New Generation LC-TOF/MS "AccuTOF™"

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Introduction

It has been a decade since Dodonov and his colleagues [1] first announced the electrospray ionization time-of-flight mass spectrometer (ESI-TOF MS). Their initial findings have been enhanced by Standing and others [2,][3], and it has been recently reported that a large TOF MS system achieved a mass resolution exceeding 10,000 [4].

Encouraged by the results acquired by Dodonov and Standing, some of the MS manufactures have produced bench top ESI-TOF MS systems. However, most of these commercial models have a narrow dynamic range and are unfit to quantitative analysis because they use a TDC (Time-to-Digital Converter) as a data acquisition system. Their applications were mainly in qualitative analysis with exact mass measurement, and are limited in such fields as environmental studies and chemical dynamics that require only qualitative analysis. Because of detector saturation, these systems only show good mass acuracy when operated within a limited analyte concentration range.

JEOL Ltd. announced a new LC-TOF MS system, the JMS-T100LC "AccuTOF™", in September 2001. The AccuTOF™ is a new generation LC-TOF MS using a continuous digital averager ADC (Analog-to-Digital Converter) as a data acquisition system to achieve a wide dynamic range. A conventional LC-TOF MS without a continuous averager as a data acquisition system is unable to achieve the wide dynamic range with high resolution and sensitivity. We optimized the design of the AccuTOF™ to achieve a dynamic range exceeding 4 orders of magnitude without forfeiting the major advantages of the conventional LC-TOF MS such as high sensitivity, high resolution, high mass accuracy, and wide mass range. These improvements include the ion detector, a key to maintaining high resolution when the continuous averager is in operation, and the ion guide that significantly affects the total ion transmission ratio and sensitivity. The AccuTOF™ also incorporates a durable

Fig. 1. External view of AccuTOF™.

Dimensions	690 mm (W) x 933 mm (D) x 1114 mm (H)
Weight	303 kg
Power supply	
Basic unit	Single phase 200/208/230 V, 30 A
LC	Single phase 100/115 V, 15 A
Data system:	Single phase 100/115 V, 15 A
Gas	Dry nitrogen gas (97% or more) 10 L/min
Solvent exhaust	Required

Table 1. Dimensions, weight, and installation requirements.

Mass range	6 to 10000 (m/z)
Resolution	6000 (FWHM, reserpine)
Sensitivity	S/N>10 (10 pg, reserpine) (S/N and RMS of mass chromatogram of protonated molecular ions)
	LC Conditions
	Column: ODS, 2.1 mm I.D. x 50 mm
	Flow rate: 0.2 mL/min
	Separating condition: Gradient with 0.1% acetic acid/methanol
Mass accuracy	5 ppm RMS
Recording speed	Maximum 10 spectra/sec (full mass range)

Table 2. The basic performance of the AccuTOF™.

orthogonal ESI ion source designed to support routine quantitative analysis.

We will introduce the features of the AccuTOF™, while outlining the principle and history of TOF MS. We will also compare the data acquisition system of the AccuTOF™, one of its most outstanding features, to the conventional TDC method.

Overview of AccuTOF™

Composition

The standard configuration of a JMS-T100LC AccuTOF™ (AccuTOF™ hereafter) is as follows:

TOFMS basic unit

- Liquid Chromatograph (Agilent 1100 series, optional depending on territory)
- ESI ion source
- Personal Computer Data System
- Printer

The AccuTOF™ has a substantially larger dataset size compared to conventional MS systems since it acquires profile data from a specified mass range. Therefore, its PC incorporates a high speed CPU and a large capacity HDD.

Optional accessories include:

- APCI ion source
- Nano ESI ion source
- Micro ESI probe (ion source shared with ESI)
- N_2 tank

The N_2 tank contains compressed dry nitrogen gas to fill the vacuum vessel if the vacuum system shuts down in emergencies such as a power failure. This prevents atmospheric moisture from infiltrating into the vacuum vessel and substantially speeds up the restarting process of the system.

External View/Installation Requirements

Figure 1 shows an external view of the AccuTOF™. LC-MS systems with an API (Atmospheric Pressure Ionization) ion source, for example ESI, need a large rotary pump. The AccuTOF™ has integrated a rotary pump into the main unit, resulting in an effective footprint equal to or smaller than a typical bench top system. The basic unit has casters at the bottom to facilitate setup, maintenance, and transport of the system. (Note: In order to prevent damage to the turbo molecular pumps, the system cannot be moved while in operation.)

Dimensions, weight, and installation requirements are as follows (**Table 1**).

Basic Performance

The basic performance of the AccuTOF™ is shown in **Table 2**.

The AccuTOF™ has sensitivity specifications appropriate for the actual operation of LC-MS.

Features of AccuTOF™

Figure 2 shows an external view of the AccuTOF™. It comprises:

- Ion source
- Ion transport
- Analyzer
- Detector
- Data acquisition system
- Data system (PC)

We will describe the functions of each component below.

Ion Source

The ion source incorporates an orthogonal electrospray (ESI) ion source specifically developed for the AccuTOF™. Compared to

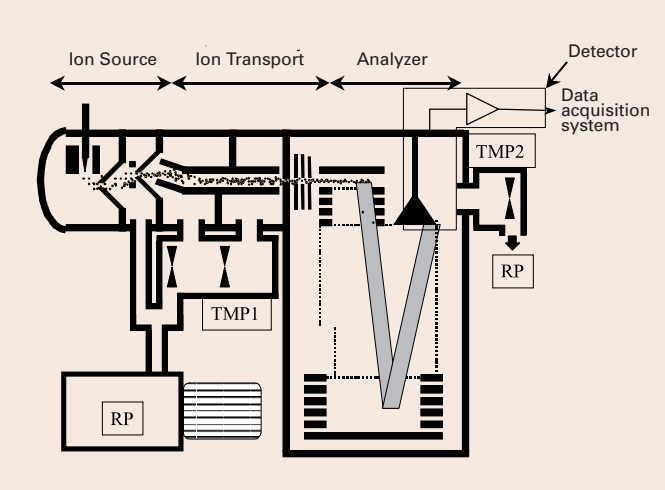

Fig. 2. Total view of AccuTOF™.

