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Identification of Small Biomolecular Compounds by MALDI-TOF-Mass Spectrometry

QING HUO[1], JIANAN LIU[2,*], SHAOXIANG XIONG[2] and RONGJIAN SHI[3]

[1]Biochemical Engineering College of Beijing Union University, Beijing 100023, P.R. China
[2]Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P.R. China
[3]Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, P.R. China

*Corresponding author: Tel: +86 10 52072259; E-mail: huo_q2002@yahoo.com.cn

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Matrix assisted laser desorption ionization time of flight mass spectrometry MALDI-TOF-MS was generally used to analyze macromolecular compounds. In this paper we study how to use the MALDI to determine the molecular weight of small molecule compounds, such as sugars, amino acids, clenbuterol, diphenylmethyl amines, plant hormones, *etc*. We also studied how to exploring the sensitivity of matrix and how to application the sensitivity. We find a macromolecular 1,2,3,4-tetrakis(3',4'-hydroxyphenyl)-thiophene as matrix, it not only absorbs the laser energy but also makes sample desorption ionization. At the same time it does not produce interfering ions to low molecular weight. In addition, perfluoro C_{60} was added in matrix sensitivity can be improved.

Key Words: Matrix-assisted laser ionization, Sensitivity, Matrix, Molecular weight.

INTRODUCTION

The MALDI-TOF systems have evolved considerably since the first commercial version was introduced by Shimadzu in 1988. Matrix-assisted laser desorption/ionization (MALDI) is a soft ionization technique used in mass spectrometry, allowing the analysis of biomolecules (biopolymers such as proteins, peptides and sugars) and large organic molecules (such as polymers, dendrimers and other macromolecules), which tend to be fragile and fragment when ionized by more conventional ionization methods. The strength of MALDI-TOF MS lies in the ability to generate a molecule profile representing a large number of molecules expressed by the specific organism tested. This introduces a high level of molecule multiplex testing with results in minutes. The molecule profile can be used for microbial identification, detecting specific strains and detection of antibiotic-resistant markers[1-3].

The MALDI process starts by adding the sample to a metal target slide. A matrix solution is then applied to the sample, resulting in formation of crystals. A focused laser beam, either in the UV or infra-red ranges, can "evaporate" compounds from the solid phase, the sample is ionized with a laser pulse where the absorption of high energy by the matrix crystals results in vaporization of the molecules that are accelerated by a voltage gradient through the Time-of- Flight tube. The velocity of the molecules through the Time-of- Flight tube is inversely proportional to the size and charge of the molecule. As the molecules separate based on size and charge, smaller molecules reach the detector first, followed by larger molecules, resulting in the creation of a series of peaks named spectra. It was said that a MALDI-TOF instrument in a shared proteomics facility can easily be set up to handle hundreds of analyses per day[4-6].

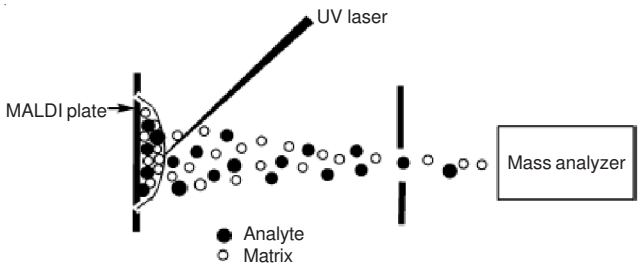


Fig. 1. Scheme graph of MALDI-TOF MS

The matrix is the core of the MALDI study. As the matrix should meet the following conditions: (1) the matrix is miscible with the measured object, the analyte is diluted, reducing the force between the molecules, preventing the formation of molecular clusters. (2) it can be stabilized under the vacuum exists. (3) it has a strong absorption of the laser light source. (4) the matrix is able to protect the sample under the laser irradiation, the energy transfer to the analyte, without destroy the structure of the analyte directly. (5) the matrix can provide the protons, by proton transfer, to promote analyte ionization.

(6) the matrix provide the volume flow, analyte molecules are thrown. The different types of compounds should be used in different matrices generally. For MALDI, the selection and use of the matrix is a significant impact on the final result of the experimental, the choice of the matrix is also related with the formation of the fragment ions. By now, there is still no perfect theoretical guidance on how to choose the matrix for the different samples[7].

EXPERIMENTAL

Matrix: 1,2,3,4-*Tetrakis*(3',4'-hydroxyphenyl)thiophene (provided by Institute of Chemistry, Chinese Academy of Sciences); α-cyano-4-hydroxy cinnamic acid (CCA) (Aldrich Co., Ltd.); perfluoro C_{60} (provided by Institute of Chemistry, Chinese Academy of Sciences).

