

Development of a highly sensitive,
quick, and easy
LC-ESI-TOF-MS method
to quantify nicotianamine and
2'-deoxymugineic acid
in plants

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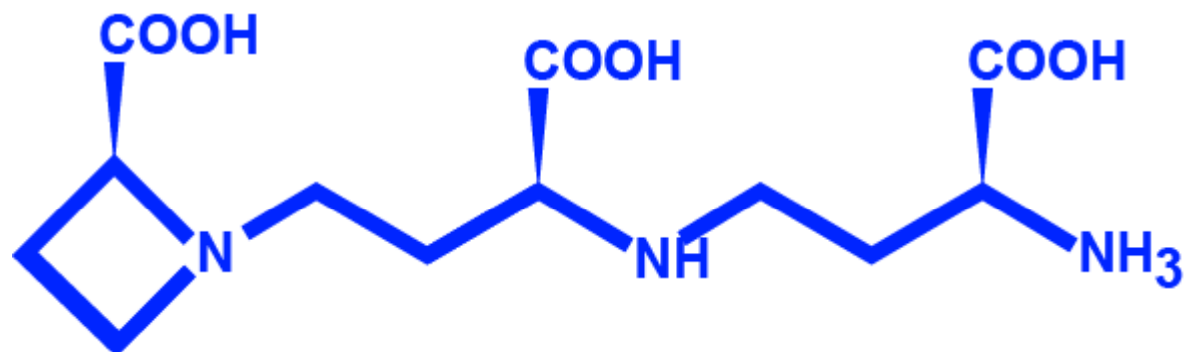
Wada et al. (2000) Biosci. Biotech. Biochem.,

Kakei et al. (Submitted) Plant Cell Physiol.

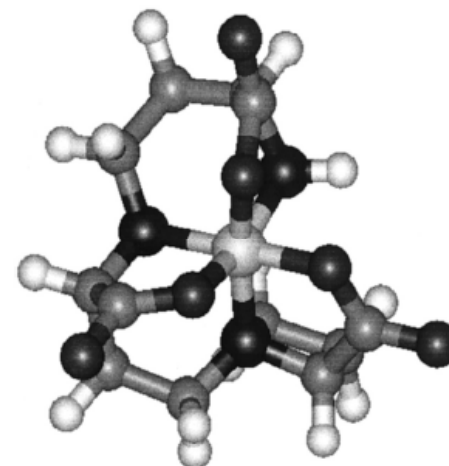


Rice plants on calcareous paddy field showing symptoms of Fe-deficiency

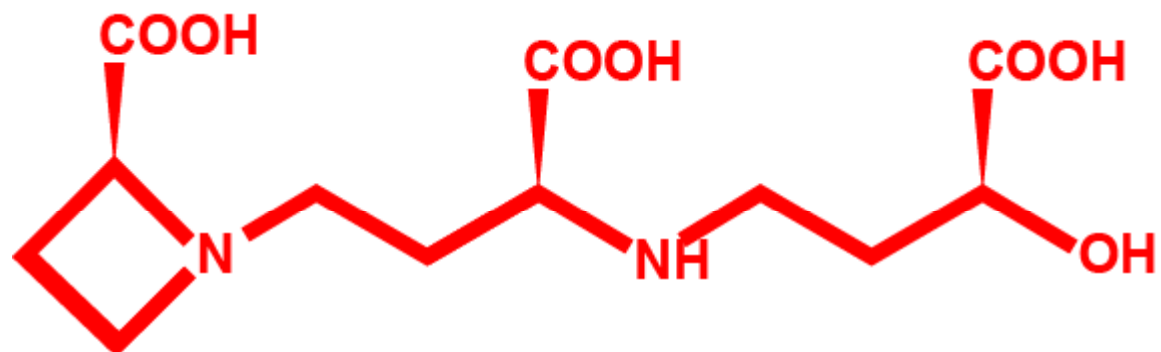
Nicotianamine and DMA are chelators of metals



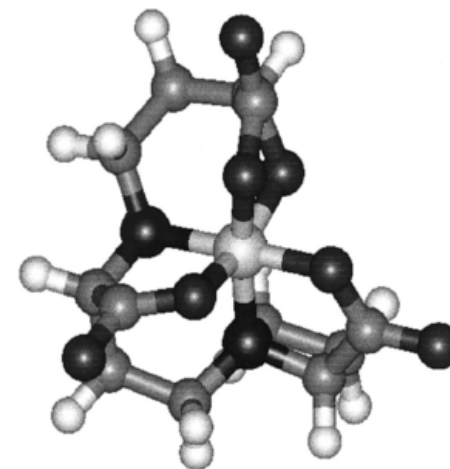
Nicotianamine (NA)



Fe^{III}NA



2'-deoxymugineic acid (DMA)



Fe^{III}DMA

von Wiren et al. (1999)

Nicotianamine(NA) is essential to metal distribution

If NA does not exist in plant,
the plant shows metal deficiency symptom.



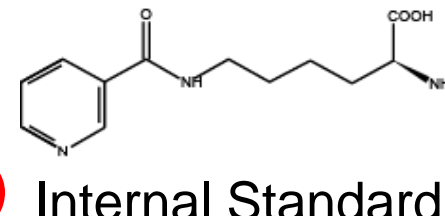
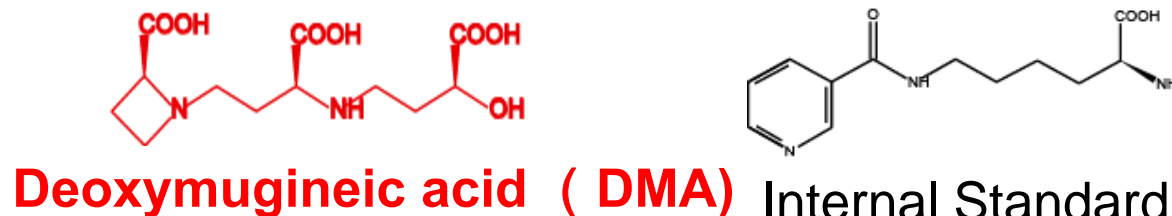
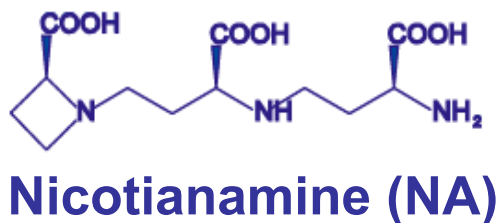
Control
Wild-type tobacco



naat-A
***naat* tobacco**
(does not have NA)

