Isomer Separation with Vocus Ion Mobility Spectrometry

Michael Kamrath, Felipe Lopez-Hilfiker
TOFWERK, Switzerland

Chemical ionization mass spectrometers reliably analyze VOCs in a variety of environments. However, when a compound is masked by either an isomeric or isobaric interference, this presents ambiguity in the analysis. Interferences introduce uncertainty in mass spectrum peak identification as there is no definitive measurement of the structures contributing to a mass spectrum peak. As an example, this is a common issue in flavor and fragrance chemistry where a single molecular formula (ion) may be present in several isomeric forms. Each ion is of importance since they uniquely contribute to the perceived flavor or fragrance. Figure 1 presents two vanillin isomers — methyl salicylate and methyl paraben.

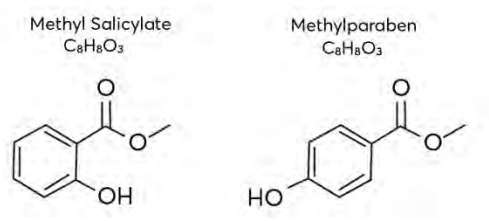

Figure 1. *The isomeric structures of methyl salicylate and methylparaben.*

To separate and differentiate between these isomers, mass spectrometry must be supplemented with another dimension of separation.

When distinct isomers need to be resolved, ion mobility spectrometry (IMS) is a technique which can be used in conjunction with mass spectrometry. IMS discriminates between ions that adopt structures which differ in their rotationally averaged collision cross sections (i.e., average molecular size). With Vocus IMS-MS, analytes are ionized by chemical ionization and the resulting ions are separated by their collision cross sections before mass analysis. This provides two-dimensional information—the mass-to-charge ratio and cross section-related IMS. This deconvolutes multiple isomeric or isobaric components present in a single mass spectrum peak.

Operation of the Vocus CI-IMS-TOF

Figure 2 presents a schematic of the TOFWERK Vocus CI-IMS-TOF. In this instrument, ions are generated by the Vocus ion source before they

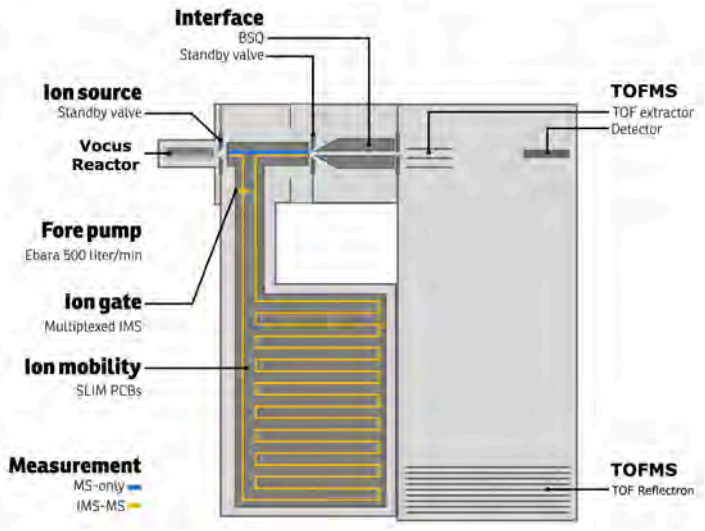

Figure 2. *Schematic representation of the TOFWERK Vocus IMS-TOF instrument. Both MS-only and IMS-MS measurements are possible as indicated by the blue and orange ion paths, respectively.*

are guided into the IMS separation chamber. The chamber is purged with an inert buffer gas (e.g., nitrogen, helium, etc.) and maintained at a pressure of 3.00 mbar. The ions are moved along a 9-meter serpentine path using technology developed at Pacific Northwest National Laboratory for lossless ion manipulations (SLIM) [1]. The movement of ions is controlled using weak electric fields. Ions with small collision cross sections travel through the buffer gas with less friction and arrive at the end of the drift region sooner than ions with larger collision cross sections. The total time an ion spends traveling through the IMS drift region is referred as the IMS drift time, which is related to a molecule's collision cross section.

the TOFWERK Vocus CI-IMS-TOF provides two modes of operation. The first mode bypasses the IMS, effectively allowing the user to perform MS-only measurements (blue path in Fig. 2). The second mode of operation directs ions through the IMS (orange path in Fig. 2) via an ion switch, producing 2-D data to deconvolute contributions from multiple isomers or isobars. IMS capabilities are easily activated on the Vocus CI-IMS-TOF when needed.

