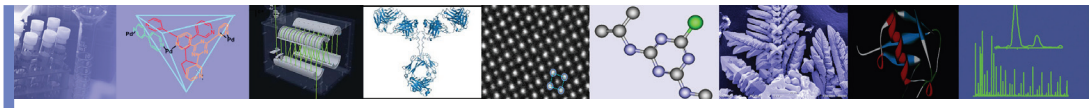

Applications Note

# SpiralTOF™

## High Sensitivity Peptide Measurement with the New Matrix α-Cyano-4-Chlorocinnamic Acid

**Introduction:**

Matrix assisted laser desorption ionization (MALDI) is a powerful and useful ionization technique that is commonly used for the analysis of biomolecules such as peptides and proteins. Typically, α-Cyano-4-hydroxycinnamic acid (CHCA) is the matrix used for MALDI peptide measurement. Recently, a new matrix "α-Cyano-4-chlorocinnamic acid (CClCA)" was investigated for peptide analysis [1]. In this study, we demonstrate the measurement of a BSA digest to evaluate the improvement in peptide sensitivity with CClCA in comparison with CHCA by using the JMS-S3000 SpiralTOF MS system.

**Experimental:**

Sample information and preparation conditions are shown in Table 1. The BSA digest was obtained from Michrom. The CHCA matrix and peptide standard were obtained from Sigma-Aldrich, and CClCA matrix was obtained from Cayman.

The BSA digest standard samples were dissolved in water containing 0.1% trifluoroacetic acid (TFA). CHCA was dissolved in 1:1 water/acetonitrile (ACN) containing 0.1% TFA. And CClCA was dissolved in 1:4 water/ACN containing 0.1% TFA. Next, the BSA digest standard solution and matrix solution were mixed together 1:1 by volume. Afterwards, 0.5 µL of this mixture was placed on the MALDI target plate. Finally, the dried sample was measured using the JMS-S3000 SpiralTOF MS system.

**Results:**

*(1) Mass resolving power and mass accuracy*

The comparison of mass resolving power and isotopic pattern for three of the peptide standards (Bradykinin1-7, Angiotensin II and ACTH18-39) with CHCA and CClCA are shown in Figure 1. The data for each matrix showed similar isotopic patterns and high mass-resolving power. The mass resolving power was approximately 23,000 at *m/z* 757.4 for Bradykinin1-7, over 30,000 at *m/z* 1046.5 for Angiotensin II and 73,000 at *m/z* 2466.2 for ACTH18-39. A residual plot for the calibration curves are shown in Figure 2. We used six peptides (Bradykinin1-7, Angiotensin II, Angiotensin I, P14R, ACTH1-17 and ACTH18-39) for the calibration. The Root Mean Squares (RMS) mass error of the actual measured *m/z* value against the calibration curve was just less than 0.4 ppm with both matrices. We achieved high mass resolving power and good mass calibration using the new matrix CClCA with SpiralTOF.

*(2) BSA digest measurements*

Next, we examined peptide sensitivity using the tryptic digest of BSA with both matrices. MALDI mass spectra of BSA digests with CClCA are shown in Figure 3. And MALDI mass spectra of BSA digests with CHCA are shown in Figure 4. There were a number of peptide peaks observed in the CClCA 250 amol/spot that had better signal-to-noise ratio than the peptide peaks observed in the CHCA 250 amol/spot (Figure 5). Additionally, the peptide mass fingerprint analysis using the peak list from CClCA 250 amol/spot mass spectrum resulted in a higher score and better coverage for the MASCOT search results (Figure 6). The protein was identified as BSA for this sample.

| Peptide | Conc. | Solvent |
|---|---|---|
| BSA digest | 100 fmol/µL | 0.1% TFA |
| | 10 fmol/µL | 0.1% TFA |
| | 1 fmol/µL | 0.1% TFA |
| Matrix | | |
| CHCA | 10 mg/mL | ACN/0.1% TFA = 1/1 (v/v) |
| CCICA | 10 mg/mL | ACN/0.1% TFA = 4/1 (v/v) |
| Sample | | |
| BSA digest/Matrix = 1/1 (v/v) | | |
| 0.5 µL of this sample solution mixture was placed on the MALDI target plate | | |

Table 1. Sample information and preparation conditions.