Takahashi et al. 2001

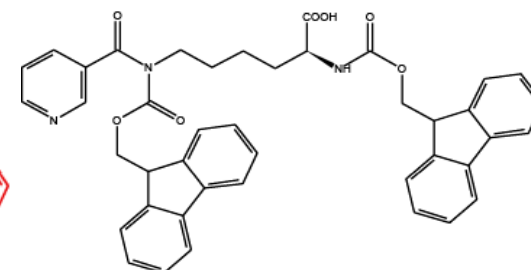
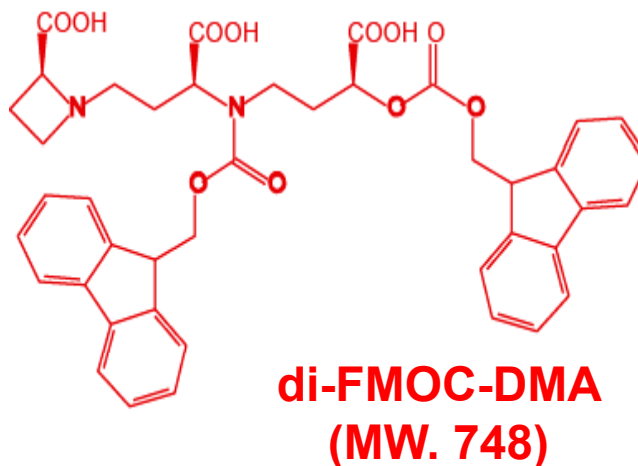
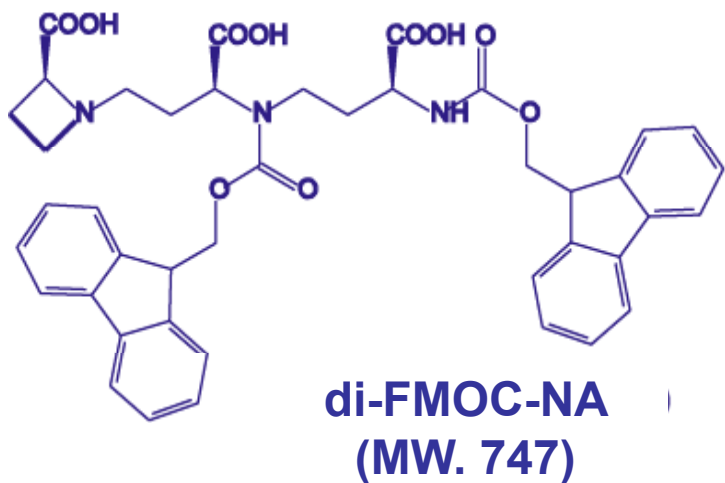
Derivatization of internal standard, NA and DMA using FMOC-Cl



↓ FMOC-Cl

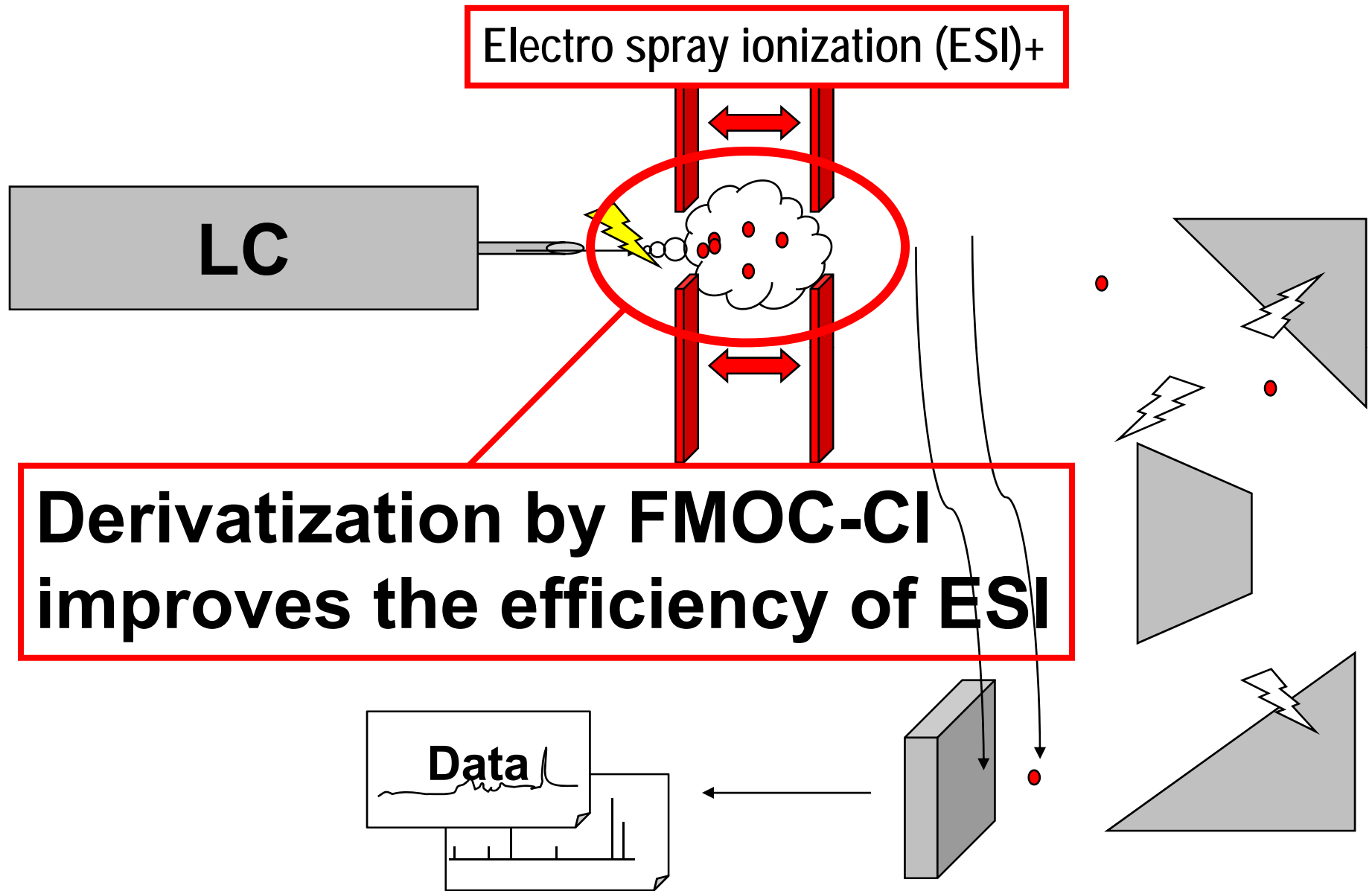
↓ FMOC-Cl

↓ FMOC-Cl

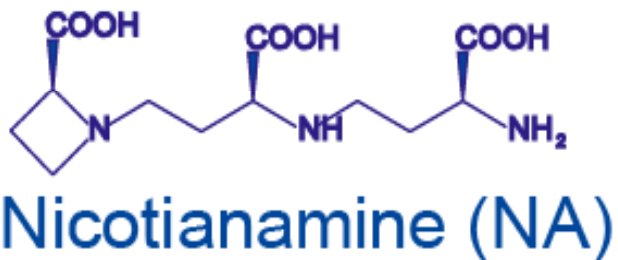


Derivatization enhances efficiency of ESI from dozens to thousands times. (pmol → fmol)

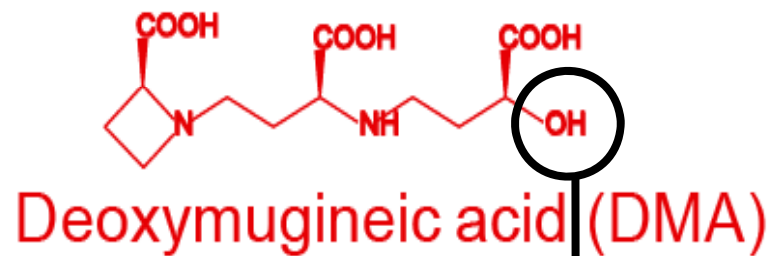
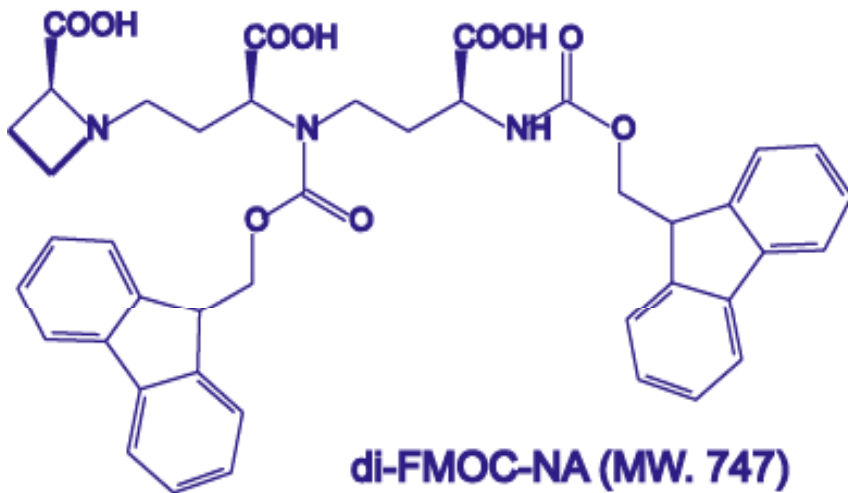
LC/ESI-TOF-MS system