Solvents: Ethanol, tetrahydrofuran (THF) (Beijing Chemical Factory); the Milli Q ultrapure water.

Ionization reagents: sodium chloride; trifluoroacetic acid (Beijing Chemical Factory).

Samples: (1) small molecule carbohydrate compounds: glucose, mannose, cellobiose, xylose, D-arabinose tree sugar (provided by the CAS Institute of Chemistry); (2) amino acid type compounds: L-glutamine, glutamate, cysteine, threonine, histidine, proline (provided by the CAS Institute of Chemistry).

Laboratory instruments and parameter settings: Bruker Company autotlex II MALDI-TOF MS equipped with a semiconductor laser, wavelength of 355 nm, the maximum power 200 μj, attenuation of 25-45 % of the laser operation. The delay leads and reflected working mode were used, the delay time 35000 ns, delayed extraction voltage 14.5 KV, acceleration voltage 19 KV, reflector voltage 20 kV, The 150 scan signal superimposed become mass spectra.

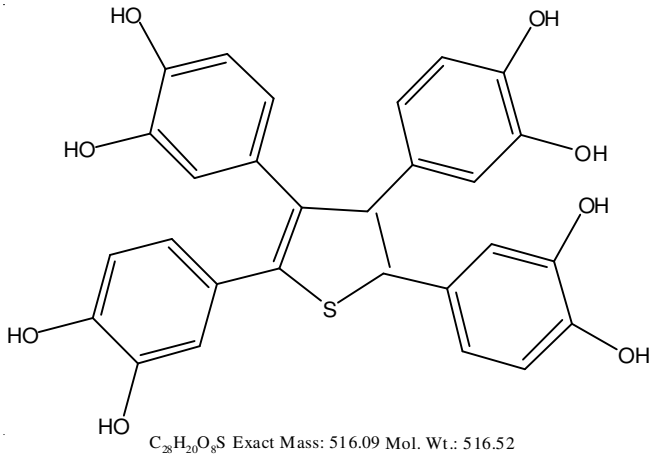
Configuration of the sample, matrix and ionized solution: Glucose, mannose, cellobiose, xylose, D-arabinose tree sugar, L-glutamine, cysteine, threonine, histidine, proline as samples were weighted. It was dissolved in aqueous ethanol (ethanol: water = 1:1) solution. The concentration of solution is 10^{-2} mol/L; 1,2,3,4-*tetrakis*(3',4'-hydroxyphenyl)thiophene or α-cyano-4-hydroxy cinnamic acid (CCA) as matrix was weighted and dissolved in aqueous ethanol (ethanol: water = 1:1) solution. The concentration of solution is 10^{-3} mol/L. Sodium chloride or trifluoroacetic acid as ionized solution was weighted, dissolved in aqueous ethanol (ethanol:water = 1:1) solution, the concentration of solution is 10^{-5} mol/L.

Sample preparation: The sample solution and the matrix solution 3 μL, ionized reagent 1 μL if necessary was added in solution, thoroughly mixed, absorb 0.5 μL mixed droplet on a clean stainless steel sample target, waiting for the solvent naturally volatile, the sample becomes crystalline, submit to the mass spectrometer for MS analysis.

RESULTS AND DISCUSSION

Matrix choice: When the molecular weight of sample is less than 400, matrix (small molecule compound) commonly used in MALDI-TOF MS will produce ionizing, lead to mass spectrum of small molecule compounds exist a large number of hetero-peaks that can not be described. Hetero-peaks have a very strong mass discrimination effect, lead to more difficult for small molecule compounds which is not easily ionized to ionize molecular ion. So we need to find a matrix, which is not only able to absorb laser energy but can also ionize sample, at the same time, does not generate interfering ions of low molecular weight. So that we are able to take advantage of MALDI-TOF MS analysis of the different types of small molecule compounds.

Fig. 2 showed that 1,2,3,4-*tetrakis* (3',4'-hydroxyphenyl)-thiophene contains eight phenolic hydroxyl group. It can provide a large number of protons in the ionization process, protons transfer to the sample molecules, so that the sample ionization occurs. Furthermore 1,2,3,4-*tetrakis* (3',4'-hydroxyphenyl)thiophene has a relatively large molecular weight (516.09), can be effectively reduced or avoided to produce a large number of hetero-peaks in the region of less than 400 Da.



$C_{28}H_{20}O_8S$ Exact Mass: 516.09 Mol. Wt.: 516.52
m/e: 516.09 (100.0%), 517.09 (32.6%), 518.09 (6.5%), 518.08 (4.4%), 519.09 (1.5%)
C, 65.11; H, 3.90; O, 24.78; S, 6.21

Fig. 2. Structure of 1,2,3,4-*tetrakis* (3',4'-hydroxyphenyl)thiophene

1,2,3,4-*Tetrakis* (3',4'-hydroxyphenyl)thiophene ultraviolet absorption diagram is shown in Fig. 3, we can see UV laser absorption in 355 nm, which can absorb energy from the laser passed to the sample molecules.

