

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] ⁺	sample No.	description	sequence	calc. [M+H] ⁺
1	NF-kB-1	CCTTGAAGGGATTCCCTCC	6045.0	12	NF-kB-2	GGAGGGAAATCCCTTCAAGG	6192.1
2	NF-kB-3'-1	CCTTGAAGGGATTCCCTC	5755.8	13	NF-kB-3'-2	GGAGGGAAATCCCTTCAAG	5862.9
3	NF-kB-33'-1	CCTTGAAGGGATTCCCT	5466.6	14	NF-kB-33'-2	GGAGGGAAATCCCTTCAA	5533.7
4	NF-kB-333'-1	CCTTGAAGGGATTCCCC	5162.4	15	NF-kB-333'-2	GGAGGGAAATCCCTTCA	5220.5
5	NF-kB-3333'-1	CCTTGAAGGGATTCC	4873.2	16	NF-kB-3333'-2	GGAGGGAAATCCCTTC	4907.3
6	NF-kB-33333'-1	CCTTGAAGGGATTTC	4584.1	17	NF-kB-33333'-2	GGAGGGAAATCCCTT	4618.1
7	NF-kB-5'-1	CTTGAAGGGATTCCCTCC	5755.8	18	NF-kB-5'-2	GAGGGAAATCCCTTCAAGG	5862.9
8	NF-kB-55'-1	TTGAAGGGATTCCCTCC	5466.6	19	NF-kB-55'-2	AGGGAAATCCCTTCAAGG	5533.7
9	NF-kB-555'-1	TGAAGGGATTCCCTCC	5162.4	20	NF-kB-555'-2	GGGAAATCCCTTCAAGG	5220.5
10	NF-kB-5555'-1	GAAGGGATTCCCTCC	4858.2	21	NF-kB-5555'-2	GGAAATCCCTTCAAGG	4891.3
11	NF-kB-55555'-1	AAGGGATTCCCTCC	4529.0	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

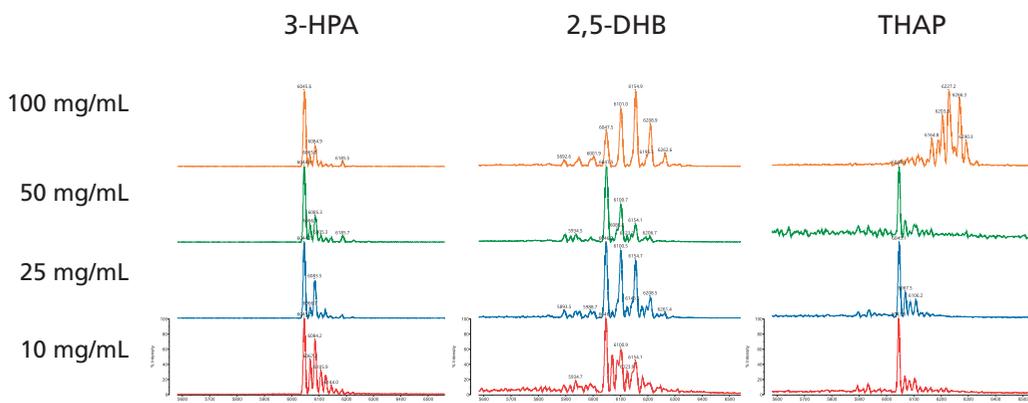


Fig.1 MS spectra of No.1 obtained with three matrices.

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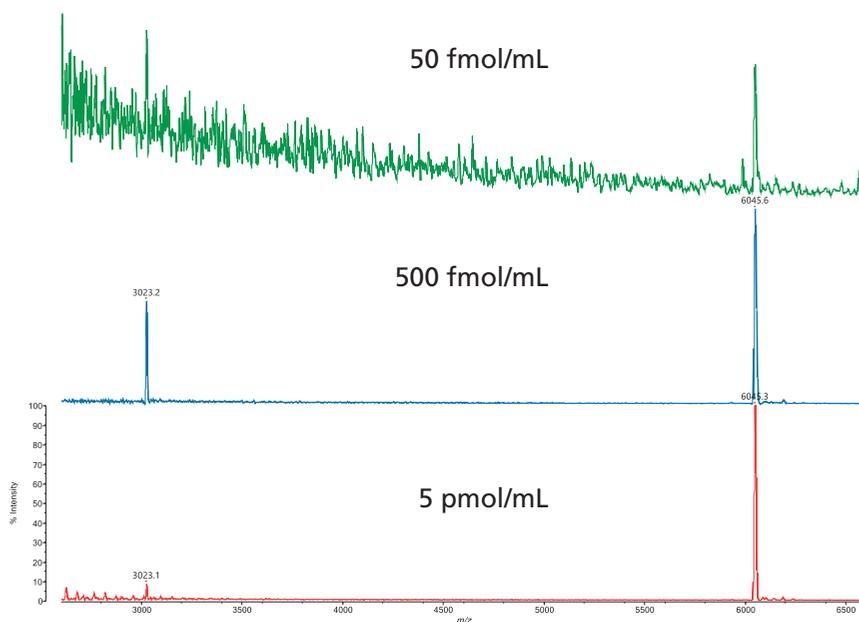


Fig.5 MS of No.1: Sensitivity with 3-HPA and AC.