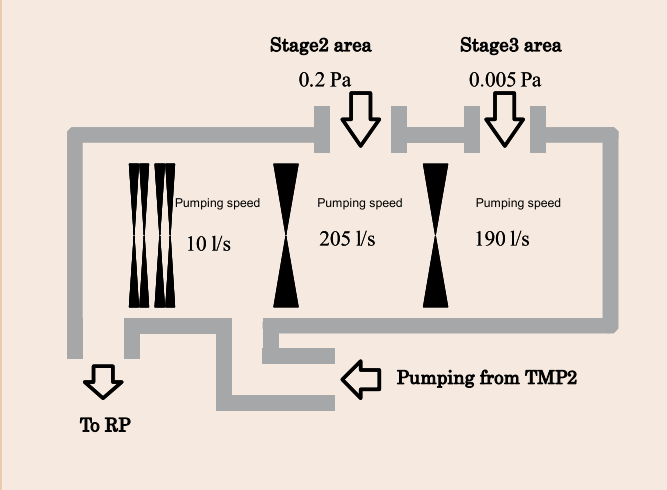

Fig. 4. Split flow TMP.

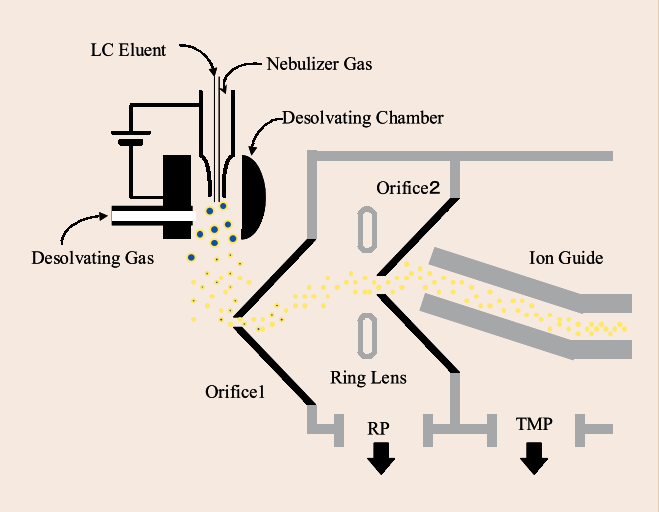

Fig. 3. Orthogonal electrospray ion source.

Type	Ion Focusing Power	Mass to Charge Ratio for Simultaneous Transmission of Ions
Quadrupole	**High**	Narrow
Hexapole		
Octupole	Low	**Wide**

Table 3. Features of different ion guides.

	JMS-700, LCmate	AccuTOF™
Type	Quadrupole	Quadrupole
Frequency	1 MHz	3 MHz
Highest voltage (peak to peak)	800 V	2,500 V

Table 4. Ion guides of AccuTOF™ and conventional models.

the conventional ESI ion source:

- The orthogonal spray prevents contamination buildup inside the system;
- The simple design facilitates maintenance;
- The new desolvating chamber enables analysis of thermally unstable non-covalent bonding complexes.
- The ion source can be used for analysis at low flow rate (<1 μL/min) by replacing the sprayer alone.
  (Option: Micro ESI probe)

Figure 3 illustrates the orthogonal electrospray ion source.

The eluent from the LC exits the sprayer from top to bottom inside the desolvating chamber. The sprayer is normally held at +2,000 V potential (in the positive ion mode), which charges the droplets. The desolvating chamber is heated from 100 to 300 °C. This heat vaporizes the solvent to generate ions. To promote solvent removal, a desolvating gas is introduced to the desolvating chamber.

Since the spray is facing downward, larger residual droplets resulting from incomplete desolvation and nonvolatile components in the eluent collect at the bottom, directing only micro droplets and ions to vacuum through the orifice 1.

The space between orifices 1 and 2 (area 1) is evacuated by a rotary pump (RP) from 200 to 300 Pa (approximately 1/300 to 1/400 atmosphere). Orifices 1 and 2 are intentionally unaligned. As a result, most of the neutral micro particles and droplets that have transmitted through the orifice 1 do not pass through the orifice 2. Only ions will be directed to the orifice 2 efficiently by setting the relative voltage between the orifice 1, orifice 2, and ring lens to an appropriate level.

Ion Transport

Ion guide

The ions that have passed through the orifice 2 are introduced to the high frequency ion guide. The ion guide is designed to efficiently transport the ions produced by the ion source to the TOF MS in high vacuum ($1x10^{-4}$ Pa). The ion guide is a critical component of the system because it controls the kinetic energy of the ions and thus determines the focus of the ions to achieve high resolution and high sensitivity of the TOF MS. The ions that have passed through the orifice 2 are captured by a high frequency electric field and focused toward the central axis of the ion guide. On the other hand, the neutral molecules that have passed through the orifice 2 (mostly nitrogen gas molecules), are not affected by the high frequency electric field and exhausted between the electrodes of the ion guide. When the pressure in the ion guide exceeds a certain level (usually 0.1 Pa), the ions will collide with the neutral molecules (nitrogen gas molecules) gradually losing kinetic energy. As a result, they will be focused toward the central axis while the kinetic energy in the direction of the axis is homogenized to a few eV [5]. This is an ideal setup for the orthogonal acceleration time-of-flight mass spectrometer (oa-TOF MS), as we will discuss later. The TOF MS itself must be kept in extremely high vacuum. To meet these apparently contradictory requirements, the ion guide is positioned through the two vacuum areas, and is evacuated by a single split flow turbo molecular pump (TMP). As a result, the front half of the ion guide is kept at a relatively high pressure appropriate for collisional focusing while the rear half is at approximately 1/100 of the pressure level of the front, facilitating combination with TOF MS.

Three different types of high frequency ion guides are generally used, that is:

- Quadrupole
- Hexapole
- Octupole

Table 3 summarizes their features.