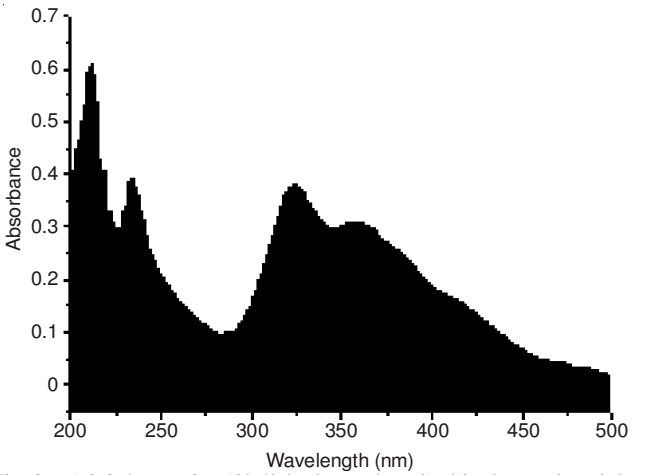


Fig. 3. 1,2,3,4-*Tetrakis* (3',4'-hydroxyphenyl) thiophene ultraviolet absorption spectra

The concentration of the matrix solution is 10^{-3} mol/L, glucose sample solution concentration is 10^{-2} mol/L, sodium

chloride concentration is 10^{-5} mol/L, solvent: ethanol: water 1:1. We can see from Fig. 4, 1,2,3,4-*tetrakis* (3',4'-hydroxyphenyl)-thiophene as a matrix, it is possible to clearly see the protonated $[M + Na]^+$ peak and we observed a large number of impurity peaks within the detection area and can not find the sample peak. Although few samples can be found in the sample peaks, but the intensity of the samples peak is lower and completely submerged in the impurity peak.

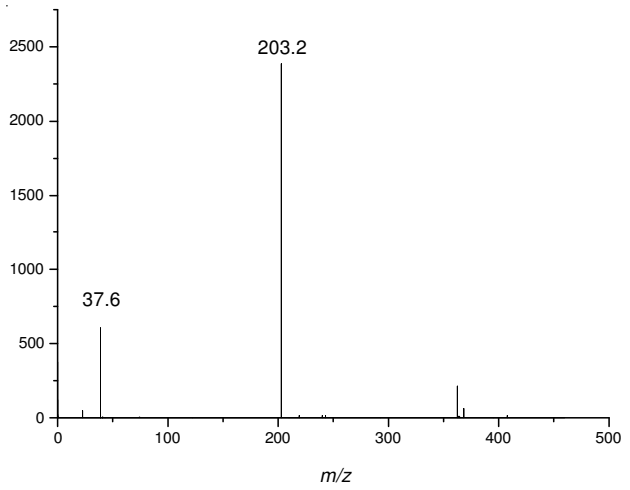


Fig. 4. MALDI-TOF mass spectrum for saccharide compounds in different matrix A: 1,2,3,4-*tetrakis*(3',4'-dihydroxyphenyl)thiophene as matrix

The concentration of the matrix solution is 10^{-3} mol/L, histidine sample solution concentration is 10^{-2} mol/L, trifluoroacetic acid concentration is 10^{-5} mol/L, solvent: ethanol:water 1:1. Fig. 5A showed 1,2,3,4-*tetrakis*(3',4'-hydroxyphenyl)thiophene as a matrix, it is possible to observe clearly the protonated $[M + H]^+$ peak and from Fig. 5B, the CCA is matrix, we see a large number of impurity peaks within the detection area and can not find the sample peak. Although few samples can be found in the sample peaks, but the intensity of the samples peak is lower and completely submerged in the impurity peak.

Improvement of the matrix sensitivity: We can see from Fig. 6A, when the sample solution was diluted to a certain concentration, sample solution concentration is 10^{-5} mol/L, under the same conditions, There is no apparent signal peak in detection area, we can not find the sample peak by using 1,2,3,4-*tetrakis*(3',4'-dihydroxyphenyl)thiophene as matrix. Although few samples can be found in the sample peaks, but the intensity of the samples peak is lower and completely submerged in the impurity peak. In Fig. 6B, we can see that when we use the mixture of 1,2,3,4-*tetrakis*(3',4'-dihydroxyphenyl)-thiophene and perfluoro C_{60} as matrix, the matrix can mix well with the sample and greatly reduce its interfering peaks in the whole region.

