Analysis of minor alkaloids in tobacco I:

Preparation of the standard solution

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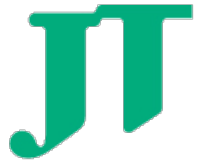


Background (1/3)

- **A collaborative study regarding analysis of minor alkaloids in tobacco was conducted by TSRC.** **(The TSRC method)**
  (Analysis of Minor Alkaloids in Tobacco: A Collaborative Study P.X. Chen et al. 2005, Beiträge zur Tabakforschung International / *Contributions to Tobacco Research*)

In that study, a mixed standard solution of 4 minor alkaloids was used.

(Nornicotine, anabasine, anatabine and myosmine)

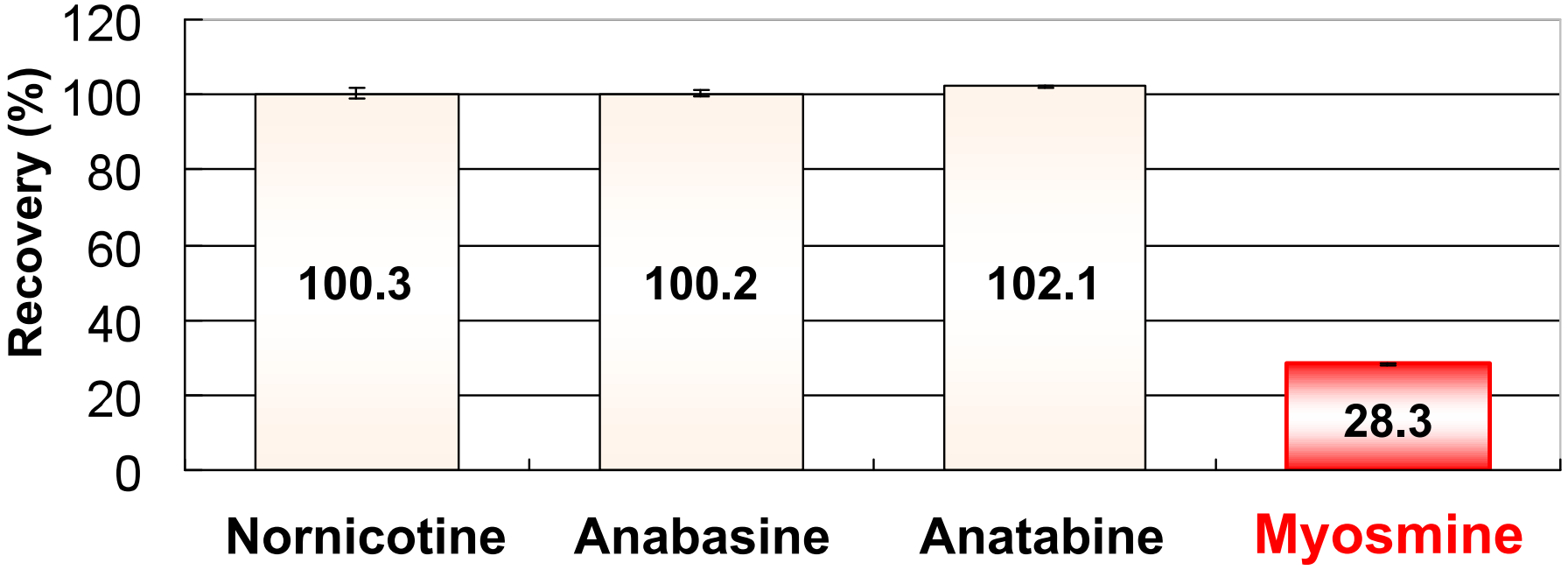
Background (2/3)

- **To verify the collaborative study, we determined the recovery rates in LFBs* of the 4 minor alkaloids one by one using the mixed standard solution.**

(*LFBs: Laboratory Fortified Blanks)

Background (3/3)

LFBs of the 4 minor alkaloids using the mixed standard solution



We found that the level of recovery of myosmine was extremely low.