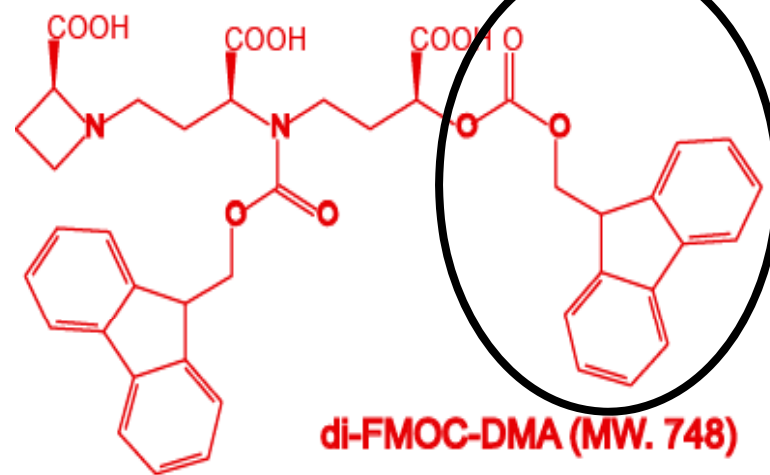
Dose hydroxyl group react with FMOC-Cl?



↓ FMOC-Cl

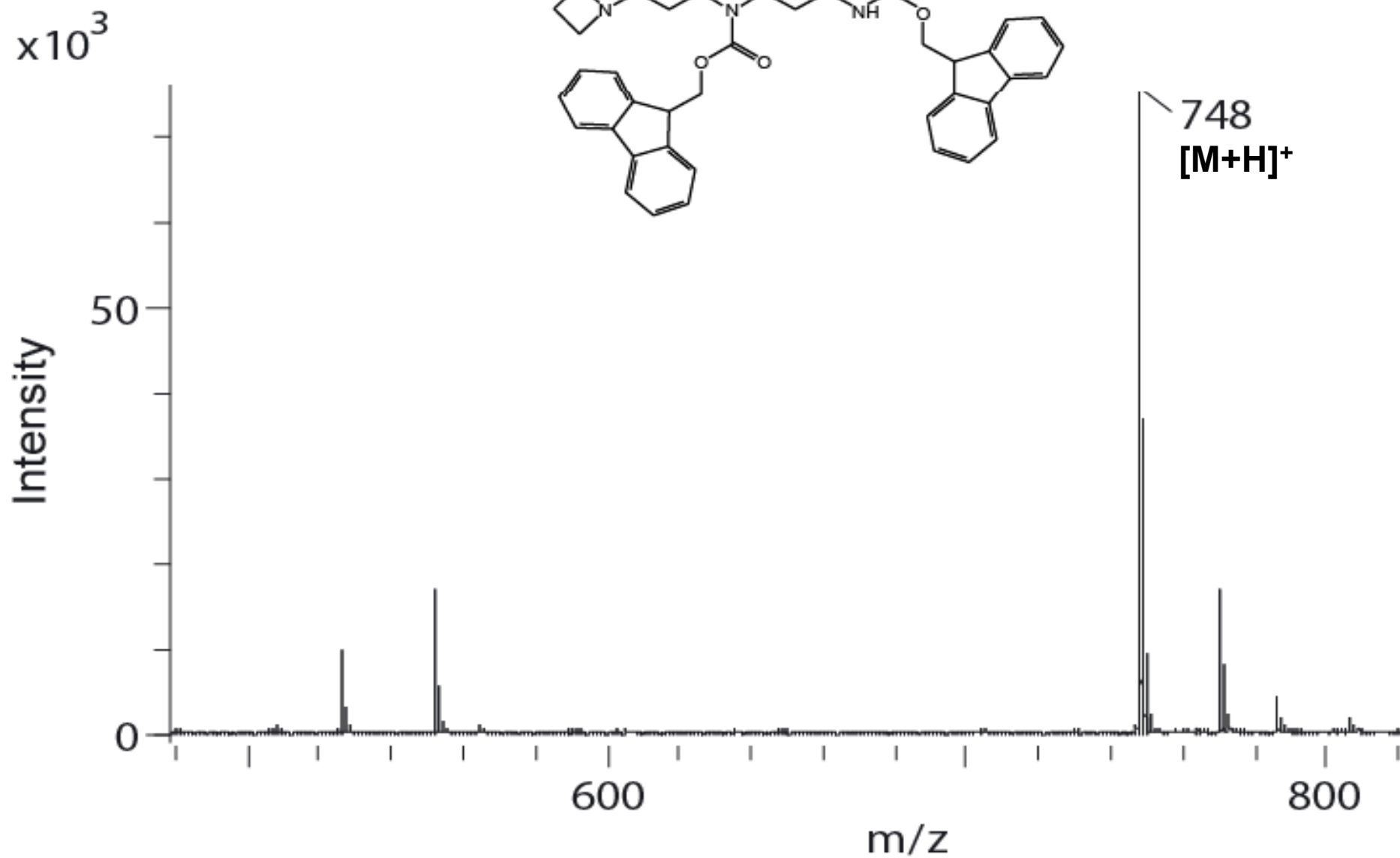
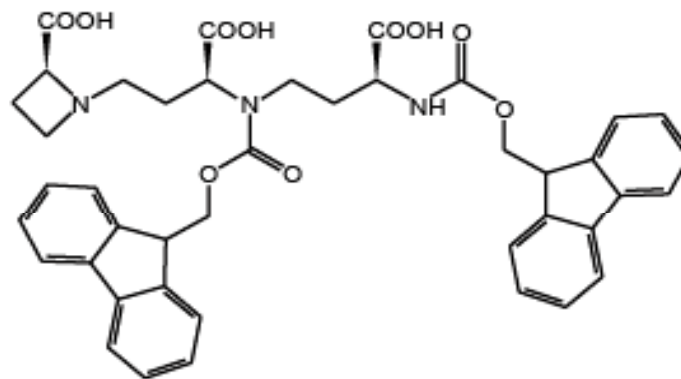


