Recent Advances in HRAM Mass Spectrometry

Christian Klein, Agilent Technologies, Inc, Santa Clara, CA-95051, USA, Christian_Klein@agilent.com

In the last 20 years, mass spectrometry has evolved from purely academic instrumentation to a technique now present in most analytical laboratories. Together with ongoing improvements in seamless software workflows and capabilities, increases in sensitivity and resolution are key drivers for this development. Herein, we describe enhancements made to the following aspects of a typical high-resolution, accurate mass (HRAM) spectrometer: ion source, ion transmission, instrument tuning (for sensitivity improvements), detector adjustments, ion optics, electronics, and detector acquisition speed (for increased resolution). In addition, we also consider whether an increase in selectivity can be obtained by ion mobility and quadrupole-based techniques.

Introduction

Thompson's first description of a mass spectrometer in 1913 [1] was eventually followed by the introduction of time-of-flight (TOF) instrumentation in the mid 60s (Bendix TOFs); however, 20 more years passed before a TOF instrument would become commercially available with broader acceptance. Magnetic sector instruments provided sufficient resolution for accurate mass measurements but were lacking sensitivity [2] in scanning mode, as the resolution is in reverse proportion to the sensitivity. Fourier-transform ion-cyclotron resonance (FT-ICR) instruments with high resolution as well as sensitivity were an alternative to the magnetic sector instruments, but the new TOF instruments were also an appropriate answer, showing a resolving power (m/Δm) approaching that of the magnetic sector instruments. However, with further improvements, including the reflector (or reflectron) and the now commonly adopted orthogonal acceleration (OA) design, TOF instrumentation has effectively replaced the once dominant magnetic sector instrumentation since the millennium.

Figure 1 shows a typical setup of a modern LC/Q-TOF. The first component of the mass spectrometer is the ion source, where analytes are ionised. The most typical ion source used is electrospray ionisation (ESI), but other techniques, such as atmospheric pressure chemical ionisation (APCI) or atmospheric pressure photo ionisation (APPI), are available. After the ionisation process, ions are guided through a capillary into the vacuum chamber. The next elements encountered in the ion optics are the quadrupole, the collision cell, the pulser, a reflector (reflectron) in the flight tube, and the detector. The separation of ions in the flight tube based on different arrival times at the detector is the fundamental principle of TOF instruments. The signal from the detector is then processed to show a final *m/z* spectrum. These elements are common to all TOF instruments but vary in the details and modifications offered by different vendors [3].

Other HRAM spectrometry instruments include FT-ICR and the electrostatic ion trap, with the latter taking a large portion of the FT-ICR market since its introduction.

Experimental

Sensitivity

Perhaps surprisingly, the first option for increasing sensitivity is made before the analyte enters the mass spectrometer. Users may select between standard flow, low/micro-flow, or nanoflow. The common understanding is that the lower the flow rate, the higher the sensitivity. The determining factor here is the size of the droplets: the smaller the droplet, the fewer Coulomb fissions are needed to reach the final ionisation state. Here, different ionisation theories come into play - either the ion evaporation model (IEM) for small molecules, or the charge residue model (CRM), which is important for large molecules and proteins. An informed decision by the user has to be made. In particular, nanoflow is a challenge for routine analysis, as leak-free control of the flow path is notoriously difficult to achieve and hard to troubleshoot [4].

Microflow, therefore, could be the answer in an MS-centric world, but historically

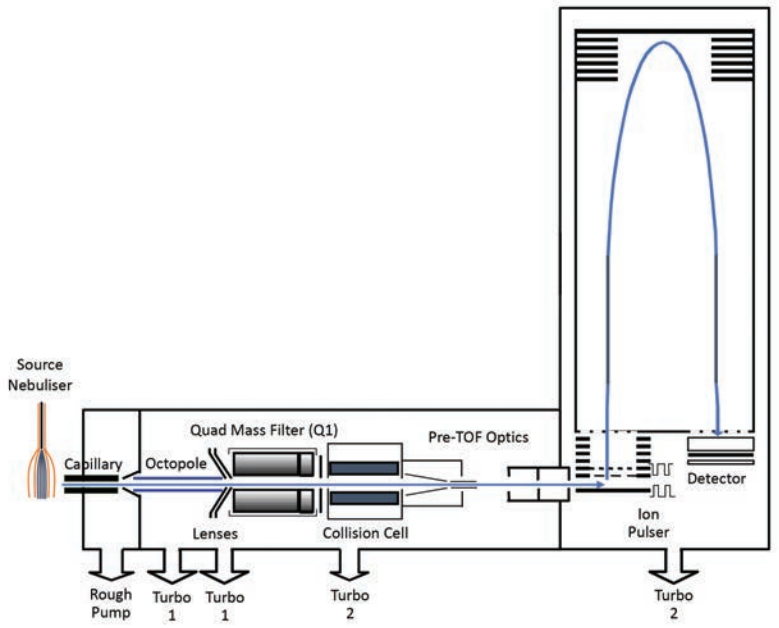

Figure 1: Schematics of a TOF instrument. Basic elements include the source, the quadrupole, collision cell, flight tune and detector