MALDI can determinate macromolecular compounds only, if the sample is small molecules, matrix must select the macromolecular compounds. But the macromolecular matrix has poor sensitivity, when the concentration of the sample solution is less than 10^{-5} mol/L, it can't produce a volume flow, significant signal peaks can't be obtained to achieve an effective measuring. In order to compensate for this deficiency,

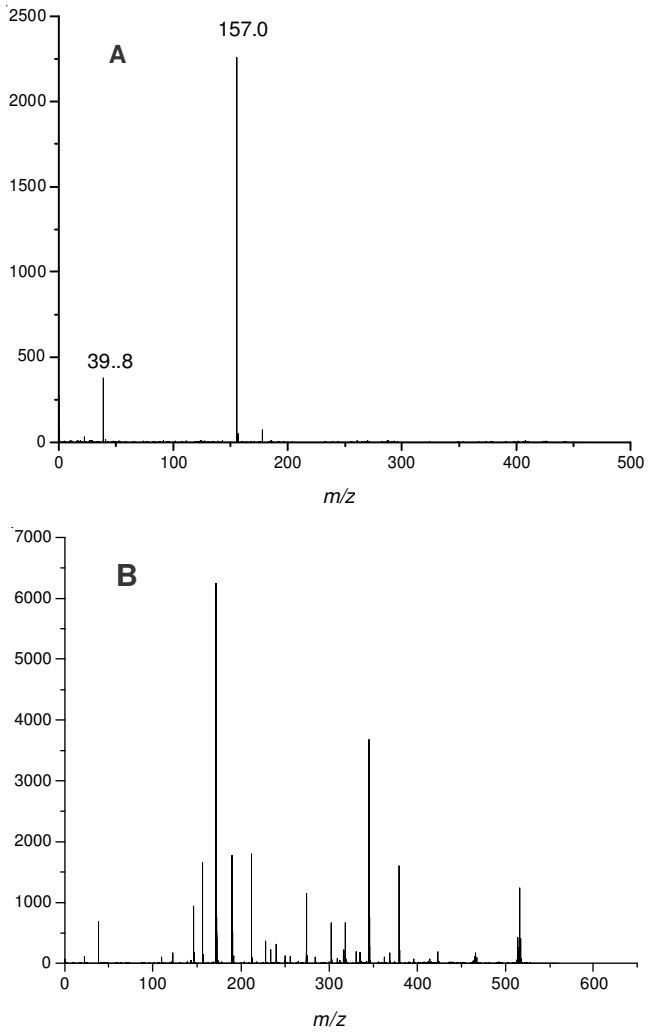


Fig. 5. MALDI-TOF mass spectrum for amino acids in different matrix A: 1,2,3,4-*tetrakis* (3',4'-dihydroxyphenyl)thiophene as matrix; B:CCA as matrix

perfluoro C_{60} is added to the matrix. Perfluoro C_{60} has higher hydrophobic characteristics, perfluoro C_{60} structure is shown in Fig. 7. It can lock sample in drops, so greatly improved the sample concentration. Because of its hydrophobic, adsorption force of the sample at the target surface becomes weak and the sample is easier sputtered from the target surface. Perfluoro C_{60} ultraviolet absorption diagram is shown in Fig. 8, we can see UV laser absorption in 355 nm, which can absorb energy from the laser passed to the sample molecules. The volume flow can be produced at lower energy. This is good solution to the problem of poor sensitivity. Therefore, we uniformly covered with a perfluoro C_{60} in the target surface, the 1,2,3,4-*tetrakis* (3',4'-hydroxyphenyl)thiophene and the sample mixed solution was added dropwise in the above, then use MALDI measure.

Analysis of saccharide compounds: Saccharide compounds analyzed in this experiment, such as glucose, xylose, etc. are important food and drug additives. The results of the saccharide compound using MALDI-TOF MS are shown in Table-1 and Fig. 9.

The concentration of the matrix solution is 10^{-3} mol/L, the polysaccharide concentration of a sample solution is 10^{-2} mol/L, the concentration of sodium chloride 10^{-5} mol/L, solvent: ethanol: water, 1:1. MALDI-TOF MS can quickly and