The quadrupole ion guide is superior to others in its power to focus ions toward the central axis, effective in achieving high resolution and high sensitivity at the TOF MS. However, the quadrupole ion guide has the narrowest mass to charge ratio (*m/z*) range for simultaneous transmission of ions. These do not present problems when the ion guide is combined with a conventional quadrupole MS (QMS) or magnetic field MS. Since the conventional MS detects ions of a certain *m/z* at one point, its ion transmission efficiency in the entire *m/z* range will be optimized by sweeping the field (quadrupole field, magnetic field) while changing the high frequency voltage applied to the ion guide. The TOF MS, on the other hand, measures the entire *m/z* range simultaneously. As a result, the ion guide is required to transmit all ions in the entire *m/z* range at a time. It is generally known that the ion guide of a certain type can transmit ions in a wider *m/z* range at a time by simultaneously increasing the high frequency voltage and frequency. The AccuTOF™ uses a quadrupole ion guide with high focusing power to achieve high mass resolution and sensitivity. The high frequency and voltage applied to the ion guide are increased by approximately 3 times to 3 MHz and 2500 V (peak to peak) respectively, enabling transmission of ions in a wide *m/z* range. **Table 4** compares the ion guides of the AccuTOF™ and JEOL's MS conventional models.

Ion focusing lens

High frequency for focusing and DC voltage (ion guide bias voltage) are applied to all electrodes of the quadrupole ion guide. The ions that have passed through the ion guide are accelerated by the potential difference between the bias voltage and the voltage on the orifice (0 V), and introduced to the analyzer through the orifice. The energy of the ions having transmitted through the orifice is nearly equal to the ion guide bias voltage due to the collisional focusing effect.

Behind the orifice is a focusing lens designed to position the ion beam in parallel and direct it to the ion accelerator of oa-TOF MS, which we will discuss later.

Analyzer

The analyzer of the AccuTOF™ is an orthogonal acceleration time-of-flight mass spectrometer (oa-TOF MS) incorporating a single stage reflectron.

Features of TOF MS

General features of TOF MS are:

- No limit on mass range in theory
- Fast mass spectrum acquisition over the full range (<1 ms)
- High ion transmission ratio; high sensitivity since most of the ions introduced to the analyzer reach the detector
- Simple relationship between mass and time of flight, resulting in less errors in mass calibration

These features, however, were not utilized in applications until 1990. We will review the history of TOF MS, emphasizing how these theoretical advantages are put into practice.

History of TOF MS

While TOF MS suddenly attracted attention when the MALDI (Matrix Assisted Laser Desorption/Ionization) method was discovered, it is actually one of the oldest MS systems. The idea of TOF MS was first proposed by Stephens et al.[6] in 1946. Two years later, in 1948, Cameron and Eggers [7] announced the first time-of-flight mass spectra, demonstrating a mass resolution of around 5. In 1955, Wiley and McLaren [8] developed a technique to focus the spread of ionization position and initial ion energy, achieving a mass resolution of 300 or higher. This modification provided TOF MS with a practical resolving power, and Bendix began distribution of commercial models [9]. In the 1970s, a reflectron TOF MS [10] was developed, featuring a resolution of a few thousand. In the late 1980s, Dawson and Guilhaus [11], and Dodonov [1] developed orthogonal acceleration, which allowed an efficient combination of TOF MS and continuous ionization source.

oa-TOF MS

The Reflectron achieved a resolution of a few thousand in TOF MS, approximately 1,000 times higher than Cameron's original design. However, the decline of ion efficiency in a system combining a continuous ionization source and TOF MS was not corrected until Dawson and Guilhaus [11] and Dodonov [1] developed orthogonal acceleration.

In the TOF MS by Cameron and Eggers, most of the ions generated from the ion source were deflected by the chopper and blocked by the slit, allowing only a small portion of the ions generated to be introduced into TOF MS. In the TOF MS by Wiley and McLaren, while most of the ions generated flew inside TOF MS and reached the detector, the duration of ion production was extremely short, and the sample molecules introduced to the ion source while the electron gate was closed were not ionized and were discarded. The utilization ratio of ions or sample molecules in these systems was probably 0.1% or less. The orthogonal acceleration method (oa) increased the ion utilization ratio to 25 to 50%. We will discuss the basic structure of an orthogonal acceleration TOF MS with reference to the analyzer of the AccuTOF™ (**Fig. 5**). The sample is continuously ionized by the ion source. The resulting ions are shaped into a parallel ion beam, which is introduced to the space between the push-out plate and grid 1 (G1) of the ion accelerator (orthogonal accelerator). Pulse voltages V_{push} and V_{pull} are applied to the push-out plate and grid 2 when ions begin their flight.

Next, we will discuss the cycle of ion flight in oa-TOF MS. Ions generated from the ion source are introduced to the space between the push-out plate and grid 1 (G1) of the ion accelerator (orthogonal accelerator) (**Fig. 6-1**). At this point the voltage on the push-out plate is the same ground level (0 V) as the grid 1. Since the potential at the ion source is V_{beam}

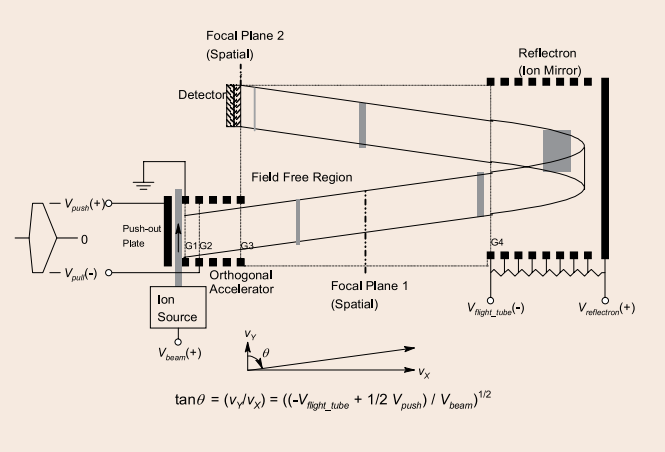

Fig. 5. Orthogonal acceleration TOF MS.