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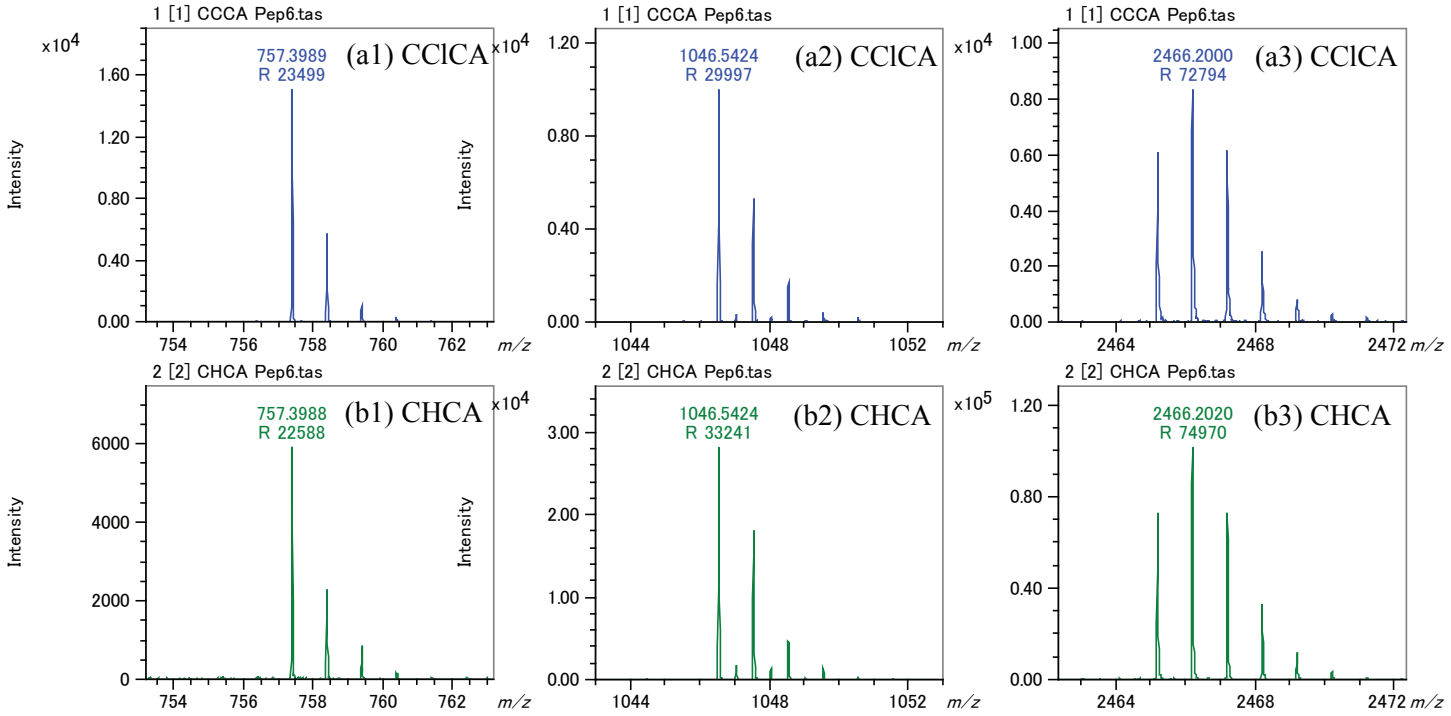


Figure 1. The comparison of mass resolving power and isotopic pattern:
(a1) Bradykinin1-7 with CClCA, (a2) Angiotensin II with CClCA and (a3) ACTH18-39 with CClCA,
(b1) Bradykinin1-7 with CHCA, (b2) Angiotensin II with CHCA and (b3) ACTH18-39 with CHCA.

