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

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Overview

Effects of MALDI matrix purity on MALDI TOF mass experiments were systematically evaluated. 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.[1-3] Matrices such as α-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SA), 3-hydroxypicolinic acid (HPA), 2, 5-dihydroxybenzoic acid (DHB), and 2, 4, 6-trihydroxyacetophenone (THAP) are among the most commonly used MALDI sample preparation reagents. It is also recognized that the quality of MALDI mass spectra is related to the quality of matrix and the failure of MALDI analysis is frequently attributed to contaminants in this critical reagent. Waters Corporation has developed a series of ultra purified MALDI matrices, MassPREP™ MALDI Matrix (Table 1), that efficiently assist in the desorption/ionization of bioanalytes from target surfaces for demanding MALDI applications. Each matrix is prepared under strict quality control. The matrices are packaged and vacuum-sealed in 1.5mL vials (10 mg/vial) for ease of use (no weigh-out needed) and to minimize reagent contamination. The influence of matrix purity on the quality of MALDI mass spectra was the subject of this presentation. 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 protein tryptic digests. The focus of the examination included the evaluation of adduct formation of metal salts (Table 2), the intensity of background ions generated, signal-to-noise ratios, and the limit of detection of proteolytic protein digests.

Methods

Matrix: Several lots of CHCA marketed as MS grade were purchased from well-known vendors and randomly coded as CHCA-1 through CHCA-6. A solution of 50% CH_3CN and 50% EtOH was used to prepare 10, 2 or 1 mg/mL of CHCA. Waters MassPREP™ matrices dissolve easily compared to other lower-purity grade matrices (few seconds vs. several minutes of vortexing).

Analytes: Peptides and Waters MassPREP™ BSA tryptic digestion standard (P/N 186002329) were dissolved in 0.3 or 0.1% of TFA.

MALDI analysis: For figures 2 to 4, equal volumes of analyte and CHCA solution were mixed, then, 1 µL of the mixture was applied onto a stainless steel MALDI target.[1] For Figure 5; 1) 0.5 µL of CHCA (1mg/mL in acetone) was applied onto a MALDI target and allowed to dry, 2) equal volumes of BSA digest (1 fmole/µL) and CHCA solution (1 mg/mL) were mixed and applied (0.5 µL) onto the target and dried at ambient temperature.[2] Waters® Micromass® M@LDI™ LR reflectron TOF mass spectrometer was used to acquire spectra.

Results

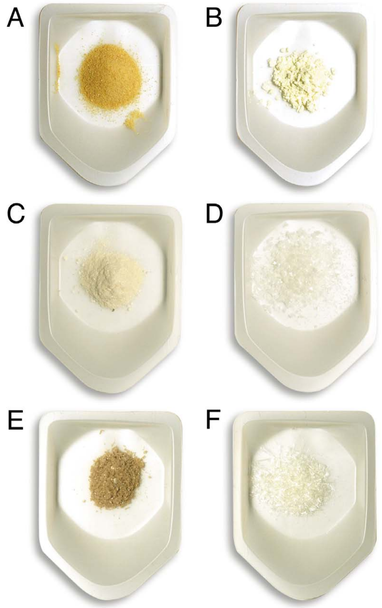

Figure 1. **Images of low and high purity MALDI matrices.** Other vendor matrixes (A) CHCA, (C) DHB, and (E) HPA; MassPREP™ MALDI Matrix CHCA (B), MassPREP™ MALDI Matrix DHB (D) and MassPREP™ MALDI Matrix HPA (F).

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.

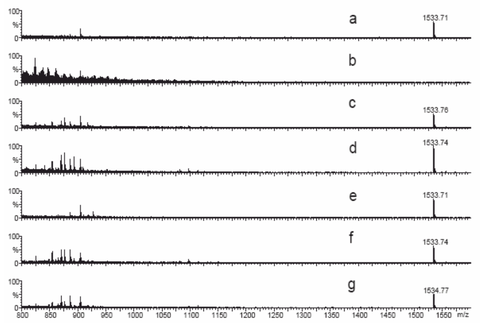

Figure 2. **MALDI spectra of 1 femtomole of peptide P14R using different brands of CHCA.** (a) Waters MassPREP™ CHCA; (b) CHCA-1; (c) CHCA-2; (d) CHCA-3; (e) CHCA-4; (f) CHCA-5; (g) CHCA-6. CHCA matrix solution was prepared as 1 mg/mL. The expected mass for the P14R ([M+H]⁺) ion peak is1533.86 Da.

