WA40075

Waters

MALDI-TOF Sample Preparation: Does Matrix Purity Really Matter?

Peter J. Lee, Weibin Chen and John C. Gebler,
Life Science Chemistry, Waters Corporation

The goal of this work was to study the effects of MALDI matrix purity on MALDI-time-of-flight (TOF) experiments. A series of MALDI matrices were evaluated systematically. The results showed that the use of ultra-purified MALDI matrices significantly improved the quality of MALDI-TOF mass analysis of peptides and enabled protein identification (PMF) at the sub-femtomole level.

Introduction

Sample preparation is recognized as a crucial step for successful MALDI-TOF analysis. A variety of MALDI sample preparation techniques have been reported to increase the sensitivity of analysis, improve the signal resolution and enhance the tolerance of MALDI analysis towards contaminants in samples, such as buffers, salts, detergents and denaturants (1–5). MALDI matrices, alpha-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SA), 2, 5-dihydroxybenzoic acid (DHB), 3-hydroxypicolinic acid (HPA) and 2, 4, 6-trihydroxyacetophenone (THAP) are among the most commonly used in these techniques. It also is recognized that the quality of MALDI mass spectra is related to the quality of matrix, and the failure of MALDI analysis frequently is attributed to contaminants in this critical reagent. Waters Corporation (Milford, MA, USA) has developed a series of ultra purified MALDI matrices, MassPREP™ MALDI Matrix (Table 1 lists part numbers). Each matrix is prepared under strict quality control constraints (NMR, MS, metal analysis and HPLC). They are packaged in 1.5-mL vials with 10 mg/vial for ease of use (no weigh-out needed) and to minimize reagent contamination (Figure 1). Excellent shelf life of MassPREP™ MALDI Matrix is obtained because each shipped vial is vacuum-sealed in a foil pouch for maximum protection from the deleterious effects of light and air.

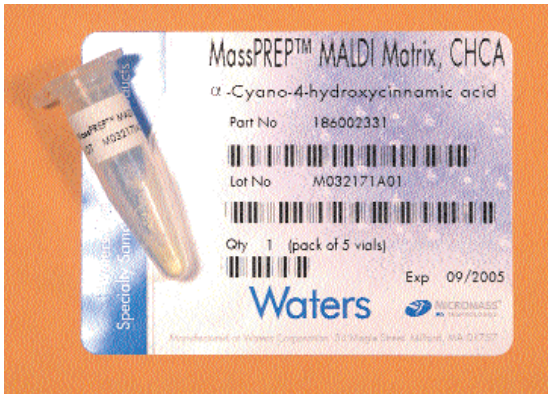

Figure 1. Waters ultra pure MassPREP™ MALDI matrices (Part numbers in Table 1). They are packaged in 1.5-mL vials (10 mg/vial) for ease of use. Each vial is vacuum-sealed in a foil pouch for protection from light and air.

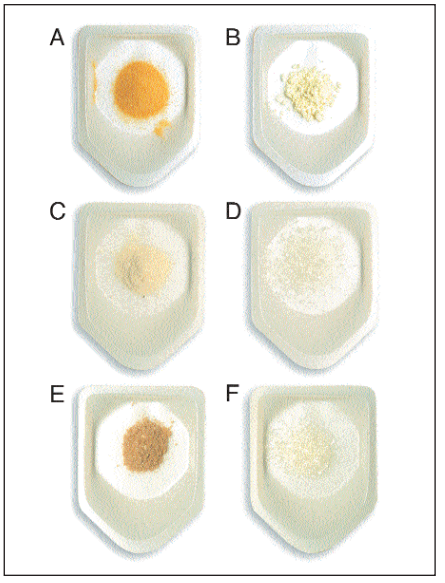

Figure 2. Images of MALDI matrices. Other vendor matrixes (A) CHCA, (C) DHB and (E) HPA; MassPREP™ MALDI Matrix CHCA (B), MassPREP™ MALDI Matrix DHB (D) and MassPREP™ MALDI Matrix HPA (F).

Since the matrix is the key component of MALDI analysis, the influence of matrix purity on the quality of MALDI mass spectra was the subject of this investigation. A variety of CHCA matrices with purity grades from 97% to ultra-pure (> 99.0%) were systematically evaluated for MALDI TOF analyses of peptides and proteins. The focus of the examination included the evaluation of adduct formation of metal salts, the intensity of background ions generated, signal-to-noise ratios and the limit of detection of proteolytic protein digests.