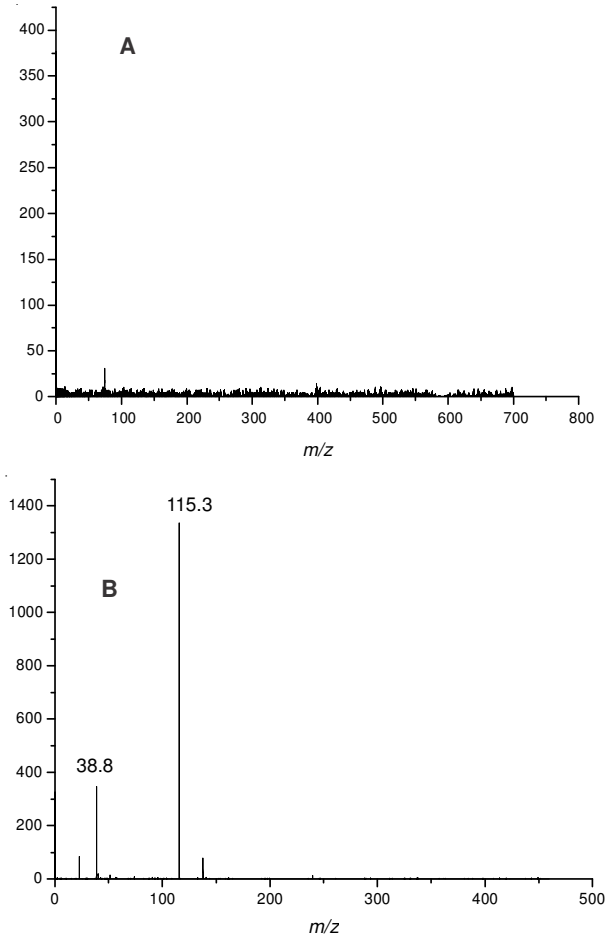


Fig. 6. MALDI-TOF mass spectrum for proline in different matrix A: 1,2,3,4-*tetrakis*(3',4'-dihydroxyphenyl)thiophene as matrix; B: Mixture of 1,2,3,4-*tetrakis*(3',4'-dihydroxyphenyl)thiophene and perfluoro C_{60} as matrix

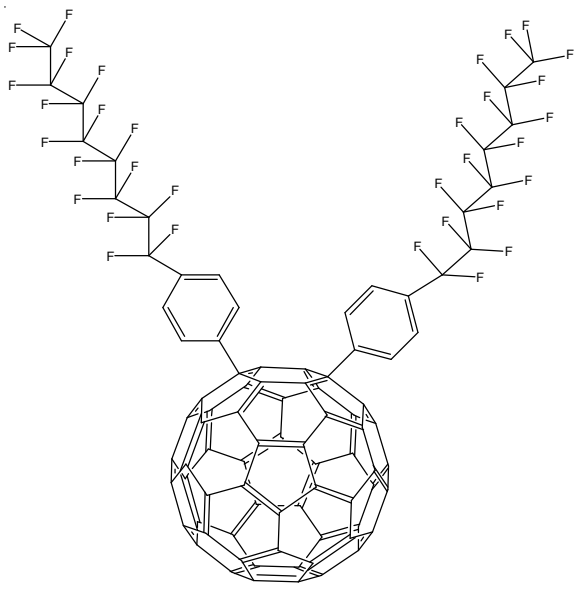


Fig. 7. Perfluoro C_{60} structure

accurately determine the molecular weight of the saccharides. It is clearly observed from the diagram of Na^+, K^+ adduct peak $[M + Na]^+$, $[M + K]^+$, impurity peaks could barely observed, MS signal is very good. The molecular weight determined accurately, the error is within the allowable range.