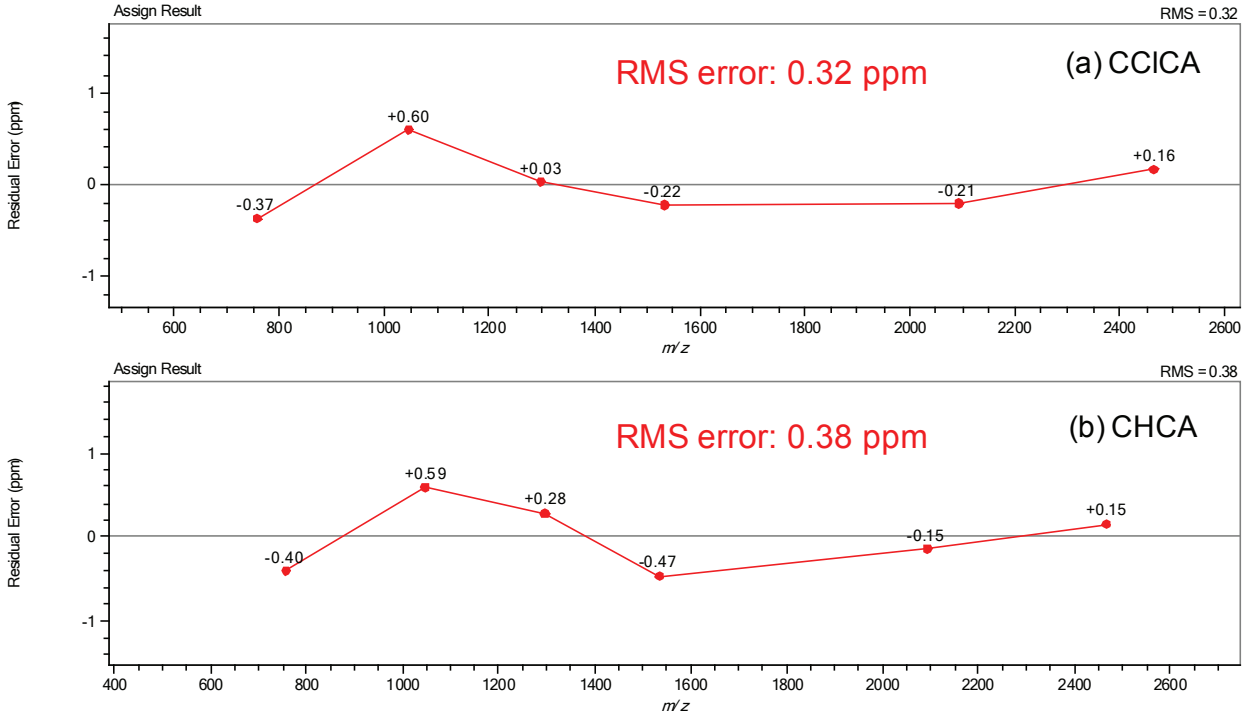


Figure 2. The residual plot for the calibration curves obtained by using six-peptide mixture:
(a) CClCA and (b) CHCA.


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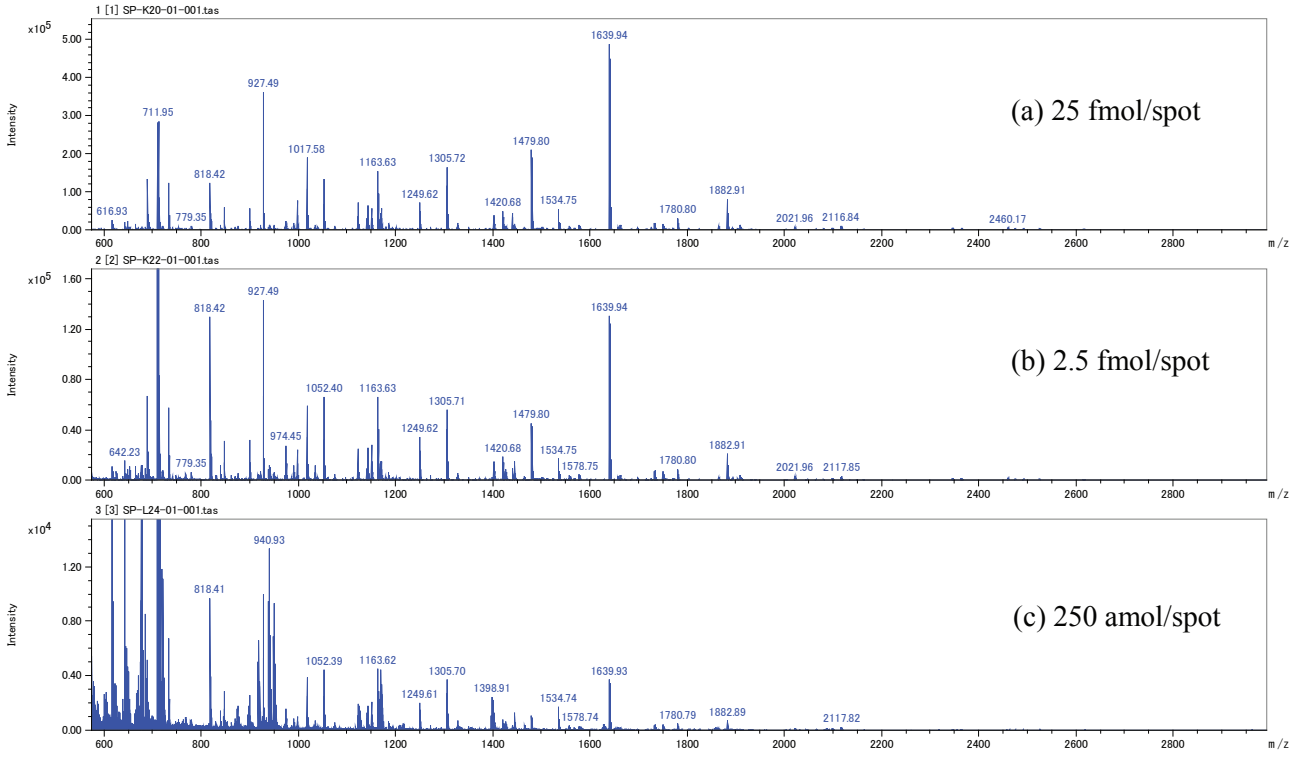