Experimental Conditions

Several lots of CHCA marketed as MS grade were purchased from well-known vendors and randomly coded as CHCA-1 through CHCA-6. Matrix solutions were prepared by dissolving 10 mg of CHCA in 1 mL of solvent containing 50% CH_3CN and 50% EtOH. These solutions were further diluted to 2 or 1 mg/mL with the same solvent. A vortex was used to assist in the dissolution of the crystalline CHCA matrices. Waters MassPREP™ MALDI Matrix CHCA was dissolved completely in less than five seconds. However, several minutes of mixing (vortexing) frequently were required to dissolve other vendor CHCA MALDI matrices. In addition, non-dissolved dark solid granules often were present in some of the non-Waters evaluated matrices. All the

peptide standards used were purchased from Sigma Chemical Company St. Louis, MO, USA) and placed in 0.3% TFA aqueous solution. Bovine serum albumin (BSA) tryptic digest standard manufactured at Waters (MassPREP™ BSA Digestion Standard, P/N 186002329) was dissolved in 0.1% TFA solution and further diluted to the desired concentrations with the same solution. Dried-droplet and thin-layer methods were used for MALDI-TOF sample preparations. In the dried-droplet method, equal volumes of analyte solution and prepared CHCA matrix solution were mixed, then, 1 µL of the sample containing matrix mixture was applied onto a stainless steel MALDI target. For the thin-layer method, the technique reported by Dai *et. al.,* was employed (4). Mass Spectra were acquired with a Waters® Micromass® MALDI™ LR reflectron time-of-flight mass spectrometer. Waters ProteinLynx™ Global SERVER 2.0 Software was used for protein identifications.

Results

For comparison, the images of lower-purity grade matrices and Waters MassPREP™ MALDI Matrices are shown in Figure 2. Generally, matrices containing even minor impurities have a darker color, as shown in Figures 2a, 2c and 2e. Waters MassPREP™ MALDI Matrix lacks color from impurities, which is consistent with the chemical makeup of pure compounds. Figure 3 shows seven MALDI mass spectra of peptide P14R (1 femtomole loading) using different sources of CHCA. The expected mass for peptide P14R ion peak at 1533.86 *m/z* could not be detected when CHCA-1 was employed. In addition, several intense CHCA-metal salt adduct clusters (6), with *m/z* in the range of 800–1100 Da, were observed when CHCA-2 to CHCA-6 were used as the matrix for mass analysis (Figure 3). The stated purity for CHCA-1 to CHCA-6 ranged from 97 to >99%. The spectrum in

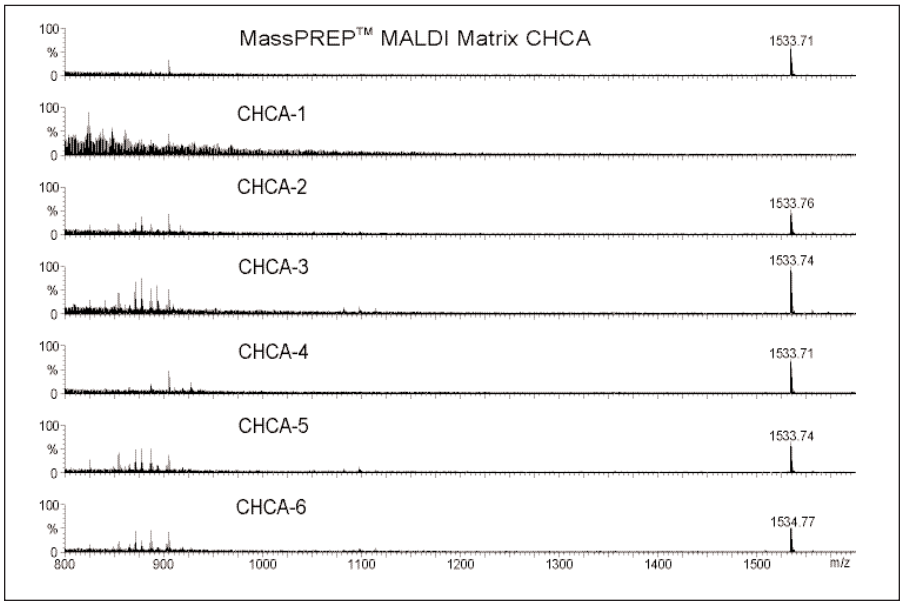

Figure 3. MALDI spectra of 1 femtomole of P14R ($[M+H]^+$ = 1533.86 Da) using different brands of CHCA matrix. Matrix solutions were prepared as 1 mg/mL.

