SHIMADZU

Application News

Liquid Chromatography Mass Spectrometry

LCMS-IT-TOF Analysis of Amoxicillin

The Shimadzu LCMS-IT-TOF incorporates the higher MS stages available with an ion trap and the high resolution and mass accuracy of a time-of-flight (TOF) mass spectrometer, leading to the only mass spectrometer in the field that allows for excellent mass accuracy for both parent and fragment ions (up to MS^{10}).

The LCMS-IT-TOF utilizes patented ion introduction techniques for both the ion trap and TOF region of the mass spectrometer. Compressed ion injection was developed to efficiently accumulate and compress the ions prior to introduction into the ion trap (Figure 1).

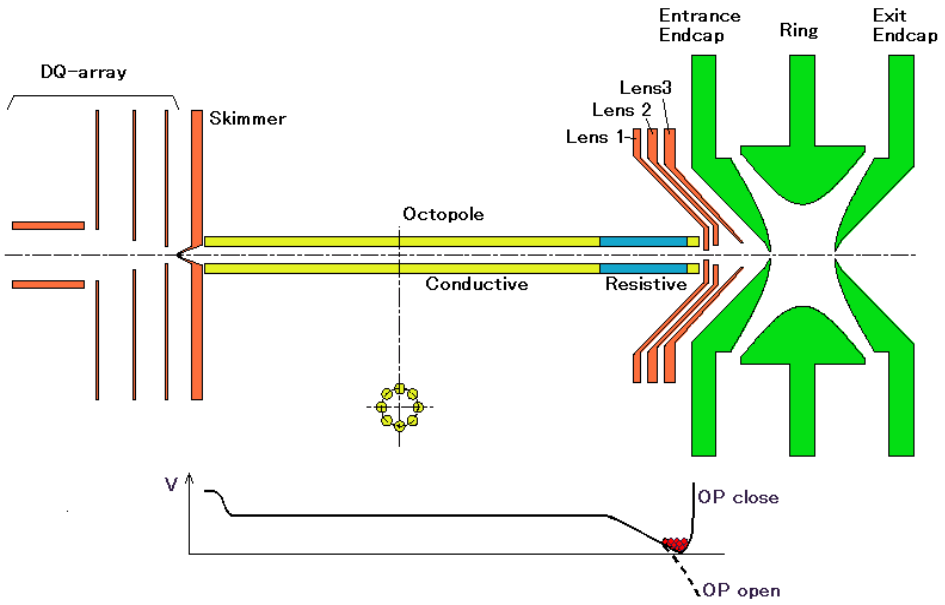

Figure 1: Schematic of the LCMS-IT-TOF MS configuration & ion introduction optics (Compressed Ion Injection) - Ions are focused in an octopole partially coated with a resistive material close to the trap. An electric potential gradient along the ion beam axis forms in the resistive region. When a voltage is applied to the electrode adjacent to the octopole, a 'potential well' forms in which ions are accumulated. The focused 'packet' of ions is rapidly pulsed into the ion trap.

Within the ion trap, all ions are simultaneously ejected into the TOF (ballistic ion extraction) instead of the traditional mode of scanning ions out of the trap region. The simultaneous extraction of the ions out of the trap region leads to lower spatial distributions for the ions as they enter the TOF region, thus leading to better resolution. Resolution is also enhanced through the use of argon gas for cooling the ions (Figure 2). Argon, a larger molecule than helium, which is traditionally used, has a higher dampening efficiency and does not lead to fragmentation. Ions entering the trap within the LCMS-IT-TOF have a lower energy than ions found within a traditional ion trap mass spectrometer experiment.

