



The Potential for Ion Mobility in Pharmaceutical and Clinical Analyses

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Abstract

The pharmaceutical and clinical industries are imperative for the maintenance of global health and welfare and require accurate, reproducible, and high throughput analyses. Technological advancements, such as the development and implementation of liquid chromatography-tandem mass spectrometry (LC-MS), have allowed for improvements in these areas, however there is still room for development. One way in which current analyses may be improved is by the implementation of ion mobility technology. Ion mobility has the capability to produce much more comprehensive data sets, by providing separation of isomers, as well as improving throughput, with separations being performed as fast as 60 ms. Here we will discuss the potential for ion mobility to assist in the two specific areas of glycosylation monitoring of biological drugs, and vitamin D analysis, as representatives of ion mobility's potential in both the pharmaceutical and clinical industries, respectively, as well as the current hurdles of ion mobility adoption in both fields.

Keywords

SLIM (Structures for Lossless Ion Manipulations) · Ion mobility · Mass spectrometry · Vitamin D · Glycosylation · Pharmaceutical · Clinical

Abbreviations

ACN	Acetonitrile
BLA	Biological License Application
CCS	Collisional cross section
CLIA	Clinical laboratory improvement amendments

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CQA	Critical quality attribute
DTIMS	Drift tube ion mobility spectrometry
FA	Formic acid
FAIMS	Field asymmetric ion mobility spectrometry
FDA	Food and Drug Administration
HILIC	Hydrophilic interaction liquid chromatography
IMS	Ion mobility spectrometry
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
MS	Mass spectrometry
RP	Reverse phase
SLIM	Structures for Lossless Ion Manipulations
TIMS	Trapped ion mobility spectrometry
TOF	Time of flight
TWIMS	Traveling wave ion mobility spectrometry

17.1 Introduction

The pharmaceutical and clinical industries are two of the most important industries in terms of global health and welfare, as they are responsible for diagnosing and monitoring diseases, and developing drugs to treat those diseases. Countless numbers of analyses are performed every day as a part of either clinical testing or drug development. In 2011, a survey of 77 clinical laboratories reported having performed a combined total of 104 million Clinical Laboratory Improvement Amendments (CLIA)-reported tests [1]. In 2017, there were 14,872 drugs in the research and development pipeline, undergoing extensive characterization to test safety, efficacy, toxicity, half-life, etc. [2]. The ways in which these analyses are performed has evolved over time with significant improvements in analytical instrumentation, such as the introduction of liquid chromatography (LC) and mass spectrometry (MS), allowing for greatly enhanced accuracy and sensitivity. However, there are still advancements that can be made to improve these analyses further. Here we will describe in more detail one common pharmaceutical analysis,

the glycosylation monitoring of biological drugs, as well as one common clinical analysis, Vitamin D testing, and will discuss current analytical techniques as well as how a more recent technology, ion mobility, can aid in these two specific areas.

17.2 Glycan Profiling of Biological Drugs

17.2.1 Background

In the pharmaceutical industry, the number of biological drugs is ever increasing, accounting for over one third of all drugs in clinical trials, or awaiting Food and Drug Administration (FDA) approval [3]. The close and consistent monitoring of these biological drugs is crucial, as very small changes, such as the change in a glycosylation site, could have large effects on potency, efficacy, and toxicity of that drug [4]. To ensure that all entities that could possibly affect the drug and its properties are monitored, a list of critical quality attributes (CQAs) are identified. The FDA defines a CQA as “a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.” This generally includes characteristics and properties that affect a drug's stability, identity, biological activity, and purity [5]. As mentioned, glycosylation plays a vital role in safety, efficacy, and potency of drugs, and is often times the most difficult to control [4]. From January of 2016 through September of 2017, 25 new drugs were granted Biological License Applications (BLAs) by the FDA, of which 24 featured glycosylation moieties of some form [6]. Because of this, the ability to very accurately, reproducibly, and quickly monitor the glycosylation of biological drugs throughout all stages of the drug development and manufacturing process is crucial. Several CQAs have been identified specifically for glycosylation and include: site-specific glycation profiles and occupancy, antennary profile, sialylation state, isoform distribution, fucosylation, acetylation, lactosamine repeats, galactosylation, high mannose residue composition, and others [4]. However, the sample preparation and analytical methods to monitor these CQAs are much less defined and often vary from lab to lab, as well as within lab, often causing inconsistent results [7]. Analytical methods currently used for glycan monitoring in biological drugs are discussed in greater detail in the following paragraph.

17.2.2 Current Technologies

Glycosylation monitoring of biological drugs can be performed at many levels including analysis of released glycans, glycosylated peptides, and glycosylated intact proteins.

Typically, glycosylation monitoring is carried out using a separation method that is coupled to a detector. Two of the most common separation methods include chromatographic and electrophoretic techniques.

Chromatographic techniques, such as high-performance liquid chromatography (HPLC), including hydrophilic interaction liquid chromatography (HILIC) and reverse phase chromatography (RPC), are often used when analyzing glycans [8]. HILIC employs the use of a polar stationary phase and mostly organic mobile phase, whereas, in RPC a non-polar stationary phase is used with a polar mobile phase to provide separation. The analytes affinity for the stationary phase, versus the changing mobile phase allows for elution of analytes at different times (retention time), providing separation of the analytes present [9]. These techniques are often coupled with an ultraviolet (UV), fluorescence, and/or a mass spectrometry detector, such as electrospray ionization-time of flight (ESI-TOF), or electrospray ionization-quadrupole time of flight (ESI-QTOF) [4]. HILIC is often the preferred technique when investigating released glycans due to high selectivity and resolution provided compared to other HPLC methods [8, 10, 11]. This was demonstrated in a study performed by Melmer et al. where they compared the performance of three chromatographic techniques (HILIC, RPC, and porous graphitic carbon), in released glycan analysis. They determined that the three methods are highly complementary to one another, but HILIC is most desirable due to its high selectivity for glycans [8]. RPC is often used when performing analyses on glycopeptides and intact glycoproteins [12–14]. Due to the complementary nature of these two methods, when performing complex glycan profile characterization, a combination of these chromatographic methods may be necessary [8].

Electrophoretic techniques, such as capillary-zone electrophoresis (CZE), capillary isoelectric focusing (CIEF), high performance capillary electrophoresis (HPCE), and capillary-gel electrophoresis (CGE), are typically used for intact-protein glycoprofiling [15–17] but can also be used in released glycan investigations as well [18–20]. Capillary electrophoresis is a technique that employs an electrical gradient applied to a sample in the liquid phase in order to separate analytes based upon their charge and size [21]. These electrophoretic separation techniques can be paired with one of many detectors including ultraviolet absorbance, fluorescence, amperometry, mass spectrometry, and others [22]. Many groups have begun using CE as a separation method for glycan analysis rather than chromatographic techniques due to its increased resolution and higher throughput [18]. For example, Balaguer et al. employed CE for the characterization of intact proteins where they were able to separate glycans differing in degrees of sialylation [23]. Additionally, Jayo et al. used CE to study N-glycans from recombinant human erythropoietin, and Chinese hamster ovary. In this study they were able to identify

over 70 glycans and concluded that CE-MS based methods could greatly complement currently established glycan characterization workflows [24].

Although these technologies are currently state-of-the-art for glycan characterization of biological drugs, they do suffer from several significant challenges which will be discussed in the following section.

17.2.3 Current Challenges

Current analytical techniques used for glycoprofiling exhibit a number of key shortcomings. These primarily include the time for analysis (throughput) and quality of data. More specifically, data often suffers from a lack of reproducibility as well as an inability to differentiate between isobaric (identical molecular weight) molecules. Two examples of limited throughput are seen in currently employed methods used to analyze high mannose structures, as well as those used to identify glycans based on glucose unit (GU) calibration from the dextran ladder standard. Both of these samples are typically run using a HILIC method that is either 34 min long in the case of the mannose standard, or 42 min long in the case of the dextran ladder [25, 26]. When pharmaceutical companies are investigating thousands of drugs, and those drugs need to be extensively monitored at every step throughout the drug development process, this creates a large bottleneck in their ability to process large sample sets. In addition, the multimodal partitioning present in HILIC often results in reproducibility problems, creating challenges for data processing and maintaining sufficient data quality across the sample set. Finally, HILIC and other LC methods often lack resolution in complex mixtures, and sometimes in clean standards as well [26]. Often chromatographic methods are unable to separate isobaric molecules, leading to isomers being present under the same chromatographic peak [25, 26]. Pharmaceutical companies must maintain the same glycan profile throughout the whole development process, and any company hoping to make a biosimilar must also mimic this profile, as the FDA states that differences in glycosylation lead to the drug no longer being biologically equivalent [4]. This is extremely disconcerting, as different isomers may have largely different effects on the drug and its safety. If companies mimic the broad glycan profile that encompasses isomers under the same chromatographic peak, differences in the isomeric composition are not necessarily taken into consideration, and thus the drugs could be biologically different, despite having identical analytical characterization profiles. The use of ion mobility technology may be incredibly helpful in alleviating many of these pain points and will be discussed in further detail in following sections. Another area of interest for potential ion mobility integration is in clinical analyses.

17.3 Vitamin D Analysis

17.3.1 Background

Vitamin D deficiency is a global epidemic with over 125 million people affected in the United States and over one billion people affected worldwide [27, 28]. Most experts define Vitamin D deficiency as having 25-hydroxyvitamin D levels less than 20 ng/ml in serum, as shown in Table 17.1 which displays different levels of 25-hydroxyvitamin D in serum and their significance [29].

The main source of vitamin D for humans is sunlight exposure [28–30]. When exposed to sunlight, the 7-dehydrocholesterol in the skin absorbs the ultraviolet B (UVB), forming previtamin D₃. Due to its inherent instability, previtamin D₃ is then quickly converted to Vitamin D₃ by a temperature dependent process [31]. The inability to properly absorb and process vitamin D leads to low levels of vitamin D in the body, and thus vitamin D deficiency. As we age, the levels of the Vitamin D₃ precursor 7-dehydrocholesterol significantly decreases, reducing the ability to make vitamin D. As a result, the elderly are one of the most at risk populations [32–35]. Vitamin D deficiency can lead to several extremely serious health issues including growth retardation in children, and cancer, osteoporosis, autoimmune diseases, and mental disorders in adults [28]. Because of its role in bone and overall health, it is recommended that everybody be tested for vitamin D deficiency [32, 34, 35]. The ability to accurately and rapidly test for vitamin D levels in human serum is critical as misdiagnosis can lead to over or under treatment, and in turn could lead to potentially life-threatening diseases [28, 32, 34, 35]. Current analytical techniques used to measure human vitamin D levels in serum will be discussed further in the following section.

17.3.2 Current Technologies

The two main techniques used for vitamin D analysis are immunoassays and LC-MS/MS. Direct immunoassays used to be the testing method traditionally used for vitamin D analyses and are in some cases still used today [32, 35]. These tests employ the use of antibodies specific to the vitamin D metabolites 25-hydroxyvitamin D₂ and 25-hydroxyvitamin

Table 17.1 25-hydroxyvitamin D levels in serum with corresponding significance

25-hydroxyvitamin D level in Serum (ng/ml)	Diagnosis
<20	Vitamin D deficiency
21–29	Insufficient vitamin D
30–149	Sufficient vitamin D
>150	Vitamin D toxicity

D₃ which are detected and quantified based on a chemiluminescent reaction. For example, two of the largest diagnostic labs, Quest Diagnostics and LabCorp, employ the use of immunoassays for total vitamin D testing and analysis [36, 37]. An additional analytical technique that has come to the forefront of clinical diagnostics testing is liquid chromatography tandem mass spectrometry (LC-MS/MS). LC-MS/MS analyses are now considered the “gold-standard” technique for vitamin D analysis based on its adoption by the National Institute of Standards and Technology (NIST) and the Centers for Disease Control and Prevention (CDC) for reference methods [32, 35]. This adoption has come as a result of the increased sensitivity, selectivity, and reproducibility obtainable with LC-MS/MS methods compared to immunoassays [35]. Nearly all major mass spectrometry instrumentation vendors have sought to demonstrate their systems’ capability to analyze the 25-hydroxyvitamin D₂ and 25-hydroxyvitamin D₃ metabolites. This not only shows the capabilities of each individual system, but also provides validation of this clinical analysis within the mass spectrometry industry. Additionally, clinical laboratories, such as Quest and LabCorp have implemented LC-MS/MS methods for vitamin D analyses in their testing as well [36, 37]. Because these tests are much more specific, there are several different tests that may be used depending on the type of vitamin D analysis needed. For example, the option to monitor 1,25-Dihydroxy-vitamin D instead of, or in addition to the 25-hydroxyvitamin D₂ and 25-hydroxyvitamin D₃ metabolites is provided. This is useful for the monitoring of vitamin D treatment in individuals with chronic kidney disease [38].