↓ FMOC-Cl

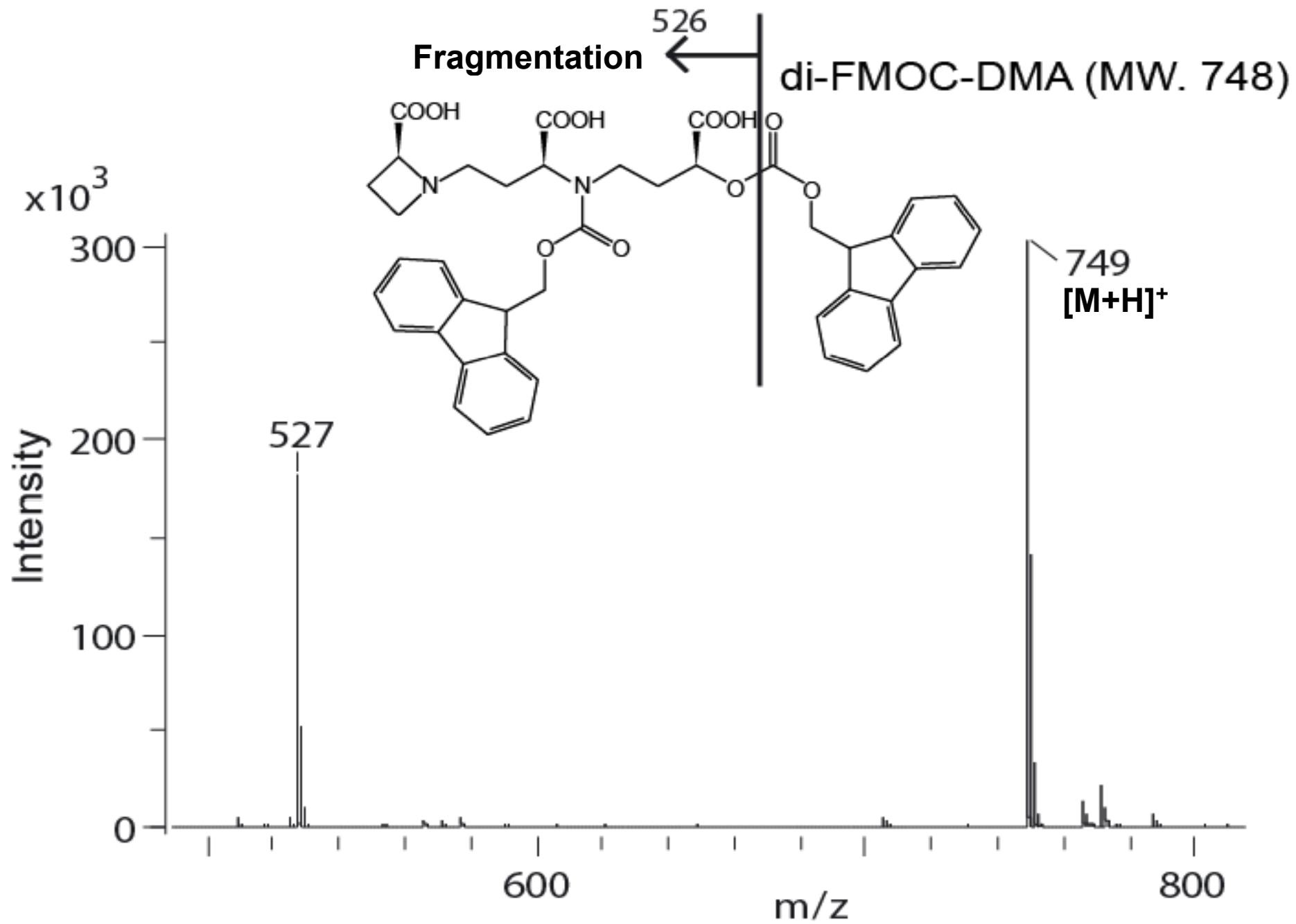


Detection of NA

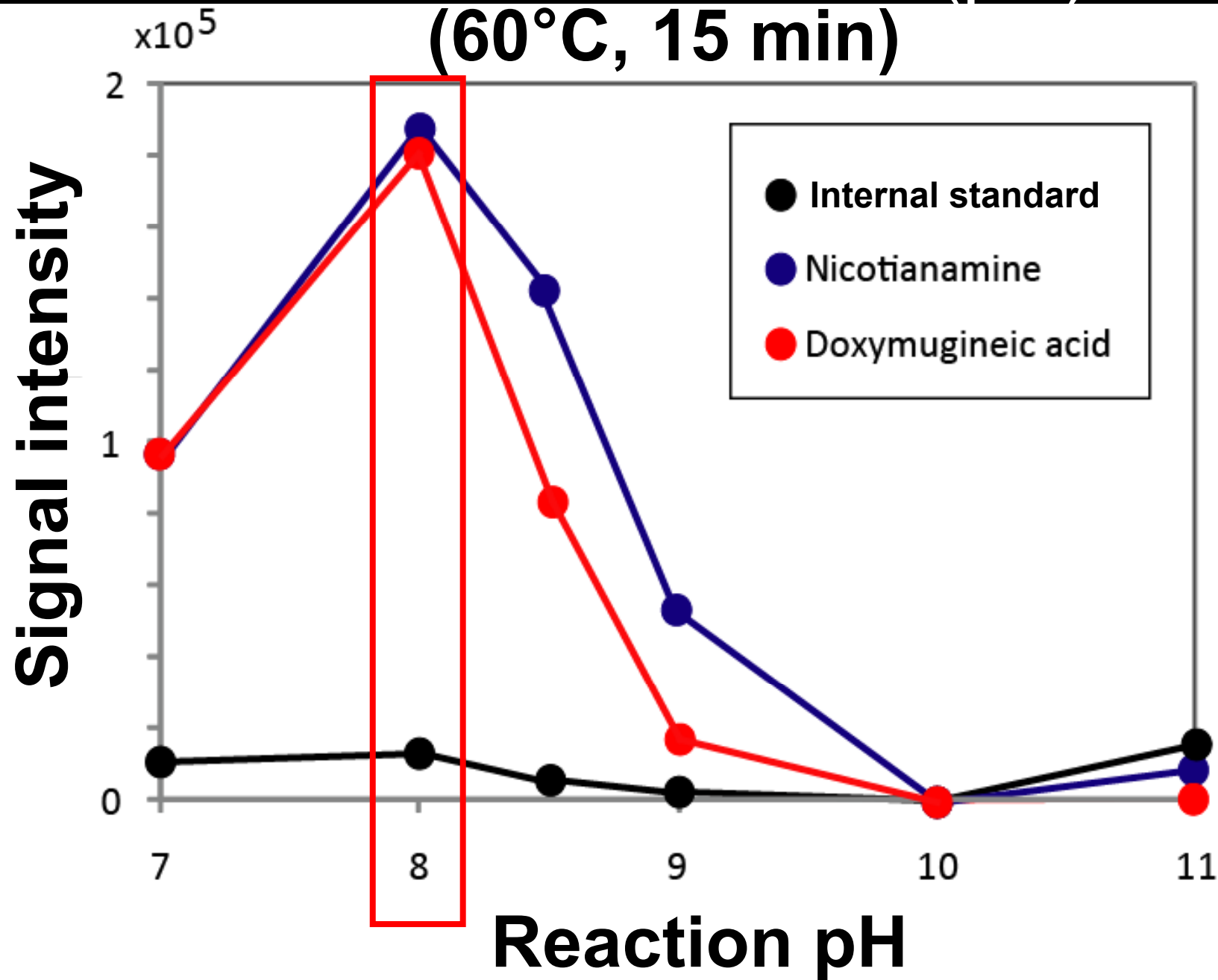
di-FMOC-NA (MW. 747)