Figure 3. MALDI mass spectra of BSA digests with CClCA: (a) 25 fmol/spot, (b) 2.5 fmol/spot, (c) 250 amol/spot.

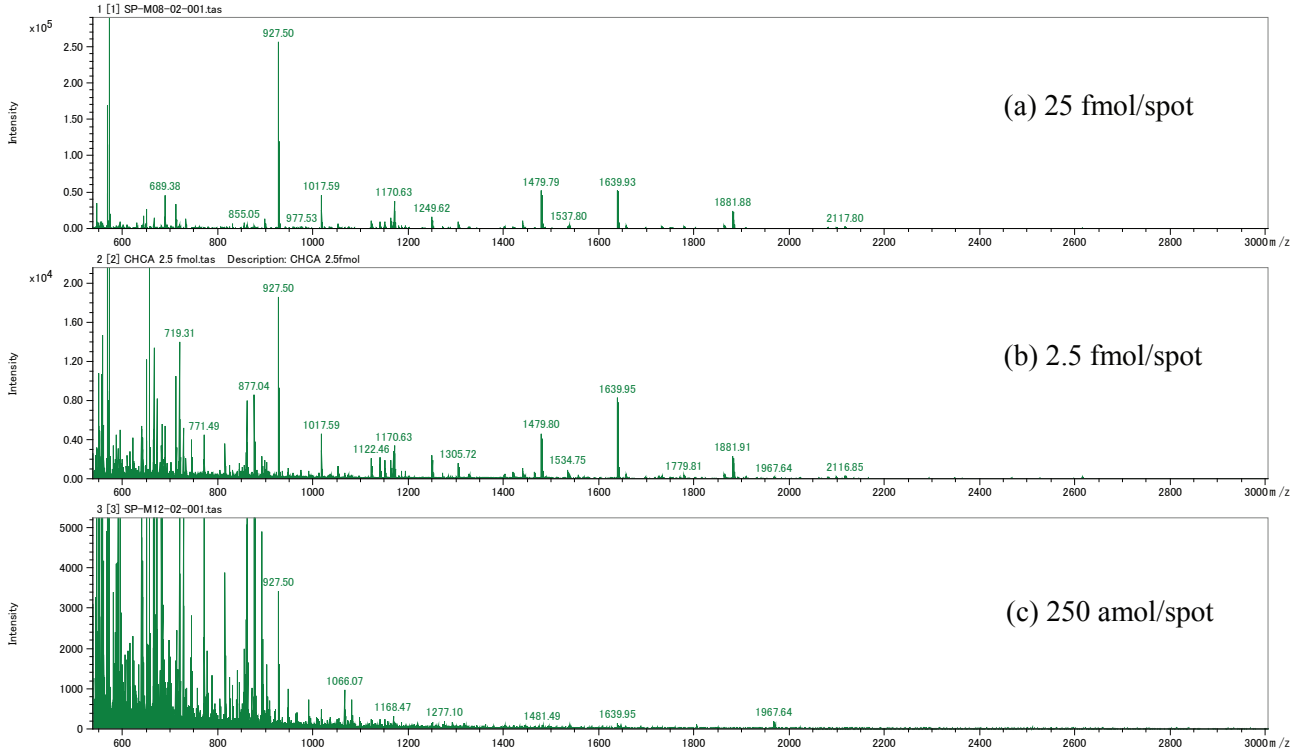


Figure 4. MALDI mass spectra of BSA digests with CHCA: (a) 25 fmol/spot, (b) 2.5 fmol/spot, (c) 250 amol/spot.