17.3.3 Current Challenges

Direct immunoassays have traditionally been used for vitamin D testing; however, they have been found to present several drawbacks. The most prominent challenge faced by immunoassay measurements is in the specificity of the antibody used. Often, rather than binding only the 25-hydroxyvitamin D₂ and 25-hydroxyvitamin D₃ metabolites, other vitamin D metabolites will bind as well, leading to inaccurate results, as one number for total vitamin D is reported, rather than separate values for each metabolite. This can unfortunately lead to misdiagnoses and insufficient treatment. Another problem that may occur is that the antibodies used often do not have 100% affinity for both 25-hydroxyvitamin D₂ and 25-hydroxyvitamin D₃. Because of this, the lack of affinity to one metabolite may lead to a lower total vitamin D being reported than what is present, leading to potential over-medication. Finally, immunoassays frequently lack sensitivity, again leading to over-medication [32, 34, 35]. The implementation of LC-MS/MS in clinical laboratories has alleviated some of these problems, allowing

for much higher specificity and higher sensitivity as well. However, there are still some challenges that are present when using LC-MS/MS, mainly the tradeoff between throughput and resolution. LC-MS/MS analyses are able to provide high throughput analyses with run time of just 2-min, as shown by Chinarkarova et al., however, the two vitamin D metabolites are separated by only 0.02 min in retention time [39]. This lack of separation fails to provide the two-point confirmation of both retention time and *m/z* preferred for diagnostic LC-MS/MS analyses. This two-point confirmation is achievable when using a longer LC gradient, as shown by Jarvis et al., but throughput must be sacrificed in this case, given the threefold increase in run time required [40]. Due to the prevalence of vitamin D deficiency, and the high quantity of vitamin D tests performed, it is imperative that the capability to perform high throughput and extremely accurate analyses is developed. Ion mobility technology offers one solution to the previously mentioned challenges.

17.4 Ion Mobility Technology Background

Ion mobility spectrometry (IMS) is a powerful analytical technique that has seen widespread growth over the last 40–50 years. IMS is a gas phase ion separation technique which takes advantage of differences in the mobilities of ions under the influence of an electric field. As will be discussed, ion mobility separations can be achieved under a variety of conditions, including ambient pressure or vacuum, static or dynamic electric fields, moving or stationary gas flow, all producing separations of molecules which occur in space, time, or both. IMS has several capabilities as a stand-alone instrument and has been used to achieve detection of atmospheric compounds [41–43], explosives [44–46], chemical warfare agents (CWAs) [47, 48], and petrochemical reagents [41]. In recent years, the coupling of IMS with MS has extended the capabilities and applications of the technique tremendously. IMS–MS is extremely useful for obtaining structural information on everything from small polyatomic ions [49, 50] to macromolecular ions, such as proteins [51–53] and even viruses [53]. IMS–MS instruments can be operated in modes which take advantage of IMS as a separation device allowing complex mixtures to be investigated and low-abundance species to be detected owing to the removal of chemical noise. Furthermore, IMS–MS provides fast measurements which allow it to be compatible with other front-end analytical separations, such as liquid chromatography (LC) [54–56]. Here we will briefly describe the main ion mobility techniques and how ion mobility can assist in the previously mentioned pharmaceutical and clinical fields, with specific examples provided for glycan profile monitoring of biological drugs and vitamin D analyses.

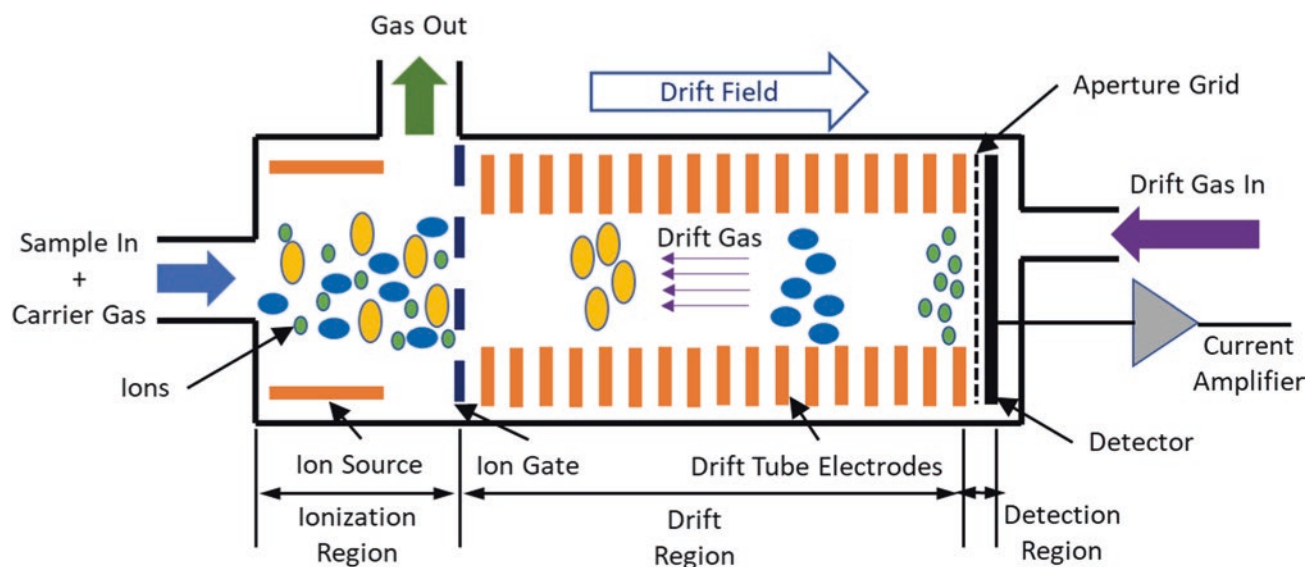


Fig. 17.1 Schematic of a traditional DTIMS system showing three ions of different size in the reaction region and then migrating at different velocities in the drift region

17.4.1 Drift Tube Ion Mobility Spectrometry

The oldest and simplest ion mobility technique is drift tube ion mobility spectrometry (DTIMS) [57]. In DTIMS, illustrated in Fig. 17.1, the sample is introduced to a region where ionization can take place by several methods including β -emission from a ^{63}Ni corona discharge, photo-ionization, electrospray, etc. The newly created ions are accelerated through an electric shutter grid, while neutrals remain in the ionization source and the measurement time is initiated. The drift-tube can vary in length from 5 cm to 2 m or more [58, 59]. An electric field gradient, typically from 2.5 to -20 V cm^{-1} , from the ionization source to the detector causes the ions to traverse the drift-tube at a constant velocity [60, 61]. A neutral drift gas is introduced counter to the flow of ions [57, 62–66]. The ions collide with neutral buffer gas molecules and exit via a detector such as a Faraday plate. The gas collisions in combination with the electric field provides separation of the ions based on size and charge. Smaller ions will reach the detector first, as they do not have as many collisions and interactions with the gas while larger ions with more gas collisions will exit the drift tube at a later point in time [62]. The inert gas used also serves to keep the drift tube free of background compounds which could interfere with the separation, though sometimes dopant compounds can be used advantageously [46, 67].

Although DTIMS suffers from an inherent lack of sensitivity, due to the fact that it is a pulsed analysis (where ions are measured in packets), and results in a subsequent loss in duty cycle, as the time between packets of ion is not utilized, it does have a significant advantage in that it is able to directly calculate collisional cross section (CCS) without calibration

and it provides extremely high resolving power [68]. The CCS Ω_r , at a temperature T can be obtained by measuring the velocity of the ions and solving the Mason-Schamp equations at weak electrical fields ($5\text{--}100 \text{ V cm}^{-1}$)

$$v_d = KE \quad (17.1)$$

where v_d is the drift velocity of the ion, K is the ion mobility constant, and E is the imposed electric field, and

$$\Omega_r = \left(\frac{3ze}{16N} \right) \left(\frac{2\pi}{\mu kT} \right)^{\frac{1}{2}} \left(\frac{1}{K} \right) \quad (17.2)$$

where z is the numerical charge of the ion, e is the elementary charge, N is the number density of the buffer gas, μ is the reduced mass of the ion-buffer gas neutral pair, k is the Boltzmann constant, and T is the temperature in Kelvin. CCS can be an extremely useful measurement as it correlates to the shape of an ion, and therefore can be used as an independent identification parameter, and as a tool to provide researchers with structural information for their analytes [69].

17.4.2 Trapped Ion Mobility Spectrometry

Trapped ion mobility spectrometry (TIMS) was previously described by Fernandez-Lima and co-workers using a stacked ring ion trap in a mobility-selective mode [70–72]. In contrast to conventional drift tube ion mobility spectrometry (DTIMS) in which ions are pushed through a stationary gas, in TIMS the analyte ions are held in place against a moving gas. Therefore, the achieved mobility separation and analysis range in a TIMS device depends on the bath gas drift

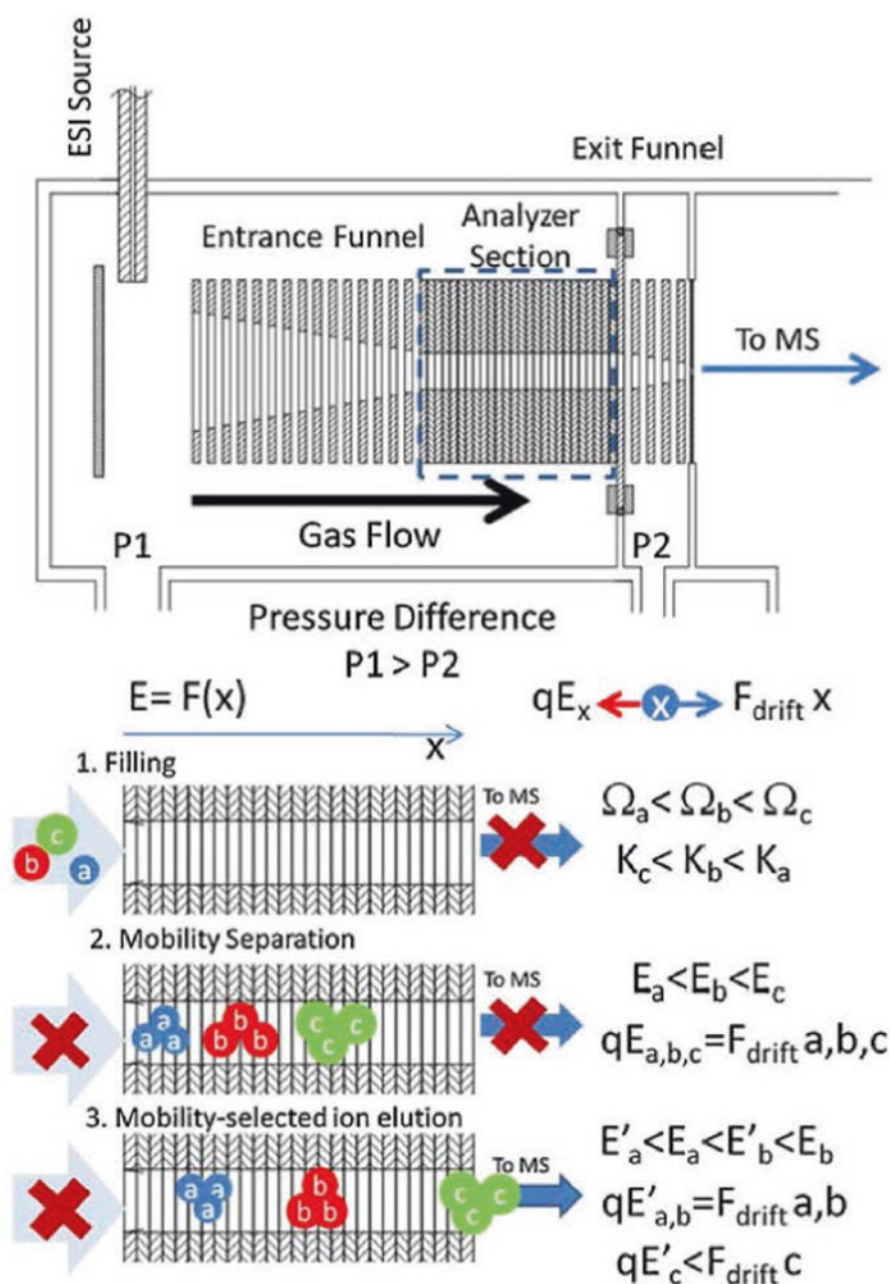
velocity, ion confinement and ion elution parameters [70]. The TIMS funnel is comprised of three main regions: the entrance funnel, the mobility analyzer section, and the exit funnel [72]. In TIMS analyzer section, ions are pushed forward using a defined gas flow and trapped against a counter potential. Each ring electrode is divided into quadrants where a quadrupolar confining-RF potential is applied on each of the four quadrants to radially confine the ions during the ion trapping and analysis [71, 73].

