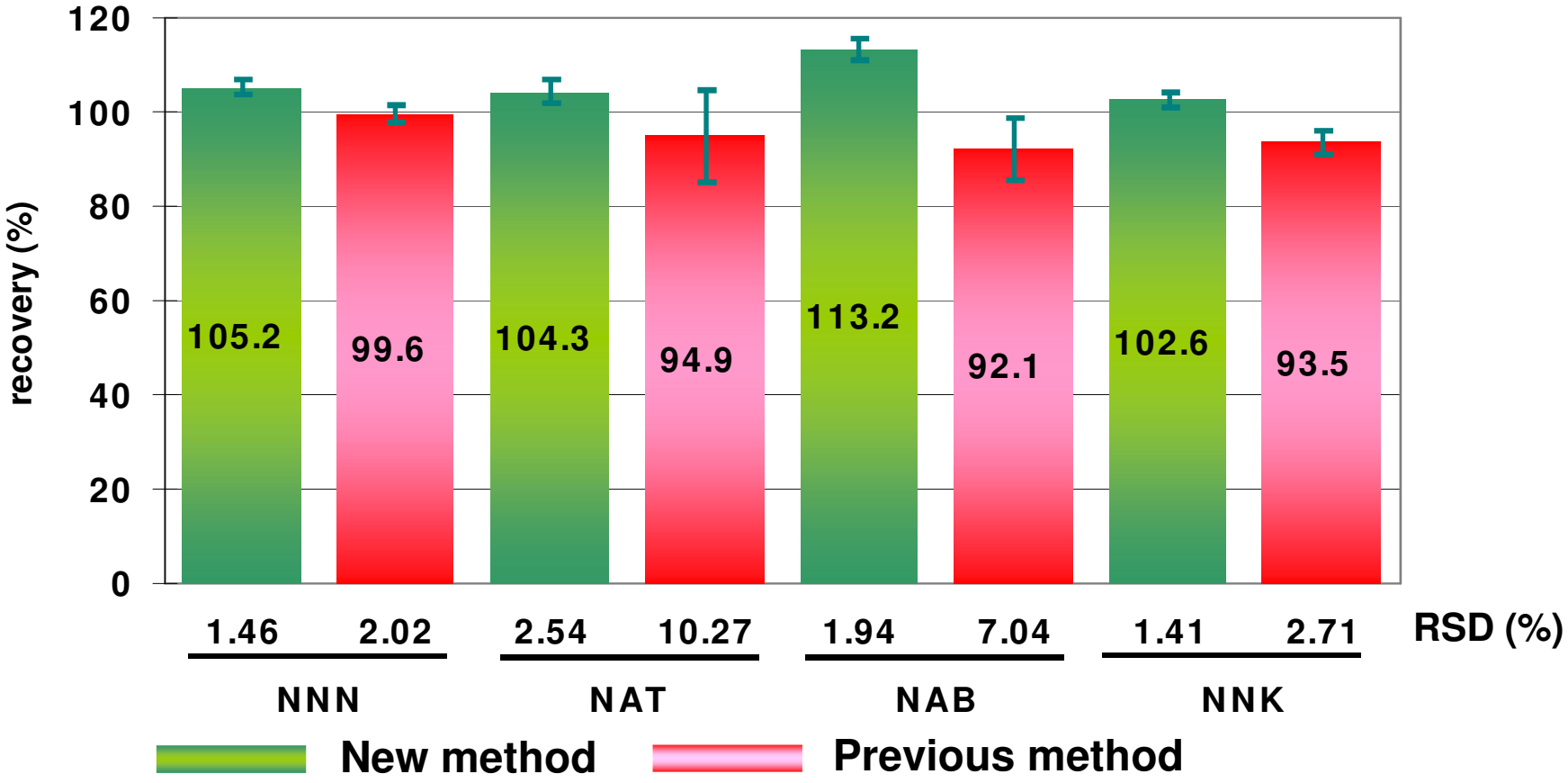
1.05μg 1.05μg 0.27μg 0.98μg

**Results with laboratory fortified blanks using the new method were satisfactory.**

# Validation 5/9



- **LFM – burley (n=6)**