method development was performed on liquid chromatography (LC)-based systems, as UV or fluorescence detection, which did not focus on the requirements of mass spectrometry. The same applies for mobile phase additives; for example, trifluoroacetic acid (TFA), utilised for its ion-pairing capabilities, is suitable for chromatography but not mass spectrometry, due to a reduction in sensitivity by ion-supression [5]. Consequently, an ESI source is ideal, exhibiting the performance of a micro/nano-flow source, but at standard flow rates.

In 2007, the concept of the Agilent Jet Stream technology (AJS) was introduced, as an extension of the classic ESI source, and soon after was widely adopted by other vendors. The critical aspects of ESI are the applied voltage and temperature. Most ESI sources have an additional gas flow in the nebuliser, assisting in the desolvation of the liquid, as well as a drying gas, typically coming in a counterflow towards the nebuliser. The innovative aspect of Jet Stream technology was to introduce a third gas stream: the sheath gas. This extremely hot gas surrounds the outcoming liquid from the nebuliser, and leads to a thermal focusing, optimising the ionisation efficiency. This results in a source so efficient that it becomes independent of the liquid flow [6] (*Figure 2*), and sensitivity levels matching those of micro-flow rates can easily be achieved.

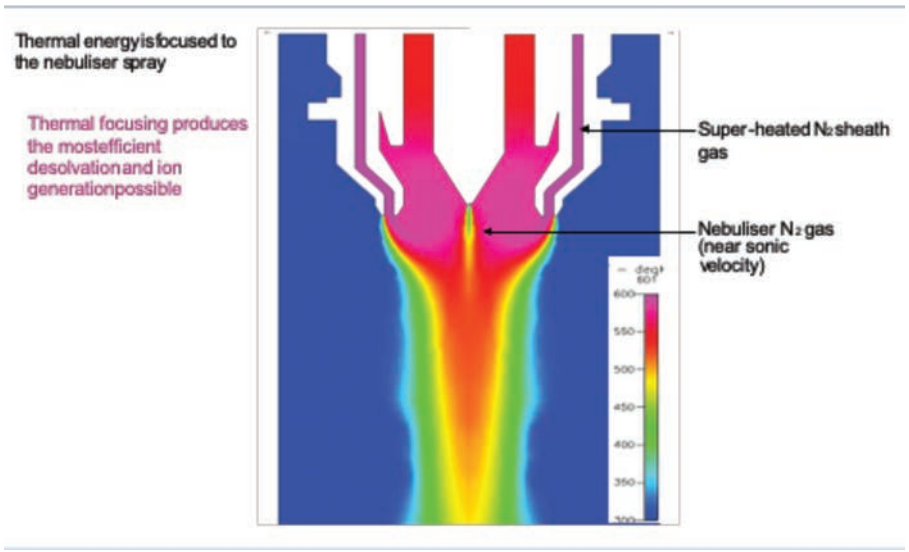

Figure 2. Simulation showing the thermal profile of the Agilent Jet Stream technology. Note the creation of a thermal confinement zone by introduction of a super-heated N_2 sheath gas.

In modern day mass spectrometry instruments, two design options are implemented following the initial analyte ionisation stage: skimmer-based entrance or ion funnels. Of these, ion funnels offer the advantage that ions are more effectively captured and guided into the high-vacuum region of the mass spectrometer, leading to improvements in sensitivity for small molecules. There is, however, a bias against high *m/z* species.

The concept of ion funnels was prototyped by R. Smith [7]. Ion funnels operate best in the low Torr range, which is relatively high for the entrance region after the capillary. As the only available gas comes from the capillary source, several modifications are needed to accommodate this factor, including a shorter capillary as well as multiple capillary inlets. Alternatively, the gas pressure can be regulated by the supply of an external gas source.

The next opportunity to gain instrument sensitivity is the trapping of ions. For some instruments, this trapping is optional, i.e., it occurs before the pulser region via lenses. In others, trapping is essential to ensure best functionality; an automatic gain control is used to avoid an overfill of the electrostatic trap and consequent space-charge effects leading to resolution loss.

