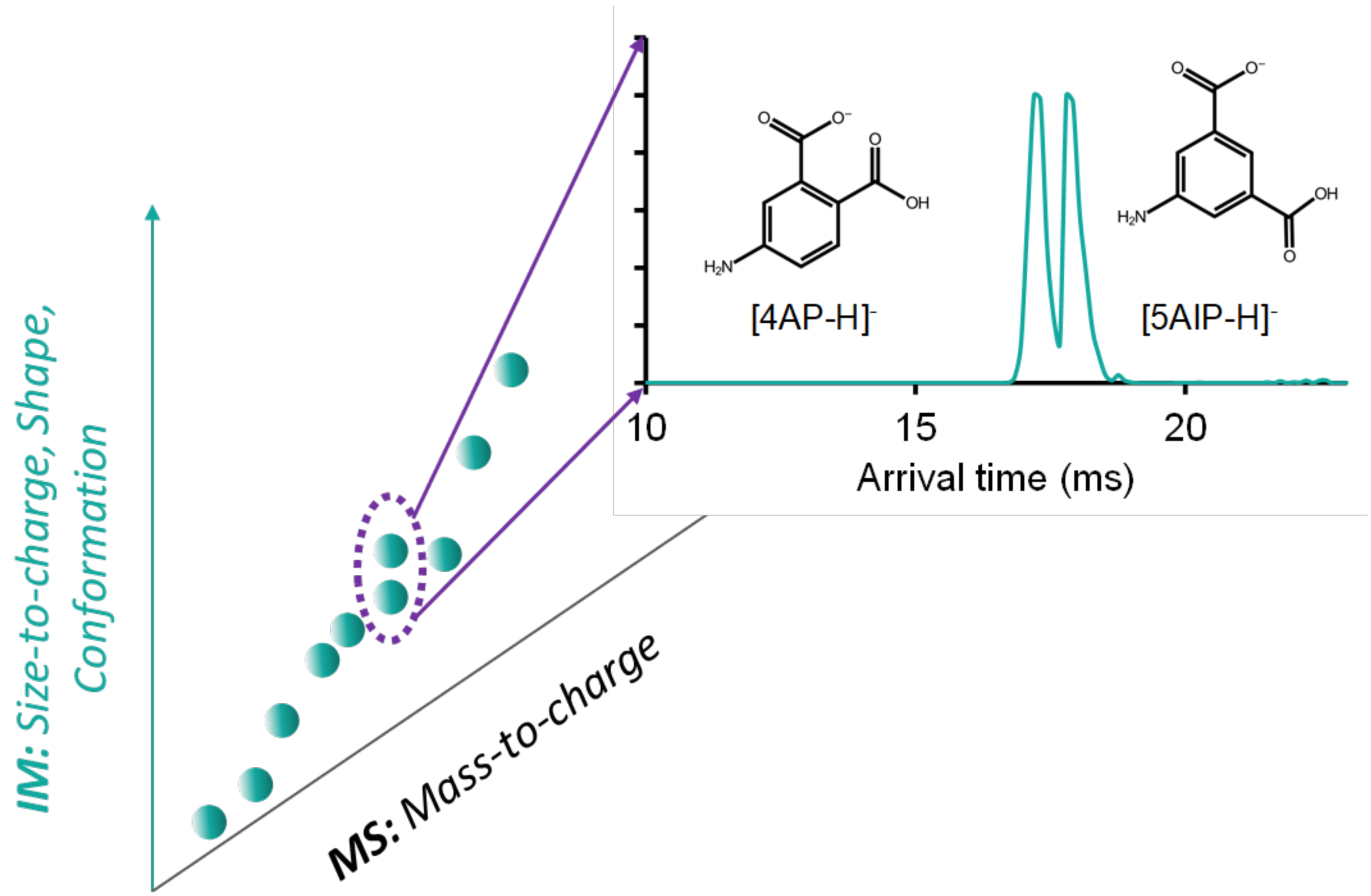
MobiliTraIN

When mass spectrometry alone is not enough: separation of isomers in the gas phase



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Appropriate for students aged

14-18 with interest in chemistry, biochemistry, environmental science, and related topics.