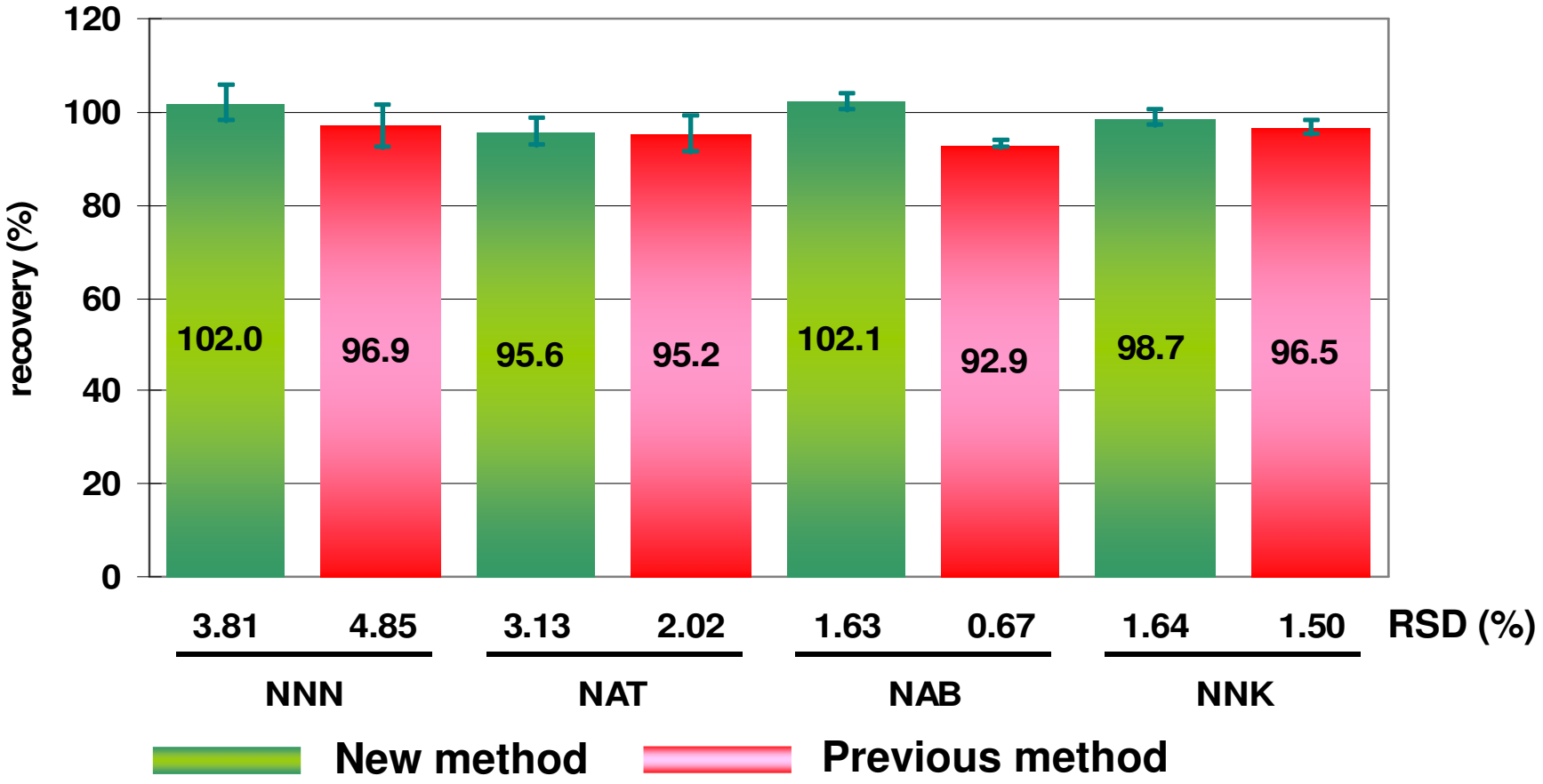



**The recovery percentage and reproducibility of the new method were equal or better level compared to those of the previous method.**