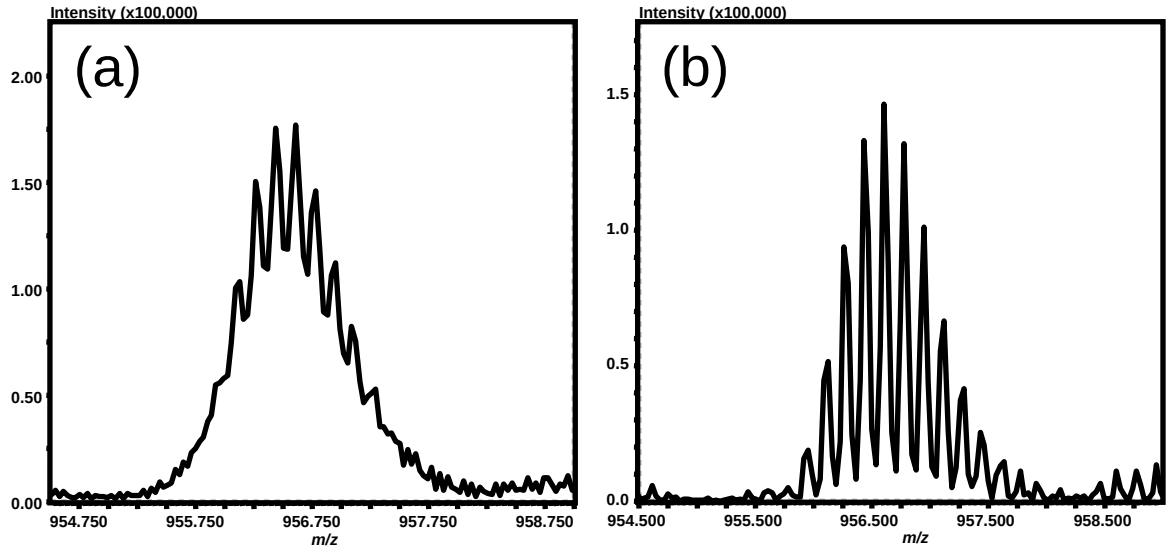

Figure 2: Resolution - Mass spectra of bovine insulin (m/z 956, $[M+6H]^{6+}$) with (a) He cooling and (b) Ar cooling. Argon is used as the cooling gas, but unlike traditional ion trap analyzers, mass resolution is not degraded. The higher dampening efficiency of Ar better localizes trapped ions and the initial space and energy distribution for the TOF analyzer are reduced, leading to higher mass resolution and accuracy.

Another key difference between the LCMS-IT-TOF and its competitors is the fast polarity switching (100 msec) found uniquely with this instrument, allowing for high mass accuracy maintained in both modes of analysis for one single run.

Amoxicillin

Amoxicillin is a drug belonging to a class of compounds known as ß-lactam antibiotics. Amoxicillin, a member of the penicillin family, is used most often to treat a number of bacterial infections including *H. influenzae, N. gonorrhoea, E. coli, Pneumococci, Streptococci*, and some strains of *Staphylococci*. It is thought that these penicillin-derived compounds work to stop the bacteria from multiplying by inhibiting its cell wall synthesis. Confirming these antibiotics in animal-related food sources is important to human health since many people can be sensitive to these drugs and/or develop resistance to the effectiveness of such antibiotics with overexposure.

A number of publications have focused on the identification and quantitation of amoxicillin and other members of the ß-lactam antibiotics family. Groups interested in confirming the presence of these antibiotics both in animal tissues and bovine milk have used ion trap mass spectrometry for its fragmentation ability.[1,2] More recently, the ability to quantitate such drugs in animal tissues has also been published utilizing triple quadrupole mass spectrometers.[3,4] In terms of qualitatively confirming the presence of such antibiotics, the Shimadzu LCMS-IT-TOF is able to generate characteristic fragmentation spectra as well as high mass accuracy information useful for confirming structure. In this experiment, the mass accuracy of amoxicillin and its fragments is showcased along with the MS^n ability of the LCMS-IT-TOF.

Experimental Methods

Amoxicillin and LC-MS grade formic acid were purchased from Sigma-Aldrich (St. Louis, MO). Burdick and Jackson HPLC water and acetonitrile were purchased through VWR (Bridgeport, NJ). LC-MS analysis was performed using the Shimadzu Prominence Series LC coupled to the LCMS-IT-TOF. Prominence Series components included two LC-20AD pumps, SIL-20A Autosampler, and a CBM-20A System Controller. The column used for reversed-phase LC analysis was a Shimadzu Shimpack VP-ODS packed with 4.6 µm particles (4.6 x 150 mm). The instrument was controlled through LCMSsolution, and data analysis was performed using the same software, but utilizing the LCMS Postrun Analysis feature.

The LC analysis consisted of the following conditions: Mobile phase A: 95% H_2O (0.1% Formic Acid) + 5% ACN; Mobile phase B: 95% ACN + 5% H_2O (0.1% Formic Acid); Flow rate: 0.300 mL/min; Gradient: 0 min 0% B, 15 min 25% B, 23 min 50% B, 25 min 50% B, 26 min 5% B. Stop time: 30 min. A 0.5 µL injection volume was used.

The LCMS-IT-TOF was operated under the following conditions: ESI in positive mode; drying gas: 1.5 L/min; CDL temperature: 200°C; interface temperature: 200°C; ion accumulation time: 50 msec; MS^1 scan range: 100 – 1000 m/z, MS^2 scan range: 101 – 400 m/z, MS^3 scan range: 97 – 400 m/z; CID parameters: Energy – 100%, collision gas – 100%, time – 30 msec.