As shown in Fig. 17.2, there are three steps that are needed to be performed for a successful TIMS-based separation which include filling, mobility separation and mobility-selected ion elution [70, 72]. During filling, ions are pushed

through the entrance funnel and trapped in the mobility analyzer section. The electric field in the mobility separation section is increased along the axial section and the electric field compensates the gas drift force. After ions are injected and thermalized, the electric field in the mobility separation section is slowly decreased, and ions elute as a function of their mobility starting with those having lowest mobility [71].

TIMS devices can be easily integrated in a mass spectrometer without a noticeable loss in ion transmission or sensitivity and can work at 100% duty cycles. They can also be easily tuned from low to high resolution achieving resolving powers of ~250 in accordance with the analytical challenge

Fig. 17.2 Schematics of a TIMS device and operation. Ions are injected using a heated capillary orthogonal and focus towards the mobility separation section by ion funnel 1 (step 1, filling). Ion packages are separated as a function of their size-to-charge ratio and will be trapped in regions where the drift force is compensated by the electric field force (step 2, separation). Noticed that the electric field increases along the device axis; i.e., ions with different size-to-charge ratios are trapped at different axial positions. When the electric field is decreased, ions packages will elute from high to small size-to-charge ratios (step 3, elution) (Source: Int J Ion Mobil Spectrom. 2011 September; 14(2-3): <https://doi.org/10.1007/s12127-011-0067-8>.)



[69, 70, 72, 74]. Similar to DTIMS, TIMS devices have the capability of generating valuable CCS data, however calibration is needed to do this [71, 75]. Because of this, TIMS devices have been utilized extensively in several separation applications, such as the identification of glycans and for the separation and identification of lasso from branched cyclic peptides, etc. [76–80].

17.4.3 Field Asymmetric Ion Mobility Spectrometry

High-field asymmetric waveform ion mobility spectrometry (FAIMS) is a technique that separates gas-phase ions at atmospheric pressure and room temperature [81, 82]. FAIMS works similarly to conventional drift tube ion mobility spectrometry (DTIMS) in that it applies the motion of ions produced by an electric field to achieve separation [83]. The major difference between the two techniques is the magnitude of and method of applying the electric field. In DTIMS, a low constant electric field is applied in the same direction of separation and is used to move ions through the drift gas inside the spectrometer, as previously described. However, in FAIMS, a much higher electric field is applied perpendicular to the direction of separation and the drift gas is used to move the ions in the direction of separation. Also, the electric field in FAIMS is not constant, with an asymmetric waveform alternating between periods of opposite-polarity (Fig. 17.3).

The principles of operation of FAIMS were first described by Buryakov et al. [84] and further detailed by Guevremont et al. [81, 82, 85]. Ions that are subjected to an electric field in a drift gas will move along the applied field with a velocity given by Eq. (17.1). The dependence of an ion's mobility on field strength provides FAIMS its separation power.

Specifically, ions are separated in FAIMS by their difference in mobility at high (K_h) and low (K) electric fields [84]. At a constant gas number density, N , the non-linear dependence of the ion's mobility in high electric fields is given by Eq. (17.3):

$$K_h(E) = K \left[1 + \alpha \left(\frac{E}{N} \right)^2 + \beta \left(\frac{E}{N} \right)^4 + \dots \right] \quad (17.3)$$

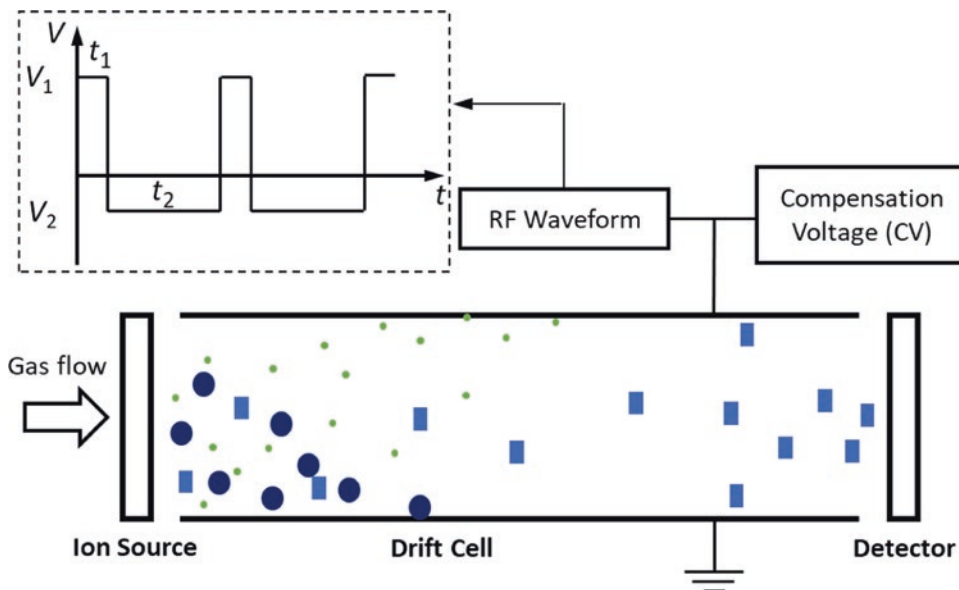
Where K is the ion mobility coefficient at low electric fields and α and β are arbitrary numbers found experimentally that describe the dependence of the ion's mobility at a high electric field in a drift gas [81, 85–87].

There are numerous geometries (planar, curved etc.) of electrode for FAIMS. In general, planar geometries typically offer higher specificity and resolving power at a cost of lower transmission, whereas curved geometries offer greater transmission due to an electrostatic focusing effect at a cost of lower resolving power [81]. For planar FAIMS (Fig. 17.3), to generate the high fields used, typically a radio frequency (RF) asymmetric waveform is applied to one plate while the other plate is held at or near ground. This asymmetric waveform, $V(t)$, is composed of a high-voltage portion, often referred to as dispersion voltage (DV, 0 to peak) applied for a short period of time (V_1 and t_1) and a lower voltage, opposite-polarity portion applied for a longer of time (V_2 and t_2). The sum of the time-voltage products of the waveform is equal to zero, as shown in Eq. (17.4) [81, 84, 85]:

$$V_1 \times t_1 + V_2 \times t_2 = 0 \quad (17.4)$$

As a result of the high-field dependence on ion mobility, ions will experience two different mobilities during one cycle of the asymmetric RF waveform, which generates two different velocities (Eq. 17.1). As ions are moved pneumatically

Fig. 17.3 Illustration of the asymmetric RF waveform (DV), compensation voltage (CV) for planar FAIMS. Ions oscillating between the FAIMS plates are transmitted through at a fixed DV and CV. Only ions in a 'balanced' condition can successfully travel through the FAIMS plates



through the cell perpendicular to the applied field. They will experience a net displacement towards one of the electrodes if there is a large enough difference between the two velocities. Once an ion experiences enough cycles of the asymmetric waveform, it will strike one of the two electrodes and be neutralized. To offset an ion's net displacement a small direct current (DC) potential is applied to one of the electrodes and is referred to as a compensation voltage (CV). Since K_h is ion-dependent, the magnitude of the CV needed to offset the net displacement will be different for different ions [88–90]. Under the conditions appropriate to transmit one ion, i.e., correct DV and CV, other ions will drift towards one of the plates and be lost. FAIMS spectrometers essentially act as ion filters which selectively transmit ions with the appropriate ratio of K_h to K . A mixture of compounds can be analyzed by scanning the CV over a range of voltages, producing a CV spectrum, the CV can also be held constant, allowing specific ions to be transmitted.

17.4.4 Traveling Wave Ion Mobility Spectrometry

Traveling wave ion mobility spectrometry (TWIMS) was introduced by Giles, et al. in 2004 and was introduced commercially by Waters Corporation in 2006 [91]. In TWIMS an

oscillatory field is traditionally applied to a stack of ring electrodes which creates a traveling wave (TW) field that pushes the ions along. In addition, an out-of-phase radiofrequency (RF) field is co-applied to adjacent electrodes for radial ion confinement [92, 93].

The ion motion depends on the ratio of ion velocity to the speed of the applied TW [94]. Accordingly, there are three general ion motion behaviors possible. If the ions have very high mobilities they 'ride' the wave and travel at the traveling wave speed and exit the device unseparated. If the ion mobility is much lower than the speed of the wave, the waves have little effect on them as waves pass underneath the ions causing slight axial displacement [94]. At intermediate TW speeds, ions separate based on their mobilities. Ions of higher mobilities (i.e. more compact structures) will move with the wave most of the time, while ions of lower mobilities (i.e. bulkier structures) will more often be rolled over by a wave and fall into the preceding traveling wave. The more rollover events ions experience, the slower they move through the device, giving rise to separations based on their mobilities. A representative apparatus that utilizes the TWIMS separation principle as well as the separation steps of a TWIMS analyzer are shown below in Fig. 17.4 [69].

Since the obtained resolving power increases separation path length, recent attempts to increase the achievable resolution with TWIMS have involved the construction of a

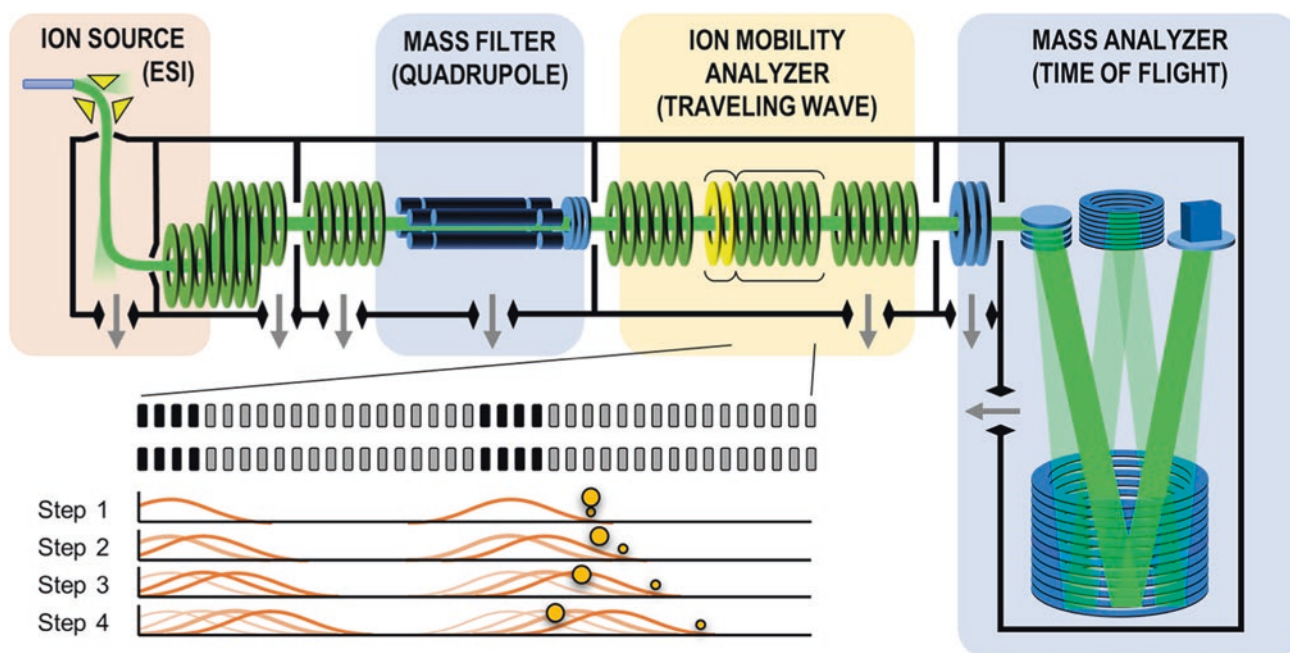


Fig. 17.4 An electrodynamic drift tube (TWIMS) arrangement similar to that described by Giles and co-workers. In both arrangements, hypothetical time courses are shown to illustrate the temporal separation of smaller

and larger collision cross section ions. (Source: [69] <https://pubs.acs.org/doi/10.1021/ac504720m>. Further permissions related to this specific material should be directed to the American Chemical Society (ACS))

cyclic ion path device where the drift path length can be increased by multiple passes through the cyclic path [95, 96]. However, this approach ultimately limits the separable range of mobilities, due to the fact that as the number of cycles increases the fastest ions start to approach the tail of the slowest, thus limiting the range of ion mobilities that can be cycled simultaneously.