# Validation 6/9



- **LFM – flue-cured (n=6)**

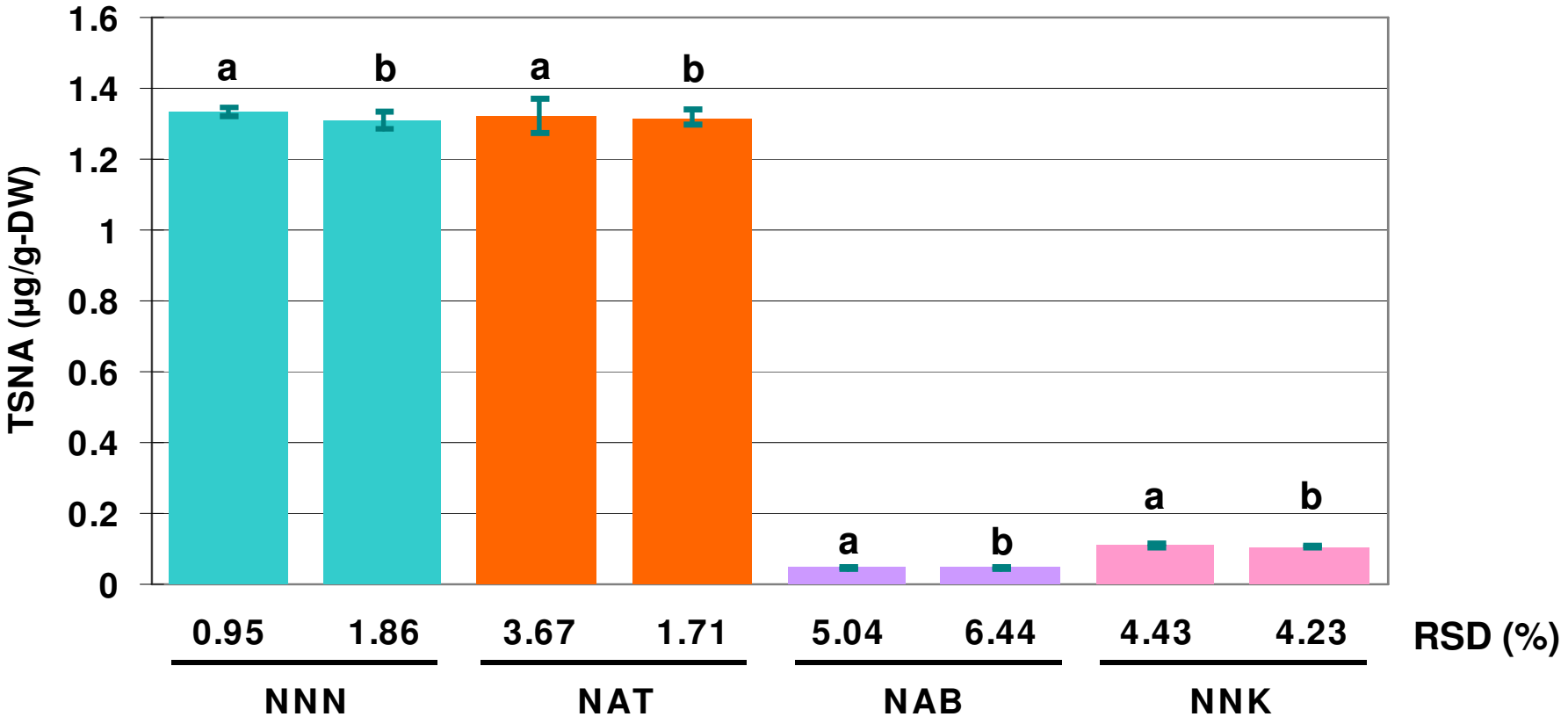



**The recovery percentage and reproducibility of the new method were equal or better level compared to those of the previous method.**