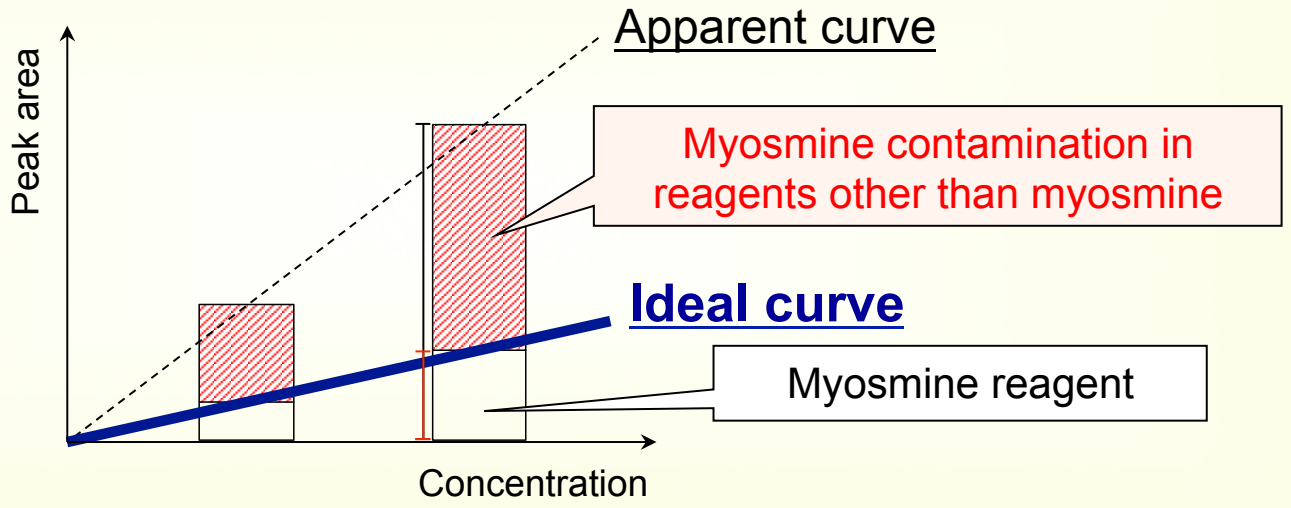
Objectives

- To determine the cause of the lower level of recovery of myosmine
- To establish a method for determining myosmine accurately

Hypothesis

"Some standard reagents of minor alkaloids contain myosmine as an impurity."

Calibration curve of myosmine using the mixed standard (image)



Verification of the hypothesis

- **To verify this hypothesis, individual analyses of the 3 minor alkaloid reagents were performed.**

<u>Minor alkaloid reagents:</u>

- **Nornicotine**
- **Anabasine**
- **Anatabine**

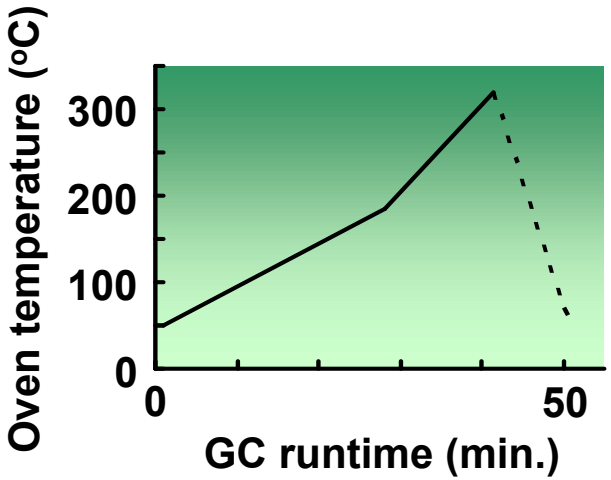
Method (1/2)

- **Equipment**
  - ✓ Agilent 6890N GC/FID system
  - ✓ Zero Air Generator
  - ✓ ChemStation Rev. B.04.02 [98]