Similar to DTIMS, one can obtain CCS information when using a TWIMS device. However, in contrast to DTIMS, in TWIMS the relationship between the drift time t and mobility K is non-linear, thus requiring calibration to determine collision cross sections [69, 97, 98].

17.4.5 Structures for Lossless Ion Manipulations (SLIM) Ion Mobility Spectrometry

A variant of the TWIMS approach based on a technology known as Structures for Lossless Ion Manipulations (SLIM) was introduced by Richard D. Smith and co-workers in 2012 [99, 100]. SLIM provides a novel paradigm for efficient, complex, and extended gas phase ion manipulations. SLIM devices seek to utilize common printed circuit board manufacturing processes to produce ion trapping devices capable

of transmitting ions very efficiently over long distances [101, 102]. A wide variety of printed electrode designs have successfully been used for this purpose. Initial prototypes developed at the laboratory were based on the electrode structure shown in Fig. 17.5a. In this design, a constant, high voltage DC field is used to drive ions from one end of the device to the other, similar to the process used in DTIMS. A key difference is that a superimposed RF field applied to the “rung” electrodes of the DC ladder serves to confine the ions between the two circuit boards, resulting in essentially lossless transmission of the ions from one end to the other. A stagnant bath gas maintained at a pressure of 0.1–5 torr provides the collisions with analyte ions necessary to achieve a mobility-based separation. Later designs of the SLIM technology have sought to separate the applied RF and DC potentials in order to simplify electrical requirements for operating the device (see Fig. 17.5b). This shift in electrode design was also accompanied by a move from using a constant DC field to drive the ions to using travelling waves (TW) of DC or AC voltages applied to a repeating pattern of electrodes within the device. In this way, ions can be pushed/pulled through the device using repulsive/attractive potentials without requiring a high voltage driving potential as shown in Fig. 17.5c. However, as described in the case of TWIMS above, when the speed of the travelling wave is

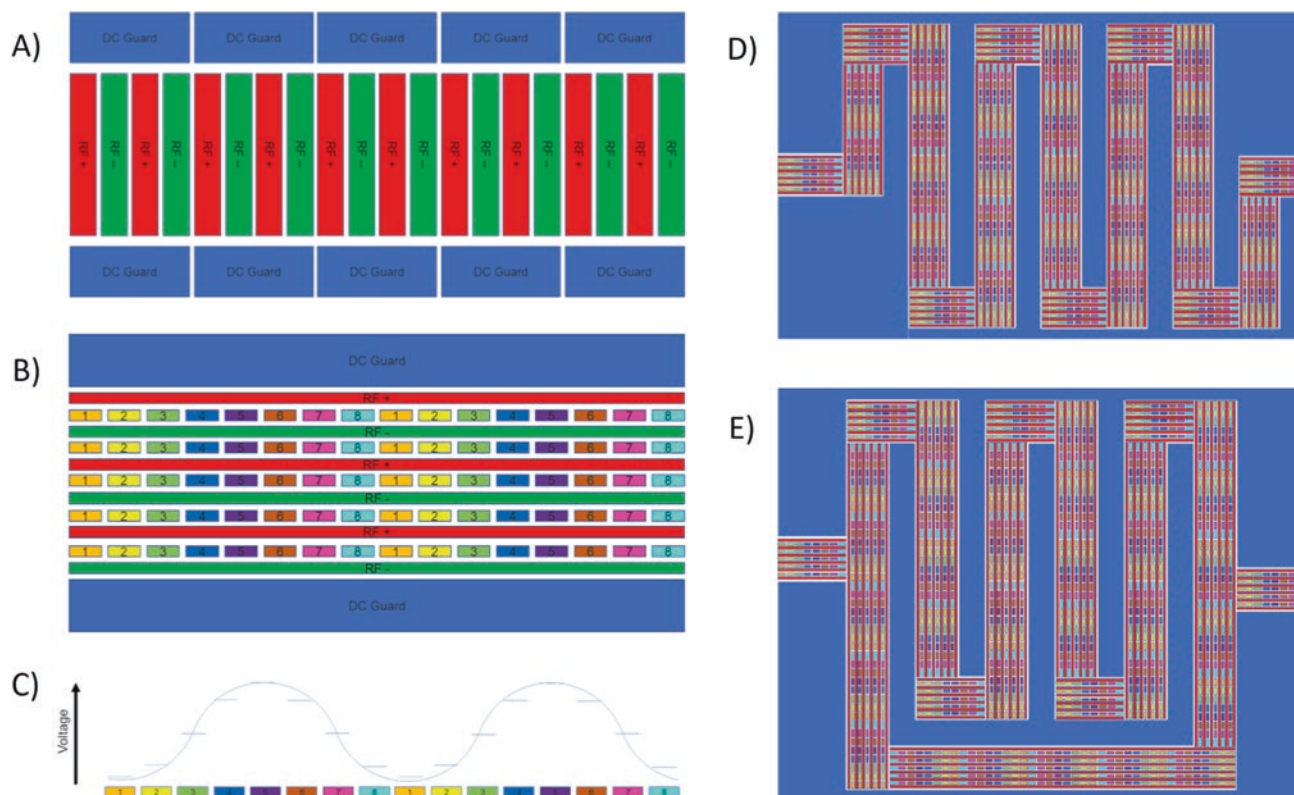


Fig. 17.5 (a) DC SLIM electrode design. (b) Travelling wave SLIM electrode design. (c) potential energy plot showing how a travelling wave is generated, (d) serpentine path SLIM device, and (e) cyclic path SLIM device for multipass separations

lower than the lowest mobility analyte to be separated, no mobility separation is achieved because all ions, regardless of their mobilities, are able to “surf” the travelling wave and move together as a packet through the device. But, when the speed of the applied waveform is greater than the highest mobility of the analytes to be separated, ions will undergo enough collisions with the bath gas such that they roll over the travelling wave peaks.

A number of other advantages to the travelling wave approach have been touted. It is particularly useful when coupling additional ion optics or ion sources upstream of the ion mobility device because they no longer need to be floated at a high electrical potential as they do for drift tube or early DC-based SLIM devices. A second advantage is that the path length of the device is no longer limited by the high voltage potential applied. With a travelling wave implementation, the mobility separation pathlength is theoretically unlimited. It also allows for one to change the direction of the separation path at will by interfacing electrode segments at a right angle to one another. This arrangement causes ions to turn the corner and coupling multiple turns together results in a serpentine path that significantly increases the distance travelled by the ions within a given linear form factor (see Fig. 17.5d). The combined attributes of flexible design, low voltage requirements and lossless ion transmission through multiple turns for the TW-SLIM module provides a basis for SLIM devices capable of achieving much greater IM resolution via greatly extended ion path lengths which typically feature compact serpentine designs [101, 103–105]. Separation pathlength can be further extended by creating a cyclical design as shown in Fig. 17.5e which allows ions to traverse the separation path multiple times before exiting the device for detection. Using this method, mobility separations with a pathlength over a kilometer in length have been performed by scientists at Pacific Northwest National Laboratory [104]. These extended SLIM path lengths have produced mobility resolving powers of over 1500. Another capability afforded by travelling wave SLIM is the ability to freeze the separation at any point in time by stalling the travelling wave. In this way, ion packets become parked at a certain position along the separation path, trapped between the repulsive electrical barrier created by the travelling wave peak potentials. These trapped ion packets can then be analyzed in a somewhat time-independent manner. To date, SLIM mobility devices have exclusively been coupled to time of flight analyzers because this is the only type of mass analyzer with an acquisition rate high enough (≥ 5 kHz) to collect multiple mass spectral scans across the millisecond wide mobility peaks as they exit the device without slowing down the separation. This allows for full resolution IMS-MS data to be collected from a single mobility separation which occurs on typical timescales of 10's of milliseconds to 10's of seconds.

The stalled travelling wave procedure also leads to another SLIM technique known as Compression Ration Ion Mobility Programming, or CRIMP, whereby one moving wave approaches a second stalled wave that only travels forward every 2, 10, 100, or more periods of the first wave [106, 107]. In this way, ion mobility peak compression can be achieved. For example, if a mobility separation is performed over a first travelling wave region such that a 10 ms wide, mobility separated peak is created. A second travelling wave region which has a speed that is 10% of the first would result in the mobility peak being transferred into ten times fewer wave troughs. By then increasing the speed of the second travelling to match that of the first travelling wave once all the ions have been transferred to the second region, the peak can be ejected from the device with 1 ms peak width, potentially resulting in a 10 \times improvement in signal to noise ratio.

Several other ion processing devices based on the SLIM technology have also been introduced. These include the ion elevator, ion escalator, SLIM switch, and flat funnel to name a few. A more exhaustive list of SLIM-based devices can be found in references [108–113]. These SLIM components enable a number of unique modes of operation that can greatly enhance the capabilities of the mass spectrometer to which it is attached, but their description is beyond the scope of this report.

17.5 Ion Mobility in Glycan Profiling of Biological Drugs

Because of the previously mentioned challenges with current methods for glycan analysis of biological drugs, research groups have started investigating ion mobility as a potential solution to some of these problems. One challenge for which ion mobility is especially well-suited is the separation of isobaric molecules and glycan isomers. Several groups have used different forms of IM-MS to demonstrate separation of the isobaric glycans cellopentaose, maltopentaose, and branched mannopentaose, whose structures are shown in Fig. 17.6a. Li et al. used TWIMS to separate these glycans [114], and Deng et al. used DTIMS and SLIM-MS to achieve separation [103]. In all cases, the glycans were separated to some degree, demonstrating ion mobility's ability to differentiate between isobaric glycans. In the SLIM-MS analysis by Deng et al., additional features were also observed, revealing the added potential for ion mobility to differentiate conformers of each glycan type [103]. Our group at MOBILion Systems Inc. is working on commercializing the previously discussed SLIM technology and has also recently demonstrated the separation power of SLIM-MS for these components, as shown in Fig. 17.6b for the individual pentasaccharides, and Fig. 17.6c for a mixture of the three pentasaccharides. These, along with the previously mentioned

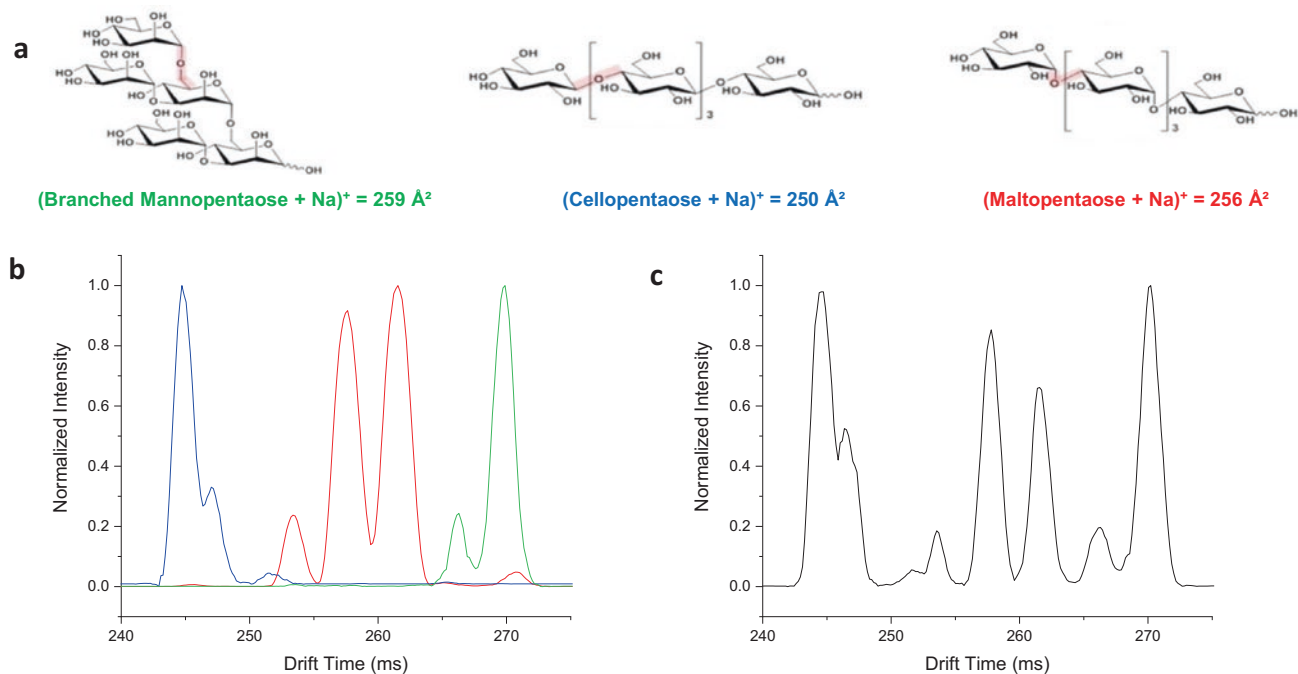


Fig. 17.6 (a) Structures of three isobaric pentasaccharides (branched mannopentaose—green, cellopentaose—blue, and maltopentaose—red), used for analyses with corresponding CCS values (REF). (b)

