ThP-337

Utilizing U-Shaped Mobility Analyzer (UMA) for High Performance Bio-molecular Analysis

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1. Overview

High resolution, wide dynamic range of filter mode and accurate CCS measurement make UMA a powerful analytical device in bio-molecular analysis.

2. Introduction

Ion mobility spectrometry (IMS) is a useful tool for separating and characterizing ions in gas phase. Recently, HPLC-IMS-MS has been widely accepted as an advanced technique for –omics studies. U-Shaped mobility analyzer (UMA) is a newly established ion mobility spectrometer which has multiple operation modes (filter mode, trap-scan mode and through mode) and high performance, as shown in Fig 1. UMA has reached high resolution of >200 for singly charged ion and ~600 for multiply charged ion. In UMA analysis of proteins, a plenty of protein conformers were resolved at several charge states. In UMA filter-scan mode, low abundant ions have lower limit of detection (LOD) than in trap-scan mode. Collision cross sections (CCS) can be accurately measured by UMA. These features make UMA a powerful analytical device in -omics studies.

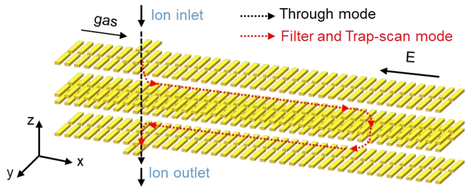

Fig 1. Structure of the U-shaped mobility analyzer

3. Methods

UMA has been coupled to a quadrupole mass spectrometer (Shimadzu LCMS8040®), as shown in Fig 2. In filter-scan mode, ions within a mobility range (defined by the electric field difference between the two ion channels in the UMA) can pass the analytical region while other ions are eliminated. In trap-scan mode, ions are first trapped for a period of time (several milliseconds up to 100ms) in channel one of UMA and then eluted out sequentially based on mobilities by a scanning electric field in channel two of UMA. As there is no ion trapping in filter-scan mode, ion capacity issue is mitigated. A range of analytes, such as Agilent tune mix solution, apo-myoglobin, Reserpine and polyethylene glycol (PEG) solutions were used for performance testing.

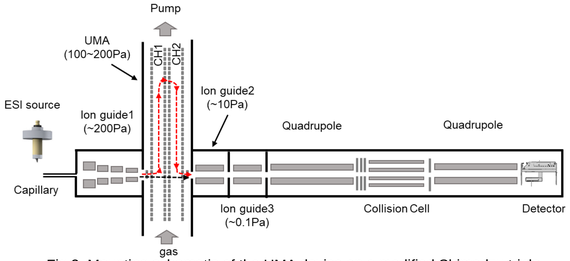

Fig 2. Mounting schematic of the UMA device on a modified Shimadzu triple quadrupole mass spectrometer LCMS8040®

4. Results

4-1. Enhanced Resolution Power

For a counter flow IMS equipment, the increase of gas flow rate can increase its resolution, as shown in Fig 3. A high gas flow (1.75 L/min with linear speed of ~300 m/s) has been established, and for singly charged ion such as hexakis(1H, 1H, 5H-octafluoropentoxy)phosphazine (m/z 1522), a high resolution of 214 has been obtained in Trap-scan mode, as shown in Fig 4. Apo-myoglobin has been utilized to test the performance of UMA to analyze proteins. At charge states from +13 to +20, a plenty of protein conformations have been resolved in filter-scan mode, as shown in Fig 5. The resolved conformers can be used for structural elucidations. Meanwhile, a high resolution of 592 has been obtained by analyzing +15 charged app-myoglobin ions in Trap-scan mode, as shown in Fig 6.

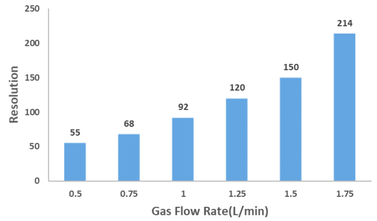

Fig 3. The influence of gas flow rate on resolution using hexakis(1H, 1H, 5H-octafluoropentoxy)phosphazine (m/z 1522)

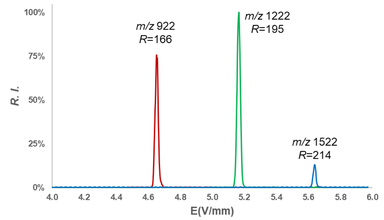

Fig 4. Representative UMA spectrum of the Agilent tune mix ions with m/z 922, 1222, and 1522, obtained at a buffer gas flow rate of 1.75 L/min in the UMA trap-scan mode.