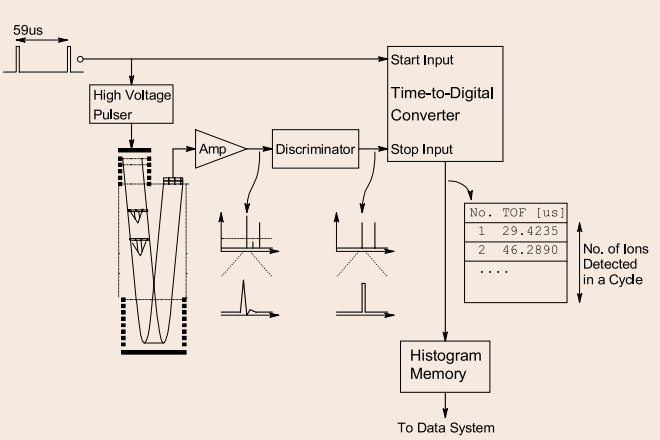

Fig. 7. TOF mass spectrum acquisition by TDC.

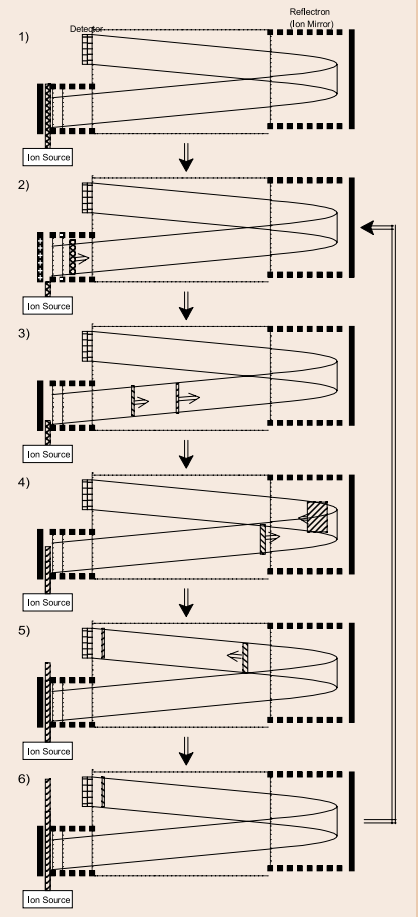

Fig. 6. Ion flight cycle in oa-TOF MS.

m/z	Time[μs]	Speed[km/s]
10	5.4	378.4
100	7.1	119.7
609	42.3	48.5
1,000	54.2	37.8
10,000	171.3	12.0

Table 5. Time/Speed of flight and mass numbers.

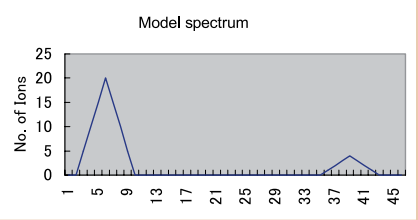

Fig. 8. Model spectrum.

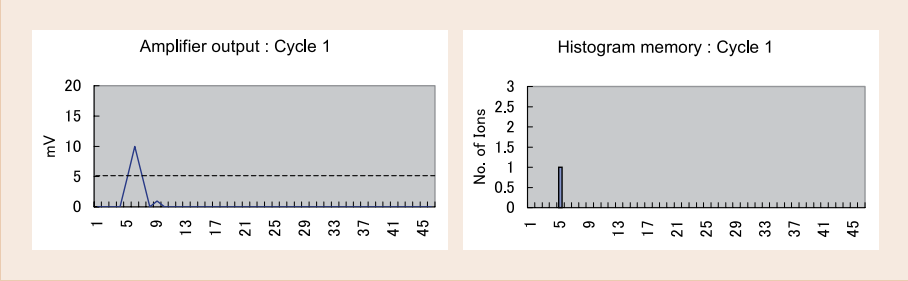

Fig. 9. TDC: Cycle 1.

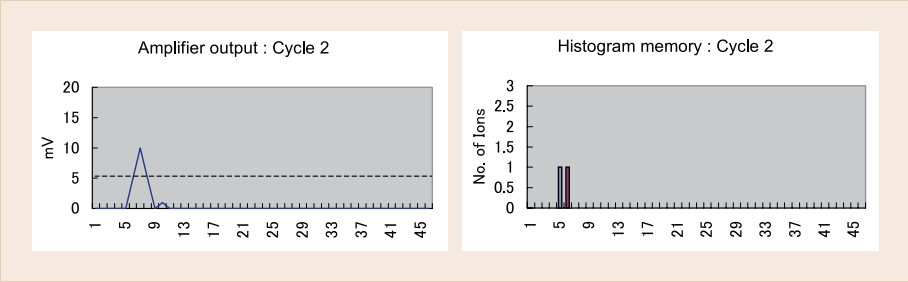

Fig. 10. TDC: Cycle 2.

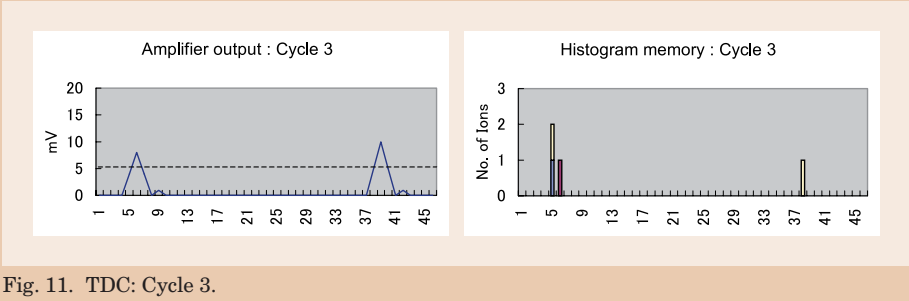

Fig. 11. TDC: Cycle 3.