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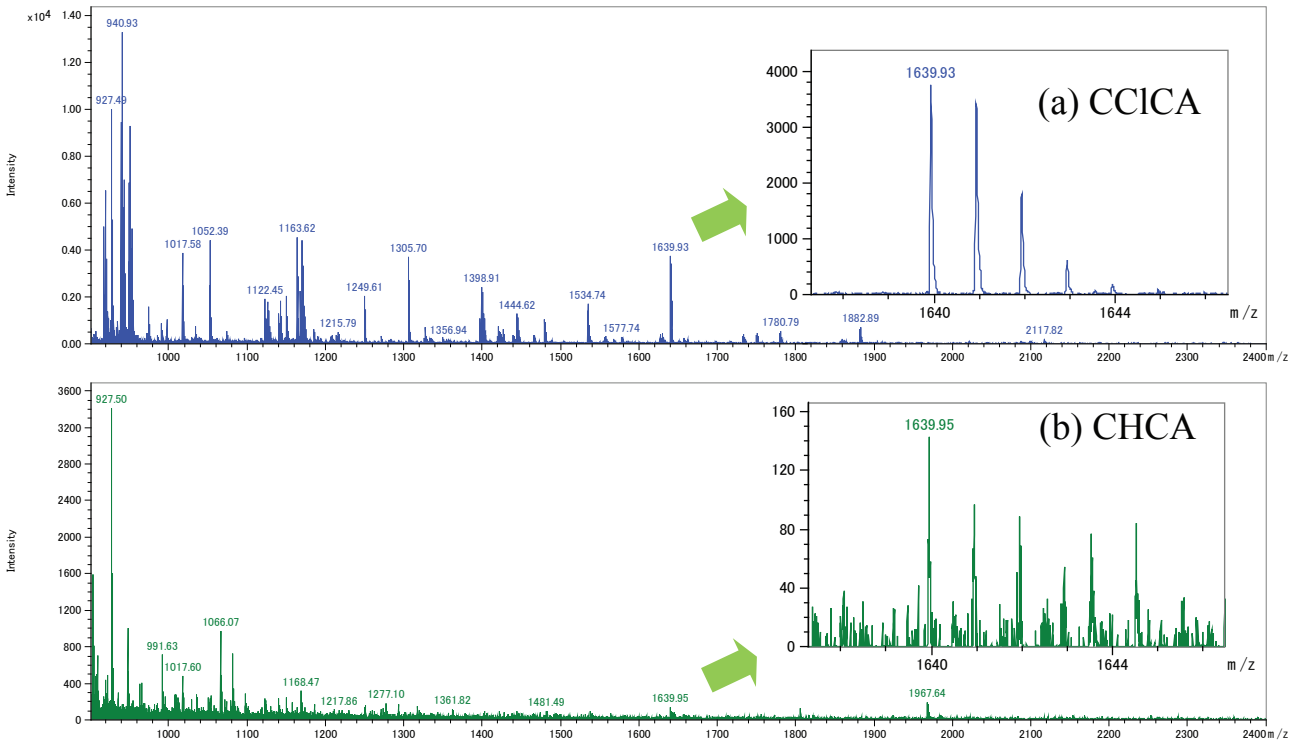
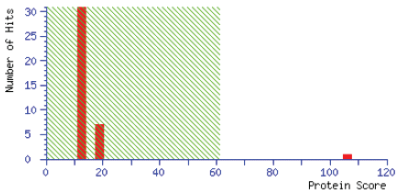



Figure 5. Comparison of BSA digests mass spectra each 250 amol/spot: (a) CClCA, (b) CHCA.

**Mascot Score Histogram**

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 61 are significant (p<0.05).




**Concise Protein Summary Report**

Format As | Concise Protein Summary | Help

Significance threshold p< 0.05 | Max. number of hits AUTO

Re-Search All | Search Unmatched

1. ALBU_BOVIN Mass: 71279 Score: 106 Expect: 1.7e-06 Matches: 28
Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4
RL27A_PIG Mass: 6179 Score: 13 Expect: 3.4e+03 Matches: 2
60S ribosomal protein L27a (Fragment) OS=Sus scrofa GN=RPL27A PE=3 SV=3

2. APOE_ZALCA Mass: 37980 Score: 19 Expect: 8.1e+02 Matches: 11
Apolipoprotein E OS=Zalophus californianus GN=APOE PE=2 SV=1

Figure 6. MASCOT search result using 250 amol/spot of CClCA.

**Conclusion:**

We have done an initial study in which the new matrix "α-cyano-4-chlorocinnamic acid (CClCA)" was coupled with the SpiralTOF. The CClCA matrix provides high mass resolving power and excellent mass calibration curves that are comparable to the CHCA matrix. Additionally, the CClCA matrix improves the sensitivity of peptides. This new matrix will be a powerful and useful tool for the analysis of peptides and proteins.

**Reference:**

[1] John D. Leszyk, Evaluation of the New MALDI Matrix 4-Chloro-α-Cyanocinnamic Acid, J. Biomol Tech. 2010 July; 21(2): 81–91.

MS-0402A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