Results and Discussion

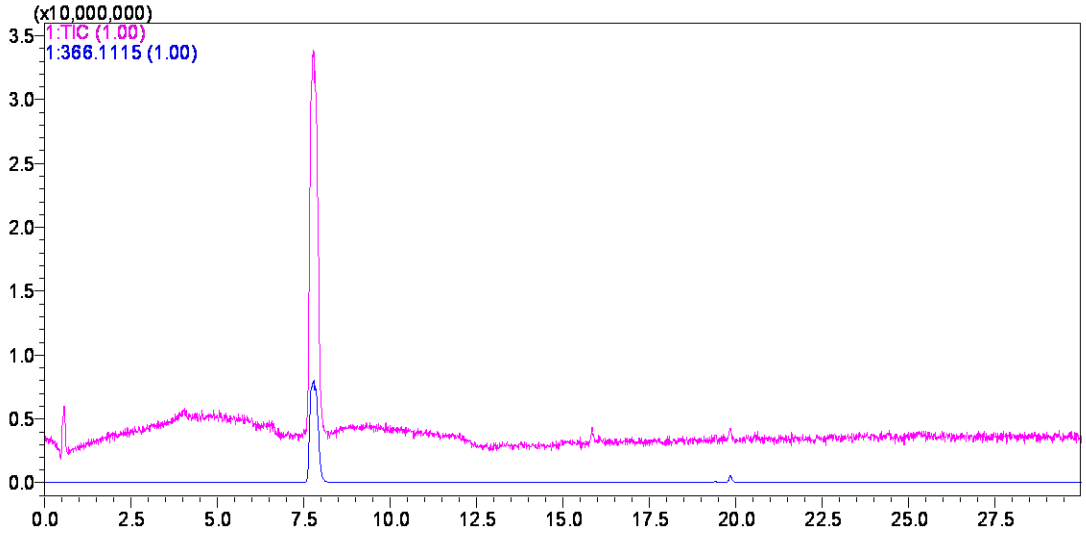

Figure 3: TIC and extracted mass chromatogram for amoxicillin.

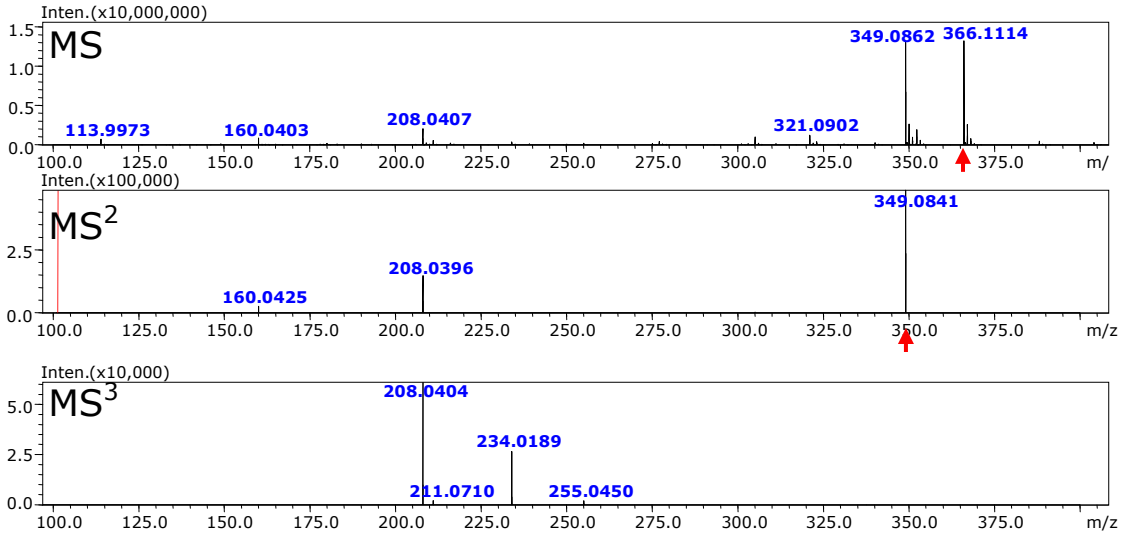

Figure 4: Analysis of amoxicillin [$(M+H)_{thr}^{+}$ = 366.1118] on the LCMS-IT-TOF. Precursor for MS^2 - 366.1114 *m/z*, Precursor for MS^3 - 349.0841 *m/z* (indicated by red arrows)