SLIM-MS combined mobiligram of overlaid individual pentasaccharide mobiligrams. (c) SLIM-MS mobiligram of the mixture of the three pentasaccharides

SLIM-MS analysis, were carried out with no LC separation prior to the ion mobility separation. This again demonstrates the potential for ion mobility to not only separate isobaric glycans, but to also separate different conformational isomers of each glycan. The separation of glycan isomers has also been demonstrated recently with the use of cyclic traveling wave ion mobility (cIM) by Li et al., where they were able to separate out isomers for numerous monosaccharides and disaccharides, including glucose, maltose, trehalose, and isomaltose. Ion mobility's ability to fully separate and distinguish glycan isomers could greatly aid pharmaceutical companies in more fully characterizing the glycan profile of biological drugs. This could further lead to the development of drugs with more specificity, less toxicity, higher efficacy, and reduced side effects, due to the fact that different isomer profiles may have large effects on these attributes. Because of the drug's specificity, the selection of participants who enter clinical trials could be much more accurate (patient stratification), allowing for a much higher success rate in clinical trials. Also, a much more comprehensive glycan profile would provide companies better protection for their drugs against biosimilars, in that specific isomeric production and characterization would need to be replicated. From a regulation point of view, this would also increase confidence that biosimilars generated are in fact biologically equivalent to the original starting material. Our group has also analyzed glycan standards commonly used in the pharmaceutical industry including a High Mannose Glycan Test

Standard and a Dextran Ladder standard (Waters Corporation, Milford, MA) [25, 26]. The high mannose test standard is used to test system suitability for monitoring high mannose structures in biopharmaceuticals manufacturing [26] and the dextran ladder is used as a calibration standard for more accurate assignment of glycan peaks [25]. SLIM-MS was used to determine whether or not ion mobility could provide mannose isomer separation in a higher throughput manner than the industry standard 34-min HILIC method [26]. Figure 17.7a shows the overlaid extracted ion mobiligram (EIM) of all mannose components in the high mannose test standard and Fig. 17.7b shows the separated EIMs for each mannose component. When employing ion mobility in the form of SLIM-MS, again, with no LC separation, we were not only able to fully separate previously identified isomers seen in the HILIC method but were also able to fully resolve additional isomers not detected via HILIC. This again demonstrates ion mobility's potential in this field to produce more comprehensive data sets in a much higher throughput manner than current LC methods, as this data was collected with just a 5-min run time. In analyzing the dextran ladder sample, we sought to compare the throughput of a SLIM-MS ion mobility-based approach with no LC separation to that of the typical 42-min HILIC method [25]. As shown in Fig. 17.8, full separation of ten different glucose units (5–14) was achieved in only 5 min. This provides eight times higher throughput for this sample compared to current methods and again demonstrates ion mobility's potential to not only

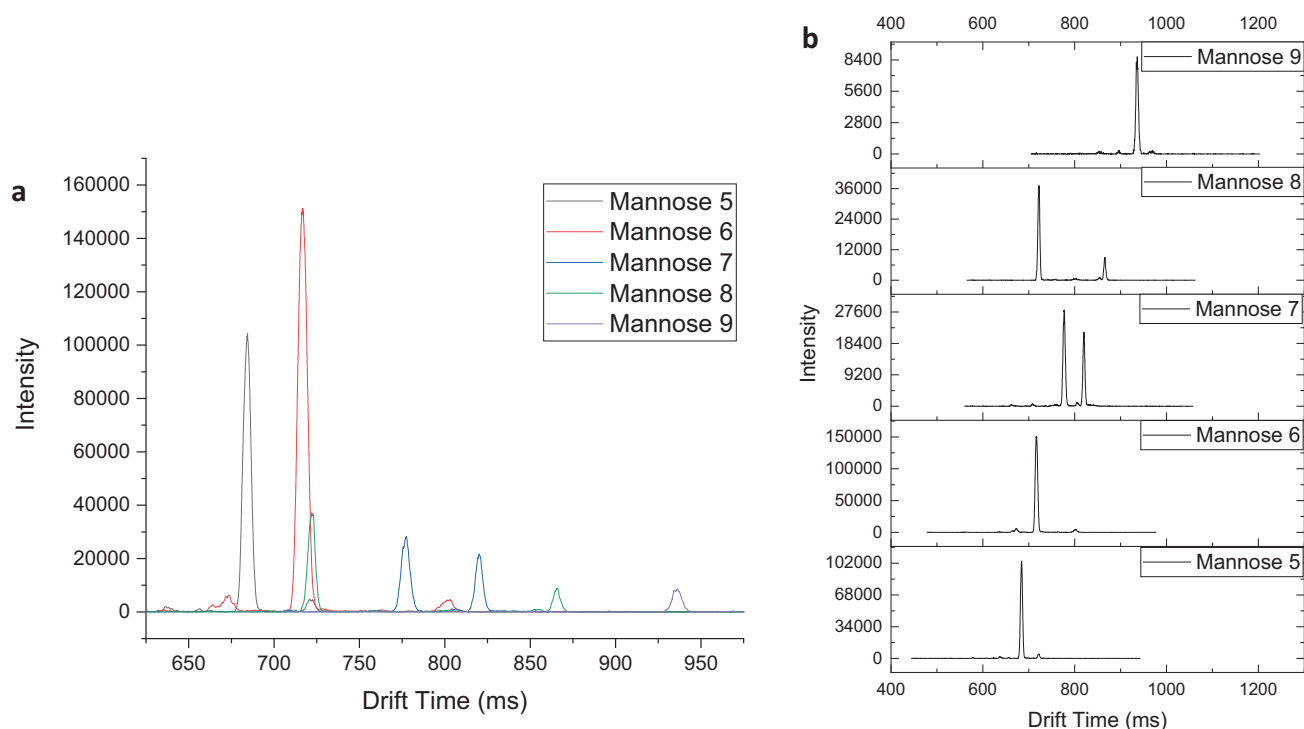
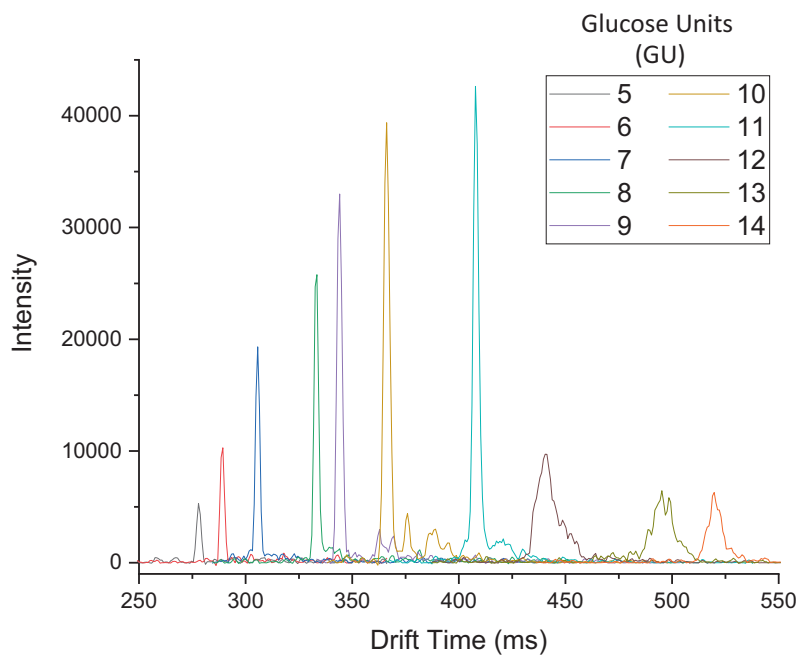


Fig. 17.7 (a) SLIM-MS overlaid extracted ion mobiligram (EIM) of all mannose components in the high mannose test standard. (b) Individual EIM's of each mannose component in the high mannose test standard

Fig. 17.8 SLIM-MS overlaid extracted ion mobiligram (EIM) of glucose unites (GU) 5 through 14 present in the dextran ladder standard



provide isomer separation, but also significantly increase sample throughput.

Another glycomics application for which ion mobility has recently been demonstrated is in the analysis of N-released glycans. Harvey et al. investigated the use of TWIMS to separate N-glycans from contaminated samples and to obtain

isomeric separation of the N-glycans. They concluded that the TWIMS method was excellent for separating the N-glycans from other contaminants present in the sample, as well as providing some isomeric resolution [115]. Damen et al. used TWIMS as well to measure lot-to-lot heterogeneity in the N-glycosylation profile of the therapeutic monoclonal

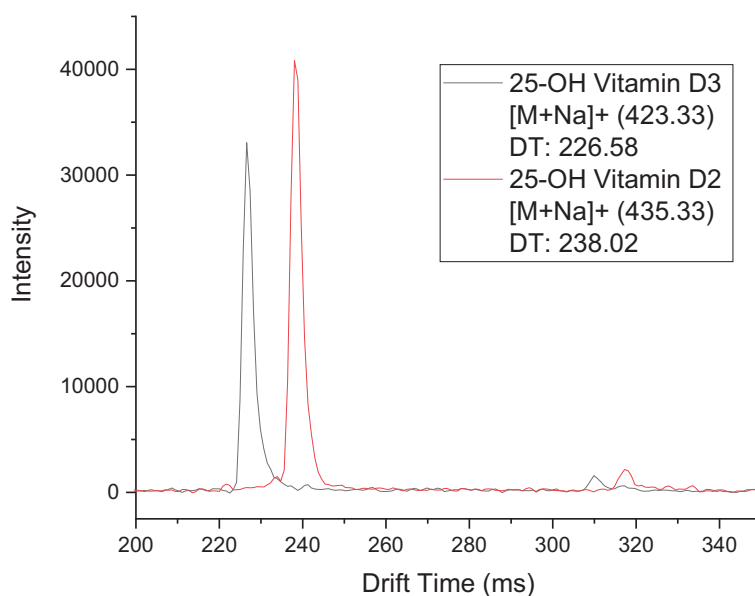
antibody trastuzumab [116]. In this study they were able to develop a high throughput method that distinguished lot-to-lot heterogeneity in trastuzumab at both the intact and reduced levels, as well as obtaining structural information for the glycans [116]. Other groups have also investigated N-glycosylation of biological drugs and antibody drug conjugates using ion mobility technologies [117, 118]. As shown by these studies, there is a significant interest in the implementation of ion mobility for glycosylation monitoring of biological drugs owing to the many demonstrated benefits of the technology. These benefits include isomeric resolution, separation of contaminants, and high throughput analyses. This leads to high throughput comprehensive data sets that can, as previously mentioned, lead to better drugs, higher success rates in clinical trials, and protection against or validation of biosimilars.