Length

Preparation & introduction: 10-15 minutes

Implementation: 15-45 minutes* (depending on extent of lab work and number of examples used)

Closing: 15-25 minutes

Hypothesis

Isomers are compounds with the same atomic composition (molecular formula), but different line formulae or different stereochemical formulae. Having different physical and/or chemical properties, the analysis of isomers is important as they have different roles and effects in complex systems. Fast separation of isomers based on differences in size and shape is one way to address their analysis.

Key vocabulary

Analytical chemistry, ion mobility, isomers, mass spectrometry, molecules.

Objectives

Upon completion of this exercise, your students will be able to:

- ✓ *Understand the difference between mass-to-charge and size-to-charge concepts*
- ✓ *Describe how isomers can be separated in the gas-phase using ion mobility spectrometry*
- ✓ *Understand why combining instrumental techniques is key for analysing complex samples*

Equipment and material

No	Material/equipment	Quantity	Role in the experiment
1	**Ion mobility-mass spectrometer**	**1**	**Instrument required for gas-phase separation of isomers.**
2	**Analytical balance**	**1**	**Accuracy to <0.1 mg desirable**
3	**Piston pipettes**	**1**	**For delivering accurate solvent volumes (e.g., 100-1000 µL)**
3	**Chemical standards**	**<5 mg**	**Compounds to be measured with IM-MS. Suggested examples included in the experiment instructions.**
4	**Solvents: LC-MS-grade**	**<50 mL**	**Necessary for dissolution of standards.**
4	**Molecular modelling**	**1**	**Optional: to illustrate differences in isomer structures in three dimensions.**

Introduction

The "**isomer problem**" presents a major challenge for many areas of research as it slows down the discovery of new molecules for life sciences, environmental chemistry and biology. One way analytical chemistry tries to address this is developing new separation technologies that can separate isomers quickly and efficiently.

Molecular mass spectrometry is the essential technology for identification of molecules in complex samples. Using the possibility to measure an accurate mass and determine the isotopologue distribution, two key pieces of information allow the molecular formula to be determined.

Isomers can play different roles in natural systems and the influence of anthropogenic chemical production has increased their prevalence enormously. Based on recent database estimates where hundreds of thousands of organic compounds are included, some 80-90% of organic formulas have known isomer forms. This "**isomer problem**" requires more than mass spectrometry alone to deliver accurate analytical results.

Ion mobility spectrometry provides a rapid separation of ions in the gas phase according to their size, shape and conformation in a millisecond timescale. For each ion, a characteristic mobility (K, $V\ m^2\ s^{-1}$) can be derived from measurements (**Figure 1**).

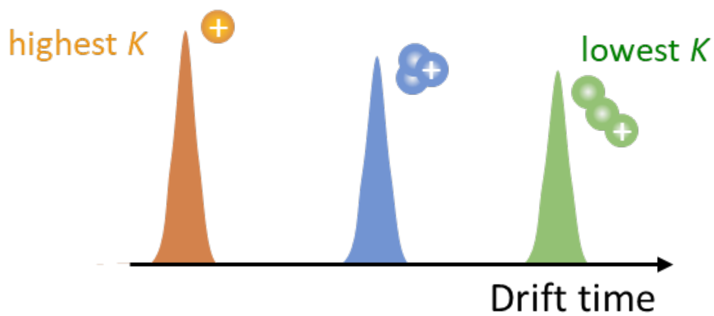


Figure 1. Illustration of an ion mobility separation of ions. The more compact (blue) structure has a higher mobility than the isomeric, more extended isomer (green).

Combining mass spectrometry and ion mobility separations in a single hybrid instrument (IM-MS) provides a means to analyse isomeric species without additional time spent on the analysis. This is briefly illustrated in **Figure 2**, whereby mass spectra of IM-separated peaks are acquired on extremely fast timescales (microseconds) providing comprehensive samples of the IM peaks (milliseconds). In this experiment, selected isomer examples are measured with IM-MS to illustrate the fundamental concept of ion mobility separation in comparison to molecular mass spectrometry.