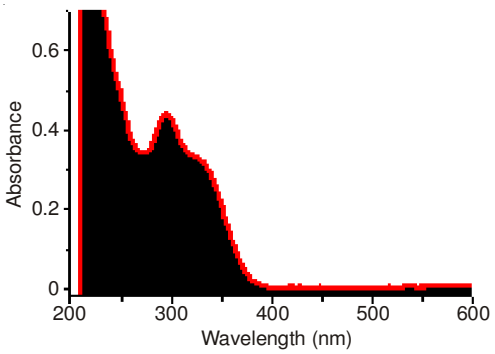


Fig. 8. Perfluoro C_{60} ultraviolet absorption spectra

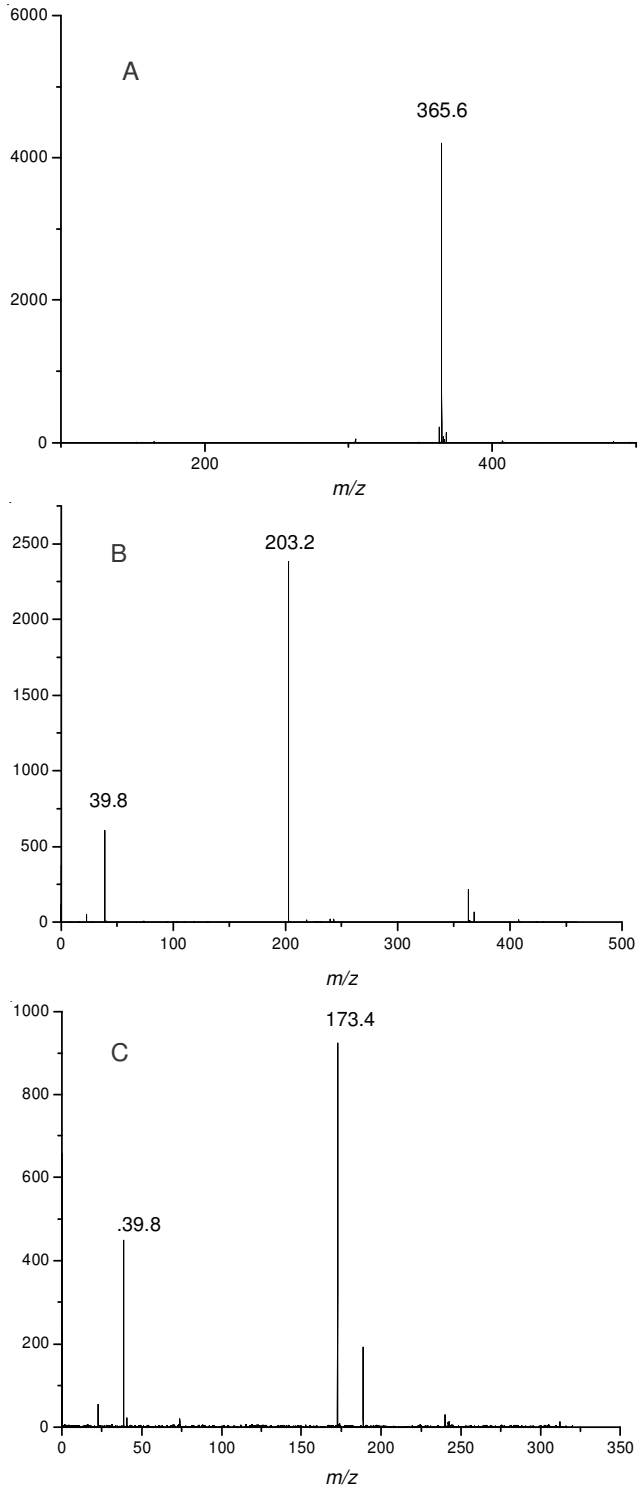
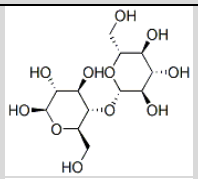
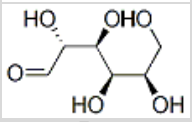
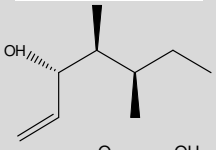
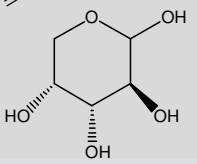
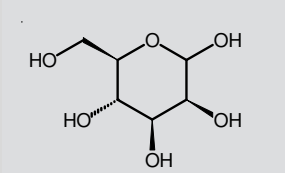


TABLE-1
DETERMINATION TABLE OF SACCHARIDE COMPOUNDS

Name	Structure	Formula	Theoretical molecular weight	Detection of molecular weight
Cellobiose		$C_{12}H_{22}O_{11}$	342.30	342.6
Glucose		$C_6H_{12}O_6$	180.16	180.2
Xylose		$C_5H_{10}O_5$	150.13	150.4
D-Arabinose		$C_5H_{10}O_5$	150?131	150.4
Mannose		$C_6H_{12}O_6$	180.16	180.5

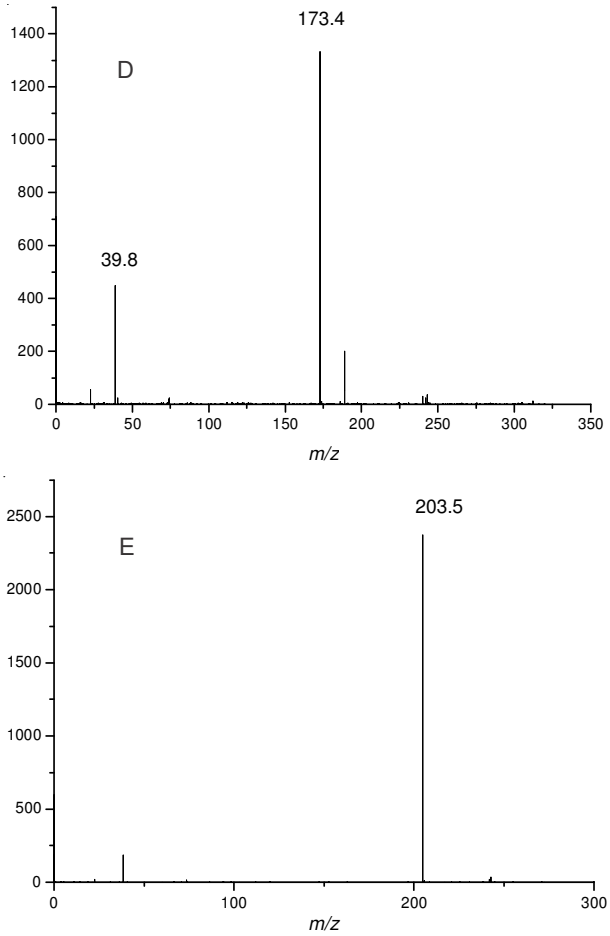


Fig. 9. Saccharide compounds MALDI-TOF mass spectrum A: fiber sugar; B: glucose; C: xylose; D: D-arabinose; E: mannose