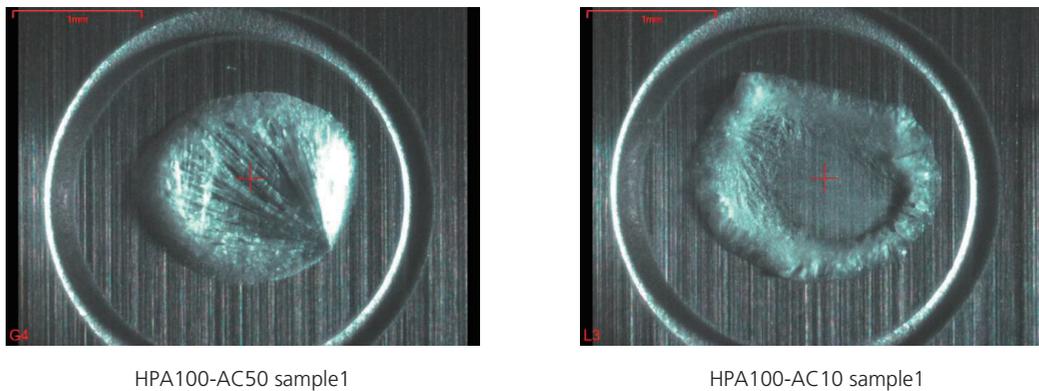


Fig.6 Photo shot by CCD camera in MALDI-TOFMS Typical difference of crystalline between two conditions

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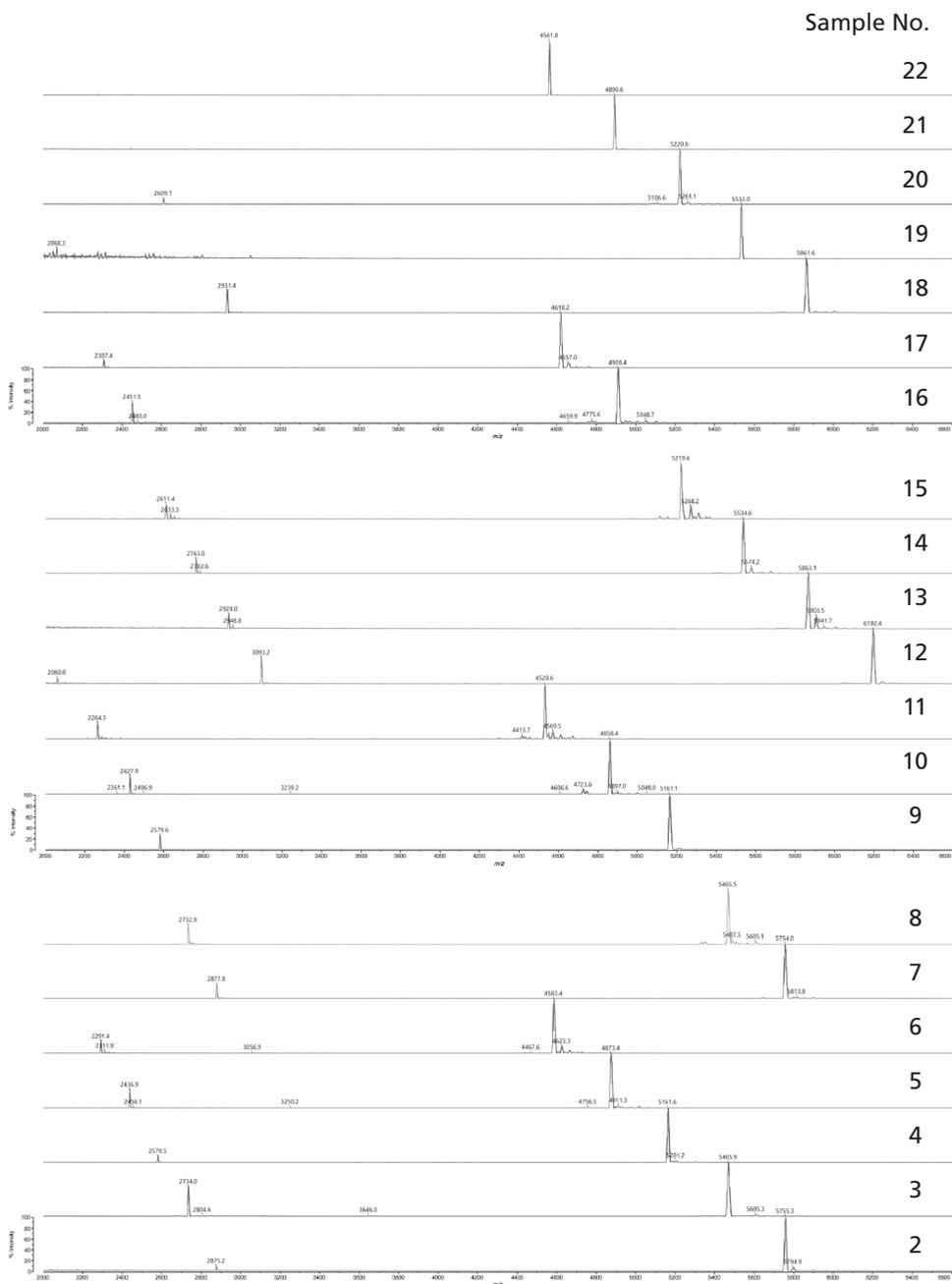


Fig.7 MS of NF- κ B and its metabolites

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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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