- **Column**
  - ✓ Varian VF-17ms Capillary column
    50% phenyl - 50% methylpolysiloxane
    30 m x 0.25 mm x 0.25 μm

Method (2/2)

- **Parameters**
  - ✓ Injection temperature: 275°C
  - ✓ Detection temperature: 320°C
  - ✓ Injection volume: 1 μL, splitless
  - ✓ Oven method



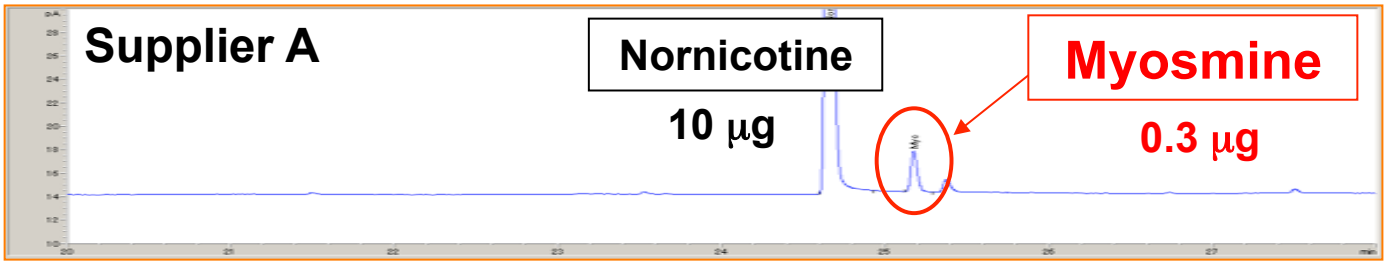
- Initial oven temperature: 50°C
- Initial hold time: 1.0 min.
- Rate of first ramp: 5°C/min.
- Final temperature: 185°C
- Hold time: 0 min.
- Rate of second ramp: 10°C/min.
- Final temperature: 320°C

< Same as in the TSRC method >

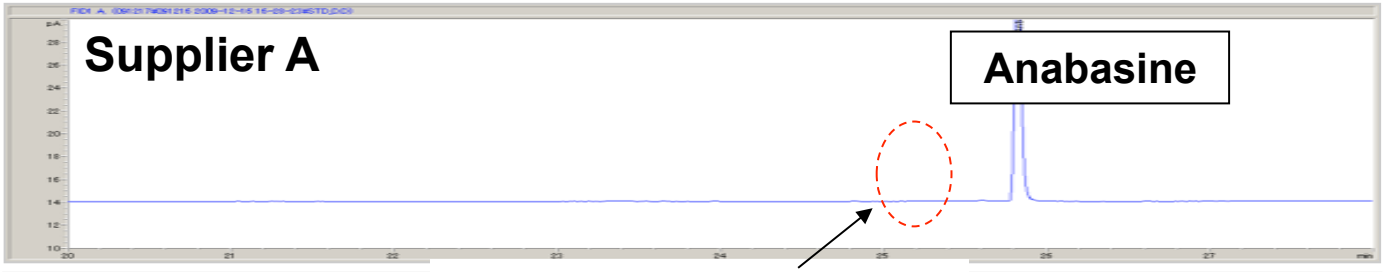
Results (1/2)

- Individual analyses of the minor alkaloid reagents

Nornicotine Std. reagent



Anabasine Std. reagent



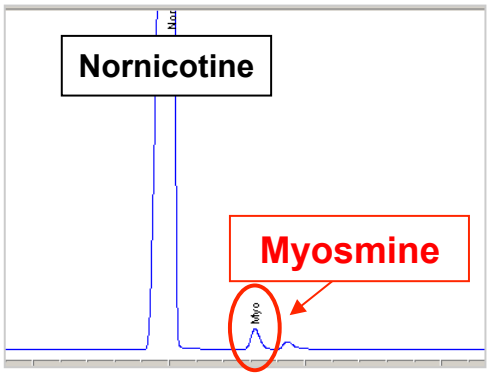
Anatabine Std. reagent

The nornicotine standard reagent contained myosmine as an impurity.

