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Investigation of matrix conditions for nucleic acid analysis in positive ion detection using a linear benchtop MALDI-TOFMS.

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Overview

- A new matrix condition for a positive ion detection in a bench-top linear mode MALDI-TOFMS was examined.
- High amount of matrix with some additives was found as much better condition for the positive ion detection.
- Those matrix conditions for a positive ion detection greatly improved sensitivity of nucleic acids.

Introduction

Nucleic acid is of interest in mass spectrometry, because it has been promising medicine to cure some diseases by working at upper stream of action mechanism with less side effect. Moreover, a molecular confirmation of nucleic acid using mass spectrometer is one of a routine analysis, where cost effectiveness of the instrument is significant factor. Generally negative ion detection is applied to the nucleic acid analysis. However, experimental conditions for positive ion detection have not been examined much, although recently a new bench-top instrument specialized to a linear mode with positive ion detection was introduced to achieve high cost-effectiveness. Here, the conditions for the positive ion detection will be reported using the benchtop mass spectrometer.

Methods

- **Synthesized DNA**

Synthesized DNAs that differ in numbers of base and their dilution series were subject to MS analysis. Nuclear factor-kappa B (NF-kB) decoy and its metabolites were obtained from NARD institute, Ltd.(Japan).

Table 1. NF-kB and its metabolites

sample No.	description	sequence	calc. $[M+H]^+$
1	NF-kB-1	CCTTGAAGGGATTTCCCTCC	6045.0
2	NF-kB-3'-1	CCTTGAAGGGATTTCCCTC	5755.8
3	NF-kB-33'-1	CCTTGAAGGGATTTCCCT	5466.6
4	NF-kB-333'-1	CCTTGAAGGGATTTCCC	5162.4
5	NF-kB-3333'-1	CCTTGAAGGGATTTCC	4873.2
6	NF-kB-33333'-1	CCTTGAAGGGATTTC	4584.1
7	NF-kB-5'-1	CTTGAAGGGATTTCCCTCC	5755.8
8	NF-kB-55'-1	TTGAAGGGATTTCCCTCC	5466.6
9	NF-kB-555'-1	TGAAGGGATTTCCCTCC	5162.4
10	NF-kB-5555'-1	GAAGGGATTTCCCTCC	4858.2
11	NF-kB-55555'-1	AAGGGATTTCCCTCC	4529.0

sample No.	description	sequence	calc. $[M+H]^+$
12	NF-kB-2	GGAGGGAAATCCCTTCAAGG	6192.1
13	NF-kB-3'-2	GGAGGGAAATCCCTTCAAG	5862.9
14	NF-kB-33'-2	GGAGGGAAATCCCTTCAA	5533.7
15	NF-kB-333'-2	GGAGGGAAATCCCTTCA	5220.5
16	NF-kB-3333'-2	GGAGGGAAATCCCTTC	4907.3
17	NF-kB-33333'-2	GGAGGGAAATCCCTT	4618.1
18	NF-kB-5'-2	GAGGGAAATCCCTTCAAGG	5862.9
19	NF-kB-55'-2	AGGGAAATCCCTTCAAGG	5533.7
20	NF-kB-555'-2	GGGAAATCCCTTCAAGG	5220.5
21	NF-kB-5555'-2	GGAAATCCCTTCAAGG	4891.3
22	NF-kB-55555'-2	GAAATCCCTTCAAGG	4562.1

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- MALDI-TOFMS

3-HPA, 2,5-DHB and THAP were examined as matrix. Various concentration of the matrices and some additives including ammonium citrate were prepared and applied to MS analysis. Matrices solutions and samples were dropped multiple times and layered on a stainless plate. MS analysis for a positive ion detection was performed with a bench-top linear mode MALDI-TOFMS (MALDI-8020, Shimadzu Corp., Japan).