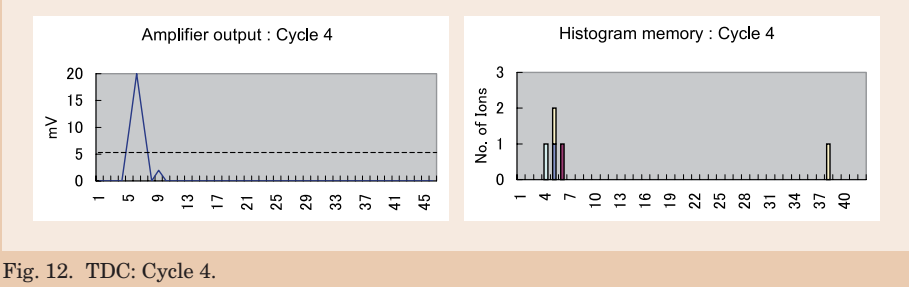

Fig. 12. TDC: Cycle 4.

[V], if the initial ion energy in the ion source is 0, the kinetic energy of these ions in the direction of Y inside in the ion accelerator will be V_{beam} [eV].

Pulse voltages V_{push} and V_{pull} will be applied to the push-out plate and grid 2 respectively. The ions between the push-out plate and grid 1 will receive a force orthogonal to the direction of advance (Y), and begin flying toward the analyzer (**Fig. 6-2**). After passing through the grid 2, the ions will be further accelerated toward the grid 3, and fly to the field-free region through the grid 3.

When all ions have moved to the field-free region through the grid 3, the voltages on the push-out plate and grid 2 will be restored to 0 [V] (**Fig. 6-3**). Then, ions from the ion source will begin filling the space between the push-out plate and grid 1 again.

The ions will continue their flight while new ions from the ion source are simultaneously filling the space between the push-out plate and grid 1, resulting in an overflow (**Fig. 6-3** to **6-5**). The "overflow" (=waste) cannot be fully eliminated because the kinetic energy in the direction of Y is conserved and because a certain distance is needed between the ion accelerator and detector. The ion utilization ratio, despite the overflow, is 25 to 50%, a considerable improvement over the conventional system.

One flight cycle will end when the ion with the highest *m/z* reaches the detector (**Fig. 6-6**). Reapplying pulse voltages to the push-out plate and grid 2 will begin another flight cycle (Fig. 6-2).

The direction of ion flight in the field free region will not be fully orthogonal to the direction of ions introduced. As Fig. 5 shows, the angle θ is determined by the ratio between the kinetic energy of ion in the directions of Y when it is introduced and the kinetic energy in the direction of X, orthogonal to Y, and is expressed as:

$$\tan\theta = \left(\frac{q \cdot \left(-V_{flight\_tube} + \frac{1}{2} V_{push} \right)}{q \cdot V_{beam}} \right)^{\frac{1}{2}} = \left(\frac{-V_{flight\_tube} + \frac{1}{2} V_{push}}{V_{beam}} \right)^{\frac{1}{2}}$$

This demonstrates:

A) For all ions to have the same θ, they must have the same kinetic energy in the direction of Y when introduced.

B) When the ion beam energy (V_{beam}) is reduced, θ will increase, narrowing the distance between the detector and ion accelerator. This will reduce the volume of ions "overflowing" from the ion accelerator at the latter half of the flight cycle (Fig. 6-5 to 6-6), increasing the ion utilization ratio (= improving sensitivity).

Lastly, we will discuss focusing of ions in oa-TOF MS with a single-stage Reflectron. As shown in Fig. 5 and 6, the ion beam from the ion source has a certain width. This spatial expansion will be temporarily focused at the focal plane 1 in Fig. 5 (spatial focusing). The ions, once having passed through the focal plane 1, will be focused again at the focal plane 2, that is, the detector surface (energy focusing), by the single-stage Reflectron.

Analyzer of AccuTOF™

The analyzer of the AccuTOF™, as Fig. 5 shows, has a simple, basic design of 2 stage acceleration and single-stage Reflectron. Three types of high resolution Reflectron TOF MS systems are available:

- 2-stage acceleration + 2-stage Reflectron
- Single-stage acceleration + 2-stage Reflectron
- 2-stage acceleration + single-stage Reflectron

We selected 2-stage acceleration + single-stage Reflectron, taking into consideration expected ion optic performance and ion transmission ratio. For the characteristics of oa-TOF MS with 2-stage acceleration + single-stage Reflectron, see a separate report by Kammei and Kato of JEOL Ltd [12].

The ion accelerator, Reflectron, and detector are positioned to apply a potential of 27 eV to the ion accelerator.

When the ion beam energy is reduced, the ion utilization ratio is expected to increase, improving sensitivity. However, if the energy is reduced excessively, slight charging of the push-out plate and grid 1 will deflect the ion beam, resulting in unstable resolution over time. Our empirical data showed that at least 20 eV is needed for the beam energy to maintain resolution over time. The AccuTOF™ uses 27 eV to include a small allowance.

Detector

As we will discuss in Data Acquisition System, when the TDC is used as a data acquisition system, the ion detection system will rarely affect the quality of the mass spectra. On the other hand, when the ADC/continuous digital averager is used as a data acquisition system, the ion detection system will directly affect the quality of the mass spectra (mass resolution, peak shape, etc.).

The detection system of the AccuTOF™, which incorporates an ADC/signal averager as its data acquisition system, is designed to minimize signal distortion.

Detector

The detector comprises a micro channel plate (MCP) and an anode.

The MCP is a glass plate approximately 0.6 mm thick having channels, that is, holes with an ID of 10 µm at intervals of 12 µm (between channel centers). Both surfaces of the MCP are metal coated, serving as electrodes. A voltage applied between the electrodes will produce an electric field gradient. When an ion hits near the inlet of the inner walls of the channel at this point, multiple secondary electrons will be emitted. These electrons will be accelerated by the electric field gradient inside the channel, hitting the walls on the opposite side and emitting secondary electrons again. The electrons will advance to the outlet while hitting the inner walls of the channel repeatedly, resulting in an electron current exponentially multiplied. These electrons will be captured by the anode, producing an electrical signal. The amplification factor of the MCP (the number of electrons emitted from the outlet when one ion is introduced to the inlet) is a few thousand at maximum. Since TOF MS needs a detector with an amplification factor of 10^6, it usually uses two MCPs layered (Dual MCP).