Results (2/2)

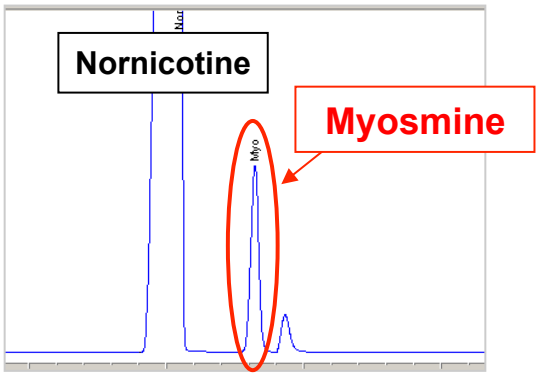
- **Analyses of nornicotine standard reagents**

Supplier B



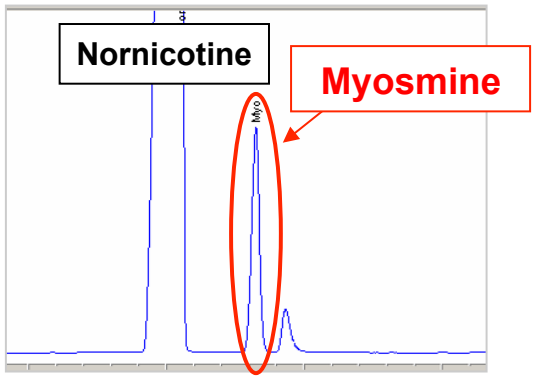
(Purity 98%)

Supplier C



(Purity not described)

Supplier D



(Purity 96%)

(Chromatograms of GC/FID analyses of 3 other nornicotine standard reagents of major suppliers in North America and Europe.)

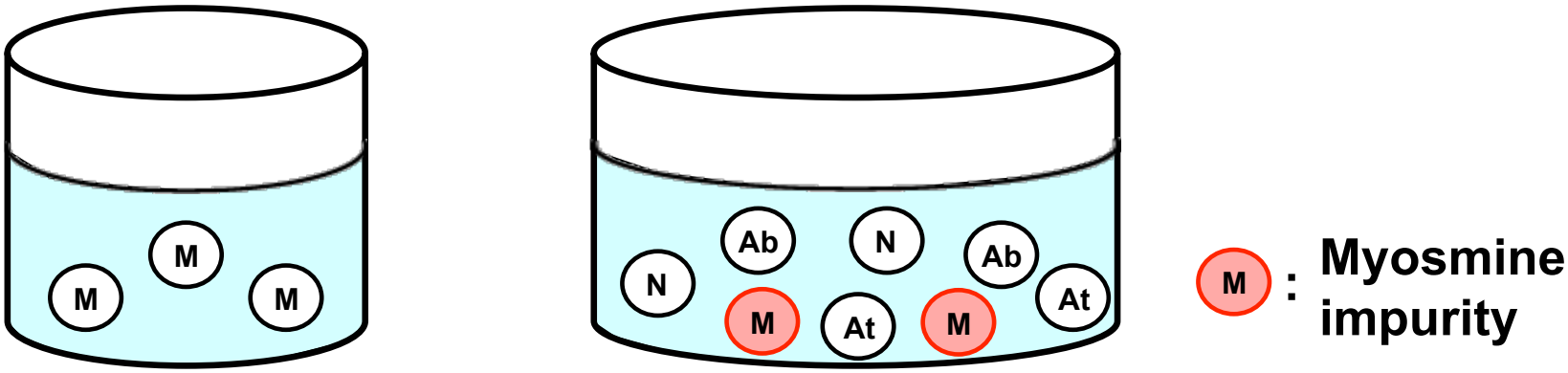
All of the nornicotine standard reagents were found to contain myosmine as an impurity.