MALDI-8020

Features:

- Compact design
- > 5,000 MS resolution
- High through-put with solid state laser and fast stage motion
- Rapid sample introduction
- UV laser-based source cleaning
- Quiet operation

Results

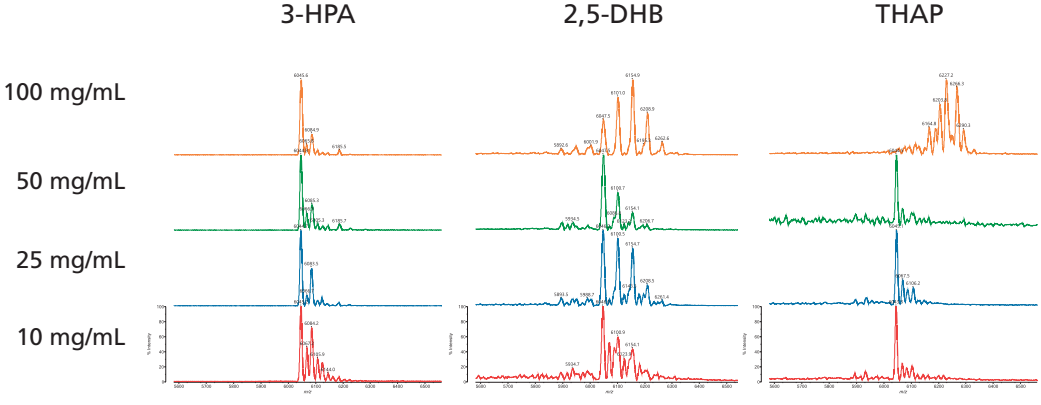

Fig.1 MS spectra of No.1 obtained with three matrices.

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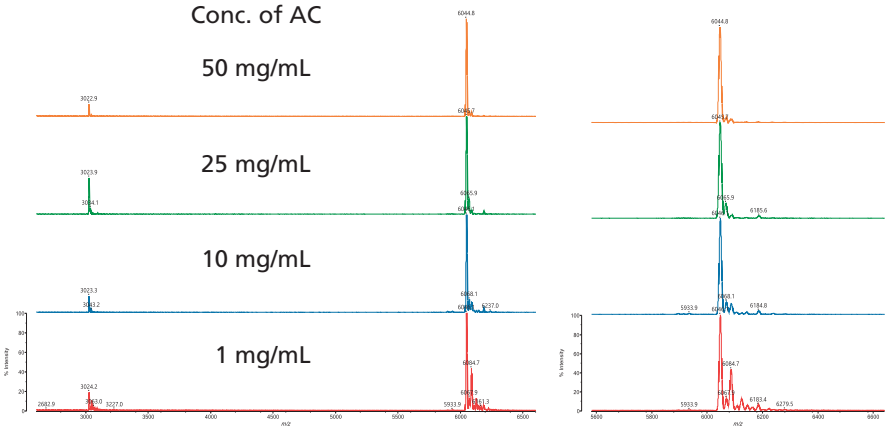

Fig.2 MS spectra of No.1: 3-HPA and various amount of AC.

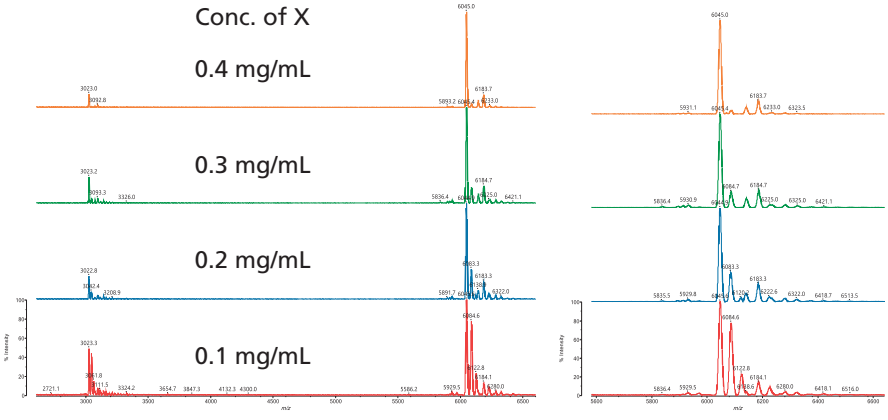

Fig.3 MS spectra of No.1: 3-HPA and various amount of X.