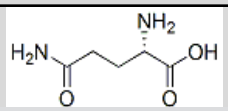
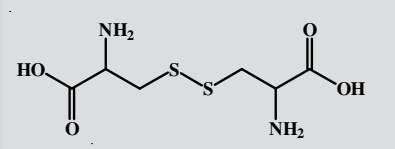
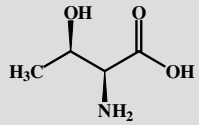
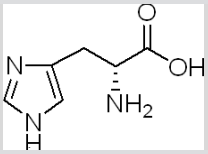
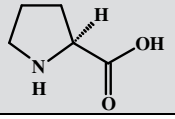
Analysis of amino acid compounds: Amino acid compounds analyzed in this experiment, such as L-glutamine, threonine, *etc*. The results of the amino acid compound using MALDI-TOF MS are shown in Table-2 and Fig. 10

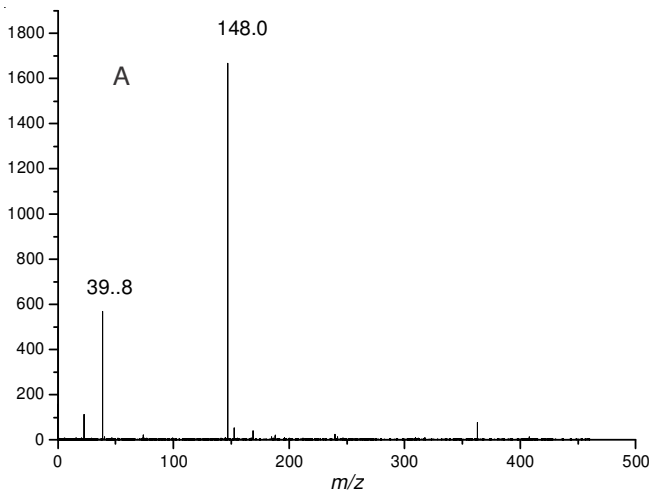
The concentration of the matrix solution is 10^{-3} mol/L, the amino acid compounds concentration of a sample solution is 10^{-2} mol/L, the concentration of trifluoroacetic acid is 10^{-5} mol/L, solvent: ethanol: water, 1:1. MALDI-TOF MS can quickly and accurately determine the molecular weight of the amino acid compounds. We can clearly see from the diagram of ions of H adduct peak $[M + H]^+$, impurity peaks could barely present, MS signal is very good. The molecular weight determined accurately, the error is within the allowable range.