Detection of DMA

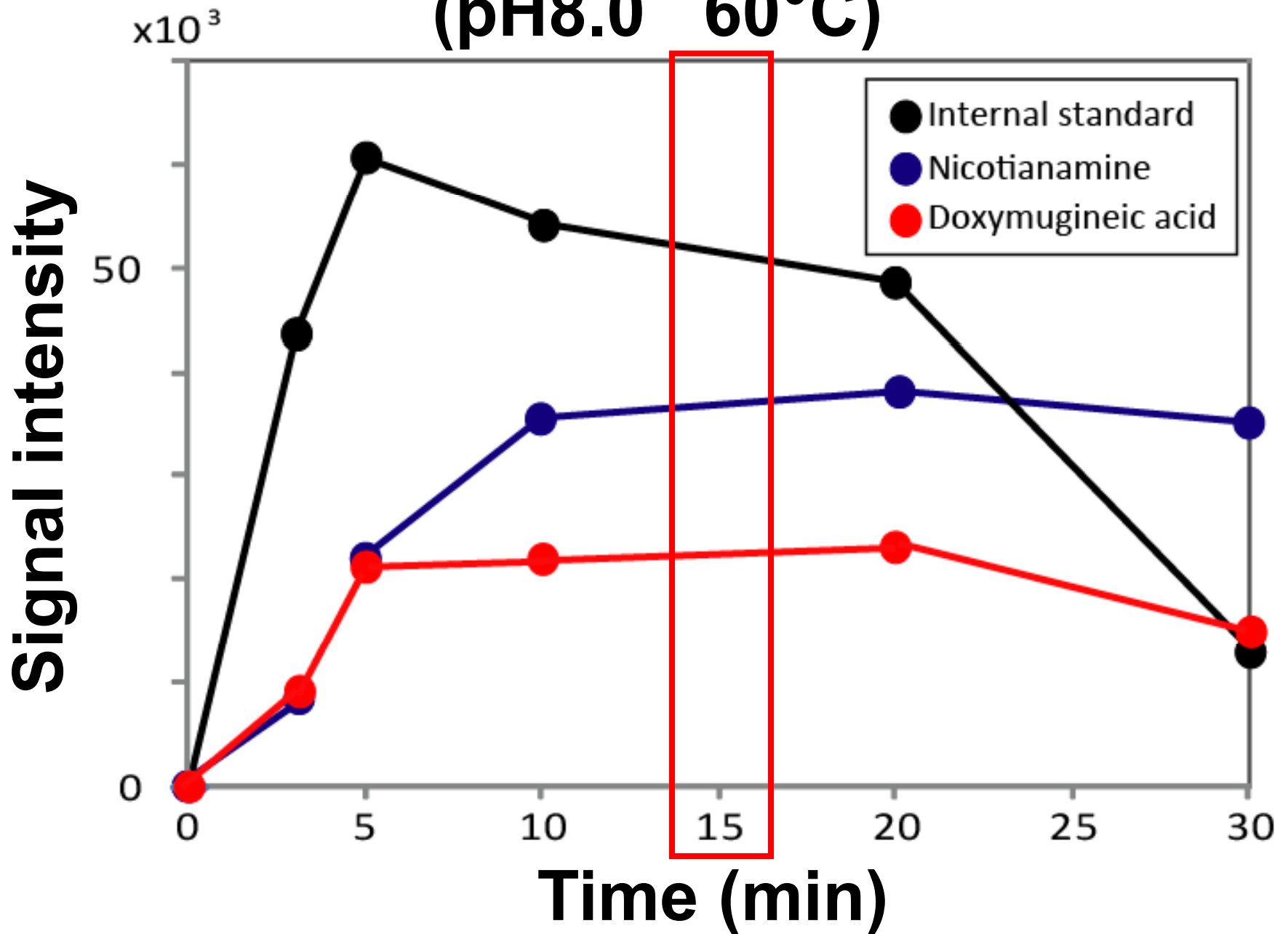


Derivatization condition (pH) (60°C, 15 min)



Derivatization condition (time)

(pH8.0 60°C)



Quantification flow

**Quantification is performed
within only 30 min. per sample.**

1. Derivatization 15 min.

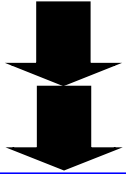
(Internal standard, EDTA, Borate buffer, FMOC-Cl, 60°C, pH8)
Stop reaction (pH 3)

2. Separation and detection 10 min.

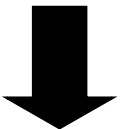
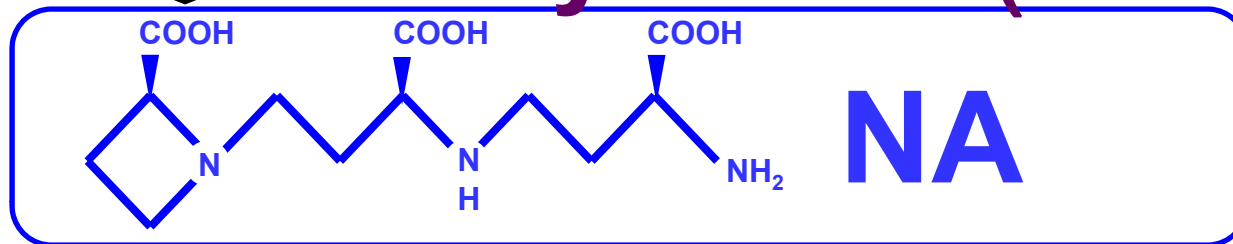
with LC/ESI-TOF-MS
(C18 column, Water 35.5%, Acetonitril 64%, Formic acid 0.5%)
(Desolvent temperature 300°C, ESI+ 2100V) .

Biosynthetic pathway of DMA

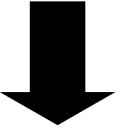
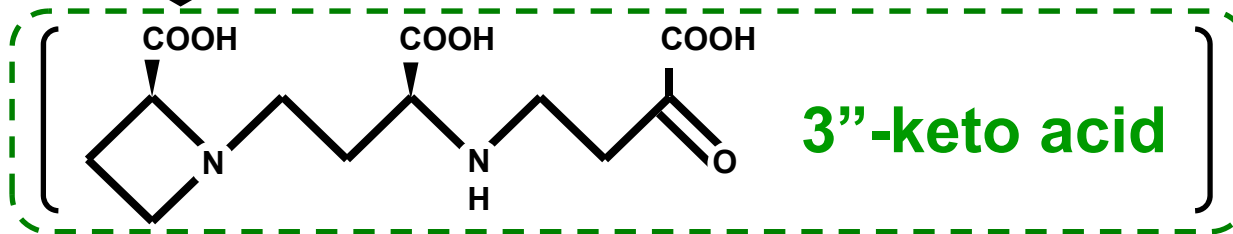
L-methionine



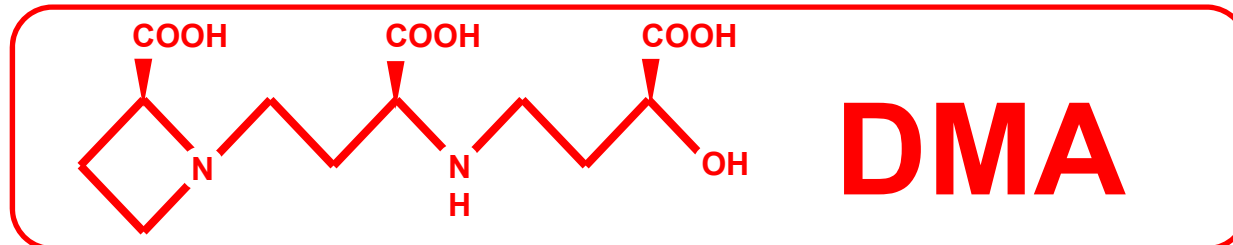
NA synthase (NAS)