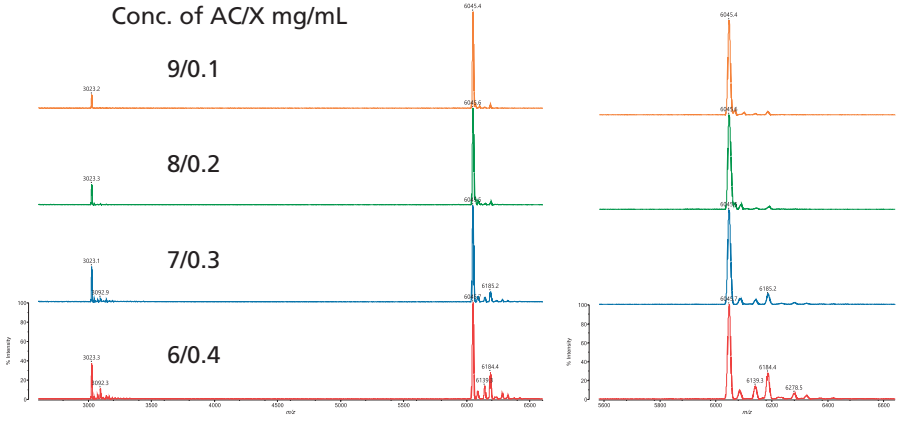

Fig.4 MS spectra of No.1: 3-HPA with AC and X.

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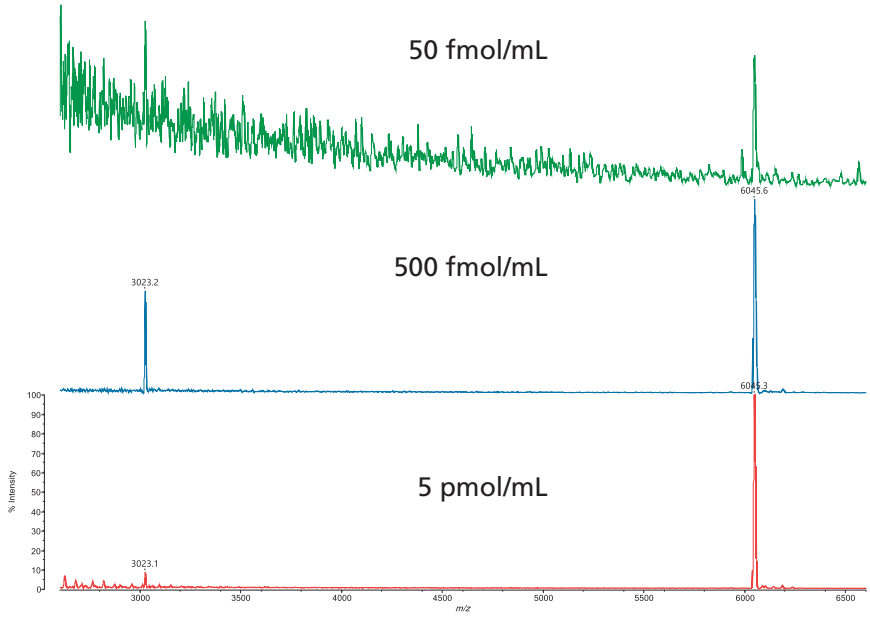
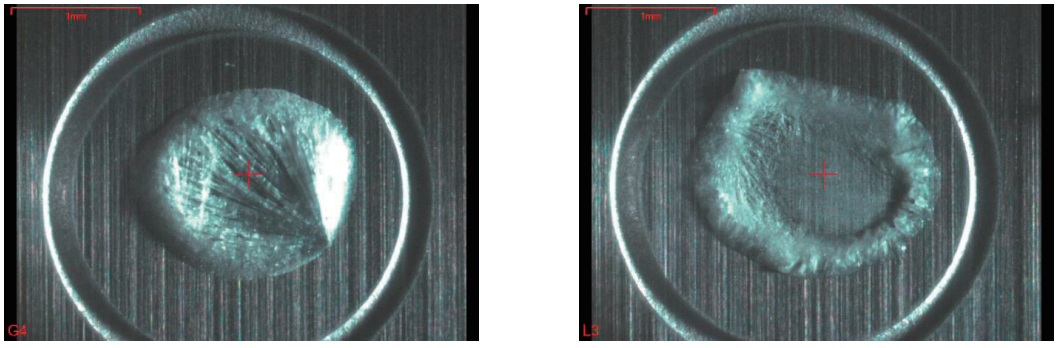


Fig.5 MS of No.1: Sensitivity with 3-HPA and AC.