Data Acquisition System

The AccuTOF™, using a continuous digital averager (ADC) as the data acquisition system, has a wider dynamic range than conventional LC-TOF MS systems with TDC. We will discuss advantages and disadvantages of TDC and continuous averager, and explain how the AccuTOF™ was designed to enhance the strengths of the continuous averager while minimizing its adverse effect.

Data acquisition system for oa-TOF MS

The data acquisition system is designed to digitize electrical signals from the detector and transfer them to the data system in a compatible format. Any data acquisition system for oa-TOF MS must meet extremely strict requirements.

Achieve extremely high time resolution

As described in oa-TOF MS, when the AccuTOF™ achieved a mass resolution of 6,000 (half width) with the protonated molecular ion of reserpine (*m/z* 609.28), the half width of the time of that peak was 3.5 ns. To identify the peak position (=flight time=mass number) as accurately as possible, it is desirable to have about 10 data points from the half width of the peak. Thus, it is ideal for the AccuTOF™'s data acquisition system to have a time resolution of 350 ps. This equals the time needed for light to advance 10.5 cm.

Continuously acquire data

As Fig. 6 shows, the system needs to begin acquiring data for a next ion flight (Fig. 6-2) as soon as one ion flight is completed (Fig. 6-6). If there is a time lag between flights, the ion utilization ratio will decline as the ions are wasted between the push-out plate and grid 1 increase, resulting in poor sensitivity.

Accumulate spectral data in real time

Table 5 shows the speed and time of ion flight calculated for the effective accelerating voltage and filght distance of the AccuTOF™. As Table 5 shows, one ion flight ends within 200 µs. If we limit the *m/z* range needed to 1000, one ion flight will be 55 µs, translating into approximately 18,000 ion flights per second. However, it is senseless to acquire 18,000 individual spectra in one second. A spectrum acquired from one ion flight has poor *S/N* ratio, unfit for analysis, because it records information from a very small amount of ions generated from the ion source for 1/18,000 second. Furthermore, saving 18,000 spectra per second on the hard disk is utterly impractical in terms of data transfer speed and data capacity. Thus, the data acquisition system needs to accumulate these spectra for a period of time specified by the user, and forward the accumulated data to the data system.

Two types of data acquisition systems meet these requirements:

A) TDC (Time-to-Digital Converter)

B) Continuous Averager (ADC)

Also called Digital Signal Averager (DSA) or Integrating Transient Recorder (ITR)

TDC (Time-to-digital converter)

TDC was originally used in high energy physics to measure the flight speed of high energy particles by counting the time needed for particles to travel between two points (time of flight). It is a kind of stop watch for ultra high speed counting. **Figure 7** illustrates the principle of TOF mass spectrum acquisition by TDC.

A pulse with a certain cycle (59 µs: is shown in the figure; this is an actual cycle used

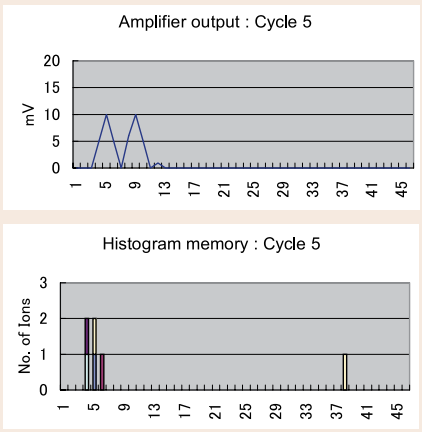

Fig. 13. TDC: Cycle 5.

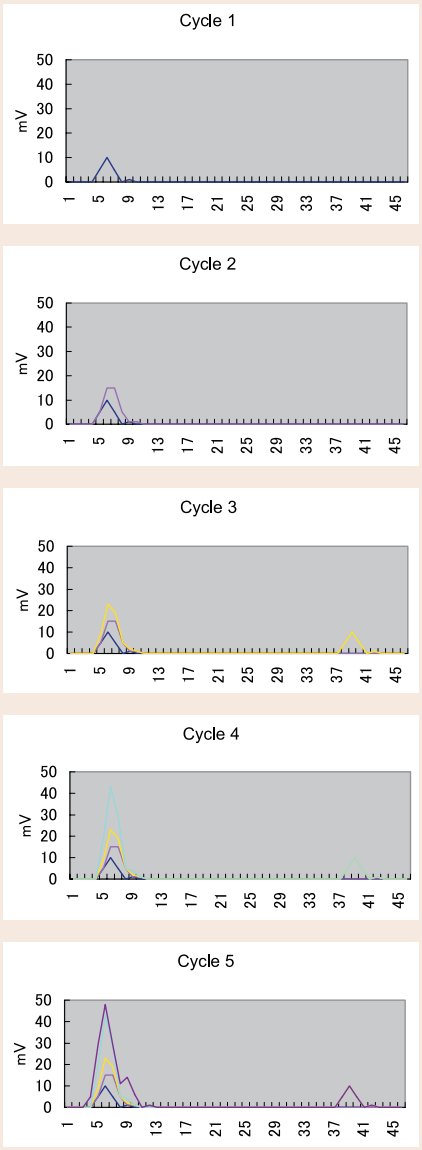

Fig. 15. TOF mass spectrum acquisition by continuous averager.