Isomer Separation With Ion Mobility

By analyzing the isomers methyl salicylate and methylparaben, we can demonstrate Vocus IMS separation. Since these compounds

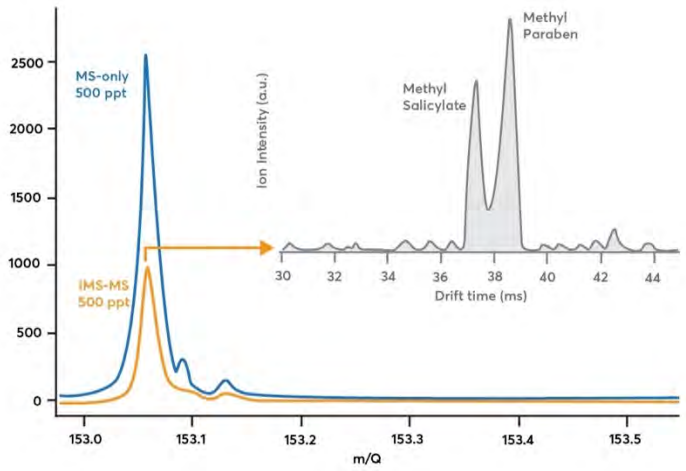

Figure 3. *Blue trace: mass spectrum obtained while sampling 500 ppt methyl salicylate and 500 ppt methylparaben in MS-only mode (blue path in Fig. 2). Orange trace: mass spectrum obtained while sampling the same methyl salicylate / methylparaben mixture in IMS-mode (orange path in Fig. 2). Gray trace: shows how the mass spectrum in orange is decomposed along the ion mobility dimension to reveal multiple components after a 90 second acquisition.*

.

have the same monoisotopic mass and only differ in the position of the hydroxyl group on the aromatic ring, they both develop the same mass spectrum peak at the same corresponding protonated mass (153.05 Th) in proton transfer reaction (PTR) chemistry. The blue trace in Figure 3 demonstrates this by experiment. This data was obtained by sampling an air mixture of methyl salicylate and methylparaben in MS-only mode at 500 ppt concentrations (blue path in Fig. 2, blue in Fig. 3). This data confirms the presence of at least one $C_8H_8O_3$ compound, but it does not indicate how many isomers contributed to the peak. This can only be accomplished by activating IMS analysis.

IMS mode activation allows for the individual components of a single mass peak to be separated. Once activated, ions are directed into the mobility region (orange path in Fig. 2) and a pseudorandom sequence (multiplexing) is applied to the ion gate, modulating the ion signal, and providing critical timing information to measure drift times. Due to ion modulation, ion losses are evident when comparing the IMS-MS mass spectrum (orange trace) to the MS-only mass spectrum (blue trace) in Figure 3. Based on the 50% duty cycle of the gate sequence, the theoretical expectation is to measure 50% of the ion signal in IMS-MS mode relative to MS-only mode. Experimentally, 40% of the ion signal is measured in IMS-MS mode

compared to MS-only mode—a 10% difference between theory and experiment—indicating that a small percentage of ions are lost either in the drift region or at the ion gate. Nevertheless, this tradeoff in ion intensity makes it possible to generate drift time distributions for a wide range of mass-to-charge ratios. Through multiplexing, the duty cycle is 50%, compared to <1% in non-multiplexing approaches. The gray trace in Figure 3 represents the drift time distribution for 153.05 Th. Averaged over 90 seconds, this trace shows successful separation of methyl salicylate from the methylparaben. This enables the presence of either species to be easily confirmed and for their relative concentrations to be monitored with time.

To better understand how the S:N ratio of IMS data degrades at concentrations below 500 ppt, we use the same dataset presented in Figure 3 and display the IMS drift distribution for the first carbon isotope ($^{13}C_1C_7H_9O_3^+$, m/Q = 154.05 Th) of the methyl salicylate and methylparaben mixture in Figure 4. This is useful because the first carbon isotope concentration is expected to be only 8% of the ^{12}C parents or 40 ppt for each compound. Aside from the lower concentration, the drift time distribution should be nearly identical to that presented for the ^{12}C parent compounds in Figure 3 (gray trace). The drift distribution

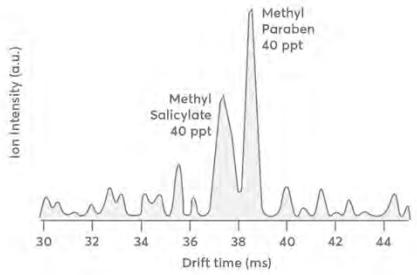

Figure 4. *Drift time distribution of the first carbon isotope (13C1C7H9O3+, m/Q 154.05) of methyl salicylate and methyl paraben sampled at 40 ppt each. The spectrum is qualitatively like that presented in Figure 3 except for the lower S:N ratio.*

presented in Figure 4 is qualitatively like that in Figure 3, however, the S:N ratio is clearly less favorable as we approach the instrument's LOD. This means that analytes having concentrations in the range of 40 ppt can be separated and detected with the Vocus CI-IMS-MS instrument but require longer averaging times.