HPA100-AC50 sample1

HPA100-AC10 sample1

Fig.6 Photo shot by CCD camera in MALDI-TOFMS Typical difference of crystalline between two conditions

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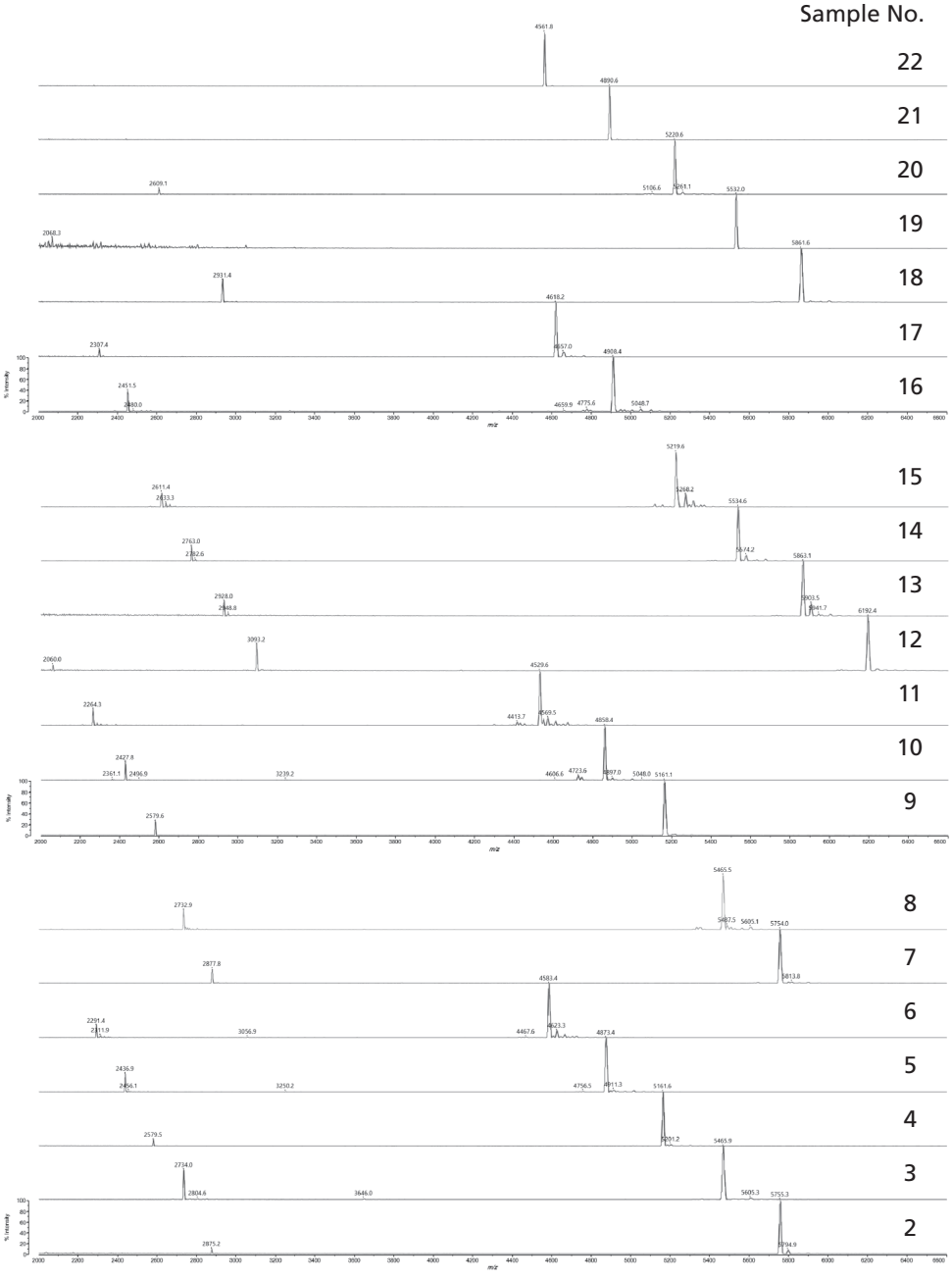

Fig.7 MS of NF-kB and its metabolites

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Conclusions

- Much more amount of matrices than general use were found to obtain higher sensitivity in a layered preparation.
- More amount additives, for instance, ammonium citrate, was responsible for an improvement of the inhomogeneous crystalline.
- More sensitivity of nucleic acid with less adduct ions were obtained due to greatly improved a shot-to-shot reproducibility.

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