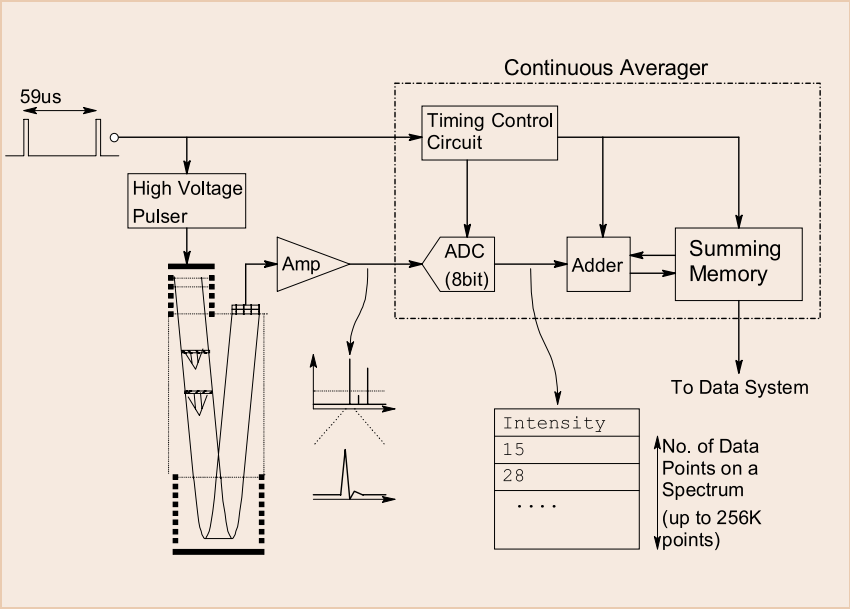

Fig. 14. TOF mass spectrum acquisition by continuous averager.

	TDC	Continuous averager (ADC)
Dynamic Range Accuracy of ion intensity axis	Only two values, 1 and 0, acquired from one flight cycle ●Maximum level in Y axis in spectral data accumulation for 1 sec is approx. 17,000 Statistical correction (dead time correction) essential ●Correction parameters determine accuracy of final results ●Unable to correct data if two or more ions are detected simultaneously ●Y level that guarantees linearity is 1/2 to 1/4 of maximum level	0 to 255 acquired from one flight cycle **Statistical correction is fundamentally unnecessary**
Time counting accuracy Mass measurement accuracy	Easier to engineer device with higher counting accuracy (time resolution) ●0.25 ns available in market Actual mass measurement accuracy is determined by parameters used for dead time correction (statistical correction)	Lower time counting accuract (time resolution than TDC ●AccuTOF™ - 0.5 ns **Statistical correction is fundamentally unnecessary**
Mass resolution	High because unaffected by response of detector or amplifier	Lower than TDC's in the same ion optic system because affected by response of detector or amplifier **Effective flight distance in AccuTOF™ - 2 m**
Effect from detection system (detector, amplifier)	Signal distortion and electric noise from detection system do not affect final spectral data as long as they are less than the threshold of discriminator	Signal distortion and electric noise from detection system directly affect the spectral quality **High quality detection system specifically developed for AccuTOF** **Ion guide featuring high ion transmission ratio assures sufficient sensitivity**
Application	Effective in accumulating weak ions for a long period of time ●Nano-ESI/MS/MS, etc.?	Wider dynamic range within one spectrum despite limited accumulation time ●**Fast LC-MS, CZE-MS, etc.**

Table 6. TDC vs. Continuous averager.

in the AccuTOF™ to analyze up to *m/z* 1,000) to initiate an ion flight cycle is forwarded to the TDC start input and the high voltage pulser to generate voltage pulse for the push-out plate/grid 2. The signal from the detector is amplified by the amp, and forwarded to the discriminator. The discriminator selects the pulse exceeding a certain threshold, and shapes it to a pulse with a certain height and width. The shaped pulse is forwarded to the TDC stop input. The TDC counts the time from the start pulse to each of the stop pulses, and forwards the resulting list to the histogram memory. The size of this list (number of lines) acquired from a single flight cycle equals the number of ions detected in that flight cycle. The histogram memory normally accumulates data of a few to 20 or 30 thousand cycles, and forwards this data as a TOF spectrum to the data system.

Since the TDC is a time counting device, the Y axis of the spectral data accumulated in the histogram memory for one flight cycle represents only two values, 1 and 0. As a result, the TDC has the serious problems below.

A) When two or more ions arrive at the detector simultaneously in one flight cycle, the TDC counts them as one ion;
B) When two ions arrive at the detector in sequence within a certain interval, the TDC does not count the latter ion (dead time loss).

We will describe these problems with reference to actual data. **Figure 8** shows a model spectrum. It is a magnified view of the protonated molecular ion (*m/z* 609.3) and its isotopic peak (*m/z* 610.3) when reserpine was analyzed in the AccuTOF™. One scale in the X axis is 1 ns, and the distance between two peaks is 33 ns.

Figure 9 shows the output from the amplifier in the flight cycle 1 and the data subsequently detected in the histogram memory. A small "child" following the peak in the amplified output waveform represents the signal distortion within the detector and amplifier (e.g., ringing, etc.). The threshold for the discriminator is 5 mV. Thus, the distortion in the detector and amplifier did not affect the results at all.

The output from the amplifier in the cycle 4 was 20 mV, approximately double the output up to the cycle 3. This suggests that two reserpine ions simultaneously arrived at the detector, while only 1 was added to the histogram memory.

The output from the amplifier in the cycle 5 has two peaks at an interval of 4 ns. This suggests that two reserpine ions with different initial energies or positions were not fully focused over time, arriving at the detector at different times. The TDC, due to its dead time, was unable to detect the latter ion.

The dead time loss of the TDC affects the intensity axis (height, area) of the mass spectrum peak as well as the peak position, that is, mass measurement accuracy. When two ions arrive at the detector during the dead time, the TDC will fail to detect the latter ion, shifting the peak position to the left (shorter flight time and lower mass number). This presents a serious problem in exact mass measurement. To analyze the mass number of reserpine within an error of 5 ppm, the AccuTOF™ should be able to determine the peak flight time within an error of 0.1 ns.

While it is known that the dead time loss of the TDC can be statistically corrected[13, 14] , incorrect parameters will produce incorrect results. It is also reported that the actual dead time is not a single "time" value, but a complex probability distribution function [13]. This is probably because the dead time function is determined by the complex factors resulting from the characteristics of the detector, discriminator, and TDC [13]. The key to accurate measurement is to define the dead time function as accurate as possible in actual operation of the TDC.

However, there is no effective correction when the ions increase in volume and multiple ions arrive at the detector simultaneously (Cycle 4 above).