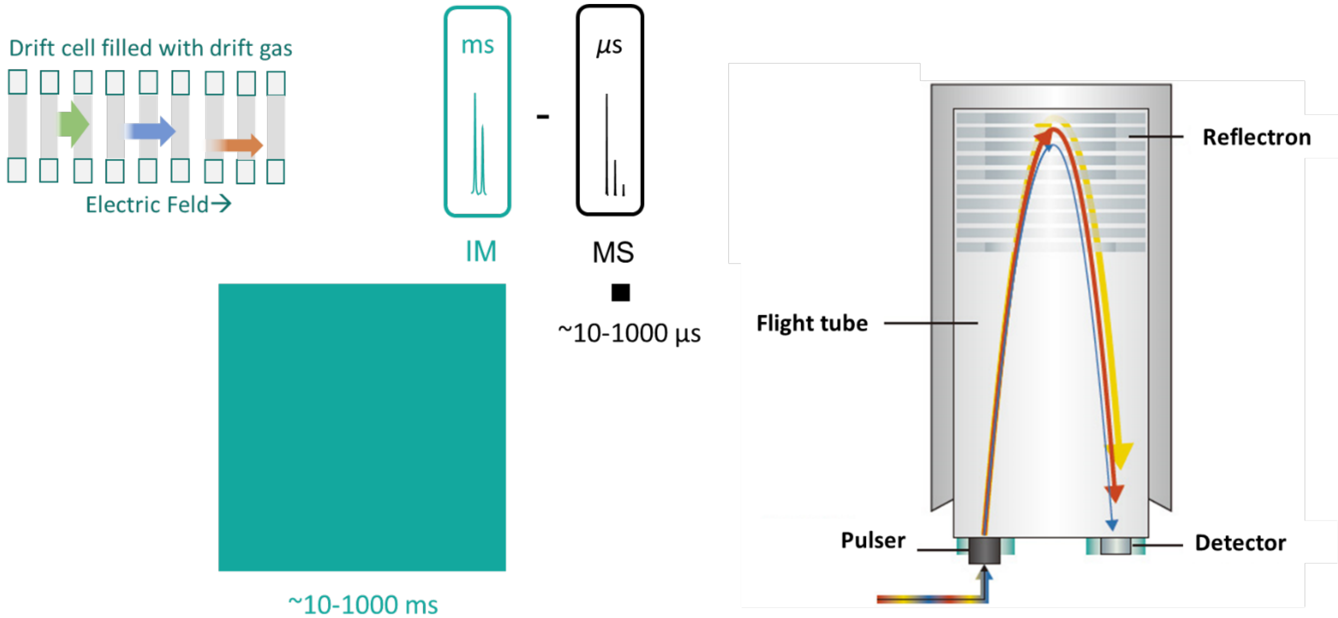


Figure 2. Illustration of the timescale of ion mobility and time-of-flight mass spectrometry separations. High-resolution time-of-flight mass spectrometry allows determination of accurate mass and the molecular formula of unknown molecules following separation with ion mobility.

Experiment instructions

General

- The range of isomers measured can be adapted according to the type of IM-MS instrument or the application focus of the laboratory used.
- While not included in this example, use of chromatographic separation prior to IM-MS will provide access to a wider range of isomer examples. This can be used when sufficient laboratory time is available for the exercise.
- One of several commercially available IM-MS instruments can be used for this exercise. Measurements of isomer examples should be evaluated by instructors in advance of the exercise.

Steps to follow

#1 Performing experiments in lab	Students meet with instructors in the lab and can be involved in the preparation of standards (time-permitting), and the measurements of isomers with IM-MS.
#2 Overview of theory and discussion of results	Presentation of topic in form of slides and discussion, followed by review of IM-MS results from isomer separations

Example system to study: isomers of aminophthalic acid in negative ionization mode. Two of these isomers (5-aminoisophthalic acid, 5-AIP and 4-aminophthalic acid, 4-AP) differ in their collision cross section (*CCS*) values by ~3.3%, allowing a moderate resolving power IM separation to yield an isomer separation (see DOI: 10.1039/d3cp01968c). A separation of 5-AIP and 4-AP achieved using drift tube IM-MS is shown in **Figure 3**.