New preparation method for standard solutions

- **Prepare 2 kinds of standard solutions:**

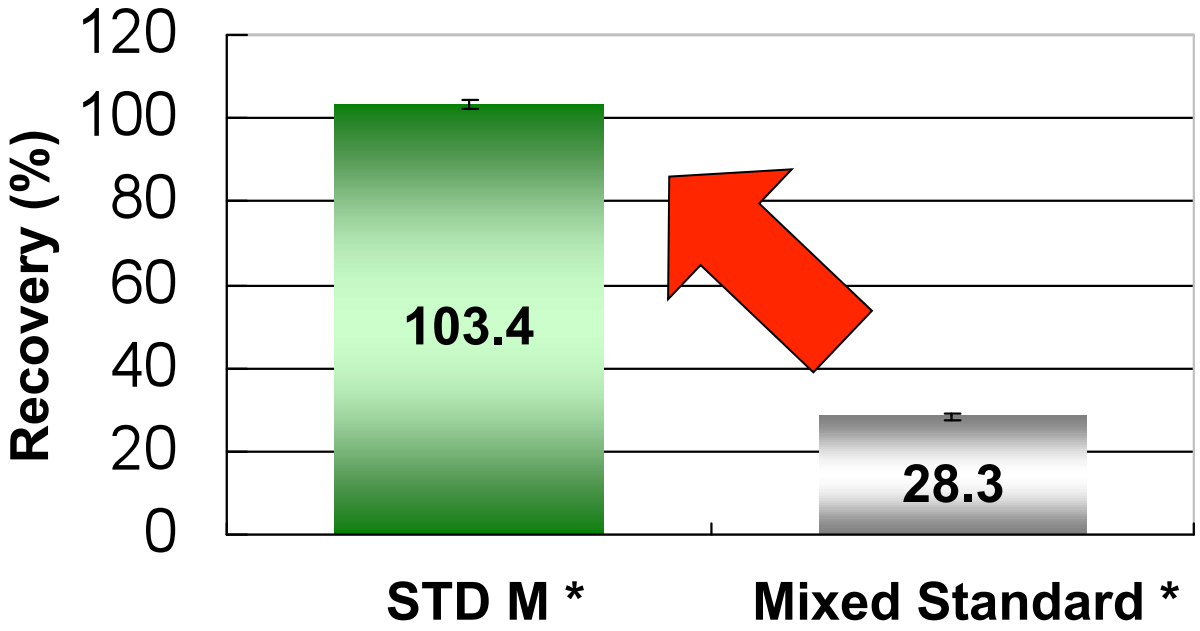
STD M: Myosmine only

STD NAA: Nornicotine + Anabasine + Anatabine



STD M **STD NAA**

Recovery of myosmine

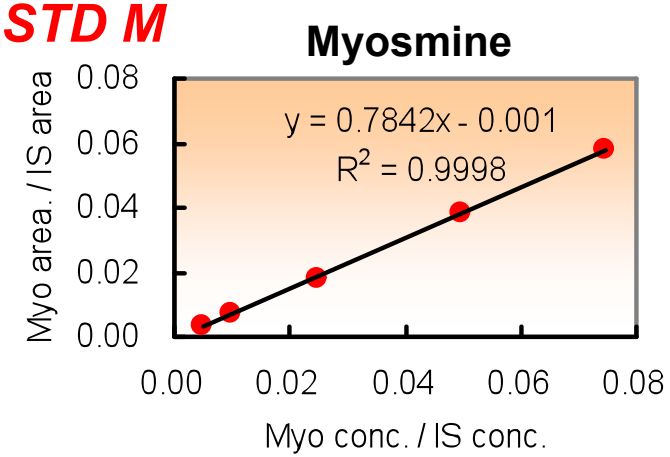


* **STD M: Myosmine only**
Mixed Standard: Nornicotine + Anabasine + Anatabine + Myosmine

The recovery in the LFB determined from the STD M was satisfactory, as it was 103.4%.