Continuous averager

Figure 14 shows the principle of TOF mass spectral acquisition with a continuous averager. The continuous averager converts a signal from the amplifier with its high speed 8 bit analog/digital converter (ADC) to a digital value of 0 to 255 ($=2^8-1$), which is accumulated in the summing memory. In the TDC, the volume of information forwarded from the TDC to the histogram memory is relatively small, since it is proportional to the number of ions detected in one cycle. In the continuous averager, on the other hand, the volume of information forwarded from the ADC to the adder is enormous, since it is unrelated to the number of ions detected, but proportional to the data points on one spectrum. For example, if the ADC sampling interval is 2 ns (sampling frequency 500 MHz) the information flow will be 500 MB/sec. As a result, continuous digital averager devices with high counting accuracy (= shorter sampling interval = higher sampling frequency) were not available until advanced digital circuit technologies emerged.

Figure 15 illustrates the accumulation of mass spectral data with a continuous averager. The signal from the amplifier is the same as the TDC example (**Fig. 9 to 15**). The spectrum from the cycle 5, compared to that of TDC (Fig. 13), demonstrates:

- The signal distortion in the detector and amplifier is reflected in the accumulated data;
- The width of the signal from the detector and amplifier is reflected in the accumulated data, resulting in a lower apparent resolution than in the TDC spectrum.

TDC vs. Continuous averager

Table 6 summarizes advantages and disadvantages of the TDC and continuous averager (ADC), as well as the solutions incorporated in the AccuTOF™ to correct the problems with the continuous averager.

Dynamic range & Accurate Mass Measurement

Dynamic Range

Reserpine was analyzed in concentrations ranging from 10 pg to 100 ng to verify the dynamic range, one of the unique features of the AccuTOF™. A calibration curve was obtained from the area of the mass chromatogram of reserpine (M+H=609).

Conditions:

LC/MS analysis

Mobile phase:	A: Water (0.1% acetic acid)
	B: MeOH
Gradient:	20-100% : B (5 min linear gradient)
Flow rate:	0.2 mL/min
Column:	Mightsil 2.0 mm (I.D.) x 50 mm (L) (Kanto Kagaku)
Concentration/injection volume:	1 ppb to 10 ppm; 10µL

The calibration curve was obtained from the mass chromatogram area. The correlation coefficient obtained from the calibration curve was R=0.998, demonstrating good linearity.

Accurate mass measurement

Erythromycin was analyzed as an unknown sample 5 times with reserpine as an internal reference.

Conditions:

Sample introduction:	Flow injection
Mobile phase:	Methanol
Flow rate:	0.2 mL/min
Sample concentration:	0.5 ppm each
Sample volume:	10 uL

Table 8 shows the results. The average error in 5 experiments was less than 1 mmu.

The results demonstrate high mass accuracy and a wide dynamic range, two of the major features of the AccuTOF™.

Summary

We have introduced the features of the AccuTOF™, while outlining the principle and history of TOF MS. We also compared the data system of the AccuTOF™, one of its most outstanding features, to the conventional TDC method.

The AccuTOF™ is a powerful tool which combines the advantages of conventional LC-TOF MS, including high sensitivity, high resolution, and high mass accuracy, with a wide dynamic range and high durability. We hope that the AccuTOF™ will be of assistance to the users in routine applications as a simple, easy to use, and versatile TOF MS.

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- New Energy and Industrial Technology Development Organization (NEDO)
- Research Association for Biotechnology

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Sample Amount (pg)	Area
10	838
100	6587
1000	56015
10000	553844
100000	6507358

Table 7. Sample amount and chromatogram.

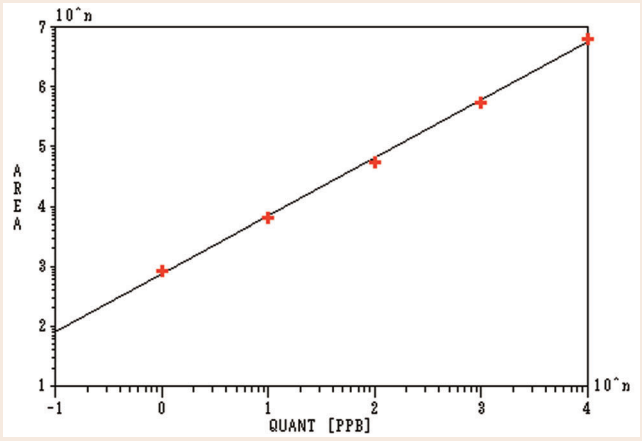

Fig. 16. Calibration curve of reserpine.

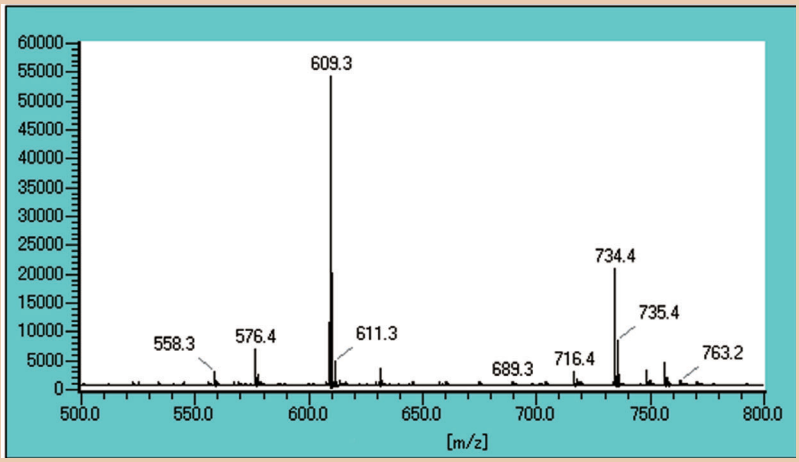

Fig. 17. Spectrum of reserpine and erythromycin.

Theoretical	Observed	Error (mmu)	Error (ppm)
734.4691	734.4700	0.94	1.28
	734.4701	0.95	1.30
	734.4699	0.84	1.14
	734.4700	0.87	1.19
	734.4700	0.88	1.20
	RMS	0.90	1.22

Table 8. Results of accarate mass analysis (5 experiments).