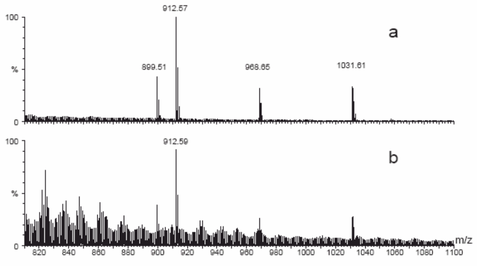

Figure 3. **MALDI spectra of 2 femtomole of angiotensin fragments using high and low purity CHCA matrix.** (a) Waters MassPREP™ CHCA; (b) CHCA-1 a 97% grade reagent. Ion peaks ([M+H]⁺) at m/z of 899.47, 912.51, 968.57, and 1031.54 are angiotensin fragment 1-7, [Sar, Gly]-angiotensin II, [Sar, Ile]-angiotensin II, and [Asn, Val]-angiotensin II fragments, respectively. CHCA matrix solution was prepared as 2 mg/mL.

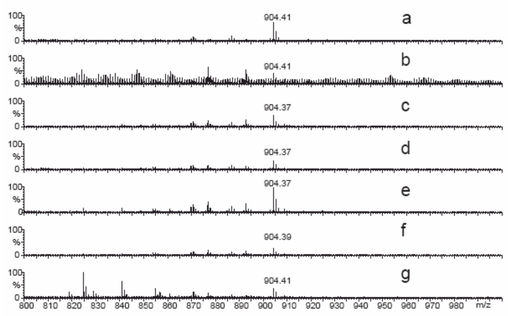

Figure 4. **MALDI spectra of 1 femtomole of bradykinin using different brands of CHCA.** (a) Waters MassPREP™ CHCA; (b) CHCA-1; (c) CHCA-2; (d) CHCA-3; (e) CHCA-4; (f) CHCA-5; (g) CHCA-6. Matrix solution was prepared as 1 mg/mL. The expected mass for the bradykinin ([M+H]⁺) ion peak is 904.47 Da.

Theoretical mass of intact CHCA and K or Na adducts[3]

$$A = nM - xH + yK + zNa$$

$$x = y + z - 1$$

$$y + z \le n + 1$$

$$x \le n$$

Where *A* is CHCA salt adduct
n = 1,2,3.....
y or *z* = 0,1,2,3....
M, H, K and *Na* stand for masses of CHCA, hydrogen, potassium and sodium

Table 2. CHCA-Na-K adducts within 800 to 900 Da

Formula of Ion	m/z	Formula of Ion	m/z
$[M_2(M\text{-}H)_2Na_2]H^{+*}$	801.14	$[(M\text{-}H)_4Na_3K]H^{+*}$	861.08
$[M(M\text{-}H)_2NaK]H^+$	817.12	$[(M\text{-}H)_4Na_4]Na^+$	867.09
$[M(M\text{-}H)_3Na_3]H^+$	823.12	$[M(M\text{-}H)_3K_3]H^{+*}$	871.05
$[M(M\text{-}H)_2K_2]H^+$	833.09	$[(M\text{-}H)_4Na_2K_2]H^{+*}$	877.06
$[M(M\text{-}H)_3Na_2K]H^+$	839.10	$[(M\text{-}H)_4Na_3K]Na^+$	883.06
$[(M\text{-}H)_4Na_4]H^+$	845.11	$[(M\text{-}H)_4NaK_3]H^{+*}$	893.03
$[M(M\text{-}H)_3NaK_2]H^+$	855.07	$[(M\text{-}H)_4Na_2K_2]Na^+$	899.04

* Most frequently observed adducts with high peak intensity

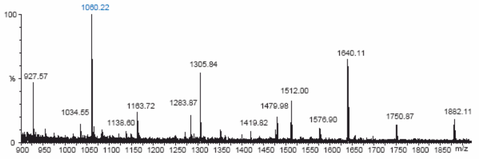

Figure 5. **Spectra of 250 attomole of BSA tryptic digest using MassPREP™ CHCA matrix.** Peptides of BSA are labeled. The peak at 1060 Da is a CHCA-potassium adduct, [5CHCA+3K-2H]⁺. Thin-layer method was used for the sample preparation.[2]

Table 3. PMF using high and low purity CHCA matrices*

Matrix source	MassPREP™ CHCA	CHCA-1
% correct protein ID	100	50
Number of peptide matched	38	30
Percentage of coverage (%)	58	48

*Average of 6 runs.
*CHCA was 10mg/mL.
*Dried-droplet method was used for the sample preparation.[1]
*BSA tryptic digest sample loading was 10 femtomole/well.
*Waters ProteinLynx™ Global SERVER 2.0 Software was used for PMF.

Conclusions

- Ultra-purified matrices such as Waters MassPREP™ MALDI matrices significantly improved the quality of MALDI mass spectra and increased limit of detection.
- Intensity of matrix background and CHCA-metal salt adduct cluster ions varied greatly among the different manufacturers of CHCA matrix. MassPREP™ CHCA has low background and adduct ions.
- 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 *et. al., Org. Mass spectrum.* **27**, 653-655 (1992)
2. M. Kussmann *et. al., J. Mass Spectrom.* **32**, 593-601 (1997)
3. B.O. Keller *et. al, J. Am. Soc. Mass Spectrom.* **11**, 88-93 (2000)