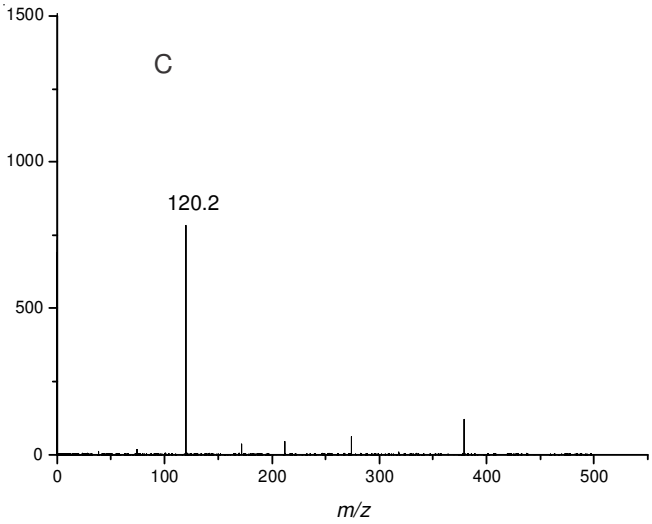
Conclusion

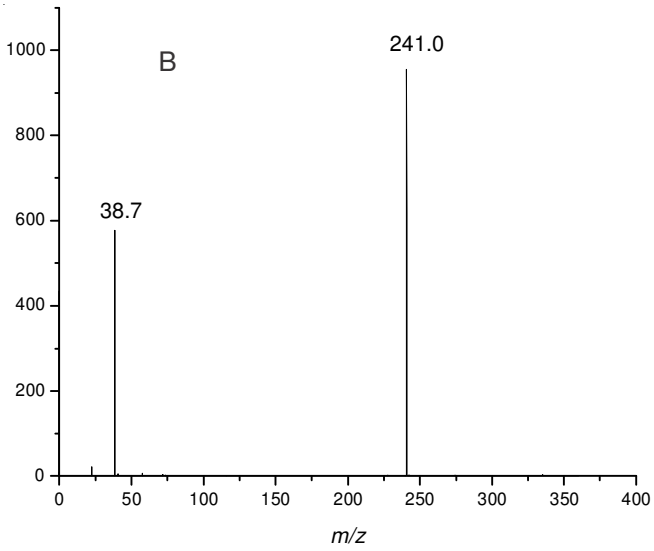
This paper explores the matrix-assisted laser ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis of small molecule compounds. MALDI-TOF MS has the advantages of high sensitivity, high throughput, its operation is simple, easy and shorter analysis time, it is suitable for analysis of a variety of compounds. Using the new macromolecular matrix 1,2,3,4-*tetrakis*(3',4'-hydroxyphenyl)thiophene, not only can be a good sample mixed, to provide a large number of protons, but also be able to transfer energy. The interference peak greatly reduced in the mass spectrum. When the sample solution was diluted to 10^{-5} mol/L, the sample is not easily to peel off from the target surface, macromolecular matrix has poor sensitivity, therefore, we introduce perfluoro C_{60}. Perfluoro C_{60} can lock sample in drops of water, thereby greatly increased sample concentration and the sample is easier sputtered from the target surface. This improves the macro-molecular matrix

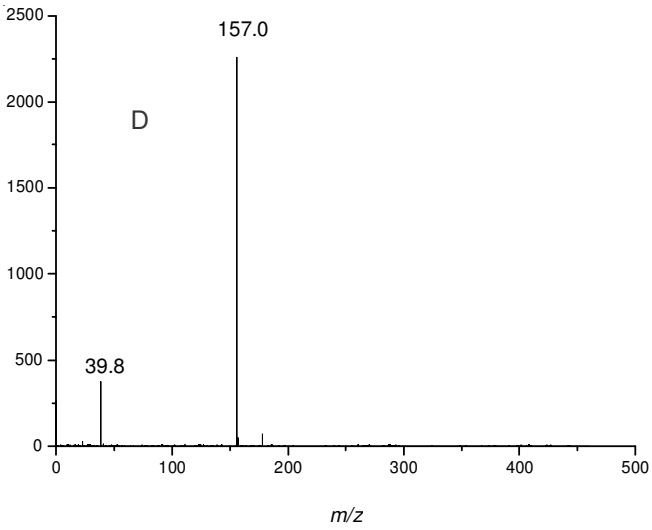
TABLE-2
DETERMINATION TABLE OF AMINO ACID COMPOUNDS

Name	Structure	Formula	Theoretical molecular weight	Detection of molecular weight
L-Glutamine		$C_5H_{10}N_2O_3$	146.15	147.0
Cystine		$C_6H_{12}N_2O_4S_2$	240?30	240.0
Threonine		$C_4H_9NO_3$	119.12	119.2
Histidine		$C_6H_9N_3O_2$	155.16	156.0
Proline		$C_5H_9NO_2$	115.13	115.3









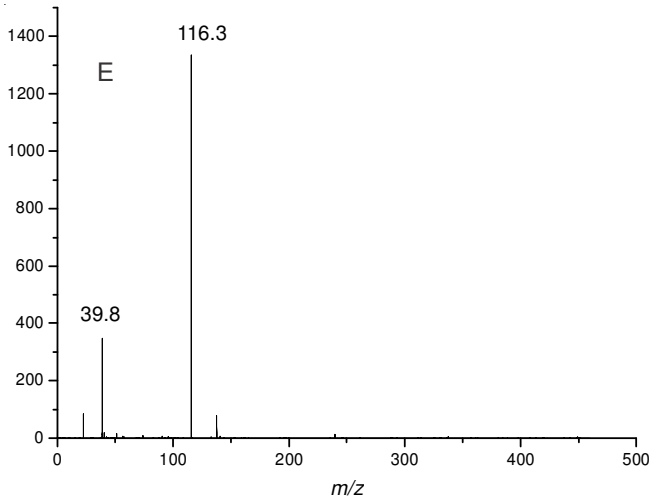


Fig. 10. Amino acid compounds MALDI-TOF mass spectrum A: L-glutamine; B: cystine; C: threonine; D: histidine; E: proline

sensitivity. The sample molecular weight is accurately measured, the error is within the allowable range, almost no impurity peaks in mass spectra. Results indicated that MALDI-TOF MS has the wide range of development and application.

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