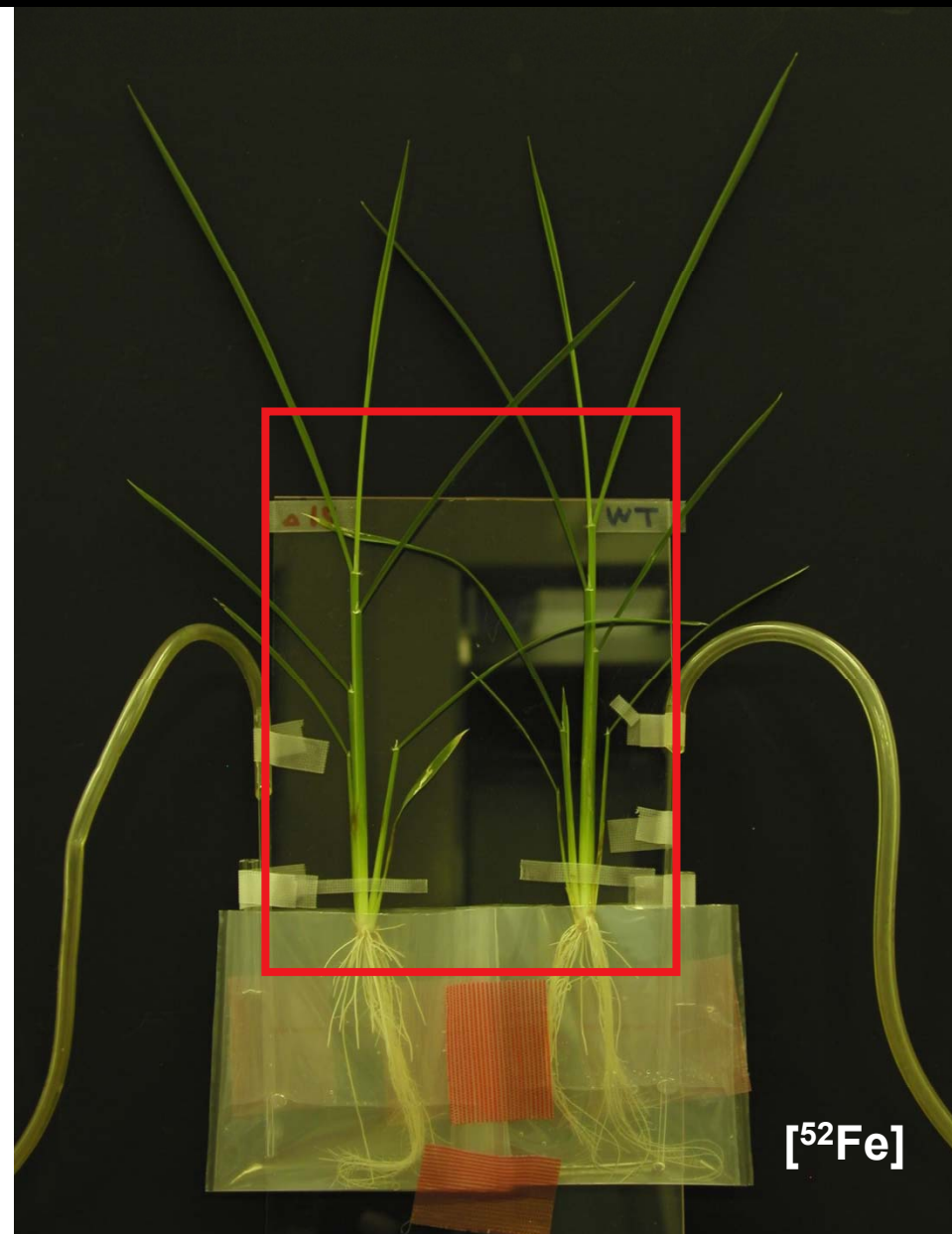
NA aminotransferase (NAAT)



DMA synthase (DMAS)