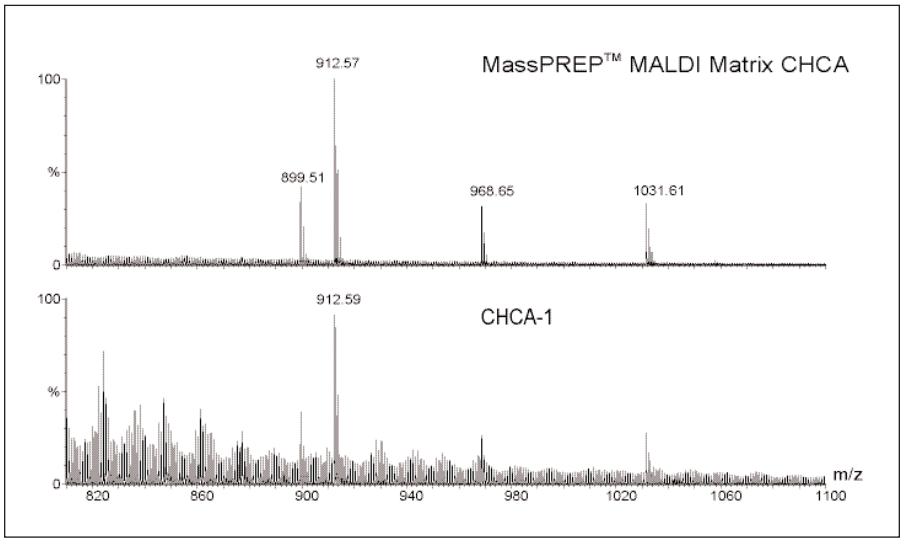

Figure 4. MALDI spectra of a mixture of 2 femtomole of angiotensin fragments using MassPREP™ MALDI Matrix CHCA and CHCA-1. Angiotensin 1–7, $[M+H]^+$ = 899.47 Da; [Sar, Gly]-Angiotensin II $[M+H]^+$ = 912.51 Da; [Sar, Ile]-Angiotensin II $[M+H]^+$ = 968.57 Da; [Asn, Val]-Angiotensin II $[M+H]^+$ = 1031.54 Da. Matrix solutions were prepared as 2 mg/mL

Figure 3 using Waters MassPREP™ MALDI Matrix CHCA has an intense molecular ion peak at 1533.71 Da, expected for peptide P14R along with the least number of CHCA-metal salt adduct peaks in the low m/z region. Figure 4 shows MALDI mass spectra from a mixture of angiotensin fragments (2 femtomole loading) using Waters MassPREP™ MALDI Matrix CHCA and CHCA-1 matrix. The spectrum in Figure 4 using the Waters material shows the expected angiotensin fragment peaks with good signal-to-noise. The spectrum CHCA-1 has a more intense background noise from CHCA-metal salt adduct cluster ions along with the other expected weak ions whose signal-to-noise

Waters Corporation
34 Maple Street,
Milford, MA 01757 USA.
Tel. (508) 478-2000, fax (508) 872-1990
www.waters.com

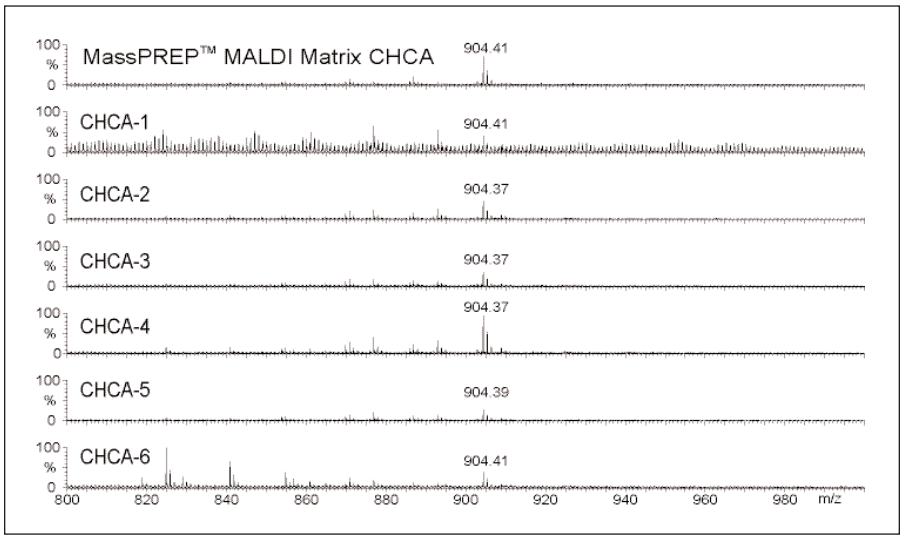

Figure 5. MALDI spectra of 1 femtomole of Bradykinin ($[M+H]^+$ = 904.47 Da) using different brands of CHCA matrix. Matrix solutions were prepared as 1 mg/mL