17.6 Ion Mobility in Clinical (Vitamin D) Analysis

There are few studies to date evaluating ion mobility analysis of vitamin D metabolites. This could be due to regulatory barriers present within the field and therefore anticipated hurdles present for introducing new technologies into a clinical laboratory setting. Despite these hurdles, we believe that ion mobility presents a viable solution to many of the previously mentioned challenges including resolution of the 25-hydroxyvitamin D₂ and 25-hydroxyvitamin D₃ vitamin metabolites, resolution of other isobaric metabolites, including the C3 epimers of the previously mentioned metabolites, and finally in increased throughput. MOBILion has performed an initial study focused on 25-hydroxyvitamin D₂

and 25-hydroxyvitamin D₃ using SLIM-MS to determine its suitability for analyzing these specific vitamin D metabolites. As shown in Fig. 17.9, full baseline separation of these samples was achieved with no LC. Two peaks are present for each metabolite, both of which are fully resolved. These likely correspond to the most stable “closed” and “open” conformations of the molecules [119]. These results demonstrate ion mobility’s potential for providing extremely precise analyses with more informative results, with each analyte being described by a characteristic drift time. In addition to this analysis performed by our group, Chouinard et al. recently used ion mobility to identify the C3 epimers of 25-hydroxyvitamin D₂ and 25-hydroxyvitamin D₃ from their original metabolites. They were also able to separate the “open” vs “closed” conformation of the 25-hydroxyvitamin D₂ and 25-hydroxyvitamin D₃ metabolites. In addition, they determined that the C3 epimers of both metabolites overlap in drift time with the closed conformations. However, because the original metabolites have an additional open conformation present that the C3 epimers do not have, the open conformation can be used to quantify the amount of 25-hydroxyvitamin D₂ or 25-hydroxyvitamin D₃ present without suffering from interference with the C3 epimer. In addition to this enhanced analytical capability, another benefit is the speed of analysis, as the ion mobility experiments were performed in approximately 60 ms [119]. With the higher specificity provided by ion mobility analyses, more accurate diagnoses can be made, leading to better diagnoses and treatments and the potential prevention of the life-threatening diseases associated with vitamin D deficiency. Because of this, the implementation of ion mobility technologies in the clinical industry is anticipated to grow significantly in the coming years.

Fig. 17.9 SLIM-MS overlaid extracted ion mobiligram (EIM) of 25-hydroxyvitamin D₂ and 25-hydroxyvitamin D₃



17.7 Hurdles of Ion Mobility Adoption in the Pharmaceutical and Clinical Fields

Despite the analytical capabilities demonstrated by the various ion mobility techniques, their adoption into pharmaceutical and clinical workflows has been relatively slow. There are a number of issues which have hindered wide-spread acceptance of this technique. Broadly speaking, these hurdles fall into five different categories, presented here in order of perceived importance:

- 1) Marginal Value Addition to Existing LC-MS Workflows
- 2) Reduction in MS sensitivity
- 3) Lack of Software Tools for Data Processing and Visualization
- 4) Ease of use
- 5) High Instrumentation Cost

17.7.1 Marginal Value Addition to Existing LC-MS Workflows

For any new technology to be accepted, the value it provides to the application must first be clearly demonstrated and understood. In the case of ion mobility, the value of adding a size-based separation technique to a workflow which already incorporates a mass-based separation is not always immediately obvious. Key data provided by ion mobility measurements include CCS measurement, biomolecule classification, separation of isobaric interferences, and reduction of background signals. Proper application of one or more of these data features to an analysis requires a deep understanding of both the application and the technology. However, early method development for IMS-MS applications often results in a lack of actionable data. In some cases, additional separation is provided simply as a means to increase the peak capacity and/or reduce the chemical background without establishing useful correlation, e.g. collision cross section, to the analytes of interest. In cases where collision cross sections are measurable, often reference values for the analytes are unavailable and computational methods of estimation lack the necessary level of accuracy.

17.7.2 Reduction in MS Sensitivity

Some of the commercially available IMS-MS systems result in a significant reduction in ion signal that is transmitted on to the mass spectrometer. Perhaps the best example of this would be the original Thermo FAIMS interface, which is

often reported as having <10% transmission for a given ion of interest [120]. This means that in the best case, sensitivity for a given analyte is often reduced by an order of magnitude. A second version of the Thermo FAIMS interface, the FAIMS Pro, sought to address this issue through a redesign that resulted in up to 50% transmission of target ions. In any situation where the researcher is forced to choose between increased sensitivity and increased specificity, a trade-off study must be performed to determine which approach actually results in improved S/N for the analyte(s) of interest at concentrations relevant to the application. Some of the newer ion mobility techniques such as TIMS and TWIMS promise increased specificity without the deleterious loss of signal intensity which is often characteristic of other approaches.

17.7.3 Lack of Software Tools for Data Processing and Visualization

Another barrier to adoption of ion mobility technologies in the pharmaceutical and clinical fields has been the lack of software tools suitable for visualizing and processing the often highly multidimensional data sets generated by LC-IMS-MS instrumentation. Programs for analyzing these 4-dimensional data sets (retention time, mobility, m/z, intensity) are still relatively uncommon, with proprietary vendor packages often being the only accessible option without the user developing custom code. However, there is an effort within the third party mass spectrometry processing software arena to support 4-dimensional data, with perhaps the most notable successes being the Skyline project (<https://skyline.ms/project/home/software/Skyline/begin.view>) and open source code developed at PNNL (<https://www.pnnl.gov/>). Looking forward, further software development to support expansion of existing LC-MS workflows to include the mobility axis will be an important focus area necessary for enabling researchers to harness the true analytical power of IMS-MS.

17.7.4 Ease of Use

LC-MS instrumentation is often the most complex instrumentation found in pharmaceutical and clinical laboratories. By taking instrumentation that requires highly skilled operators and adding an additional technological component, the result is often an analytical tool that only a handful of people in the world are capable of effectively using. This presents significant challenges in training personnel and developing methods for novel applications. Instrumentation vendors must make focused efforts to drive operational complexity out of their products if these instruments are to be successful within these fields.

17.7.5 Instrumentation Cost

Like the ease of use issue raised above, LC-MS instrumentation ranks among the most expensive analytical tools within pharma and clinical laboratories. Coupling ion mobility into this analytical workflow further exacerbates the issue by adding more cost to the commercial instrumentation. For researchers seeking to enhance the capabilities of their labs, it is often difficult to argue in favor of a \$500k-\$1M IMS-MS system when the value of such a system has not been clearly demonstrated.

17.8 Concluding Remarks

The pharmaceutical and clinical industries are two fields in which high throughput, accurate, reproducible analyses are an absolute requirement. Advances in analytical technologies have improved capabilities in these areas with the implementation of LC-MS technologies, however there is still significant room for improvement. As discussed here, one way in which pharmaceutical and clinical analyses could be improved is through the addition of ion mobility technology. Ion mobility provides separation of isobaric molecules that may be crucial in a diagnostic test, or in identifying two different drugs. It also has the ability to provide an additional point measurement (CCS) based on the shape/size of the molecule which provides additional molecular identification confirmation, as well as additional structural information. Even with the significant advantages of ion mobility in these fields, adoption will not be easy as there are many hurdles that still need to be overcome. Ion mobility vendors are presented with the challenge of developing a low cost, easy to use system in which results are easily interpretable and there is no sacrifice in sensitivity or any other area. When this happens, and ion mobility is routinely implemented in these fields, it will revolutionize what is currently classified as the gold standard and state of the art technology and revolutionize data quality in these industries.

References

1. Regenstein, M., & Andres, E. (2012). *Results from the National Survey of Independent and Community Clinical Laboratories*. Washington, DC: Department of Health Policy at the George Washington University School of Public Health and Health Services.
2. Lloyd, I., & Shimmings, A. (2017). *Pharma R&D annual review 2017*. London: Informa UK Ltd.
3. Pharmaceutical Research and Manufacturers of America (PhRMA). (2015). *Pharmaceutical profile, 2015*. Washington, DC: Pharmaceutical Research and Manufacturers of America (PhRMA).
4. Fournier, J. (2015). A review of glycan analysis requirements.
5. Food, U., & U.S. Department of Health and Human Services. (2012). Guidance for industry: Q11 development and manufacture of drug substances.
6. O'Flaherty, R., Trbojević-Akmačić, I., Greville, G., Rudd, P. M., & Lauc, G. (2018). The sweet spot for biologics: Recent advances in characterization of biotherapeutic glycoproteins. *Expert Review of Proteomics*, 15(1), 13–29.
7. Thobhani, S., Yuen, C.-T., Bailey, M. J., & Jones, C. J. G. (2008). Identification and quantification of N-linked oligosaccharides released from glycoproteins: an inter-laboratory study. *Glycobiology*, 19(3), 201–211.
8. Melmer, M., Stangler, T., Premstaller, A., & Lindner, W. (2011). Comparison of hydrophilic-interaction, reversed-phase and porous graphitic carbon chromatography for glycan analysis. *Journal of Chromatography. A*, 1218(1), 118–123.
9. Jensen, P. H., Myslasing, S., Højrup, P., & Jensen, O. N. (2013). Glycopeptide enrichment for MALDI-TOF mass spectrometry analysis by hydrophilic interaction liquid chromatography solid phase extraction (HILIC SPE). In *Mass spectrometry of glycoproteins* (pp. 131–144). New York, NY: Springer.
10. Melmer, M., Stangler, T., Schiefermeier, M., Brunner, W., Toll, H., Rupprechter, A., et al. (2010). HILIC analysis of fluorescence-labeled N-glycans from recombinant biopharmaceuticals. *Analytical and Bioanalytical Chemistry*, 398(2), 905–914.
11. Shang, T. Q., Saati, A., Toler, K. N., Mo, J., Li, H., Matlosz, T., et al. (2014). Development and application of a robust N-glycan profiling method for heightened characterization of monoclonal antibodies and related glycoproteins. *Journal of Pharmaceutical Sciences*, 103(7), 1967–1978.
12. Zhang, Z., Pan, H., & Chen, X. (2009). Mass spectrometry for structural characterization of therapeutic antibodies. *Mass Spectrometry Reviews*, 28(1), 147–176.
13. Bongers, J., Devincentis, J., Fu, J., Huang, P., Kirkley, D. H., Leister, K., et al. (2011). Characterization of glycosylation sites for a recombinant IgG1 monoclonal antibody and a CTLA4-Ig fusion protein by liquid chromatography–mass spectrometry peptide mapping. *Journal of Chromatography. A*, 1218(45), 8140–8149.
14. Gong, B., Cukan, M., Fisher, R., Li, H., Stadheim, T. A., & Gerngross, T. (2009). Characterization of N-linked glycosylation on recombinant glycoproteins produced in *Pichia pastoris* using ESI-MS and MALDI-TOF. In *Glycomics* (pp. 213–223). New York, NY: Springer.
15. Dotz, V., Haselberg, R., Shubhakar, A., Kozak, R. P., Falck, D., Rombouts, Y., et al. (2015). Mass spectrometry for glycosylation analysis of biopharmaceuticals. *TrAC Trends in Analytical Chemistry*, 73, 1–9.
16. Little, M. J., Paquette, D. M., & Roos, P. K. J. E. (2006). Electrophoresis of pharmaceutical proteins: Status quo. *Electrophoresis*, 27(12), 2477–2485.
17. Staub, A., Guillaume, D., Schappler, J., Veuthey, J.-L., Rudaz, S., & analysis, b. (2011). Intact protein analysis in the biopharmaceutical field. *Journal of Pharmaceutical and Biomedical Analysis*, 55(4), 810–822.
18. Lu, G., Crieffield, C. L., Gattu, S., Veltri, L. M., & Holland, L. A. (2018). Capillary electrophoresis separations of glycans. *Chemical Reviews*, 118(17), 7867–7885.
19. Szabo, Z., Guttman, A., Bones, J., & Karger, B. L. (2011). Rapid high-resolution characterization of functionally important monoclonal antibody N-glycans by capillary electrophoresis. *Analytical Chemistry*, 83(13), 5329–5336.
20. Zhong, X., Chen, Z., Snovidia, S., Liu, Y., Rogers, J. C., & Li, L. (2015). Capillary electrophoresis-electrospray ionization-mass spectrometry for quantitative analysis of glycans labeled with multiplex carbonyl-reactive tandem mass tags. *Analytical Chemistry*, 87(13), 6527–6534.