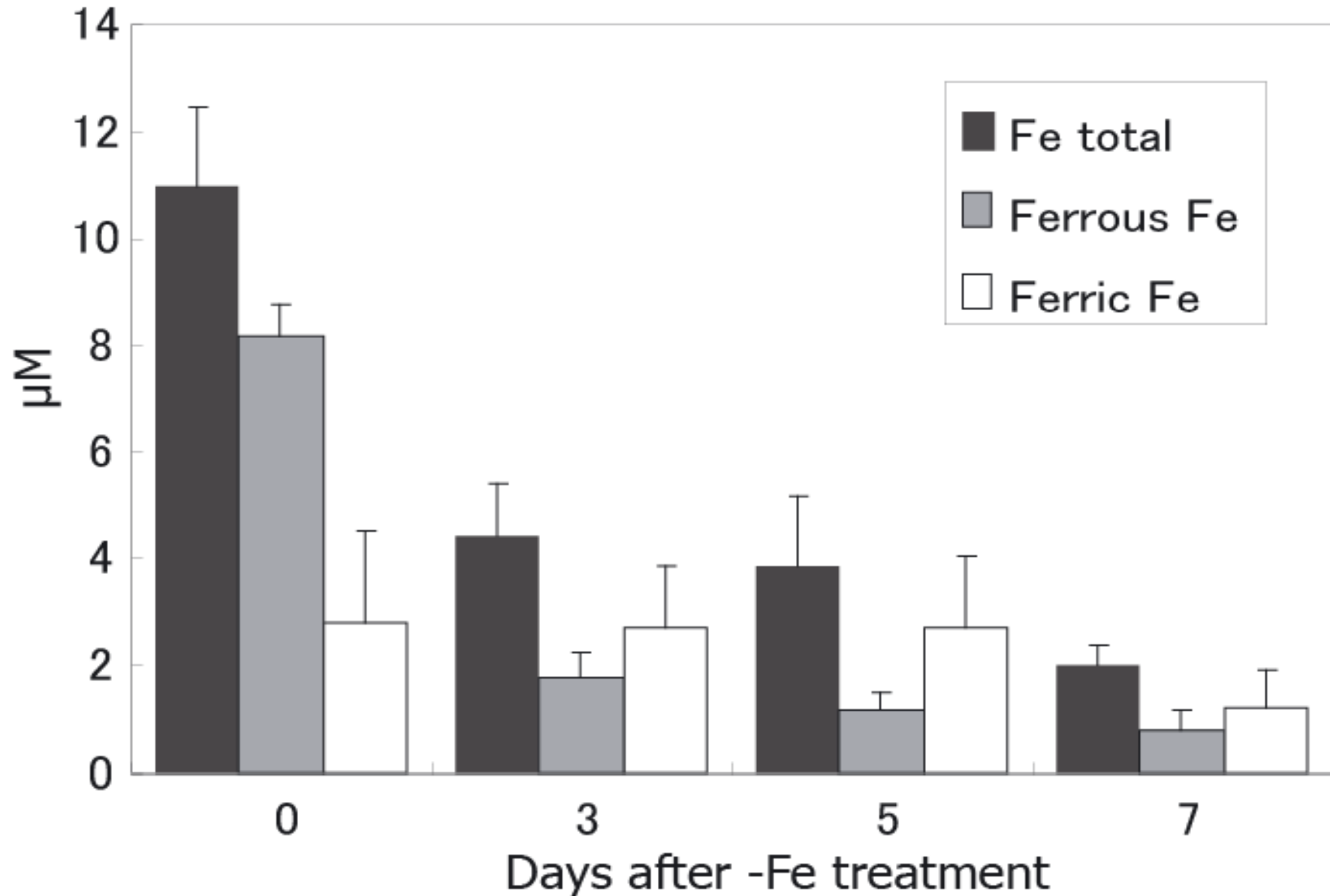


Transport of $[^{52}\text{Fe}]$ in rice detected by PETIS



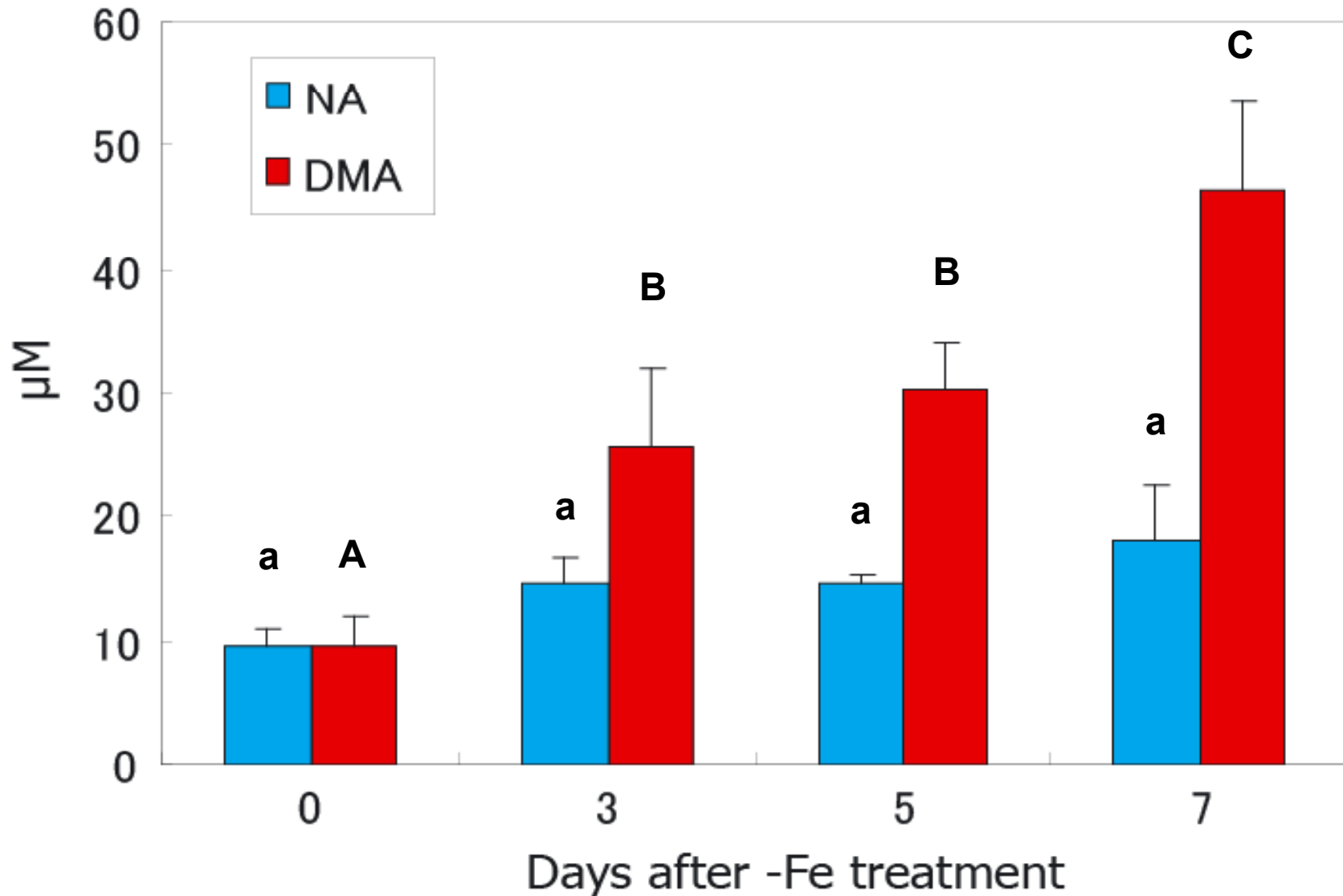
Concentration of Fe in rice xylem sap with -Fe treatment

Xylem sap was collected in 30 min.



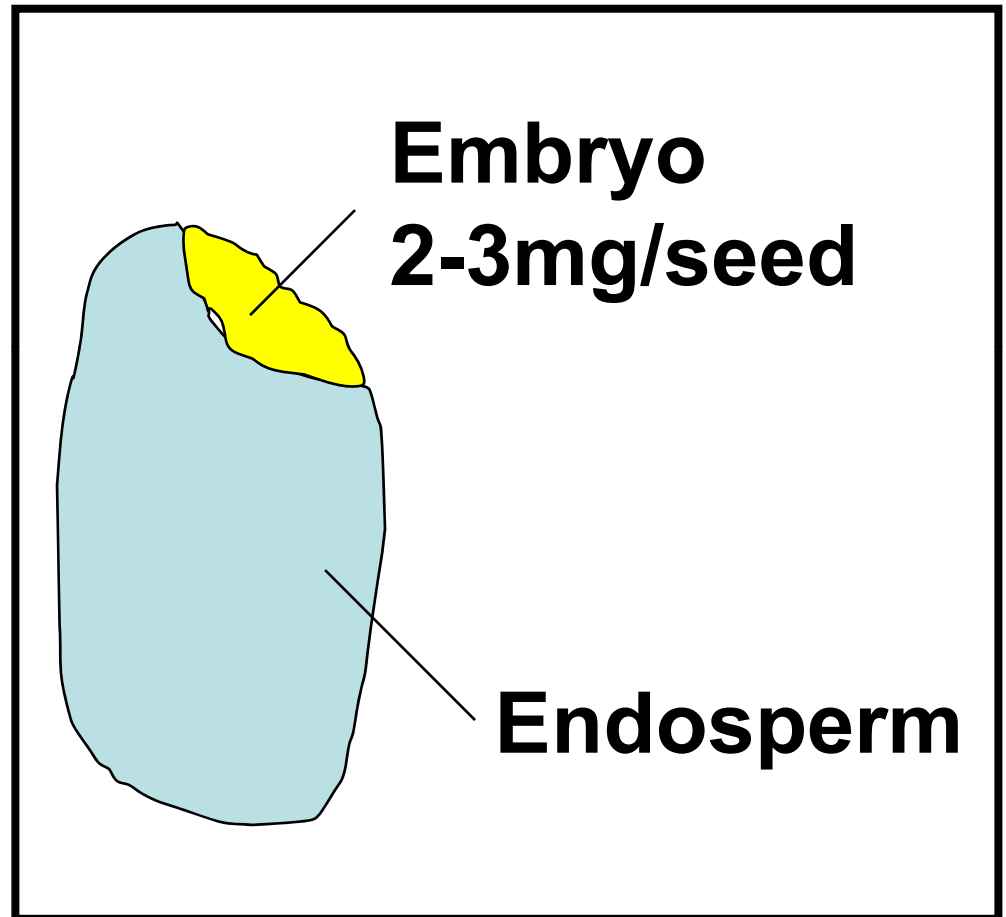
Concentration of NA and DMA in rice xylem sap with -Fe treatment

NA and DMA were quantified from 10 μl of xylem sap.