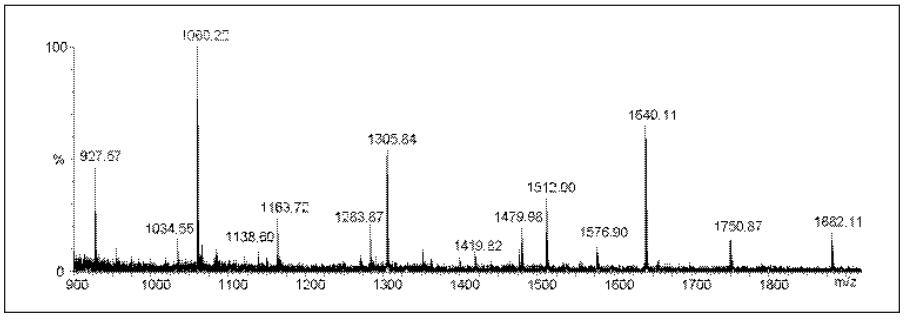

Figure 6. Spectrum of 250 attomole of MassPREP™ BSA Digestion Standard using MassPREP™ MALDI Matrix CHCA matrix by thin-layer MALDI sample preparation method. The peptide fragments generated from a tryptic digest of BSA are labeled. The peak at 1060.22 Da is a CHCA-potassium adduct, $[5CHCA+3K-2H]^+$.

ratios were substantially less than that obtained with the MassPREP MALDI matrix. Figure 5 shows seven MALDI mass spectra of bradykinin (1 femtomole loading) using different sources of CHCA. There are many intense background ions, including CHCA-sodium(Na)-potassium(K) salt adducts in the spectra CHCA-1 to CHCA-6. Among them, the most discernible peaks of CHCA-Na-K adducts are as follows: $[4CHCA+3Na-2H]^+$= 823.12, $[4CHCA+2Na+K-2H]^+$= 839.10, $[4CHCA+Na+2K-2H]^+$= 855.07, $[4CHCA+3K-2H]^+$= 871.05, $[4CHCA+2Na+2K-3H]^+$= 877.05 and $[4CHCA+Na+3K-3H]^+$= 893.03. The spectrum using MassPREP™ MALDI Matrix CHCA (Figure 5) gave the most intense bradykinin molecular ion peak (904.41 Da), highest signal-to-noise ratio and least CHCA-metal salt adducts and background ions.

In an effort to demonstrate high sensitivity, a trypsin digestion of BSA (MassPREP™ BSA Digestion Standard) was deposited onto a MALDI target with Waters MassPREP™ MALDI Matrix CHCA employing the thin-layer method. Figure 6 shows the mass spectra results from the analysis of 250 attomole of BSA digest, where an abundance of BSA peptide fragments were observed with good signal-to-noise values. The results were attributed to the combination of ultra-pure CHCA matrix and clean peptide sample.

Conclusions

The use of Waters MassPREP™ MALDI matrices significantly improved the quality of MALDI-TOF spectra and increased limits of detection. The intensity of matrix background and CHCA-metal salt adduct cluster ions varied greatly among the different manufacturers of CHCA matrix. These variations in matrix composition can significantly influence the quality of MALDI mass analysis results. Using Waters MassPREP™ MALDI Matrices, peptide and PMF identifications at low femtomole and even sub-femtomole loadings can be routinely achieved.

Reference

1. R.C. Beavis, T. Chaudhary and B.T. Chait, *Org. Mass Spectrum*. 27, 653–655 (1992)
2. O. Vorm, P. Roepstroff and M. Mann, *Anal. Chem*. 66, 3281–3287 (1994)
3. M. Kussmann et. al., *J. Mass Spectrom*. 32, 593–601 (1997)
4. Y. Dai, R.M. Whittal and L. Li, *Anal. Chem.* 71, 1087–1091 (1999)
5. S. Laugesen and P. Roepstrof, *J. Am. Soc. Mass Spectrom.* 14, 992–1002 (2003)
6. B.O. Keller, L. Li, *J. Am. Soc. Mass Spectrom.* 11, 88–93 (2000).

Table 1. Waters MassPREP™ Ultra-Pure MALDI Matrices

Description	Part Number
MassPREP™ MALDI Matrix CHCA (1 pk)*	186002331
MassPREP™ MALDI Matrix SA (1 pk)*	186002332
MassPREP™ MALDI Matrix DHB (1 pk)*	186002333
MassPREP™ MALDI Matrix HPA (1 pk)*	186002334
MassPREP™ MALDI Matrix THAP (1 pk)*	186002335
MassPREP™ MALDI Matrix Kit (1 pk)**	186002336

* 5 vials/pack (10 mg per vial)
** 5 vials/pack. One vial from each matrix type