Ion mobility adds another dimension of separation, and, as a result of removing background from different regions, sensitivity. The main advantage of ion mobility is the ability to separate molecules based on their collision cross section (CCS) or ion structure. Molecules can have the same *m/z* value, but very different structures, so ion mobility can distinguish between isomeric molecules [8]. The main techniques include: differential (ion) mobility spectrometry (DMS, commercialised by Owlstone as FAIMS), travelling wave (Waters), drift tube-based IM (Agilent), and trapped ion mobility spectrometry (TIMS, Bruker Daltonics).

Another important aspect for gaining sensitivity relies on instrument tuning. Manually tuning an instrument may be optional for advanced users to obtain the best results for resolution or sensitivity. However, this strategy is not suitable for an ever-growing market size with a finite number of specialists.

Automatic tuning of the instrument is therefore a valid solution. Nevertheless, determining the right boundaries and tunable parameter space proves challenging today. Multiple parameters impact the resolution, including grids in the pulser region, grids at the reflector, and voltages prior to entering the pulser. The complexity of these elements no longer permits multiple iterative cycles to determine the best combination, but rather requires tuning multiple elements simultaneously.

All TOF instruments increase in resolution as the *m/z* value increases. With a typical mass range of 3000 *m/z* for most TOF instruments, instruments are typically tuned for resolution where the impact is the highest, namely for high *m/z* species. However, metabolomics researchers and pesticide and environmental laboratories are often less interested in the best resolution at high *m/z*, preferring high sensitivity and good resolution at low *m/z*. An application tune needs to consider this performance requirement. Electrostatic traps, on the other hand, have excellent resolution at low mass, and is optimised for this range, but require different settings for larger molecules.

Resolution

As previously mentioned, the resolving power is defined as m/Δm between two peaks. In mass spectrometry, resolving power and resolution are frequently used interchangeably, and all instruments are specified by their resolution, which is R=m/FWHM, assuming that the peak width at half maximum of a single peak corresponds to the ability to separate two neighbouring peaks. The resolution on TOF instruments, defined in the time domain, is approximately R=TOF/Δt. To define Δt, the following equation is used: $\Delta t^2 = \tau TA^2 + \tau PW^2 + \tau R^2$, with TA for the turnaround time, PW for the detector pulse width, and R for residual terms.

In 2008, Bruker Daltonics introduced an instrument with a resolution of 40,000 (mass 2,722), which more than doubled the resolution possible with instruments from other vendors [3]. This was achieved by increasing the length of the flight tube with a single reflector. The three-meter-long flight tube, resulting in a nearly six-meter-long flight time of ions, is in direct relation to the turnaround time in the above equation. A similar resolution was then achieved by Waters using the W-mode, with multiple passes through grid-based reflectors. However, each pass through the reflectron grids leads to a loss in sensitivity. Agilent changed both the detector and ion optics, addressing opportunities in the detector peak width as well as the residual term. Several years ago, Sciex introduced an instrument using N-optics, resulting in an instrument balancing sensitivity loss and resolution increase.

Two main principles are used for detecting and converting the ions arriving at the detector: analogue-to-digital conversion (ADC) and time-to-digital conversion (TDC).

The TDC detector is an ion-counting detector. The basic principle is that they register the arrival of a single ion at discrete time bins, and the obtained counts are then summed together for all consecutive spectra. The pulse rate is typically in the kHz range, allowing thousands of spectra to be summed together.

On the other hand, ADC detectors digitise the pulsed ion current from the detector at discrete time intervals. The time intervals are in direct proportion to the sampling rate of the acquisition board. Whereas (for example) a 2 GHz acquisition board allows a maximum time interval of 500 picoseconds, this can be substantially reduced by higher acquisition boards. The 10 GHz acquisition boards of Agilent's latest GC and LC/Q-TOF (7250 and 6546) allow for time intervals of 100 picoseconds. It is noteworthy that the higher acquisition rates are only useful if the detector pulse width is as narrow as possible; otherwise users are exposed to a risk of oversampling. The most prominent effect of the faster digitisation occurs at low *m/z* species, because of the lower arrival times. A peak at low arrival times has, in absolute value, much narrower peak width compared to later arrival times/higher *m/z*. Due to this narrow peak, slow digitisation would lead to undersampling of the peak, and therefore lower resolution. The impact of the fast 10 GHz acquisition board is that previously undersampled low *m/z* peaks now have enough datapoints over the peak, and therefore a resolution at full width at half maximum (FWHM) reflective of their real peak shape. In general, based on the summation of ion currents, ADC detectors show a wider dynamic range and better isotope fidelity compared to TDC detectors, as the ion current is a better measure at high and low ion intensities.