Rice seed

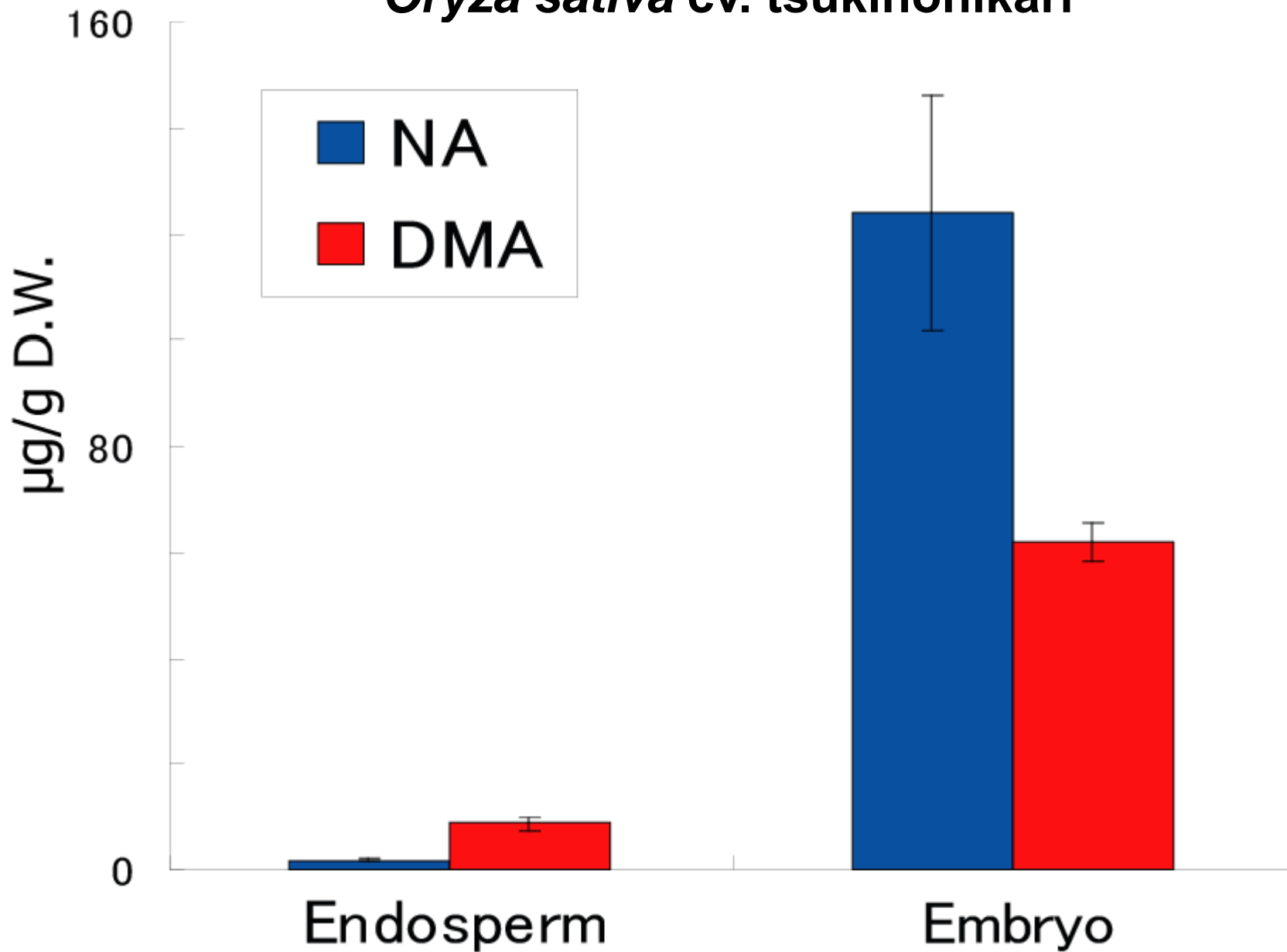
Oryza sativa cv. tsukinohikari



20mg/seed

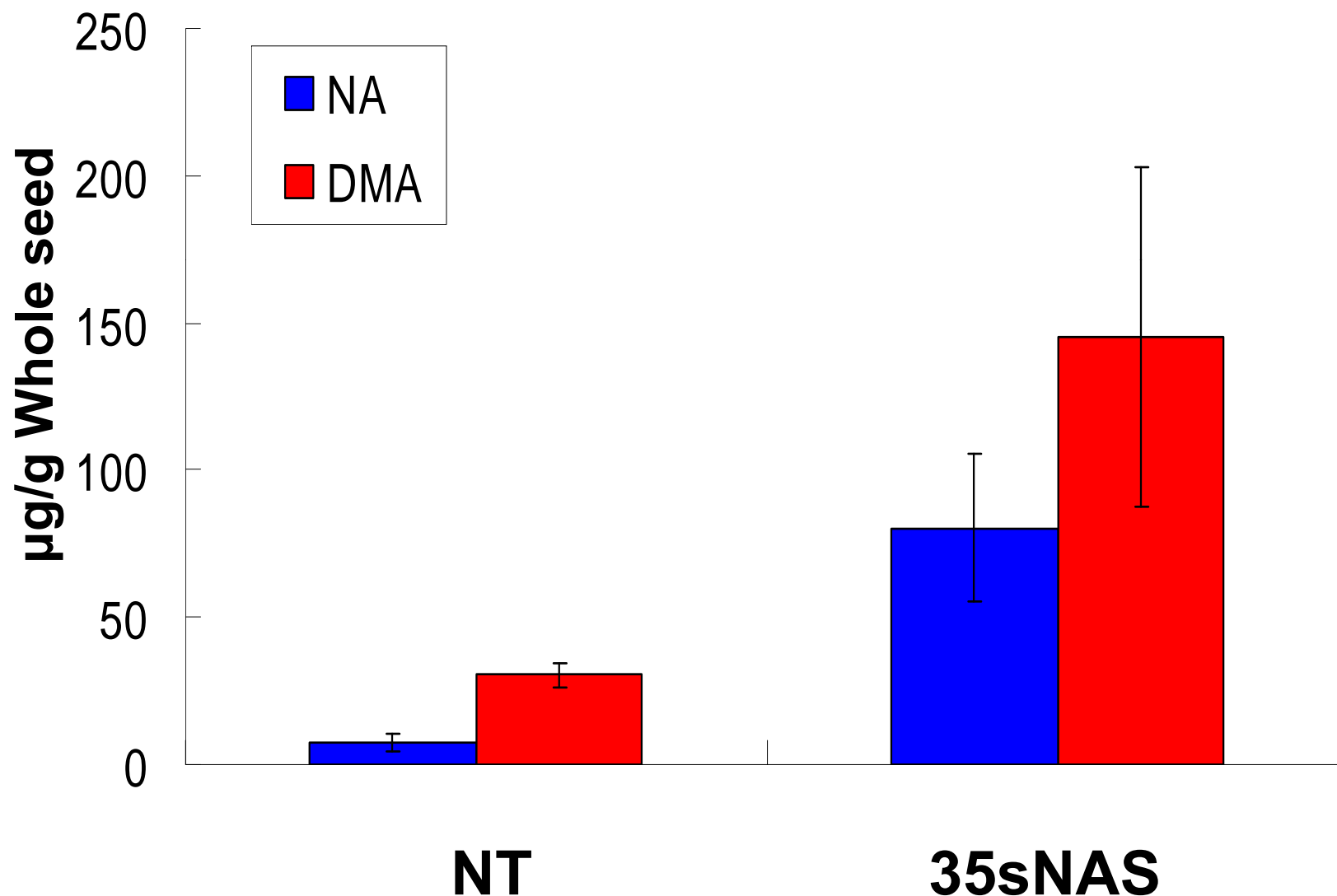
Concentration of NA and DMA in rice seed

Oryza sativa cv. tsukinohikari



Concentration of NA and DMA in 35sNAS rice seed

35sNAS – Rice which has high expression HvNAS



Conclusion

- **NA and DMA were detected simultaneously in 30 min.**
- **This method is about thousand times more sensitive than HPLC.**
- **An embryo, 10 μ l of xylem sap was enough to quantify NA and DMA.**