# Validation 7/9



- **Intermediate precision – burley (n=6)**



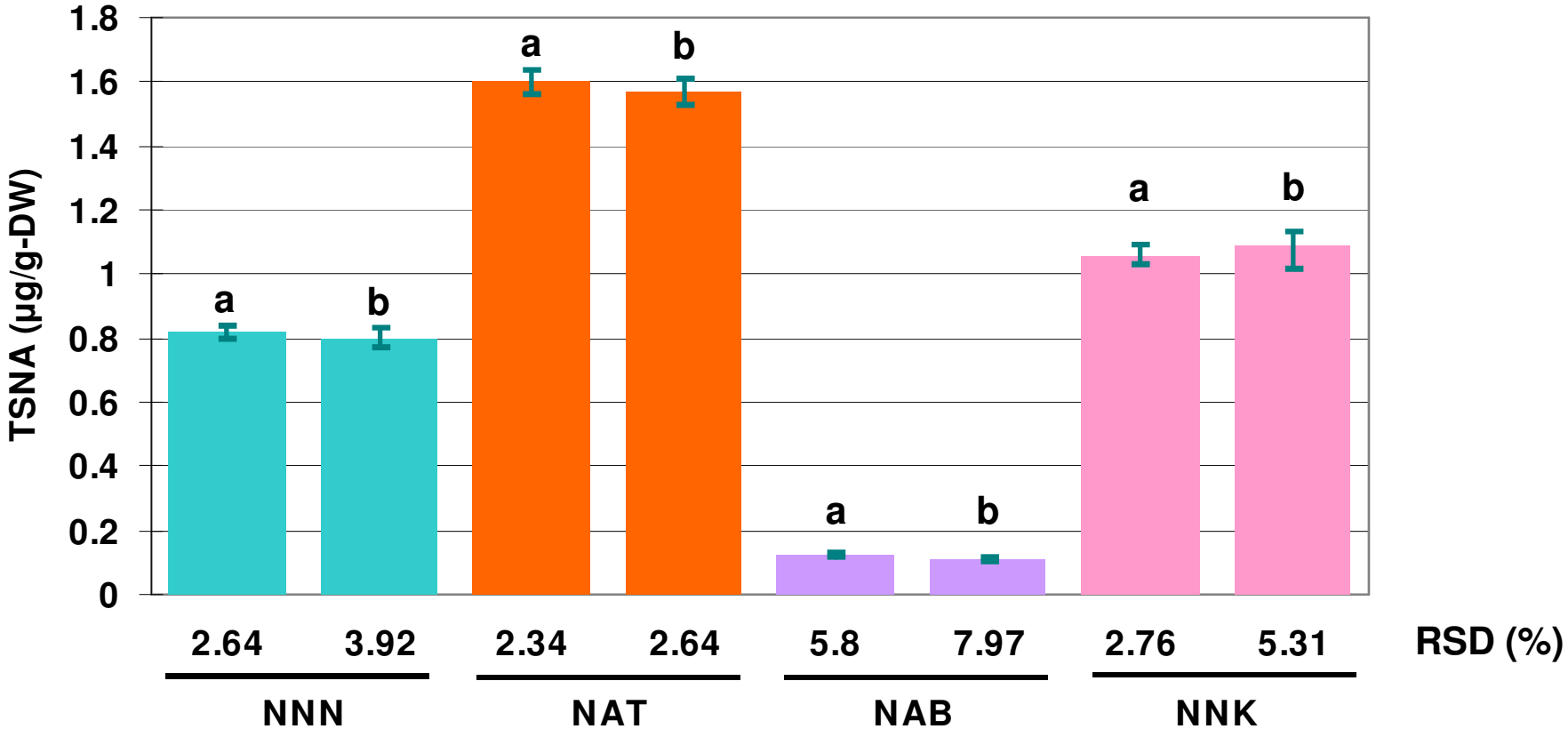

* a - 12/16/2008, b - 12/18/2008

**Repeatability and precision were satisfactory.**

# Validation 8/9



- **Intermediate precision – flue-cured (n=6)**




* a - 12/16/2008, b - 12/18/2008

**Repeatability and precision were satisfactory.**

# Validation 9/9



- **Range**

| analyte | Range (µg/g) |
|---|---|
| NNN | 0.118 - 12 |
| NAT | 0.184 - 12 |
| NAB | 0.040 - 3 |
| NAK | 0.115 - 12 |

- **LOD, LOQ**

(µg/g)

| New method | | |
|---|---|---|
| analyte | LOD | LOQ |
| NNN | 0.035 | 0.118 |
| NAT | 0.055 | 0.184 |
| NAB | 0.012 | 0.040 |
| NAK | 0.035 | 0.115 |

(µg/g)

| Previous method | | |
|---|---|---|
| analyte | LOD | LOQ |
| NNN | 0.044 | 0.145 |
| NAT | 0.045 | 0.151 |
| NAB | 0.021 | 0.071 |
| NAK | 0.038 | 0.125 |

**The LODs and LOQs of the new method were almost same as those of the previous method.**

# Conclusion



- **The runtime of the new method was 65% faster than that of the previous method.**
- **The level of accuracy and reproducibility of the new method were equal to or better than those of the previous method.**
- **Matrix effects were adequately suppressed by appropriately setting the column equilibrium time.**

# Thank you
# for your time.