One of the preferential cleavages for amoxicillin is the loss of NH_3 observed in both the MS and the MS^2 spectra. Another important characteristic cleavage among ß-lactam antibiotics is also the opening of the ß-lactam ring (160 m/z) (Figure 5). In terms of screening, differentiating the ß-lactam antibiotics containing an amino group from other antibiotics within the ß-lactam family is possible using the fragments at 349 and 208 m/z.[1]

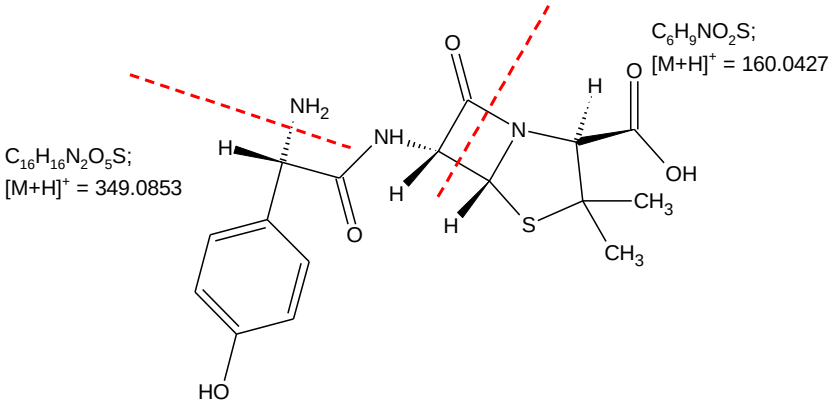

Figure 5: Structure of Amoxicillin, $C_{16}H_{19}N_3O_5S$; $[M+H]^+$ = 366.1118 with cleavages and the corresponding fragment masses.

The LCMS-IT-TOF does not rely on an internal standard to give high mass accuracy data. Simply performing a shortened version of the autotuning function built into LCMSsolution allows one to routinely achieve mass accuracy measurements well below 10 ppm. Similar studies that show the quality of mass accuracy data that can be acquired using an Agilent LC/MSD TOF utilize a dual-sprayer source for the simultaneous measurement of a reference ion (internal standard), an approach utilized by a number of mass spectrometry manufacturers. Comparing data published by Nägele et. al. on the measurement of amoxicillin using the LC/MSD TOF, mass measurements acquired on the LCMS-IT-TOF are comparable if not better *(see Table 1).*

Formula	*Calculated mass $(M+H)^+$*	*Measured mass $(M+H)^+$*	*Mass accuracy (ppm)*
$C_{16}H_{19}N_3O_5S$	366.1118	366.1114	1.1
$C_{16}H_{16}N_2O_5S$	349.0853	349.0841	3.4
$C_6H_9NO_2S$	160.0427	160.0425	1.3

Table 1: Mass accuracy values for amoxicillin and its CID fragments using the LCMS-IT-TOF. Note: Analysis performed without an internal standard.

The mass accuracy for the same fragments reported by Nägele et al. was found to be 1.4, 1.7, and 5.0 ppm for the same ions when accounting for the electron (an error found in the published report lists the mass accuracy at 0.18, 0.23, and 1.71 respectively). Important to note, these mass accuracy measurements were acquired with the use of the reference ion mass as discussed earlier.[5]

Conclusions

Amoxicillin was successfully separated and detected under gradient conditions using a Shimadzu Prominence series LC coupled to the LCMS-IT-TOF. Data acquired on the LCMS-IT-TOF allows for MSn and excellent mass accuracy within one experiment. Mass accuracy data obtained from the LCMS-IT-TOF is comparable to data reported by other vendors requiring the use of an internal standard or a dual-sprayer configuration.

References

1. Heller DN, Ngoh MA. *Rapid Commun. Mass Spectrom*. 1998; 12: 2031-2040.
2. Fagerquist CK, Light.eld AR. *Rapid Commun. Mass Spectrom*. 2003; 17: 660-671.
3. Baere SD, Cherlet M, Baert K, De Backer P. *Anal Chem*. 2002; 74: 1393-1401.
4. Fagerquist CK, Light.eld AR, Lehotay SJ. *Anal Chem*. 2005; 77: 1473-1482.
5. Näegele, E., Moritz, R. Structure elucidation of degradation products of the antibiotic amoxicillin with ion trap MS^n and accurate mass determination by ESI TOF. *J. Am Soc Mass Spectrom* 2005, 16: 1670-1676.

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