21. Landers, J. P. (1996). *Handbook of capillary electrophoresis*. Boca Raton, FL: CRC press.
22. Grossman, P. D., & Colburn, J. C. (2012). *Capillary electrophoresis: Theory and practice*. New York, NY: Academic Press.
23. Balaguer, E., & Neusüss, C. (2006). Glycoprotein characterization combining intact protein and glycan analysis by capillary electrophoresis-electrospray ionization-mass spectrometry. *Analytical Chemistry*, 78(15), 5384–5393.
24. Jayo, R. G., Thaysen-Andersen, M., Lindenburg, P. W., Haselberg, R., Hankemeier, T., Ramautar, R., et al. (2014). Simple capillary electrophoresis–mass spectrometry method for complex glycan analysis using a flow-through microvial interface. *Analytical Chemistry*, 86(13), 6479–6486.
25. Matthew, A., Lauber, M. F. M., Brousmiche, D. W., & Koza, S. M. (2015). Robustness of RapiFluor-MS N-glycan sample preparations and glycan BEH amide HILIC chromatographic separations. In *Water application notes: Glycans*.
26. McCall, S. A., Lauber, M. A., Koza, S. M., & Chambers, E. E. *Profiling released high mannose and complex N-glycan structures from monoclonal antibodies using optimized hydrophilic interaction chromatography*.
27. Forrest, K. Y., & Stuhldreher, W. L. (2011). Prevalence and correlates of vitamin D deficiency in US adults. *Nutrition Research*, 31(1), 48–54.
28. Holick, M. F., & Chen, T. C. (2008). Vitamin D deficiency: A worldwide problem with health consequences. *The American Journal of Clinical Nutrition*, 87(4), 1080s–1086s.
29. Holick, M. F. (2007). Vitamin D deficiency. *The New England Journal of Medicine*, 357(3), 266–281.
30. Brot, C., Vestergaard, P., Koltzoff, N., Gram, J., Hermann, A. P., & Sørensen, O. H. (2001). Vitamin D status and its adequacy in healthy Danish perimenopausal women: Relationships to dietary intake, sun exposure and serum parathyroid hormone. *The British Journal of Nutrition*, 86(S1), S97–S103.
31. Holick, M. F. (2006). Resurrection of vitamin D deficiency and rickets. *The Journal of Clinical Investigation*, 116(8), 2062–2072.
32. Chen, H., McCoy, L. F., Schleicher, R. L., & Pfeiffer, C. M. (2008). Measurement of 25-hydroxyvitamin D3 (25OHD3) and 25-hydroxyvitamin D2 (25OHD2) in human serum using liquid chromatography-tandem mass spectrometry and its comparison to a radioimmunoassay method. *Clinica Chimica Acta*, 391(1–2), 6–12.
33. Holick, M., Matsuoka, L., & Wortsman, J. (1989). Age, vitamin D, and solar ultraviolet. *Lancet*, 334(8671), 1104–1105.
34. Reid, I. R., Bolland, M. J., & Grey, A. (2014). Effects of vitamin D supplements on bone mineral density: A systematic review and meta-analysis. *Lancet*, 383(9912), 146–155.
35. Tai, S. S.-C., Bedner, M., & Phinney, K. W. (2010). Development of a candidate reference measurement procedure for the determination of 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 in human serum using isotope-dilution liquid chromatography– tandem mass spectrometry. *Analytical Chemistry*, 82(5), 1942–1948.
36. America, L.C.o. (2018). *Vitamin D, 25-hydroxy test, in test: 081950*.
37. Diagnostics, Q. (2018). *Vitamin D, 25-hydroxy, total, immunoassay*.
38. Diagnostics, Q. (2018). *Vitamin D deficiency and toxicity: Laboratory support of diagnosis and management*. Available from: https://www.questdiagnostics.com/testcenter/testguide.action?dc=CF_VitaminD#Table_4.
39. Chindarkarova, B., & Kazak, M. (2010). Quantitative analysis of 25-OH-D2 and 25-OH-D3 in plasma using LC-MS/MS.
40. Jarvis, M., McClure, E., Leigh, D., Blake, D., & Koerner, P. (2012). Measurement of 25-OH-vitamin D3 and 3-Epi-25-OHVitamin D3 by LC/MS/MS.
41. Armenta, S., Alcalá, M., & Blanco, M. (2011). A review of recent, unconventional applications of ion mobility spectrometry (IMS). *Analytica Chimica Acta*, 703(2), 114–123.
42. Eiceman, G. A. (2002). Ion-mobility spectrometry as a fast monitor of chemical composition. *TrAC Trends in Analytical Chemistry*, 21(4), 259–275.
43. Márquez-Sillero, I., Aguilera-Herrador, E., Cárdenas, S., & Valcárcel, M. (2011). Ion-mobility spectrometry for environmental analysis. *TrAC Trends in Analytical Chemistry*, 30(5), 677–690.
44. Ewing, R. G., Atkinson, D. A., Eiceman, G. A., & Ewing, G. J. (2001). A critical review of ion mobility spectrometry for the detection of explosives and explosive related compounds. *Talanta*, 54(3), 515–529.
45. Mäkinen, M., Nousiainen, M., & Sillanpää, M. (2011). Ion spectrometric detection technologies for ultra-traces of explosives: A review. *Mass Spectrometry Reviews*, 30(5), 940–973.
46. Tam, M., & Hill, H. H. (2004). Secondary electrospray ionization-ion mobility spectrometry for explosive vapor detection. *Analytical Chemistry*, 76(10), 2741–2747.
47. Kolakowski, B. M., & Mester, Z. (2007). Review of applications of high-field asymmetric waveform ion mobility spectrometry (FAIMS) and differential mobility spectrometry (DMS). *Analyst*, 132(9), 842–864.
48. Mäkinen, M. A., Anttalainen, O. A., & Sillanpää, M. E. (2010). *Ion mobility spectrometry and its applications in detection of chemical warfare agents*. ACS Publications.
49. Kaplan, K., & Hill Jr., H. (2011). *Ion mobility spectrometry-mass spectrometry*. Boca Raton, FL: CRC Press.
50. O'Donnell, R. M., Sun, X., & Harrington, P. B. (2008). Pharmaceutical applications of ion mobility spectrometry. *TrAC Trends in Analytical Chemistry*, 27(1), 44–53.
51. Jurneczko, E., & Barran, P. E. J. A. (2011). How useful is ion mobility mass spectrometry for structural biology? The relationship between protein crystal structures and their collision cross sections in the gas phase. *Analyst*, 136(1), 20–28.
52. McLean, J. A., Ruotolo, B. T., Gillig, K. J., & Russell, D. H. (2005). Ion mobility–mass spectrometry: a new paradigm for proteomics. *International Journal of Mass Spectrometry*, 240(3), 301–315.
53. Uetrecht, C., Rose, R. J., van Duijn, E., Lorenzen, K., & Heck, A. J. (2010). Ion mobility mass spectrometry of proteins and protein assemblies. *Chemical Society Reviews*, 39(5), 1633–1655.
54. Baker, E. S., Livesay, E. A., Orton, D. J., Moore, R. J., Danielson III, W. F., Prior, D. C., et al. (2010). An LC-IMS-MS platform providing increased dynamic range for high-throughput proteomic studies. *Journal of Proteome Research*, 9(2), 997–1006.
55. Sowell, R. A., Koeniger, S. L., Valentine, S. J., Moon, M. H., & Clemmer, D. E. (2004). Nanoflow LC/IMS-MS and LC/IMS-CID/MS of protein mixtures. *Journal of the American Society for Mass Spectrometry*, 15(9), 1341–1353.
56. Zheng, X., Wojcik, R., Zhang, X., Ibrahim, Y. M., Burnum-Johnson, K. E., Orton, D. J., et al. (2017). Coupling front-end separations, ion mobility spectrometry, and mass spectrometry for enhanced multidimensional biological and environmental analyses. *Annual Review of Analytical Chemistry (Palo Alto, California)*, 10, 71–92.
57. McAfee Jr., K. B., Sipler, D., & Edelson, D. (1967). Mobilities and reactions of ions in argon. *Physical Review*, 160(1), 130.
58. Koeniger, S. L., Merenbloom, S. I., Valentine, S. J., Jarrold, M. F., Udseth, H. R., Smith, R. D., et al. (2006). An IMS–IMS Analogue of MS–MS. *Analytical Chemistry*, 78(12), 4161–4174.
59. Wytenbach, T., Kemper, P. R., & Bowers, M. T. (2001). Design of a new electrospray ion mobility mass spectrometer. *International Journal of Mass Spectrometry*, 212(1–3), 13–23.
60. Borsdorf, H., Mayer, T., Zarejousheghani, M., & Eiceman, G. A. (2011). Recent developments in ion mobility spectrometry. *Applied Spectroscopy Reviews*, 46(6), 472–521.