In the electrostatic trap, a completely different principle for ion detection is used. The oscillation of ions is measured, converted into an *m/z* spectrum via Fourier transform (FT), and a single scan is used for the assembly of a spectrum. Here, the resolution is directly dependent upon the time allowed to measure the oscillation; the longer the measuring time, the better the resolution. An instrument can have a resolution at 1 Hz of 240,000, but at 3 Hz the resolution will drop to 70,000, and at 10 Hz to 10,000 (at *m/z* 200).

Acquisition modes

For a long time, mass spectrometry had two basic modes of operation: MS-only and tandem MS. The latter was split into two variants: auto MS/MS, which is a data-dependent acquisition (DDA) method, and targeted MS/MS. Targeted MS/MS is a mode similar to single reaction monitoring (SRM) or multiple reaction monitoring (MRM) on a triple quadrupole instrument. A variation of this mode, used on electrostatic traps, is called single ion monitoring (SIM), where a selected *m/z* species is isolated in the quadrupole and then accumulated in the C-trap. The ion of interest can then either be scanned directly, or fragmented and then analysed. In general, tandem MS follows the principle of precursor selection in the quadrupole followed by fragmentation (a collision cell in all TOF instruments, or ion trap-based fragmentation and/or collision cell). Over 10 years ago, Sciex introduced a new acquisition mode called SWATH - where instead of a distinct precursor isolation, a wide band of *m/z* species was isolated by the quadrupole (*Figure 3*). This new mode has the advantage that fragment information in intervals over the whole *m/z* range can be obtained. The most prominent application was proteomics; post-fragmentation, each peptide generates a sufficient number of fragmented ions used for the subsequent identification. Chromatography results typically showed broader peaks in nano-LC ranges, therefore this mode was widely accepted because users were able to identify and quantitate purer analytes. Versions of it are now adopted by all vendors.

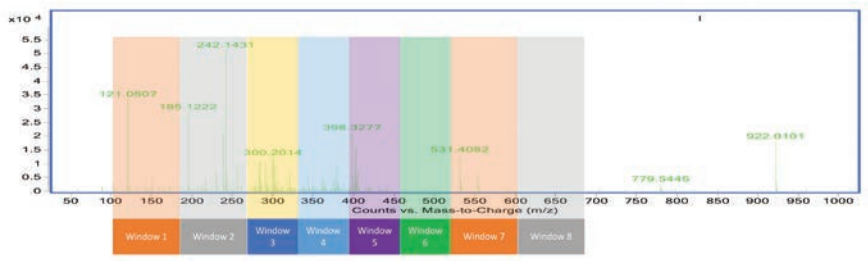

Figure 3: Schematics of a wide band quadrupole experiment. Similar to All Ions, but using the quadrupole to take sequential windows of the mass range to reduce complexity of the MS/MS spectra.

MS^E (Waters) and All Ions (Agilent) are instrument acquisition modes that are capable of fragmenting all analytes without any prior quadrupole isolation. Both wideband isolation and full-spectrum fragmentation are part of the data-independent acquisition (DIA) modes.

The most sophisticated acquisition mode is auto MS/MS, a DDA mode where several variables must be confirmed prior to selecting the precursor. These include charge state determination, abundance threshold/ranking, isotope grouping, neighbouring peaks, chromatographic apex prediction, number of selected precursors per cycle, precursor exclusion, and abundance-dependent acquisition time to name a few, followed by prediction of the most suitable collision energy (typically in proteomics experiments).

Results and discussion

Each acquisition mode described above has a defined application role: an MS-only run is the method of choice for screening applications, while auto MS/MS is used in proteomics and other identification-based workflows, and targeted MS/MS is used for quantitation.

Waters MS^E, with the ability to deconvolute fragments in the MS/MS domain and align with one (or more) coeluting precursor ions from the MS domain, overcomes the limitations of a precursor selection-based approach, and the limitation of the duty cycle. However, the identification process is solely dependent upon the SW algorithm. With several thousand peaks per spectrum, the correct assignment of fragment ions to a molecular ion is challenging. SCIEX's SWATH increases the selectivity by using multiple quadrupole precursor ion windows, producing significantly less complex fragment ion spectra. However, proteomics remains the key application.