Real-Time Monitoring of Isomer Populations

Although the data in Figures 3 and 4 were averaged over 90 seconds, typical drift times occur within a 20–150 ms timescale. Since mobility separations occur on such short timescales, it is possible to monitor isomer populations in real time. This is useful when monitoring processes where isomer ratios fluctuate within timescales < 60 seconds, which is a challenge for fast gas chromatography (GC) instruments.

Figure 5 presents results from an experiment demonstrating real-time measurement of isomer populations. Here a small amount of a methyl salicylate solution is injected into a liquid calibration system (LCS) and the instrument response is recorded. In this experiment, an initial spike of methyl salicylate signal is expected and then decays as it is flushed out of the system by the methylparaben solution. The maximum concentration of methylparaben is 2.5 ppb toward the end of the run when it is no longer diluted by the methyl salicylate injection.

The top panel in Figure 5 presents three IMS spectra "snapshots" taken at different times during the experiment, each averaged over 2.9 seconds. The green and red dots identify the mass spectra peaks associated with methyl salicylate and methylparaben, respectively. The relative intensities of these features change with time, which implies a corresponding change in the relative population of the two species. Changes in isomer populations can be monitored by integrating the individual IMS signals and dividing the measured ion current by the ratio of the integrated areas. This method of analysis was performed for the methyl salicylate and methylparaben IMS signals – the results are presented in the bottom panel of Figure 5. Here the methyl

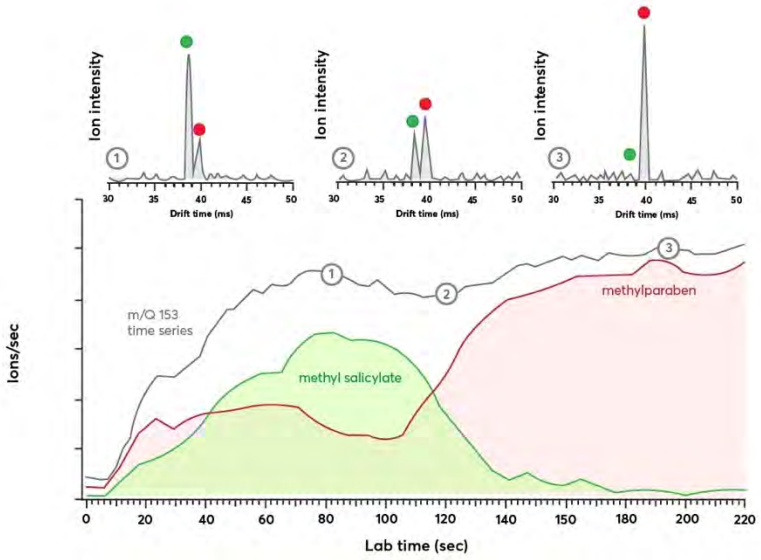

Figure 5. *Bottom panel: time series data for m/Q = 153 Th showing the evolution of the ion current with time (in gray). The IMS dimension reveals the transient response of the isomers methyl salicylate (in green) and methylparaben (in red). Top panel: Shows the IMS spectra recorded at three different time points during the m/Q = 153 Th time series as indicated by the numbers 1, 2, and 3. The IMS spectra were each averaged for 2.9 seconds and show a clear exchange of ion population from methyl salicylate to methylparaben.*

salicylate signal (green) rises quickly until it reaches a maximum signal at 80 seconds after which it decreases. The methylparaben signal (red) remains low until later in the run.

The m/Q Th channel presented in gray includes contributions from both methyl salicylate and methylparaben time series data. Here the signal quickly increases and then stabilizes after ~70 seconds. After this point, there is no clear indication that the isomer populations change. The labels "1", "2", and "3" along the time series trace correspond to the time points where the IMS spectra in the top panel were acquired as indicated by the matching labels.

While it may be possible to collect gas chromatography data like that presented in Figures 3 and 4, it would be challenging to reproduce the real-time data presented in Figure 5. The TOFWERK Vocus CI-IMS-TOF conducts isomer separations on a much faster timescale than GC, providing powerful utility for those needing fast analysis with isomer separation.

Reference

[1] https://www.pnnl.gov/available-technologies/structures-lossless-ion-manipulation-slim

Acknowledgment

MOBILion is the exclusive licensee of the SLIM technology for commercialization purposes. TOFWERK has been authorized to implement the proprietary technology in select products.

Contact

sales@tofwerk.com