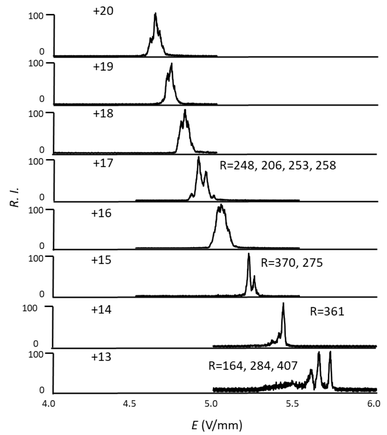

Fig 5 IMS spectrum for ions with +13 to +20 charge state of apo-myoglobin protein ions in the filter-scan mode

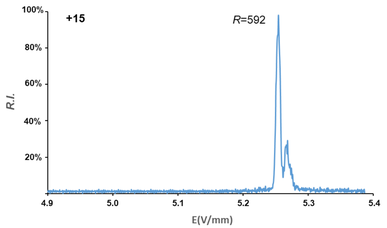

Fig 6 IMS spectrum for ions with +15 charge state of apo-myoglobin protein ions in the trap-scan mode

4-2. Wide dynamic range in filter mode

In UMA filter-scan mode, ions are not trapped in the analytical region, such that low abundant ion are not prone to loss by high abundant ions due to ion capacity issue. Two low abundant ions have lower LOD in UMA filter-scan mode than in trap-scan mode in highly interfering solution. During the analysis of a low concentration Agilent tune mix (1 ppb) doped with a high concentration of reserpine (10 ppm in solution), two phosphazine derivatives (m/z 622 and 922), could not be detected by the UMA trap-scan mode (Fig 7b). In the analysis of the same sample in the filter-scan mode, the detection of the two phosphazine derivatives (m/z 622 and 922) was successful(Fig 7a). The UMA filter mode can more effectively detect trace amounts of targeted molecules in complicated matrices or under severely interfering conditions, thus leading to a wider in-spectra dynamic range.

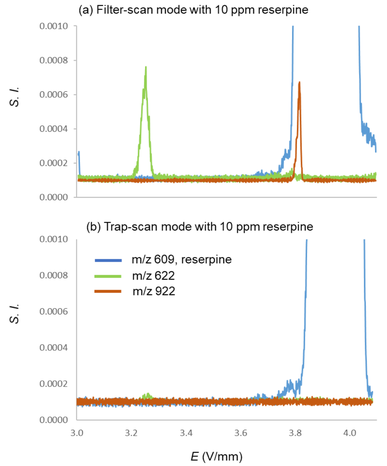

Fig 7. IMS spectrum for two phosphazine derivatives in the Agilent tune mix with inference ions from reserpine: (a) in filter mode, (b) in trap-scan mode.

4-3. Accurate CCS measurement

Collisional cross section (CCS) of molecules can be accurately measured by UMA. PEG has been used as calibrants for instrument calibration and the ion CCS measurement accuracy is around 0.5%~2% for different ion groups with large structural diversity, as shown in Table 1.

Table 1 CCS values of PEG and Agilent tuning mix determined by UMA.

m/z	E(V/mm)	CCS_{N2}(Å²)	CCS_E(Å²)	Dev.(%)	
652	3.280	240	241.1	0.46	PEG
696	3.411	251	251.0	0.0	
740	3.526	261	259.8	-0.46	
784	3.672	271	270.9	-0.04	
828	3.791	280	280.0	0.0	
872	3.916	290	289.5	-0.17	
916	4.038	299	298.8	-0.07	
960	4.158	307	307.9	0.29	
622	2.794	203	203.1	0.05	Agilent tuning mix
922	3.372	243	246.7	1.52	
1222	3.906	281	287.3	2.24	
1522	4.402	316	325.2	2.91	

5. Conclusions

UMA has been coupled to a Shimadzu quadrupole mass spectrometer. UMA has reached high resolution of >200 for singly charged ion and ~600 for multiply charged ion. Low abundant ions have lower limit of detection (LOD) in filter-scan mode. Around 0.5%~2% collision cross sections (CCS) measurement accuracy was obtained in UMA. These features make UMA a powerful analytical device in bio-molecular analysis.

Acknowledgement

The authors wish to thank Shimadzu Cooperation for funding this project.