Updates to European food safety guidelines (SANTE/11813/2017) [9] require analyte peaks from precursors and/or product ion(s) to fully overlap in the extracted ion chromatograms of HRAM spectrometry instruments. This is a substantial shift from previous MS-only screening, whereby the identification was traditionally done by mass accuracy, retention time (RT), and where applicable, isotope fidelity. Classic compound ID workflows using a single spectrum for identification were also not acceptable by the new guidelines. As quantitative experiments require at least 12 datapoints over the chromatographic peak, targeted MS/MS experiments are theoretically possible, but would require accurate RT knowledge of the compound. In contrast, for targeted quantitation, standards are needed to build a concentration curve from which the RT is apparent. This is not always the case for suspect screening or nontargeted screening, where the RTs are either totally unknown, based on a prediction, or merely suspected [10].

In such cases, depending on the size of the database that the suspect screening is performed against, too many targeted precursors may be coeluting, and the requirements of the 12 datapoints over the chromatographic peak are limited by the duty cycle. The only viable options for dealing with this are a DIA workflow, using either an MS^E/All Ions workflow, or a SWATH-like quadrupole wideband selection. For the latter, the number of possible windows (and used collision energies) are limited again by the duty cycle. Even a moderate number of six windows and a fixed acquisition rate of 6 Hz in MS would require about 20 Hz in MS, and 10 windows, about 30 Hz. A TOF instrument under these fast acquisition rates would reveal a lower dynamic range compared to 3 Hz acquisition rates, and electrostatic trap instruments would exhibit lower resolution.

Conclusion

High resolution accurate mass spectrometry shows the fastest growth rate of all mass spectrometer techniques. This is due to substantial increase in both resolution and sensitivity, accompanied by acquisition modes and workflows suitable for nearly all applications in research and routine analysis. With all vendors competing in the space, no single specification can be used to determine suitability; rather, the whole performance spectrum needs to be considered.

Acknowledgement

Special thanks to Pat Perkins, David Weil, and George Stafford for providing critical insights to this publication and careful review.

References

1. *Thompson, J.J. (1913). 'Rays of Positive Electricity and Their Application to Chemical Analyses'. Longmans, Green and Company*
2. *Pramanik, B.N., Ganguly A.K., Gross, M.L. (2002) 'Applied Electrospray Mass Spectrometry' in Practical Spectroscopy Series Volume 32, 36-37*
3. *Gelpi, E. 'From large analogical instruments to small digital black boxes: 40 years of progress in mass spectrometry and its role in proteomics. Part II 1985–2000'. J. Mass Spectrom. (2009) 44, 1137-1161*
4. *Noga, M., Sucharski, F., Suder, P., Silberring, J. 'A practical guide to nano-LC troubleshooting' J. Sep. Sci. 2007, 30, 2179 – 2189*
5. *Eshraghi, J., Chowdhury, S.K. 'Factors affecting electrospray ionization of effluents containing trifluoroacetic acid for high-performance liquid chromatography/mass spectrometry'. Anal. Chem. (1993) 65, 3528-3533*
6. *Mordehai, A., Fjelsted, J 'Agilent Jet Stream Thermal Gradient Focusing Technology' (2009) Agilent publication 5990-3494EN*
7. *Shaffer, S.A., Tang, K., Anderson, G.A., Prior, D.C., Udseth, H.R., and Smith, R.D. (1997). 'A Novel Ion Funnel for Focusing Ions at Elevated Pressure using Electrospray Ionization Mass Spectrometry'. Rapid Communications in Mass Spectrometry. 11 (16): 1813–1817*
8. *May, J.C., Goodwin, C.R., Lareau, N.M., Leaptrot, K.L., Morris, C.B., Kurulugama, R.T., Mordehai, A., Klein, C., Barry, W., Darland, E., Overney, G., Imatani, K., Stafford, G.C., Fjeldsted, J.C., and McLean, J.A. (2014). 'Conformational Ordering of Biomolecules in the Gas-Phase: Nitrogen Collision Cross Sections Measured on a Prototype High Resolution Drift Tube Ion Mobility-Mass Spectrometer'. Analytical Chemistry 86(4), 2107–2116.*
9. *https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf*
10. *Schymanski, E.L.1, Singer, H.P., Slobodnik, J., Ipolyi, I.M., Oswald, P., Krauss, M., Schulze, T., Haglund, P., Letzel, T., Grosse, S., Thomaidis, N.S., Bletsou, A., Zwiener, C., Ibáñez, M., Portolés, T., de Boer, R., Reid, M.J., Onghena, M., Kunkel, U., Schulz, W., Guillon, A., Noyon, N., Leroy, G., Bados, P., Bogialli, S., Stipani ev, D., Rostkowski, P., and Hollender, J. (2015). 'Non-target Screening with High-Resolution Mass Spectrometry: Critical Review using a Collaborative Trial on Water Analysis'. Anal Bioanal Chem. Aug; 407(21):6237–55.*