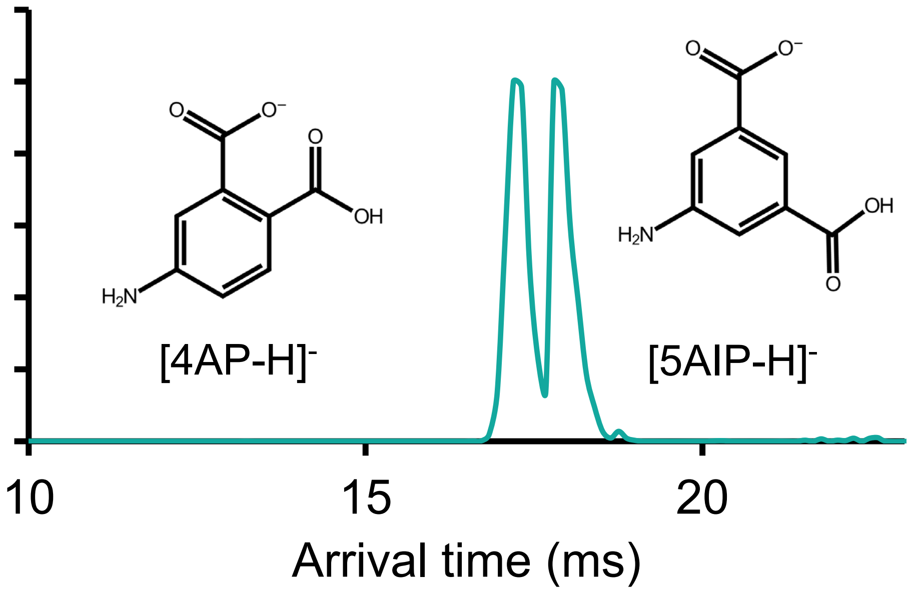


Figure 3. Drift tube IM-MS separation of deprotonated ions of 5-AP and 4-AIP.

Another suggested examples to consider for this exercise is carbohydrates (e.g. sodium adducts of raffinose and melezitose).

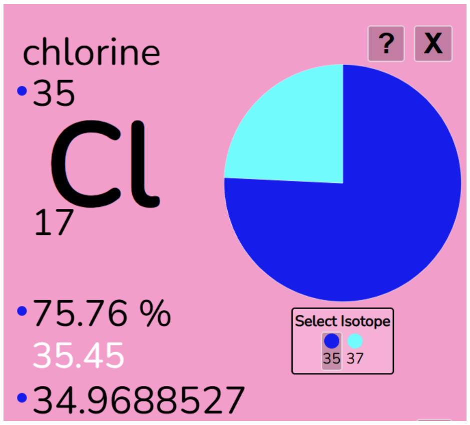
Safety measures

No	Risk	Safety measure
1	**Leaks occurring in liquid infusiong or chromatography system**	**Safety glasses, lab coat, gloves, paper towel**
2	**Exposure to chemical solvents during preparation of standards**	**Safety glasses, gloves, lab coat**

Evaluation

- A follow-up quiz using a suitable app (e.g., Kahoot) is used for the students to self-evaluate what they have learned. Examples of questions are provided below with correct answers underlined and boldened.

    1) What is the monoisotopic mass of chlorine gas (Cl_2)?



A: 70.90, B: 70, C: 52 or **D: 69.9377054**

Explanation: Monoisotopic mass = sum of the exact masses of the most abundant isotopes.

2) In which order will these ions be separated by ion mobility spectrometry?

A B C D

A: 1st: A, then B, C, D.; B: A, then C, B, D.; **C: A, then D, B, C**.; or D: A, then D, C, B.

Explanation: A has the highest mobility and C has the lowest mobility according to their large differences in mass-to-charge. B and D are isomers with intermediate mobilities. D is more compact than B.

3) Which analytical method delivers the fastest separation of molecules or molecular ions?

A: Ion mobility spectrometry; **B: Mass spectrometry**, C: Sedimentation, D: Chromatography

Explanation: Mass spectrometry must be the fastest dimension when used in combination with IM and/or MS to provide adequate data points per peak.