61. Kanu, A. B., Dwivedi, P., Tam, M., Matz, L., & Hill Jr., H. H. (2008). Ion mobility–mass spectrometry. *Journal of Mass Spectrometry*, 43(1), 1–22.
62. Asbury, G. R., & Hill, H. H. (2000). Using different drift gases to change separation factors (α) in ion mobility spectrometry. *Analytical Chemistry*, 72(3), 580–584.
63. Clemmer, D. E., & Jarrold, M. F. (1997). Ion mobility measurements and their applications to clusters and biomolecules. *Journal of Mass Spectrometry*, 32(6), 577–592.
64. Howdle, M. D., Eckers, C., Laures, A. M.-F., & Creaser, C. S. (2010). The effect of drift gas on the separation of active pharmaceutical ingredients and impurities by ion mobility–mass spectrometry. *Journal of Mass Spectrometry*, 298(1–3), 72–77.
65. McKnight, L., McAfee, K., & Sipler, D. J. (1967). Low-field drift velocities and reactions of nitrogen ions in nitrogen. *Physics Review*, 164(1), 62.
66. von Helden, G., Wyttenbach, T., & Bowers, M. T. (1995). Inclusion of a MALDI ion source in the ion chromatography technique: Conformational information on polymer and biomolecular ions. *International Journal of Mass Spectrometry and Ion Processes*, 146, 349–364.
67. Eiceman, G., & Stone, J. (2004). *Peer reviewed: Ion mobility spectrometers in national defense*. ACS Publications.
68. Stow, S. M., Causon, T. J., Zheng, X., Kurulugama, R. T., Mairinger, T., May, J. C., et al. (2017). An interlaboratory evaluation of drift tube ion mobility–mass spectrometry collision cross section measurements. *Analytical Chemistry*, 89(17), 9048–9055.
69. May, J. C., & McLean, J. A. (2015). Ion mobility–mass spectrometry: Time-dispersive instrumentation. *Analytical Chemistry*, 87(3), 1422–1436.
70. Fernandez-Lima, F., Kaplan, D. A., Suetering, J., & Park, M. A. (2011). Gas-phase separation using a trapped ion mobility spectrometer. *International Journal for Ion Mobility Spectrometry*, 14(2–3), 93–98.
71. Michelmann, K., Silveira, J. A., Ridgeway, M. E., & Park, M. A. (2015). Fundamentals of trapped ion mobility spectrometry. *Journal of the American Society for Mass Spectrometry*, 26(1), 14–24.
72. Ridgeway, M. E., Lubeck, M., Jordens, J., Mann, M., & Park, M. A. (2018). Trapped ion mobility spectrometry: A short review. *International Journal of Mass Spectrometry*, 425, 22.
73. Hernandez, D. R., DeBord, J. D., Ridgeway, M. E., Kaplan, D. A., Park, M. A., & Fernandez-Lima, F. (2014). Ion dynamics in a trapped ion mobility spectrometer. *Analyst*, 139(8), 1913–1921.
74. Silveira, J. A., Ridgeway, M. E., Laukien, F. H., Mann, M., & Park, M. A. (2017). Parallel accumulation for 100% duty cycle trapped ion mobility–mass spectrometry. *International Journal of Mass Spectrometry*, 413, 168–175.
75. Silveira, J. A., Ridgeway, M. E., & Park, M. A. (2014). High resolution trapped ion mobility spectrometry of peptides. *Analytical Chemistry*, 86(12), 5624–5627.
76. Castellanos, A., Benigni, P., Hernandez, D., DeBord, J., Ridgeway, M., Park, M., et al. (2014). Fast screening of polycyclic aromatic hydrocarbons using trapped ion mobility spectrometry–mass spectrometry. *Analytical Methods*, 6(23), 9328–9332.
77. Dit Fouque, K. J., Moreno, J., Hegemann, J. D., Zirah, S., Rebuffat, S., & Fernandez-Lima, F. (2018). Identification of lasso peptide topologies using native nano-electrospray ionization–trapped ion mobility spectrometry–mass spectrometry. *Analytical Chemistry*, 90(8), 5139–5146.
78. Liu, F. C., Kirk, S. R., & Bleiholder, C. (2016). On the structural denaturation of biological analytes in trapped ion mobility spectrometry–mass spectrometry. *Analyst*, 141(12), 3722–3730.
79. Pu, Y., Ridgeway, M. E., Glaskin, R. S., Park, M. A., Costello, C. E., & Lin, C. (2016). Separation and identification of isomeric glycans by selected accumulation–trapped ion mobility spectrometry–electron activated dissociation tandem mass spectrometry. *Analytical Chemistry*, 88(7), 3440–3443.
80. Zietek, B. M., Mengerink, Y., Jordens, J., Somsen, G. W., Kool, J., & Honing, M. (2017). Adduct-ion formation in trapped ion mobility spectrometry as a potential tool for studying molecular structures and conformations. *International Journal for Ion Mobility Spectrometry*, 21, 1–14.
81. Guevremont, R. (2004). High-field asymmetric waveform ion mobility spectrometry: a new tool for mass spectrometry. *Journal of Chromatography. A*, 1058(1–2), 3–19.
82. Purves, R. W., & Guevremont, R. (1999). Electrospray ionization high-field asymmetric waveform ion mobility spectrometry–mass spectrometry. *Analytical Chemistry*, 71(13), 2346–2357.
83. Eiceman, G. A., Karpas, Z., & Hill Jr, H. H. (2013). *Ion mobility spectrometry*. Boca Raton, FL: CRC press.
84. Buryakov, I., Krylov, E., Nazarov, E., & Rasulev, U. K. (1993). A new method of separation of multi-atomic ions by mobility at atmospheric pressure using a high-frequency amplitude-asymmetric strong electric field. *International Journal of Mass Spectrometry and Ion Processes*, 128(3), 143–148.
85. Purves, R. W., Guevremont, R., Day, S., Pipich, C. W., & Matyjaszczyk, M. S. (1998). Mass spectrometric characterization of a high-field asymmetric waveform ion mobility spectrometer. *The Review of Scientific Instruments*, 69(12), 4094–4105.
86. Guevremont, R., Barnett, D. A., Purves, R. W., & Viehland, L. A. (2001). Calculation of ion mobilities from electrospray ionization high-field asymmetric waveform ion mobility spectrometry mass spectrometry. *The Journal of Chemical Physics*, 114(23), 10270–10277.
87. Guevremont, R., Ding, L., Ells, B., Barnett, D. A., & Purves, R. W. (2001). Atmospheric pressure ion trapping in a tandem FAIMS-FAIMS coupled to a TOFMS: Studies with electrospray generated gramicidin S ions. *Journal of the American Society for Mass Spectrometry*, 12(12), 1320–1330.
88. Barnett, D. A., Ells, B., Guevremont, R., & Purves, R. W. (1999). Separation of leucine and isoleucine by electrospray ionization–high field asymmetric waveform ion mobility spectrometry–mass spectrometry. *Journal of the American Society for Mass Spectrometry*, 10(12), 1279–1284.
89. Barnett, D. A., Purves, R. W., Ells, B., & Guevremont, R. (2000). Separation of o-, m- and p-phthalic acids by high-field asymmetric waveform ion mobility spectrometry (FAIMS) using mixed carrier gases. *Journal of Mass Spectrometry*, 35(8), 976–980.
90. Rorrer III, L., Prieto, M., & Yost, R. (2008). Evaluation of linear injection and orthogonal injection into Planr FAIMS-MS. In *56th ASMS Conference on Mass spectrometry and allied topics*, Denver, CO. Chicago, IL: ASMS.
91. Giles, K., Pringle, S. D., Worthington, K. R., Little, D., Wildgoose, J. L., & Bateman, R. H. (2004). Applications of a travelling wave-based radio-frequency-only stacked ring ion guide. *Rapid Communications in Mass Spectrometry*, 18(20), 2401–2414.
92. Giles, K., Wildgoose, J. L., Langridge, D. J., & Campuzano, I. (2010). A method for direct measurement of ion mobilities using a travelling wave ion guide. *International Journal of Mass Spectrometry*, 298(1–3), 10–16.
93. Pringle, S. D., Giles, K., Wildgoose, J. L., Williams, J. P., Slade, S. E., Thalassinou, K., et al. (2007). An investigation of the mobility separation of some peptide and protein ions using a new hybrid quadrupole/travelling wave IMS/oa-ToF instrument. *International Journal of Mass Spectrometry*, 261(1), 1–12.
94. Shvartsburg, A. A., & Smith, R. D. (2008). Fundamentals of traveling wave ion mobility spectrometry. *Analytical Chemistry*, 80(24), 9689–9699.
95. Giles, K., Wildgoose, J., Pringle, S., Garside, J., Carney, P., Nixon, P., et al. (2014). *62nd ASMS conference on mass Spectrometry and allied topics* (pp. 15–19). Chicago, IL: ASMS.

96. Giles, K., Wildgoose, J., Pringle, S., Langridge, D., Nixon, P., Garside, J., et al. (2015). *63rd ASMS conference on mass spectrometry and allied topics*. St. Louis, MO. Chicago, IL: ASMS.
97. Forsythe, J. G., Petrov, A. S., Walker, C. A., Allen, S. J., Pellissier, J. S., Bush, M. F., et al. (2015). Collision cross section calibrants for negative ion mode traveling wave ion mobility-mass spectrometry. *Analyst*, *140*(20), 6853–6861.
98. Lietz, C. B., Yu, Q., & Li, L. (2014). Large-scale collision cross-section profiling on a traveling wave ion mobility mass spectrometer. *Journal of the American Society for Mass Spectrometry*, *25*(12), 2009–2019.
99. Ibrahim, Y. M., Hamid, A. M., Deng, L., Garimella, S. V., Webb, I. K., Baker, E. S., et al. (2017). New frontiers for mass spectrometry based upon structures for lossless ion manipulations. *Analyst*, *142*(7), 1010–1021.
100. Tolmachev, A. V., Webb, I. K., Ibrahim, Y. M., Garimella, S. V. B., Zhang, X., Anderson, G. A., et al. (2014). Characterization of ion dynamics in structures for lossless ion manipulations. *Analytical Chemistry*, *86*(18), 9162–9168.
101. Hamid, A. M., Ibrahim, Y. M., Garimella, S. V., Webb, I. K., Deng, L., Chen, T.-C., et al. (2015). Characterization of traveling wave ion mobility separations in structures for lossless ion manipulations. *Analytical Chemistry*, *87*(22), 11301–11308.
102. Webb, I. K., Garimella, S. V., Tolmachev, A. V., Chen, T.-C., Zhang, X., Norheim, R. V., et al. (2014). Experimental evaluation and optimization of structures for lossless ion manipulations for ion mobility spectrometry with time-of-flight mass spectrometry. *Analytical Chemistry*, *86*(18), 9169–9176.
103. Deng, L., Ibrahim, Y. M., Baker, E. S., Aly, N. A., Hamid, A. M., Zhang, X., et al. (2016). Ion mobility separations of isomers based upon long path length structures for lossless ion manipulations combined with mass spectrometry. *ChemistrySelect*, *1*(10), 2396–2399.
104. Deng, L., Ibrahim, Y. M., Hamid, A. M., Garimella, S. V., Webb, I. K., Zheng, X., et al. (2016). Ultra-high resolution ion mobility separations utilizing traveling waves in a 13 m serpentine path length structures for lossless ion manipulations module. *Analytical Chemistry*, *88*(18), 8957–8964.
105. Hamid, A. M., Garimella, S. V., Ibrahim, Y. M., Deng, L., Zheng, X., Webb, I. K., et al. (2016). Achieving high resolution ion mobility separations using traveling waves in compact multiturn structures for lossless ion manipulations. *Analytical Chemistry*, *88*(18), 8949–8956.
106. Deng, L., Garimella, S. V. B., Hamid, A. M., Webb, I. K., Attah, I. K., Norheim, R. V., et al. (2017). Compression ratio ion mobility programming (CRIMP) accumulation and compression of billions of ions for ion mobility-mass Spectrometry using traveling waves in structures for lossless ion manipulations (SLIM). *Analytical Chemistry*, *89*(12), 6432–6439.
107. Garimella, S. V. B., Hamid, A. M., Deng, L., Ibrahim, Y. M., Webb, I. K., Baker, E. S., et al. (2016). Squeezing of ion populations and peaks in traveling wave ion mobility separations and structures for lossless ion manipulations using compression ratio ion mobility programming. *Analytical Chemistry*, *88*(23), 11877–11885.
108. Chen, T.-C., Ibrahim, Y. M., Webb, I. K., Garimella, S. V. B., Zhang, X., Hamid, A. M., et al. (2016). Mobility-selected ion trapping and enrichment using structures for lossless ion manipulations. *Analytical Chemistry*, *88*(3), 1728–1733.
109. Chen, T.-C., Webb, I. K., Prost, S. A., Harrer, M. B., Norheim, R. V., Tang, K., et al. (2015). Rectangular ion funnel: A new ion funnel Interface for structures for lossless ion manipulations. *Analytical Chemistry*, *87*(1), 716–722.
110. Deng, L., Ibrahim, Y. M., Garimella, S. V. B., Webb, I. K., Hamid, A. M., Norheim, R. V., et al. (2016). Greatly increasing trapped ion populations for mobility separations using traveling waves in structures for lossless ion manipulations. *Analytical Chemistry*, *88*(20), 10143–10150.
111. Garimella, S. V. B., Ibrahim, Y. M., Webb, I. K., Ipsen, A. B., Chen, T.-C., Tolmachev, A. V., et al. (2015). Ion manipulations in structures for lossless ion manipulations (SLIM): Computational evaluation of a 90° turn and a switch. *The Analyst*, *140*(20), 6845–6852.
112. Garimella, S. V. B., Webb, I. K., Prabhakaran, A., Attah, I. K., Ibrahim, Y. M., & Smith, R. D. (2017). Design of a TW-SLIM module for dual polarity confinement, transport, and reactions. *Journal of the American Society for Mass Spectrometry*, *28*(7), 1442–1449.
113. Webb, I. K., Garimella, S. V. B., Norheim, R. V., Baker, E. S., Ibrahim, Y. M., & Smith, R. D. (2016). A structures for lossless ion manipulations (SLIM) module for collision induced dissociation. *Journal of the American Society for Mass Spectrometry*, *27*(7), 1285–1288.
114. Li, H., Bendiak, B., Siems, W. F., Gang, D. R., & Jr, H. H. H. (2013). Carbohydrate structure characterization by tandem ion mobility mass spectrometry (IMMS) 2. *Analytical Chemistry*, *85*(5), 2760–2769.
115. Harvey, D. J., Scarff, C. A., Edgeworth, M., Struwe, W. B., Pagel, K., Thalassinou, K., et al. (2016). Travelling-wave ion mobility and negative ion fragmentation of high-mannose N-glycans. *Journal of Mass Spectrometry*, *51*(3), 219–235.
116. Damen, C. W., Chen, W., Chakraborty, A. B., van Oosterhout, M., Mazzeo, J. R., Gebler, J. C., et al. (2009). Electrospray ionization quadrupole ion-mobility time-of-flight mass spectrometry as a tool to distinguish the lot-to-lot heterogeneity in N-glycosylation profile of the therapeutic monoclonal antibody trastuzumab. *Journal of the American Society for Mass Spectrometry*, *20*(11), 2021–2033.
117. Marcoux, J., Champion, T., Colas, O., Wagner-Roussel, E., Corvaña, N., Van Dorsselaer, A., et al. (2015). Native mass spectrometry and ion mobility characterization of trastuzumab emtansine, a lysine-linked antibody drug conjugate. *Protein Science*, *24*(8), 1210–1223.
118. Pagel, K., & Harvey, D. J. (2013). Ion mobility–mass Spectrometry of complex carbohydrates: Collision cross sections of Sodiated N-linked Glycans. *Analytical Chemistry*, *85*(10), 5138–5145.
119. Chouinard, C. D., Cruzeiro, V. W. D., Beekman, C. R., Roitberg, A. E., & Yost, R. A. (2017). Investigating differences in gas-phase conformations of 25-hydroxyvitamin D3 sodiated epimers using ion mobility-mass spectrometry and theoretical modeling. *Journal of the American Society for Mass Spectrometry*, *28*(8), 1497–1505.
120. Bonislowski, A. (2018). Thermo Fisher gives FAIMS second try with new release.