Linearity

STD M

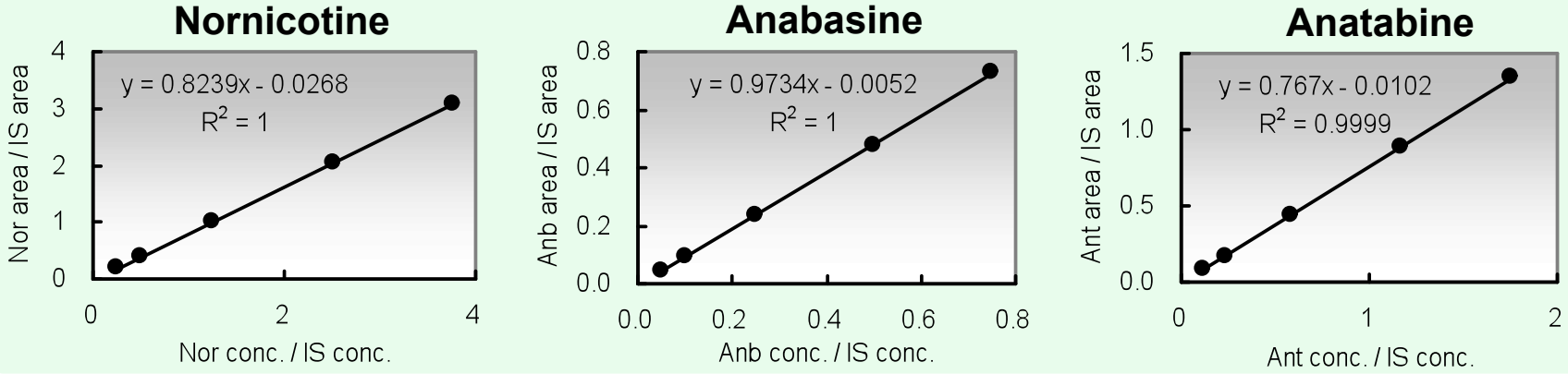


	Myo (mg/L)	Nor (mg/L)	Anb (mg/L)	Ant (mg/L)
STD1	0.2	10	2	4
STD2	0.4	20	4	8
STD3	1	50	10	20
STD4	2	100	20	40
STD5	3	150	30	60

STD M

STD NAA

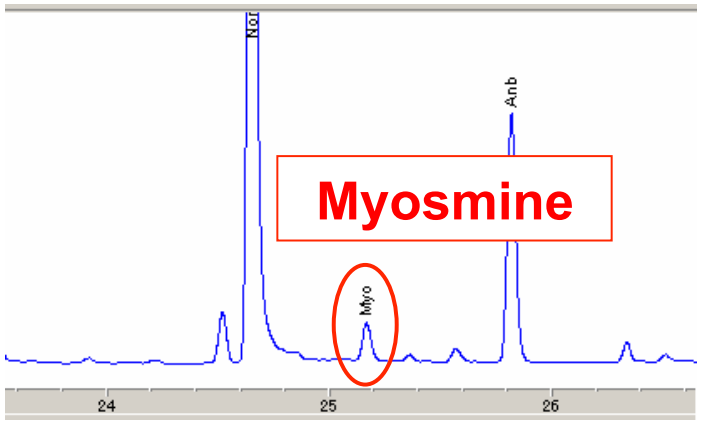
STD NAA *(Nornicotine + Anabasine + Anatabine)*



The calibration curve of each alkaloid showed good linearity.

Effect of standard solutions

- **Analysis of a flue-cured tobacco sample**



Std. used for calculation	Myosmine (μg/g)
STD M	**43**
Mixed Standard	9

There was a large difference between the levels of myosmine found when using the STD M and the Mixed standard solutions.

Conclusions

- **The lower level of recovery of myosmine was caused by the contamination of myosmine (as an impurity) in Nornicotine standard reagents.**
- **For the accurate determination of myosmine, a myosmine standard solution should be prepared separately from the other minor alkaloid reagents.**

Thank you for your time.